07 715272
EXPRESS MAIL LABEL NO. B59937585
DATE MAILED: June 14, 1991

MAIL ROOM
JUN 14 1991
29
PAT. & TRADEMARK OFF.

GENENTECH, INC.
460 Point San Bruno Boulevard, South San Francisco, CA 94080
(415) 266-1000

Docket No. 709

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

SIR:

Transmitted herewith for filing is the patent application of inventor(s): PAUL J. CARTER ET AL.

Title: IMMUNOGLOBULIN VARIANTS

CERTIFICATION UNDER 37 CFR §1.10

I hereby certify that this New Application and the documents referred to as enclosed herein are being deposited with the United States Postal Service on this date June 14, 1991, in an envelope bearing "Express Mail Post Office To Addressee" Mailing Label Number B59937585 addressed to: Patent Application, Honorable Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Carolyn R. Adler
(Name of person mailing paper)

Carolyn R. Adler
Signature

Enclosed are:

1. The papers required for filing date under CFR §1.53(b):
106 Pages of specification (including claims); 5 Sheets of drawings (_ formal / x informal)
2. x Declaration/Oath/Power of Attorney
3. __ Assignment of the invention to GENENTECH, INC.

4. Fee Calculation

CLAIMS AS FILED

	Number Filed	Number Extra	Rate	Basic Fee $630
Total Claims	16 - 20 =	*	x $20.00	630.
Indep. Claims	8 - 3 =	* 5	x $60.00	300.
__	Multiple dependent claim(s), if any		$200.00	

*If less than zero, enter "0".

7. __ Recording Assignment [$8.00] . . . . $
Total Fees Enclosed $930.00

8. Payment of Fees
x Charge Account No. 07-0630 in the amount of $__. A duplicate of this transmittal is attached.
9. x Authorization to Charge Additional Fees
The Commissioner is hereby authorized to charge any additional fees (or credit any overpayment) associated with this communication and which may be required under 37 CFR §1.16 or §1.17 to Account No. 07-0630. A duplicate sheet is attached.

10. __ Information Disclosure Statement
11. x Return Receipt Postcard

By: Carolyn R. Adler
Name: Carolyn R. Adler
Registration No. 32,324

Dated June 14, 1991

07 715272

FIGURE 1A: V_L DOMAIN

```
                     10        20        30        40        50
4D5         DIVMTQSHKFMSTSVGDRVSITCKASQDVNTAVAWYQQKPGHSPKLLIYSASFRYT
              |    |||| |      |   |                 ||          |||
HU4D5       DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLES
                                         ||||                |  |
HUVLκI      DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLES
                                   -----------               -------
                                      -------                 --
                                      VL-CDR1                VL-CDR2
```

```
              60        70         80       90       100
4D5         GVPDRFTGNRSGTDFTFTISSVQAEDLAVYYCQQHYTTPPTFGGGTKLEIKRA
               |  | |       |    | |  | |              |   |    |
HU4D5       GVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT
                     |                        |||| |
HUVLκI      GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT
                                            ---------
                                              ------
                                             VL-CDR3
```

07 715272

FIGURE 1B: V_H DOMAIN

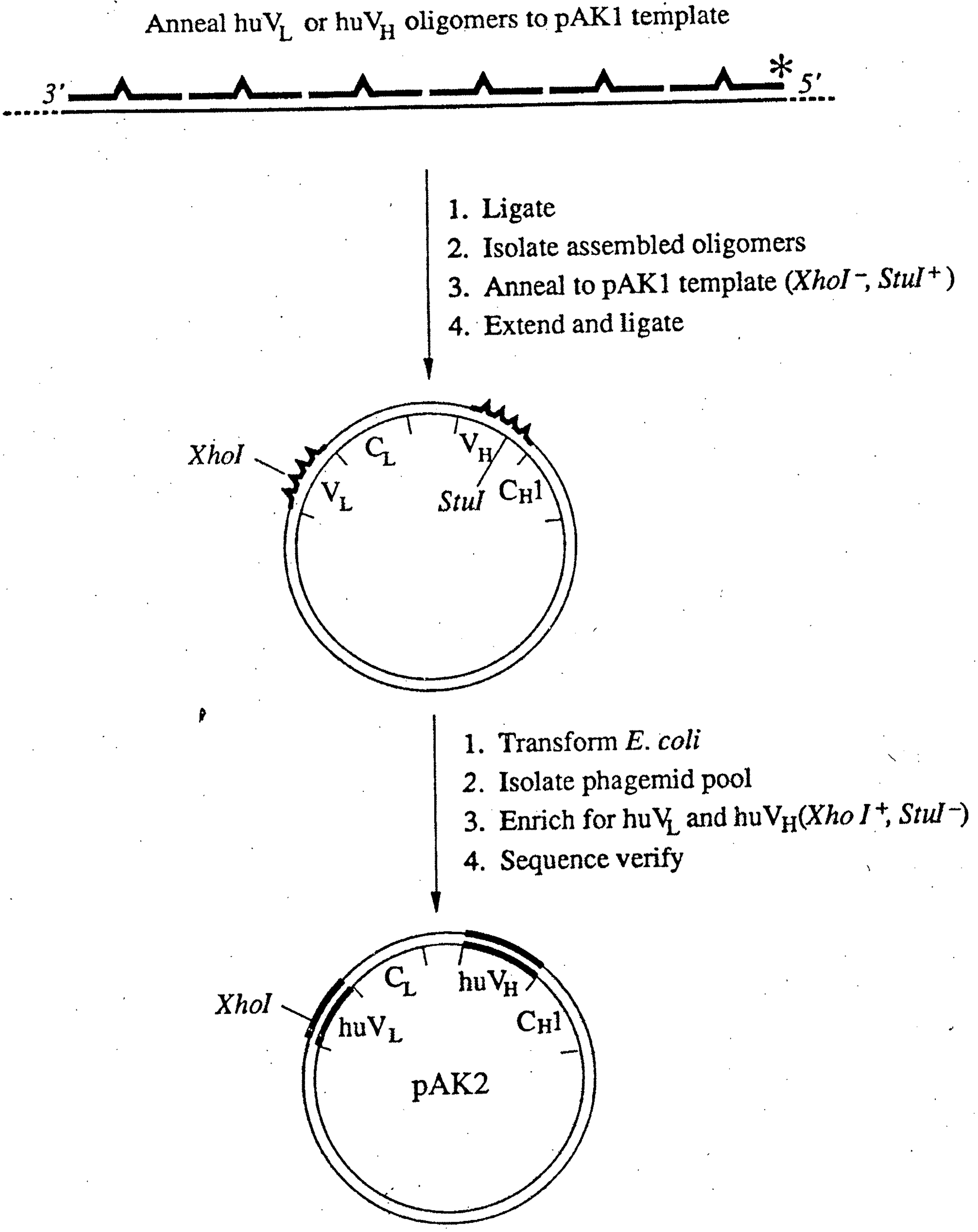
```
                    10        20        30        40        50  A
4D5         EVQLQQSGPELVKPGASLKLSCTASGFNIKDTYIHWVKQRPEQGLEWIGRIYPTN
                ||  ||  |  |  |   |              | | ||    ||
HU4D5       EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTN
                                       ||| ||||              | ||||
HUVHIII     EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENG
                                     ----------              ------
                                     ---------                 ----
                                      VH-CDR1               VH-CDR2


                60        70        80  ABC      90       100ABC
4D5         GYTRYDPKFQDKATITADTSSNTAYLQVSRLTSEDTAVYYCSRWGGDGFYAMDYW
                 ||||||||  |    |      ||| ||                    |
HU4D5       GYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVW
            || |            | |    |                 | || ||||||
HUVHIII     SDTYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARDRGGAVSYFDVW
            -----------                                -----------
                                                        ---------
                                                         VH-CDR3


                110
4D5         GQGASVTVSS
               ||
HU4D5       GQGTLVTVSS

HUVHIII     GQGTLVTVSS
```

FIGURE 2


Anneal huV$_L$ or huV$_H$ oligomers to pAK1 template
3'
*
5'
1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*XhoI*$^-$, *StuI*$^+$)
4. Extend and ligate
XhoI
C$_L$
V$_H$
V$_L$
StuI
C$_H$1
1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV$_L$ and huV$_H$(*Xho I*$^+$, *StuI*$^-$)
4. Sequence verify
XhoI
C$_L$
huV$_H$
huV$_L$
C$_H$1
pAK2

715272

FIGURE 3

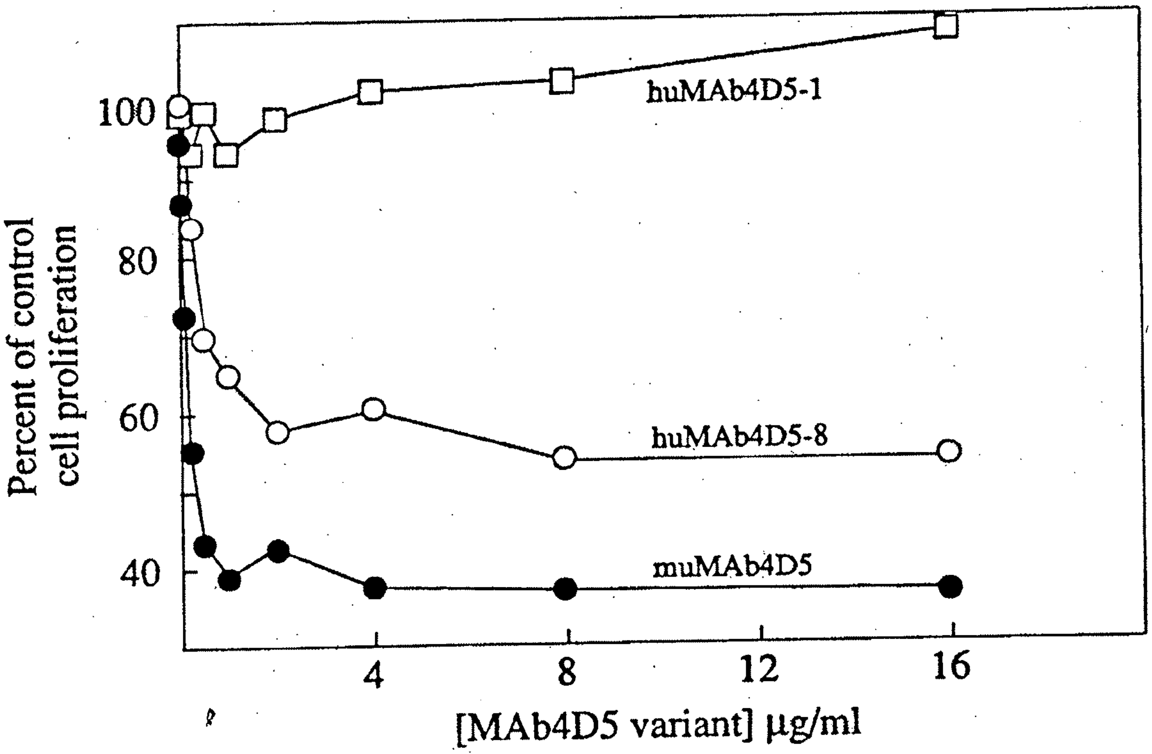
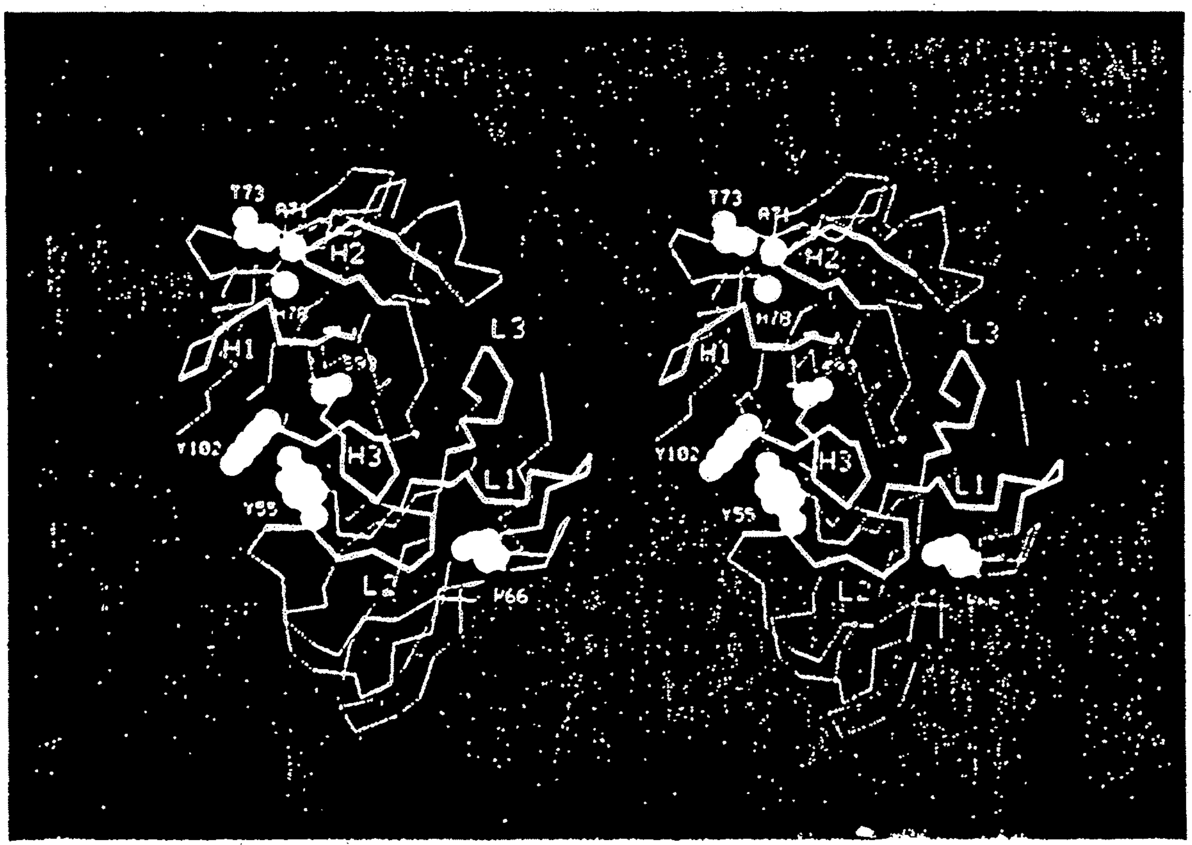


FIGURE 4



6

MAIL ROOM
JUN 14 1991
29
PAT. & TRADEMARK OFF.

A715272

DOCKET 709
EXPRESS MAIL NO. B59937585
MAILED 14 JUNE 1991

561

IMMUNOGLOBULIN VARIANTS

Field of the Invention

This invention relates to methods for the preparation and use of variant antibodies and finds application particularly in the fields of immunology and cancer diagnosis and therapy.

Background of the Invention

Naturally occurring antibodies (immunoglobulins) comprise two heavy chains linked together by disulfide bonds and two light chains, one light chain being linked to each of the heavy chains by disulfide bonds. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain (V_L) at one end and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains, see e.g. Chothia *et al.*, *J. Mol. Biol.* 186:651-663 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci.*

USA 82:4592-4596 (1985).

The constant domains are not involved directly in binding the antibody to an antigen, but are involved in various effector functions, such as participation of the antibody in antibody-dependent cellular cytotoxicity. The variable domains of each pair of light and heavy chains are involved directly in binding the antibody to the antigen. The domains of natural light and heavy chains have the same general structure, and each domain comprises four framework (FR) regions, whose sequences are somewhat conserved, connected by three hyper-variable or complementarity determining regions (CDRs) (see Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, MD, (1987)). The four framework regions largely adopt a β-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site.

Widespread use has been made of monoclonal antibodies, particularly those derived from rodents including mice, however they are frequently antigenic in human clinical use. For example, a major limitation in the clinical use of rodent monoclonal antibodies is an anti-globulin response during therapy (Miller, R. A. *et al., Blood* 62:988-995 (1983); Schroff, R. W. *et al., Cancer Res.* 45:879-885 (1985)).

The art has attempted to overcome this problem by constructing "chimeric" antibodies in which an animal antigen-binding variable domain is coupled to a human constant domain (Cabilly *et al.*, U.S. patent No. 4,816,567; Morrison, S. L. *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Boulianne, G. L. *et al., Nature* 312:643-646 (1984); Neuberger, M. S. *et al., Nature* 314:268-270 (1985)). The term "chimeric" antibody is used herein to describe a polypeptide comprising at least the antigen binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

The isotype of the human constant domain may be selected to tailor the chimeric antibody for participation in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (see e.g. Brüggemann, M. *et al.*, *J. Exp. Med.* **166**:1351-1361 (1987); Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Love *et al.*, *Methods in Enzymology* 178:515-527 (1989); Bindon *et al.*, *J. Exp. Med.* 168:127-142 (1988).

In the typical embodiment, such chimeric antibodies contain about one third rodent (or other non-human species) sequence and thus are capable of eliciting a significant anti-globulin response in humans. For example, in the case of the murine anti-CD3 antibody, OKT3, much of the resulting anti-globulin response is directed against the variable region rather than the constant region (Jaffers, G. J. *et al.*, *Transplantation* **41**:572-578 (1986)).

In a further effort to resolve the antigen binding functions of antibodies and to minimize the use of heterologous sequences in human antibodies, Winter and colleagues (Jones, P. T. *et al.*, *Nature* **321**:522-525 (1986); Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Verhoeyen, M. *et al.*, *Science* 239:1534-1536 (1988)) have substituted rodent CDRs or CDR sequences for the corresponding segments of a human antibody. As used herein, the term "humanized" antibody is an embodiment of chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The therapeutic promise of this approach is supported by the clinical efficacy of a humanized antibody specific for the CAMPATH-1 antigen with two non-Hodgkin lymphoma patients, one of whom had previously developed an anti-globulin response to the parental rat antibody (Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Hale, G. *et al.*, *Lancet* i:1394-1399 (1988)). A murine antibody to the interleukin 2 receptor has also recently been humanized (Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* **86**:10029-10033 (1989)) as a potential immunosuppressive reagent. Additional references related to humanization of antibodies include Co *et al.*, *Proc. Natl. Acad. Sci.*

USA 88:2869-2873 (1991); Gorman *et al., Proc. Natl. Acad. Sci. USA* 88:4181-4185 (1991); Daugherty *et al., Nucleic Acids Research* 19(9):2471-2476 (1991); Brown *et al., Proc. Natl. Acad. Sci. USA* 88:2663-2667 (1991); Junghans *et al., Cancer Research* 50:1495-1502 (1990).

In some cases, substituting CDRs from rodent antibodies for the human CDRs in human frameworks is sufficient to transfer high antigen binding affinity (Jones, P. T. *et al., Nature* 321:522-525 (1986); Verhoeyen, M. *et al., Science* 239:1534-1536 (1988)), whereas in other cases it has been necessary to additionally replace one (Riechmann, L. *et al., Nature* 332:323-327 (1988)) or several (Queen, C. *et al., Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)) framework region (FR) residues. See also Co *et al., supra*.

For a given antibody a small number of FR residues are anticipated to be important for antigen binding. Firstly for example, certain antibodies have been shown to contain a few FR residues which directly contact antigen in crystal structures of antibody-antigen complexes (e.g., reviewed in Davies, D. R. *et al., Ann. Rev. Biochem.* 59:439-473 (1990)). Secondly, a number of FR residues have been proposed by Chothia, Lesk and colleagues (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987); Chothia, C. *et al., Nature* 342:877-883 (1989); Tramontano, A. *et al., J. Mol. Biol.* 215:175-182 (1990)) as critically affecting the conformation of particular CDRs and thus their contribution to antigen binding. See also Margolies *et al., Proc. Natl. Acad. Sci. USA* 72:2180-2184 (1975).

It is also known that, in a few instances, an antibody variable domain (either V_H or V_L) may contain glycosylation sites, and that this glycosylation may improve or abolish antigen binding, Pluckthun, *Biotechnology* 9:545-51 (1991); Spiegelberg *et al., Biochemistry* 9:4217-4223 (1970); Wallic *et al., J. Exp. Med.* 168:1099-1109 (1988); Sox *et al., Proc. Natl. Acad. Sci. USA* 66:975-982 (1970); Margni *et al., Ann. Rev. Immunol.* 6:535-554 (1988). Ordinarily, however, glycosylation has no influence on the antigen-binding properties of an antibody, Pluckthun, *supra*, (1991).

The three-dimensional structure of immunoglobulin chains has been studied, and crystal structures for intact immunoglobulins, for a variety of immunoglobulin fragments, and for antibody-antigen complexes have been published (see e.g., Saul *et al.*, *Journal of Biological Chemistry* 25:585-97 (1978); Sheriff *et al.*, *Proc. Natl. Acad. Sci. USA* 84:8075-79 (1987); Segal *et al.*, *Proc. Natl. Acad. Sci. USA* 71:4298-4302 (1974); Epp *et al.*, *Biochemistry* 14(22):4943-4952 (1975); Marquart *et al.*, *J. Mol. Biol.* 141:369-391 (1980); Furey *et al.*, *J. Mol. Biol.* 167:661-692 (1983); Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267-279, Alan R. Liss, Inc. pubs. (1986); Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987); Chothia *et al.*, *Nature* 342:877-883 (1989); Chothia *et al.*, *Science* 233:755-58 (1986); Huber *et al.*, *Nature* 264:415-420 (1976); Bruccoleri *et al.*, *Nature* 335:564-568 (1988) and *Nature* 336:266 (1988); Sherman *et al.*, *Journal of Biological Chemistry* 263:4064-4074 (1988); Amzel and Poljak, *Ann. Rev. Biochem.* 48:961-67 (1979); Silverton *et al.*, *Proc. Natl. Acad. Sci. USA* 74:5140-5144 (1977); and Gregory *et al.*, *Molecular Immunology* 24:821-829 (1987). It is known that the function of an antibody is dependent on its three dimensional structure, and that amino acid substitutions can change the three-dimensional structure of an antibody, Snow and Amzel, *supra*. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. *et al.*, *Nature* 332:323-327 (1988); Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)).

Humanizing an antibody with retention of high affinity for antigen and other desired biological activities is at present difficult to achieve using currently available procedures. Methods are needed for rationalizing the selection of sites for substitution in preparing such antibodies and thereby increasing the efficiency of antibody humanization.

The proto-oncogene *HER2* (human epidermal growth factor receptor 2) encodes a protein tyrosine kinase ($p185^{HER2}$) that is related to and somewhat homologous to the human epidermal growth factor receptor (see Coussens, L. *et al.*, *Science* 230:1132-1139 (1985); Yamamoto, T. *et al.*, *Nature* 319:230-234 (1986); King, C. R. *et al.*, *Science* 229:974-976 (1985)). *HER2* is also known in the field as *c-erbB-2*, and sometimes by the name of the rat homolog, *neu*. Amplification and/or overexpression of *HER2* is associated with multiple human malignancies and appears to be integrally involved in progression of 25-30% of human breast and ovarian cancers (Slamon, D. J. *et al.*, *Science* 235:177-182 (1987), Slamon, D. J. *et al.*, *Science* 244:707-712 (1989)). Furthermore, the extent of amplification is inversely correlated with the observed median patient survival time (Slamon, *supra*, Science 1989).

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. *et al.*, *Cancer Res.* 50:1550-1558 (1990)), directed against the extracellular domain (ECD) of p185^{HER2}, specifically inhibits the growth of tumor cell lines overexpressing p185^{HER2} in monolayer culture or in soft agar (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* 9:1165-1172 (1989); Lupu, R. *et al.*, *Science* 249:1552-1555 (1990)). MuMAb4D5 also has the potential of enhancing tumor cell sensitivity to tumor necrosis factor, an important effector molecule in macrophage-mediated tumor cell cytotoxicity (Hudziak, *supra*, 1989; Shepard, H. M. and Lewis, G. D. *J. Clinical Immunology* 8:333-395 (1988)). Thus muMAb4D5 has potential for clinical intervention in and imaging of carcinomas in which p185^{HER2} is overexpressed. The muMAb4D5 and its uses are described in copending U.S. patent applications 07/143,912 and 07/147,461, and in corresponding PCT application WO 89/06692 published 27 July 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. However, this antibody may be immunogenic in humans.

It is therefore an object of this invention to provide methods for the preparation of antibodies which are less antigenic in humans than non-human antibodies but have desired antigen binding and other characteristics and activities.

It is a further object of this invention to provide methods for the efficient humanization of antibodies, i.e. selecting non-human amino acid residues for importation into a human antibody background sequence in such a fashion as to retain or improve the affinity of the non-human donor antibody for a given antigen.

It is another object of this invention to provide humanized antibodies capable of binding p185^{HER2}.

Other objects, features, and characteristics of the present invention will become apparent upon consideration of the following description and the appended claims.

Summary of the Invention

The objects of this invention are accomplished by a method for making a humanized antibody comprising amino acid sequence of an import, non-human antibody and a human antibody, comprising the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

    1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L - V_H interface; and

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, the method of this invention comprises the additional steps of determining if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), retaining the consensus residue.

Additionally, in certain embodiments the method of this invention comprises the feature wherein the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)).

In certain embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). If the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human sequence if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another embodiment of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

Certain alternate embodiments of the methods of this invention comprise obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

In preferred embodiments, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody.

Optionally, this just-recited embodiment comprises the additional steps of following the method steps appearing at the beginning of this summary and determining whether a particular amino acid residue can reasonably be expected to have undesirable effects.

This invention also relates to a humanized antibody comprising the CDR sequence of an import, non-human antibody and the FR sequence of a human antibody, wherein an amino acid residue within the human FR sequence located at any one of the sites 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H has been substituted by another residue. In preferred embodiments, the residue substituted at the human FR site is the residue found at the corresponding location of the non-human antibody from which the non-human CDR was obtained. In other embodiments, no human FR residue other than those set forth in this group has been substituted.

This invention also encompasses specific humanized antibody variable domains, and isolated polypeptides having homology with the following sequences.

1. SEQ. ID NO. 1, which is the light chain variable domain of a humanized version of muMAb4D5:
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP
KLLIYSASFLESGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHY
TTPPTFGQGTKVEIKRT

2. SEQ. ID NO. 2, which is the heavy chain variable domain of a humanized version of muMAb4D5):
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE
WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT
AVYYCSRWGGDGFYAMDVWGQGTLVTVSS

In another aspect, this invention provides a consensus human antibody variable domain amino acid sequence for use in the preparation of humanized antibodies, methods for obtaining, using, and storing a computer representation of such a consensus sequence, and computers comprising the sequence data of such a sequence. In one embodiment, the following consensus human antibody variable domain amino acid sequences are provided:

SEQ. ID NO. 3 (light chain):
DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPK
LLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYN
SLPYTFGQGTKVEIKRT, and

SEQ. ID NO. 4 (heavy chain):
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKG
LEWVAVISENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAE
DTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS

Brief Description of the Drawings

FIGURE 1A shows the comparison of the V_L domain amino acid residues of muMAb4D5, huMAb4D5, and a consensus human sequence (Fig. 1A, SEQ.ID NO. 5, SEQ. ID NO. 1 and SEQ. ID NO. 3, respectively). FIGURE 1B shows the comparison between the V_H domain amino acid residues of the muMAb4d5, huMAb4D5, and a consensus human sequence (Fig. 1B, SEQ. ID NO. 6, SEQ. ID NO. 2 and SEQ. ID NO. 4, respectively). Both Figs 1A and 1B use the generally accepted numbering scheme from Kabat, E. A., *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD (1987)). In both Fig. 1A and Fig. 1B, the CDR residues determined according to a standard sequence definition (as in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) are indicated by the first underlining beneath the sequences, and the CDR residues determined according to a structural definition (as in Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)) are indicated by the second, lower underlines.

a The mismatches between ~~genes~~ sequences are shown by the vertical lines.

FIGURE 2 shows a scheme for humanization of muMAb4D5 V_L and V_H by gene conversion mutagenesis.

FIGURE 3 shows the inhibition of SK-BR-3 proliferation by MAb4D5 variants. Relative cell proliferation was determined as described (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* 9:1165-1172 (1989)) and data (average of triplicate determinations) are presented as a percentage of results with untreated cultures for muMAb4D5 (l), huMAb4D5-8 (n) and huMAb4D5-1 (l).

FIGURE 4 shows a stereo view of α-carbon tracing for model of huMAb4D5-8 V_L and V_H. The CDR residues (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) are shown in bold and side chains of V_H residues A71, T73, A78, S93, Y102 and V_L residues Y55 plus R66 (see Table 1) are shown.

Detailed Description of the Invention

Definitions

In general, the following words or phrases have the indicated definitions when used in the description, examples, and claims:

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. *et al.*, *Cancer Res.* 50:1550-1558 (1990)) is directed against the extracellular domain (ECD) of $p185^{HER2}$. The muMAb4D5 and its uses are described in copending U.S. patent applications 07/143,912 and 07/147,461, and in corresponding PCT application WO 89/06692 published 27 July 1989. This murine antibody was deposited with the ATCC and designated ATCC CRL 10463. In this description and claims, the terms muMAb4D5, chMAb4D5 and huMAb4D5 represent murine, chimerized and humanized versions of the monoclonal antibody 4D5, respectively.

A humanized antibody for the purposes herein is an immunoglobulin amino acid sequence variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a FR region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin.

In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (Fab) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain.

The humanized antibody will be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG_1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art..

The FR and CDR regions of the humanized antibody need not correspond precisely to the parental sequences, e.g., the import CDR or the consensus FR may be mutagenized by substitution, insertion or deletion of a residue so that the CDR or FR residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often 90%, and most preferably greater than 95%.

In general, humanized antibodies prepared by the method of this invention are produced by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen.

Residues that influence antigen binding are defined to be residues that are substantially responsible for the antigen affinity or antigen specificity of a candidate immunoglobulin, in a positive or a negative sense. The object here is to select FR residues from the consensus and import sequence so that the desired immunoglobulin characteristic is achieved. Such desired characteristics include increases in affinity and greater specificity for the target antigen, although it is conceivable that in some circumstances the opposite effects might be desired. In general, the CDR residues are directly and most substantially involved in influencing antigen binding (although not all CDR residues are so involved and therefore need not be substituted into the consensus sequence). However, FR residues also have a significant effect and can exert their influence in at least three ways: They may noncovalently directly bind to antigen, they may interact with CDR residues and they may affect the interface between the heavy and light chains.

A residue that noncovalently directly binds to antigen is one that, by three dimensional analysis, is reasonably expected to noncovalently directly bind to antigen. Typically, it is necessary to impute the position of antigen from the spatial location of neighboring CDRs and the dimensions and structure of the target antigen. In general, only those humanized antibody residues that are capable of forming salt bridges, hydrogen bonds, or hydrophobic interactions are likely to be involved in non-covalent antigen binding, however residues which are separated spatially by 3.2 Angstroms or less may also non-covalently interact. Such residues typically are the relatively larger amino acids, such as tyrosine, arginine, and lysine. Antigen-binding FR residues also typically will have side chains that are oriented into an envelope surrounding the solvent oriented face of a CDR which extends about 7 Angstroms into the solvent from the CDR domain and about 7 Angstroms on either side of the CDR domain, again as visualized by three dimensional modeling.

A residue that interacts with a CDR generally is a residue that either affects the conformation of the CDR polypeptide backbone or forms a noncovalent bond with a CDR residue side chain. Conformation-affecting residues ordinarily are those that change the spatial position of any CDR backbone atom (N, Cα, C, O, Cβ) by more than about 0.2 Angstroms. Backbone atoms of CDR sequences are displaced for example by residues that interrupt or modify organized structures such as beta sheets, helices or loops. Residues that can exert a profound affect on the conformation of neighboring sequences include proline and glycine, both of which are capable of introducing bends into the backbone. Other residues that can displace backbone atoms are those that are capable of participating in salt bridges and hydrogen bonds.

A residue that interacts with a CDR side chain is one that is reasonably expected to form a noncovalent bond with a CDR side chain, generally either a salt bridge or hydrogen-bond. Such residues are identified by three dimensional positioning of their side chains. A salt or ion bridge could be expected to form between two side chains positioned within about 2.5 - 3.2 Angstroms of one another that bear opposite charges, for example a lysinyl and a glutamyl pairing. A hydrogen bond could be expected to form between the side chains of residue pairs such as seryl or threonyl with aspartyl or glutamyl (or other hydrogen accepting residues). Such pairings are well known in the protein chemistry art and will be apparent to the artisan upon three dimensional modeling of the candidate immunoglobulin.

Immunoglobulin residues that affect the interface between heavy and light chain variable regions ("the V_L - V_H interface") are those that affect the proximity or orientation of the two chains with respect to one another.

Certain residues involved in interchain interactions are already known and include V_L residues 34, 36, 38, 44, 46, 87, 89, 91, 96, and 98 and V_H residues 35, 37, 39, 45, 47, 91, 93, 95, 100, and 103 (utilizing the nomenclature set forth in Kabat *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)). Additional residues are newly identified by the inventors herein, and include 43L, 85L, 43H and 60H. While these residues are indicated for IgG only, they are applicable across species. In the practice of this invention, import antibody residues that are reasonably expected to be involved in interchain interactions are selected for substitution into the consensus human sequence. It is believed that heretofore no humanized antibody has been prepared with an ~~intrachain~~ interchain-affecting residue selected from an import antibody sequence.

Since it is not entirely possible to predict in advance what the exact impact of a given substitution will be it may be necessary to make the substitution and assay the candidate antibody for the desired characteristic. These steps, however, are *per se* routine and well within the ordinary skill of the art.

CDR and FR residues are determined according to a standard sequence definition (Kabat *et al., Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda MD (1987), hereby specifically incorporated by reference), and a structural definition (as in Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987), hereby specifically incorporated by reference). Where these two methods result in slightly different identifications of a CDR, the structural definition is preferred, but the residues identified by the alternate method are considered important FR residues for determination of which framework residues to import into a consensus sequence.

The terms "consensus sequence" and "consensus antibody" as used herein refers to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins of any particular subclass. In preferred embodiments, the

consensus human variable domain sequences are derived from the most abundant subclasses in the sequence compilation of Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda MD (1987), namely V_L κ subgroup I and V_H group III. In such preferred embodiments, the V_L consensus domain has the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT (SEQ. ID NO. 3);
the V_H consensus domain has the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS (SEQ. ID NO. 4).
While not wishing to be limited to any particular theories, it may be that these preferred embodiments are less likely to be immunogenic in an individual than less abundant subclasses. However, in other embodiments, the consensus sequence is derived from human constant domains, or from other subclasses of human immunoglobulin variable domains.

Identity or homology with respect to a specified amino acid sequence of this invention is defined herein as the percentage of amino acid residues in a candidate sequence that are identical with the specified residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the specified sequence shall be construed as affecting homology. All sequence alignments called for in this invention are such maximal homology alignments.

definition of homology.

"Non-homologous" import antibody residues are those residues which are not identical to the amino acid residue at the analogous or corresponding location in a consensus sequence, after the import and consensus sequences are aligned.

The term "computer representation" refers to information which is in a form that can be manipulated by a computer. The act of storing a computer representation refers to the act of placing the information in a form suitable for manipulation by a computer.

This invention is also directed to novel polypeptides, and in certain aspects, isolated novel humanized anti-p185^{HER2} antibodies are provided. These novel anti-p185^{HER2} antibodies are sometimes collectively referred to herein as huMAb4D5, and also sometimes as the light or heavy chain variable domains of huMAb4D5, and are defined herein to be any polypeptide sequence which possesses a biological property of a polypeptide comprising the following polypeptide sequence:

> DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLESGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT (SEQ. ID NO. 1, which is the light chain variable domain of huMAb4D5); or
>
> EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS (SEQ. ID NO. 2, which is the heavy chain variable domain of huMAb4D5).

"Biological property" for the purposes herein means an *in vivo* effector or antigenic function or activity that is directly or indirectly performed by huMAb4D5 (whether in its native or denatured conformation). Effector functions include receptor binding, any enzyme activity or enzyme modulatory activity, any carrier binding activity, any hormonal activity, any mitogenic or angiogenic activity, any cytotoxic activity, any activity in promoting or inhibiting adhesion of cells to extracellular matrix or cell surface molecules, or any structural role. However, effector functions do not include possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against huMAb4D5. An antigenic function means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against the polypeptide sequence of huMAb4D5.

Biologically active huMAb4D5 is defined herein as a polypeptide that shares an effector function of huMAb4D5 and which may (but need not) in addition possess an antigenic function. A principal known effect or function of huMAb4D5 is its ability to bind to $p185^{HER2}$.

Antigenically active huMAb4D5 is defined as a polypeptide that possesses an antigenic function of huMAb4D5 and which may (but need not) in addition possess an effector function.

In preferred embodiments, antigenically active huMAb4D5 is a polypeptide that binds with an affinity of at least about 10-9 l/mole to an antibody capable of binding huMAb4D5. Ordinarily the polypeptide binds with an affinity of at least about 10-8 l/mole. Isolated antibody capable of binding huMAb4D5 is an antibody which is identified and separated from a component of the natural environment in which it may be present. Most preferably, antigenically active huMAb4D5 is a polypeptide that binds to an antibody capable of binding huMAb4D5 in its native conformation. HuMAb4D5 in its native conformation is huMAb4D5 as recovered according to the methods described in Example 1 below, which has not been denatured by chaotropic agents, heat or other treatment that substantially modifies the three dimensional structure of huMAb4D5 as determined for example by migration on nonreducing, nondenaturing sizing gels. Antibody used in this determination is rabbit polyclonal antibody raised by formulating native huMAb4D5 in Freund's complete adjuvant, subcutaneously injecting the formulation, and boosting the immune response by intraperitoneal injection of the formulation until the titer of anti-huMAb4D5 antibody plateaus.

Ordinarily, biologically or antigenically active huMAb4D5 will have an amino acid sequence having at least 75% amino acid sequence identity with the huMAb4D5 amino acid sequence, more preferably at least 80%, more preferably at least 90%, and most preferably at least 95%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the huMAb4D5 residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the huMAb4D5 sequence shall be construed as affecting homology.

Thus, the biologically active and antigenically active huMAb4D5 polypeptides that are the subject of certain embodiments of this invention include the sequence of the entire translated nucleotide sequence of huMAb4D5; mature huMAb4D5; fragments thereof having a consecutive sequence of at least 5, 10, 15, 20, 25, 30 or 40 amino acid residues from huMAb4D5; amino acid sequence variants of huMAb4D5 wherein an amino acid residue has been inserted N- or C-terminal to, or within, huMAb4D5 or its fragment as defined above; amino acid sequence variants of huMAb4D5 or its fragment as defined above wherein an amino acid residue of huMAb4D5 or its fragment as defined above has been substituted by another residue, including predetermined mutations by, e.g., site-directed or PCR mutagenesis; derivatives of huMAb4D5 or its fragments as defined above wherein huMAb4D5 or its fragments have been covalent modified, by substitution, chemical, enzymatic, or other appropriate means, with a moiety other than a naturally occurring amino acid; and glycosylation variants of huMAb4D5 (insertion of a glycosylation site or deletion of any glycosylation site by deletion, insertion or substitution of suitable residues). Such fragments and variants exclude any polypeptide heretofore identified, including muMAb4D5 or any known polypeptide fragment, which are anticipatory order 35 U.S.C.102 as well as polypeptides obvious thereover under 35 U.S.C. 103.

"Isolated" huMAb4D5 means huMAb4D5 which has been identified and separated and/or recovered from a component of its natural cell culture environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for huMAb4D5, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, huMAb4D5 will be purified (1) to greater than 95% by weight of protein as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated huMAb4D5 includes huMAb4D5 in situ within recombinant cells since at least one component of the huMAb4D5 natural environment will not be present. Ordinarily, however, isolated huMAb4D5 will be prepared by at least one purification step.

In accordance with this invention, huMAb4D5 nucleic acid is RNA or DNA containing greater than ten bases that encodes a biologically or antigenically active huMAb4D5, is complementary to nucleic acid sequence encoding such huMAb4D5, or hybridizes to nucleic acid sequence encoding such huMAb4D5 and remains stably bound to it under stringent conditions.

Preferably, the huMAb4D5 nucleic acid encodes a polypeptide sharing at least 75% sequence identity, more preferably at least 80%, still more preferably at least 85%, even more preferably at 90%, and most preferably 95%, with the huMAb4D5 amino acid sequence. Preferably, a nucleic acid molecule that hybridizes to the huMAb4D5 nucleic acid contains at least 20, more preferably 40, and most preferably 90 bases. Such hybridizing or complementary nucleic acid, however, is further defined as being novel under 35 U.S.C. 102 and unobvious under 35 U.S.C. 103 over any prior art nucleic acid.

Stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0/1% $NaDodSO_4$ at 50° C; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0/1% Ficoll/0/1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42° C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42 C, with washes at 42 C in 0.2 x SSC and 0.1% SDS.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. However enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used in accord with conventional practice.

An "exogenous" element is defined herein to mean nucleic acid sequence that is foreign to the cell, or homologous to the cell but in a position within the host cell nucleic acid in which the element is ordinarily not found.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are commercially available, are publicly available on an unrestricted basis, or can be constructed from such available plasmids in accord with published procedures. In addition, other equivalent plasmids are known in the art and will be apparent to the ordinary artisan.

"Restriction Enzyme Digestion" of DNA refers to catalytic cleavage of the DNA with an enzyme that acts only at certain locations in the DNA. Such enzymes are called restriction endonucleases, and the sites for which each is specific is called a restriction site. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors, and other requirements as established by the enzyme suppliers are used. Restriction enzymes commonly are designated by abbreviations composed of a capital letter followed by other letters representing the microorganism from which each restriction enzyme originally was obtained and then a number designating the particular enzyme. In general, about 1 μg of plasmid or DNA fragment is used with about 1-2 units of enzyme in about 20 μl of buffer solution. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation of about 1 hour at 37°C is ordinarily used, but may vary in accordance with the supplier's instructions. After incubation, protein or polypeptide is removed by extraction with phenol and chloroform, and the digested nucleic acid is recovered from the aqueous fraction by precipitation with ethanol. Digestion with a restriction enzyme may be followed with bacterial alkaline phosphatase hydrolysis of the terminal 5' phosphates to prevent the two restriction cleaved ends of a DNA fragment from "circularizing" or forming a closed loop that would impede insertion of another DNA fragment at the restriction site. Unless otherwise stated, digestion of plasmids is not followed by 5' terminal dephosphorylation. Procedures and reagents for dephosphorylation are conventional as described in sections 1.56-1.61 of Sambrook *et al.* (Molecular Cloning: A Laboratory Manual New York: Cold

Spring Harbor Laboratory Press, 1989).

"Recovery" or "isolation" of a given fragment of DNA from a restriction digest means separation of the digest on polyacrylamide or agarose gel by electrophoresis, identification of the fragment of interest by comparison of its mobility versus that of marker DNA fragments of known molecular weight, removal of the gel section containing the desired fragment, and separation of the gel from DNA. This procedure is known generally. For example, see Lawn *et al.*, Nucleic Acids Res., 9: 6103-6114 (1981), and Goeddel *et al.*, Nucleic Acids Res. 8: 4057 (1980).

"Southern blot analysis" is a method by which the presence of DNA sequences in a restriction endonuclease digest of DNA or DNA-containing composition is confirmed by hybridization to a known, labeled oligonucleotide or DNA fragment. Southern analysis typically comprises electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, or another suitable membrane supports for analysis with a radiolabeled, biotinylated or enzyme-labeled probe as described in sections 9.37-9.52 of Sambrook et al, *supra*.

"Northern analysis" is a method used to identify RNA sequences that hybridize to a known probe such as an oligonucleotide, DNA fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as 32-P, or by biotinylation, or with an enzyme. The RNA to be analyzed is usually electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook et al., *supra*.

"Ligation" refers to the process of forming phosphodiester bonds between two nucleic acid fragments. To ligate the DNA fragments together, the ends of the DNA fragments must be compatible with each other. In some cases, the ends will be directly compatible after endonuclease digestion. However, it may be necessary to first convert the staggered ends commonly produced after endonuclease digestion to blunt ends to make them compatible for ligation. To blunt the ends, the DNA is treated in a suitable buffer for at least 15 minutes at 15°C with about 10 units of the Klenow fragment of DNA polymerase I or T4 DNA polymerase in the presence of the four deoxyribonucleotide triphosphates. The DNA is then purified by phenol-chloroform extraction and ethanol precipitation. The DNA fragments that are to be ligated together are put in solution in about equimolar amounts. The solution will also contain ATP, ligase buffer, and a ligase such as T4 DNA ligase at about 10 units per 0.5 μg of DNA. If the DNA is to be ligated into a vector, the vector is first linearized by digestion with the appropriate restriction endonuclease(s). The linearized fragment is then treated with bacterial alkaline phosphatase, or calf intestinal phosphatase to prevent self-ligation during the ligation step.

"Preparation" of DNA from cells means isolating the plasmid DNA from a culture of the host cells. Commonly used methods for DNA preparation are the large and small scale plasmid preparations described in sections 1.25-1.33 of Sambrook *et al., supra*. After preparation of the DNA, it can be purified by methods well known in the art such as that described in section 1.40 of Sambrook *et al., supra*.

"Oligonucleotides" are short-length, single- or double-stranded polydeoxynucleotides that are chemically synthesized by known methods (such as phosphotriester, phosphite, or phosphoramidite chemistry, using solid phase techniques such as described in EP 266,032 published 4 May 1988, or via deoxynucleoside H-phosphonate intermediates as described by Froehler *et al.*, Nucl. Acids Res., 14: 5399-5407 [1986]). They are then purified on polyacrylamide gels.

The technique of "polymerase chain reaction," or "PCR," as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis *et al.*, Cold Spring Harbor Symp. Quant. Biol., 51: 263 (1987); Erlich, ed., PCR Technology, (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample, comprising the use of a known nucleic acid (DNA or RNA) as a primer and utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

Suitable Methods for Practicing the Invention

Some aspects of this invention include obtaining an import, non-human antibody variable domain, humanizing the antibody sequence, and producing the humanized antibody. Methods for determining a desired humanized antibody sequence and for humanizing an antibody gene sequence are described below. A particularly preferred method of gene conversion from a non-human or consensus sequence into a humanized nucleic acid sequence is described in Example 1. Additionally, methods are given for obtaining and producing antibodies generally, which apply equally to native non-human antibodies as well as to humanized antibodies.

Generally, the antibodies and antibody variable domains of this invention are conventionally prepared in recombinant cell culture, as described in more detail below. Recombinant synthesis is preferred for reasons of safety and economy, but it is known to prepare peptides by chemical synthesis and to purify them from natural sources; such preparations are included within the definition of antibodies herein.

Molecular Modeling

Where it is desired to prepare molecular models for the antibodies of this invention, one may utilize any of the commercially available modeling programs described in the literature cited in the Background above.

Generally, models for a particular antibody domains, for example non-human, import antibody variable V_H and V_L domains, are constructed separately from consensus coordinates based upon FAb structures which have similar sequences. Models of consensus human antibody sequences are similarly created.

For example, in modeling the muMAb4d5, the models were constructed based upon seven Fab structures from the Brookhaven protein data bank (entries 1FB4, 2RHE, 2MCP, 3FAB, 1FBJ, 2HFL and 1REI). The Fab fragment KOL (Marquart, M. *et al.*, *J. Mol. Biol.* **141**:369-391 (1980)) was first chosen as a template for V_L and V_H domains and additional structures were then superimposed upon this structure using their main chain atom coordinates (INSIGHT program, Biosym Technologies). Similar programs and techniques are utilized for modeling the desired antibody.

The distance from the template $C\alpha$ to the analogous $C\alpha$ in each of the superimposed structures is calculated for each residue position. Generally, if all (or nearly all) $C\alpha$-$C\alpha$ distances for a given residue are $\leq$ 1Å, then that position is included in the consensus structure. In some cases the β-sheet framework residues will satisfy these criteria whereas the CDR loops may not. For each of these selected residues the average coordinates for individual N, $C\alpha$, C, O and $C\beta$ atoms are calculated and then corrected for resultant deviations from non-standard bond geometry by 50 cycles of energy minimization using a commercially available program such as the DISCOVER program (Biosym Technologies) with the AMBER forcefield (Weiner, S. J. *et al.*, *J. Amer. Chem. Soc.* **106**:765-784 (1984)), and the $C\alpha$ coordinates are fixed. The side chains of highly conserved residues, such as the disulfide-bridged cysteine residues, are then incorporated into the resultant consensus structure. Next the sequences of the particular antibody V_L and V_H domains are incorporated starting with the CDR residues and using the tabulations of CDR conformations from Chothia et al. (Chothia, C. *et al.*, *Nature* 342:877-883 (1989)) as a guide. Side-chain conformations are chosen on the basis of Fab crystal structures, rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* 193:775-791 (1987)) and packing considerations. Since V_H-CDR3 typically cannot be assigned a definite backbone conformation from these criteria, models may be created from a search of similar sized loops using the INSIGHT program, derived using packing and solvent exposure considerations, or created using other routine and commercially available techniques. It is preferable to subject the model to 5000 cycles of energy minimization.

Methods for Obtaining a Humanized Antibody Sequence

In humanizing muMAb4D5, consensus human sequences are first derived, and then a molecular model is generated for these sequences using the methods described above. In certain embodiments of this invention, the consensus human sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)), namely V_L κ subgroup I and V_H group III, and have the sequences indicated in the definitions above.

While these steps may be taken in different order, typically a structure for the candidate humanized antibody is created by transferring the CDRs from the non-human, import sequence into the consensus human structure. The humanized antibody may contain human replacements of the non-human import residues at positions within CDRs as defined by sequence variability (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) or as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)). For example, huMAb4D5 contains human replacements of the muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)): V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S.

Differences between the non-human import and the human consensus framework residues are individually investigated to determine their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is desirably performed through modeling, by examination of the characteristics of the amino acids at particular locations, or determined experimentally through evaluating the effects of substitution or mutagenesis of particular amino acids.

In certain preferred embodiments of this invention, a humanized antibody is made comprising amino acid sequence of an import, non-human antibody and a human antibody, utilizing the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

    1. non-covalently binds antigen directly,
    2. interacts with a CDR; or

3. participates in the V_L - V_H interface; and

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

Optionally, one determines if any non-homologous residues identified in step (e) are exposed on the surface of the domain or buried within it, and if the residue is exposed but has none of the effects identified in step (f), one may retain the consensus residue.

Additionally, in certain embodiments the corresponding consensus antibody residues identified in step (e) above are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H (utilizing the numbering system set forth in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)).

In preferred embodiments, the method of this invention comprises the additional steps of searching either or both of the import, non-human and the consensus variable domain sequences for glycosylation sites, determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody (i.e., determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity). If the import sequence bears the glycosylation site, it is preferred to substitute that site for the corresponding residues in the consensus human sequence if the glycosylation site is reasonably expected to be important. If only the consensus sequence, and not the import, bears the glycosylation site, it is preferred to eliminate that glycosylation site or substitute therefor the corresponding amino acid residues from the import sequence.

Another preferred embodiment of the methods of this invention comprises aligning import antibody and the consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

In certain alternate embodiments, one need not utilize the modeling and evaluation steps described above, and may instead proceed with the steps of obtaining the amino acid sequence of at least a portion of an import, non-human antibody variable domain having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

a. (in the FR of the variable domain of the light chain) 4L, 35L, 36L, 38L, 43L, 44L, 58L, 46L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, or

b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

Preferably, the non-CDR residue substituted at the consensus FR site is the residue found at the corresponding location of the non-human antibody. If desired, one may utilize the other method steps described above for determining whether a particular amino acid residue can reasonably be expected to have undesirable effects, and remedying those effects.

If after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one preferably reexamines the potential effects of the amino acids at the specific locations recited above. Additionally, it is desirable to reinvestigate any buried residues which are reasonably expected to affect the V_L - V_H interface but may not directly affect CDR conformation. It is also desirable to reevaluate the humanized antibody utilizing the steps of the methods claimed herein.

In certain embodiments of this invention, amino acid residues in the consensus human sequence are substituted for by other amino acid residues. In preferred embodiments, residues from a particular non-human import sequence are substituted, however there are circumstances where it is desired to evaluate the effects of other amino acids. For example, if after making a humanized antibody according to the steps above and testing its activity one is not satisfied with the humanized antibody, one may compare the sequences of other classes or subgroups of human antibodies, or classes or subgroups of antibodies from the particular non-human species, and determine which other amino acid side chains and amino acid residues are found at particular locations and substituting such other residues.

Antibodies

Certain aspects of this invention are directed to natural antibodies and to monoclonal antibodies, as illustrated in the Examples below and by antibody hybridomas deposited with the ATCC (as described below). Thus, the references throughout this description to the use of monoclonal antibodies are intended to include the use of natural or native antibodies as well as humanized and chimeric antibodies. As used herein, the term "antibody" includes the antibody variable domain and other separable antibody domains unless specifically excluded.

In accordance with certain aspects of this invention, antibodies to be humanized (import antibodies) are isolated from continuous hybrid cell lines formed by the fusion of antigen-primed immune lymphocytes with myeloma cells.

In certain embodiments, the antibodies of this invention are obtained by routine screening. Polyclonal antibodies to an antigen generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the antigen and an adjuvant. It may be useful to conjugate the antigen or a fragment containing the target amino acid sequence to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N = C = NR$, where R and R^1 are different alkyl groups.

The route and schedule of the host animal or cultured antibody-producing cells therefrom are generally in keeping with established and conventional techniques for antibody stimulation and production. While mice are frequently employed as the test model, it is contemplated that any mammalian subject including human subjects or antibody-producing cells obtained therefrom can be manipulated according to the processes of this invention to serve as the basis for production of mammalian, including human, hybrid cell lines.

Animals are typically immunized against the immunogenic conjugates or derivatives by combining 1 mg or 1 μg of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of conjugate in Freund's complete adjuvant (or other suitable adjuvant) by subcutaneous injection at multiple sites. 7 to 14 days later animals are bled and the serum is assayed for antigen titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to enhance the immune response.

After immunization, monoclonal antibodies are prepared by recovering immune lymphoid cells--typically spleen cells or lymphocytes from lymph node tissue--from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Kohler and Milstein, *Eur. J. Immunol.* 6:511 (1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

It is possible to fuse cells of one species with another. However, it is preferable that the source of the immunized antibody producing cells and the myeloma be from the same species.

The hybrid cell lines can be maintained in culture *in vitro* in cell culture media. The cell lines of this invention can be selected and/or maintained in a composition comprising the continuous cell line in hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody. The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, Ion exchange chromatography, affinity chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM as the case may be that heretofore have been used to purify these immunoglobulins from pooled plasma, e.g. ethanol or polyethylene glycol precipitation procedures. The purified antibodies are sterile filtered, and optionally are conjugated to a detectable marker such as an enzyme or spin label for use in diagnostic assays of the antigen in test samples.

While routinely rodent monoclonal antibodies are used as the source of the import antibody, the invention is not limited to any species. Additionally, techniques developed for the production of chimeric antibodies (Morrison *et al.*, *Proc. Natl. Acad. Sci.*, 81:6851 (1984); Neuberger *et al.*, *Nature* 312:604 (1984); Takeda *et al.*, *Nature* 314:452 (1985)) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity (such as ability to activate human complement and mediate ADCC) can be used; such antibodies are within the scope of this invention.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules (known as Fab fragments) which bypass the generation of monoclonal antibodies are encompassed within the practice of this invention. One extracts antibody-specific messenger RNA molecules from immune system cells taken from an immunized animal, transcribes these into complementary DNA (cDNA), and clones the cDNA into a bacterial expressions system. One example of such a technique suitable for the practice of this invention was developed by researchers at Scripps/Stratagene, and incorporates a proprietary bacteriophage lambda vector system which contains a leader sequence that causes the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional FAb fragments for those which bind the antigen. Such FAb fragments with specificity for the antigen are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

Amino Acid Sequence Variants

Amino acid sequence variants of the antibodies and polypeptides of this invention (referred to in herein as the target polypeptide) are prepared by introducing appropriate nucleotide changes into the DNA encoding the target polypeptide, or by *in vitro* synthesis of the desired target polypeptide. Such variants include, for example, humanized variants of non-human antibodies, as well as deletions from, or insertions or substitutions of, residues within particular amino acid sequences. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the target polypeptide, such as changing the number or position of glycosylation sites, altering any membrane anchoring characteristics, and/or altering the intra-cellular location of the target polypeptide by inserting, deleting, or otherwise affecting any leader sequence of the native target polypeptide.

In designing amino acid sequence variants of target polypeptides, the location of the mutation site and the nature of the mutation will depend on the target polypeptide characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1-3. In certain embodiments, these choices are guided by the methods for creating humanized sequences set forth above.

A useful method for identification of certain residues or regions of the target polypeptide that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (Science, 244: 1081-1085 [1989]). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to optimize the performance of a mutation at a given site, ala scanning or random mutagenesis may be conducted at the target codon or region and the expressed target polypeptide variants are screened for the optimal combination of desired activity.

There are two principal variables in the construction of amino acid sequence variants: the location of the mutation site and the nature of the mutation. In general, the location and nature of the mutation chosen will depend upon the target polypeptide characteristic to be modified.

Amino acid sequence deletions of antibodies are generally not preferred, as maintaining the generally configuration of an antibody is believed to be necessary for its activity. Any deletions will be selected so as to preserve the structure of the target antibody.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within the target polypeptide sequence) may range generally from about 1 to 10 residues, more preferably 1 to 5, most preferably 1 to 3. Examples of terminal insertions include the target polypeptide with an N-terminal methionyl residue, an artifact of the direct expression of target polypeptide in bacterial recombinant cell culture, and fusion of a heterologous N-terminal signal sequence to the N-terminus of the target polypeptide molecule to facilitate the secretion of the mature target polypeptide from recombinant host cells. Such signal sequences generally will be obtained from, and thus homologous to, the intended host cell species. Suitable sequences include STII or lpp for *E. coli*, alpha factor for yeast, and viral signals such as herpes gD for mammalian cells.

Other insertional variants of the target polypeptide include the fusion to the N- or C-terminus of the target polypeptide of immunogenic polypeptides, e.g., bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli trp* locus, or yeast protein, and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, as described in WO 89/02922 published 6 April 1989.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the target polypeptide molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s) of the target polypeptide, and sites where the amino acids found in the target polypeptide from various species are substantially different in terms of side-chain bulk, charge, and/or hydrophobicity. Other sites for substitution are described infra, considering the effect of the substitution of the antigen binding, affinity and other characteristics of a particular target antibody.

Other sites of interest are those in which particular residues of the target polypeptides obtained from various species are identical. These positions may be important for the biological activity of the target polypeptide. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. If such substitutions result in a change in biological activity, then other changes are introduced and the products screened until the desired effect is obtained.

Substantial modifications in function or immunological identity of the target polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;
(2) neutral hydrophilic: cys, ser, thr;
(3) acidic: asp, glu;
(4) basic: asn, gln, his, lys, arg;
(5) residues that influence chain orientation: gly, pro; and
(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another. Such substituted residues may be introduced into regions of the target polypeptide that are homologous with other antibodies of the same class or subclass, or, more preferably, into the non-homologous regions of the molecule.

Any cysteine residues not involved in maintaining the proper conformation of target polypeptide also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.

DNA encoding amino acid sequence variants of the target polypeptide is prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the target polypeptide. A particularly preferred method of gene conversion mutagenesis is described below in Example 1. These techniques may utilized target polypeptide nucleic acid (DNA or RNA), or nucleic acid complementary to the target polypeptide nucleic acid.

Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion, and insertion variants of target polypeptide DNA. This technique is well known in the art as described by Adelman *et al.*, DNA, 2: 183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the target polypeptide. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in the target polypeptide DNA.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea *et al.* (Proc. Natl. Acad. Sci. USA, 75: 5765 [1978]).

Single-stranded DNA template may also be generated by denaturing double-stranded plasmid (or other) DNA using standard techniques.

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of the target polypeptide, and the other strand (the original template) encodes the native, unaltered sequence of the target polypeptide. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as *E. coli* JM101. After the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabeled with 32-phosphate to identify the bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for protein production, generally an expression vector of the type typically employed for transformation of an appropriate host.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single-stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTTP), is combined with a modified thio-deoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham Corporation). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion.

After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell such as *E. coli* JM101, as described above.

DNA encoding target polypeptide variants with more than one amino acid to be substituted may be generated in one of several ways. If the amino acids are located close together in the polypeptide chain, they may be mutated simultaneously using one oligonucleotide that codes for all of the desired amino acid substitutions. If, however, the amino acids are located some distance from each other (separated by more than about ten amino acids), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods may be employed.

In the first method, a separate oligonucleotide is generated for each amino acid to be substituted. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template will encode all of the desired amino acid substitutions.

The alternative method involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for the single mutants: wild-type DNA is used for the template, an oligonucleotide encoding the first desired amino acid substitution(s) is annealed to this template, and the heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

PCR mutagenesis is also suitable for making amino acid variants of target polypeptide. While the following discussion refers to DNA, it is understood that the technique also finds application with RNA. The PCR technique generally refers to the following procedure (see Erlich, *supra*, the chapter by R. Higuchi, p. 61-70): When small amounts of template DNA are used as starting material in a PCR, primers that differ slightly in sequence from the corresponding region in a template DNA can be used to generate relatively large quantities of a specific DNA fragment that differs from the template sequence only at the positions where the primers differ from the template. For introduction of a mutation into a plasmid DNA, one of the primers is designed to overlap the position of the mutation and to contain the mutation; the sequence of the other primer must be identical to a stretch of sequence of the opposite strand of the plasmid, but this sequence can be located anywhere along the plasmid DNA. It is preferred, however, that the sequence of the second primer is located within 200 nucleotides from that of the first, such that in the end the entire amplified region of DNA bounded by the primers can be easily sequenced. PCR amplification using a primer pair like the one just described results in a population of DNA fragments that differ at the position of the mutation specified by the primer, and possibly at other positions, as template copying is somewhat error-prone.

If the ratio of template to product material is extremely low, the vast majority of product DNA fragments incorporate the desired mutation(s). This product material is used to replace the corresponding region in the plasmid that served as PCR template using standard DNA technology.

Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the vector fragment in a three (or more)-part ligation.

In a specific example of PCR mutagenesis, template plasmid DNA (1 μg) is linearized by digestion with a restriction endonuclease that has a unique recognition site in the plasmid DNA outside of the region to be amplified. Of this material, 100 ng is added to a PCR mixture containing PCR buffer, which contains the four deoxynucleotide tri-phosphates and is included in the GeneAmp® kits (obtained from Perkin-Elmer Cetus, Norwalk, CT and Emeryville, CA), and 25 pmole of each oligonucleotide primer, to a final volume of 50 μl. The reaction mixture is overlayed with 35 μl mineral oil. The reaction is denatured for 5 minutes at 100°C, placed briefly on ice, and then 1 μl *Thermus aquaticus (Taq)* DNA polymerase (5 units/μl, purchased from Perkin-Elmer Cetus, Norwalk, CT and Emeryville, CA) is added below the mineral oil layer. The reaction mixture is then inserted into a DNA Thermal Cycler (purchased from Perkin-Elmer Cetus) programmed as follows: 2 min. at 55°C, then 30 sec. at 72°C, then 19 cycles of the following: 30 sec. at 94°C, 30 sec. at 55°C, and 30 sec. at 72°C.

At the end of the program, the reaction vial is removed from the thermal cycler and the aqueous phase transferred to a new vial, extracted with phenol/chloroform (50:50:vol), and ethanol precipitated, and the DNA is recovered by standard procedures. This material is subsequently subjected to the appropriate treatments for insertion into a vector.

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells *et al.* (Gene, 34: 315 [1985]). The starting material is the plasmid (or other vector) comprising the target polypeptide DNA to be mutated. The codon(s) in the target polypeptide DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the target polypeptide DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated target polypeptide DNA sequence.

Insertion of DNA into a Cloning Vehicle

The cDNA or genomic DNA encoding the target polypeptide is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available, and selection of the appropriate vector will depend on 1) whether it is to be used for DNA amplification or for DNA expression, 2) the size of the DNA to be inserted into the vector, and 3) the host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the host cell for which it is compatible. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(a) Signal Sequence Component

In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector.

The target polypeptides of this invention may be expressed not only directly, but also as a fusion with a heterologous polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. Included within the scope of this invention are target polypeptides with any native signal sequence deleted and replaced with a heterologous signal sequence. The heterologous signal sequence selected should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native target polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native target polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

(b) <u>Origin of Replication Component</u>

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, i.e. they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using *Bacillus* species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in *Bacillus* genomic DNA. Transfection of *Bacillus* with this vector results in homologous recombination with the genome and insertion of the target polypeptide DNA. However, the recovery of genomic DNA encoding the target polypeptide is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the target polypeptide DNA.

(c) Selection Gene Component

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g. ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern *et al.*, J. Molec. Appl. Genet., 1: 327 [1982]), mycophenolic acid (Mulligan *et al.*, Science, 209: 1422 [1980]) or hygromycin (Sugden *et al.*, Mol. Cell. Biol., 5: 410-413 [1985]). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the target polypeptide nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes the target polypeptide. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the target polypeptide are synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216 [1980]. The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the target polypeptide. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the target polypeptide, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3′ phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, Nature, 282: 39 [1979]; Kingsman *et al.*, Gene, 7: 141 [1979]; or Tschemper *et al.*, Gene, 10: 157 [1980]). The *trp*1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 (Jones, Genetics, 85: 12 [1977]). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu*2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu*2 gene.

(d) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the target polypeptide nucleic acid. Promoters are untranslated sequences located upstream (5′) to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence, such as that encoding the target polypeptide, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g. the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to DNA encoding the target polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native target polypeptide promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target polypeptide DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of expressed target polypeptide as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems (Chang *et al.*, Nature, 275: 615 [1978]; and Goeddel *et al.*, Nature, 281: 544 [1979]), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, Nucleic Acids Res., 8: 4057 [1980] and EP 36,776) and hybrid promoters such as the tac promoter (deBoer *et al.*, Proc. Natl. Acad. Sci. USA, 80: 21-25 [1983]). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to DNA encoding the target polypeptide (Siebenlist *et al.*, Cell, 20: 269 [1980]) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also generally will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the target polypeptide.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255: 2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7: 149 [1968]; and Holland, Biochemistry, 17: 4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into mammalian expression vectors.

Target polypeptide transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the target polypeptide sequence, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209: 1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78: 7398-7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway *et al.*, Gene, 18: 355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295: 503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297: 598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79: 5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79: 6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

(e) Enhancer Element Component

Transcription of DNA encoding the target polypeptide of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10-300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' (Laimins *et al.*, Proc. Natl. Acad. Sci. USA, 78: 993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3: 1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33: 729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4: 1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297: 17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5′ or 3′ to the target polypeptide DNA, but is preferably located at a site 5′ from the promoter.

(f) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5′ and, occasionally 3′ untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the target polypeptide. The 3′ untranslated regions also include transcription termination sites.

Construction of suitable vectors containing one or more of the above listed components the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9: 309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65: 499 (1980).

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding the target polypeptide. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention for purposes of identifying analogs and variants of the target polypeptide that have target polypeptide-like activity.

Other methods, vectors, and host cells suitable for adaptation to the synthesis of the target polypeptide in recombinant vertebrate cell culture are described in Gething *et al.*, Nature, 293: 620-625 [1981]; Mantei *et al.*, Nature, 281: 40-46 [1979]; Levinson *et al.*; EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of the target polypeptide is pRK5 (EP pub. no. 307,247) or pSVI6B (U.S. Ser. No. 07/441,574 filed 22 November 1989, the disclosure of which is incorporated herein by reference).

Selection and Transformation of Host Cells

Suitable host cells for cloning or expressing the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *E. coli*, *Bacilli* such as *B. subtilis*, *Pseudomonas* species such as *P. aeruginosa*, *Salmonella typhimurium*, or *Serratia marcescans*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* χ1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Preferably the host cell should secrete minimal amounts of proteolytic enzymes. Alternatively, *in vitro* methods of cloning, e.g. PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for target polypeptide-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe* [Beach and Nurse, Nature, 290: 140 (1981); EP 139,383 published May 2, 1985], *Kluyveromyces* hosts (U.S. 4,943,529) such as, e.g., *K. lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *K. fragilis, K. bulgaricus, K. thermotolerans*, and *K. marxianus, yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070; Sreekrishna *et al.*, J. Basic Microbiol., 28: 265-278 (1988)], *Candida, Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76: 5259-5263 (1979)], and filamentous fungi such as, e.g, *Neurospora, Penicillium, Tolypocladium* [WO 91/00357 published 10 January 1991], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112: 284-289 (1983); Tilburn *et al.*, Gene, 26: 205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81: 1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4: 475-479 (1985)].

Suitable host cells for the expression of glycosylated target polypeptide are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6: 47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315: 592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the target polypeptide DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding target polypeptide is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the target polypeptide DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1: 561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. See EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36: 59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216 [1980]); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23: 243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383: 44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al., supra*, is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23: 315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30-16.37 of Sambrook *et al, supra,* is preferred. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216, issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130: 946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76: 3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or protoplast fusion may also be used.

Culturing the Host Cells

Prokaryotic cells used to produce the target polypeptide of this invention are cultured in suitable media as described generally in Sambrook *et al.*, *supra*.

The mammalian host cells used to produce the target polypeptide of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58: 44 (1979), Barnes and Sato, Anal. Biochem., 102: 255 (1980), U.S. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Pat. Re. 30,985; or copending U.S.S.N. 07/592,107 or 07/592,141, both filed in 3 October 1990, the disclosures of all of which are incorporated herein by reference, may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The host cells referred to in this disclosure encompass cells in *in vitro* culture as well as cells that are within a host animal.

It is further envisioned that the target polypeptides of this invention may be produced by homologous recombination, or with recombinant production methods utilizing control elements introduced into cells already containing DNA encoding the target polypeptide currently in use in the field. For example, a powerful promoter/enhancer element, a suppressor, or an exogenous transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired target polypeptide. The control element does not encode the target polypeptide of this invention, but the DNA is present in the host cell genome. One next screens for cells making the target polypeptide of this invention, or increased or decreased levels of expression, as desired.

Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77: 5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75: 734-738 (1980).

Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native target polypeptide or against a synthetic peptide based on the DNA sequences provided herein as described further in Section 4 below.

Purification of The Target polypeptide

The target polypeptide preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal.

When the target polypeptide is expressed in a recombinant cell other than one of human origin, the target polypeptide is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the target polypeptide from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the target polypeptide. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The membrane and soluble protein fractions are then separated. The target polypeptide may then be purified from the soluble protein fraction and from the membrane fraction of the culture lysate, depending on whether the target polypeptide is membrane bound. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove contaminants such as IgG.

Target polypeptide variants in which residues have been deleted, inserted or substituted are recovered in the same fashion, taking account of any substantial changes in properties occasioned by the variation. For example, preparation of a target polypeptide fusion with another protein or polypeptide, e.g. a bacterial or viral antigen, facilitates purification; an immunoaffinity column containing antibody to the antigen (or containing antigen, where the target polypeptide is an antibody) can be used to adsorb the fusion. Immunoaffinity columns such as a rabbit polyclonal anti-target polypeptide column can be employed to absorb the target polypeptide variant by binding it to at least one remaining immune epitope. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for native target polypeptide may require modification to account for changes in the character of the target polypeptide or its variants upon expression in recombinant cell culture.

Covalent Modifications of Target Polypeptides

Covalent modifications of target polypeptides are included within the scope of this invention. One type of covalent modification included within the scope of this invention is a target polypeptide fragment. Target polypeptide fragments having up to about 40 amino acid residues may be conveniently prepared by chemical synthesis, or by enzymatic or chemical cleavage of the full-length target polypeptide or variant target polypeptide. Other types of covalent modifications of the target polypeptide or fragments thereof are introduced into the molecule by reacting specific amino acid residues of the target polypeptide or fragments thereof with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

Cysteinyl residues most commonly are reacted with *α*-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, *α*-

bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'-N=C=N-R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking target polypeptide to a water-insoluble support matrix or surface for use in the method for purifying anti-target polypeptide antibodies, and vice versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the target polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. By altering is meant deleting one or more carbohydrate moieties found in the native target polypeptide, and/or adding one or more glycosylation sites that are not present in the native target polypeptide.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the target polypeptide is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the native target polypeptide sequence (for O-linked glycosylation sites). For ease, the target polypeptide amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above under the heading of "Amino Acid Sequence Variants of Target Polypeptide".

Another means of increasing the number of carbohydrate moieties on the target polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- and O- linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published 11 September 1987, and in Aplin and Wriston (CRC Crit. Rev. Biochem., pp. 259-306 [1981]).

Removal of carbohydrate moieties present on the native target polypeptide may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin *et al.* (Arch. Biochem. Biophys., 259:52 [1987]) and by Edge *et al.* (Anal. Biochem., 118:131 [1981]). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.* (Meth. Enzymol., 138:350 [1987]).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin *et al.* (J. Biol. Chem., 257:3105 [1982]). Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of the target polypeptide comprises linking the target polypeptide to various nonproteinaceous polymers, e.g. polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in U.S. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The target polypeptide also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-[methylmethacylate] microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A., Ed., (1980).

Target polypeptide preparations are also useful in generating antibodies, for screening for binding partners, as standards in assays for the target polypeptide (e.g. by labeling the target polypeptide for use as a standard in a radioimmunoassay, enzyme-linked immunoassay, or radioreceptor assay), in affinity purification techniques, and in competitive-type receptor binding assays when labeled with radioiodine, enzymes, fluorophores, spin labels, and the like.

Since it is often difficult to predict in advance the characteristics of a variant target polypeptide, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. For example, a change in the immunological character of the target polypeptide molecule, such as affinity for a given antigen or antibody, is measured by a competitive-type immunoassay. The variant is assayed for changes in the suppression or enhancement of its activity by comparison to the activity observed for the target polypeptide in the same assay. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, stability in recombinant cell culture or in plasma, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

Diagnostic and Related Uses of the Antibodies

The antibodies of this invention are useful in diagnostic assays for antigen expression in specific cells or tissues. The antibodies are detectably labeled and/or are immobilized on an insoluble matrix.

The antibodies of this invention find further use for the affinity purification of the antigen from recombinant cell culture or natural sources.

Suitable diagnostic assays for the antigen and its antibodies depend on the particular antigen or antibody. Generally, such assays include competitive and sandwich assays, and steric inhibition assays. Competitive and sandwich methods employ a phase-separation step as an integral part of the method while steric inhibition assays are conducted in a single reaction mixture. Fundamentally, the same procedures are used for the assay of the antigen and for substances that bind the antigen, although certain methods will be favored depending upon the molecular weight of the substance being assayed. Therefore, the substance to be tested is referred to herein as an analyte, irrespective of its status otherwise as an antigen or antibody, and proteins that bind to the analyte are denominated binding partners, whether they be antibodies, cell surface receptors, or antigens.

Analytical methods for the antigen or its antibodies all use one or more of the following reagents: labeled analyte analogue, immobilized analyte analogue, labeled binding partner, immobilized binding partner and steric conjugates. The labeled reagents also are known as "tracers."

The label used (and this is also useful to label antigen nucleic acid for use as a probe) is any detectable functionality that does not interfere with the binding of analyte and its binding partner. Numerous labels are known for use in immunoassay, examples including moieties that may be detected directly, such as fluorochrome, chemiluminescent, and radioactive labels, as well as moieties, such as enzymes, that must be reacted or derivatized to be detected. Examples of such labels include the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Conventional methods are available to bind these labels covalently to proteins or polypeptides. For instance, coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine, and the like may be used to tag the antibodies with the above-described fluorescent, chemiluminescent, and enzyme labels. See, for example, U.S. Pat. Nos. 3,940,475 (fluorimetry) and 3,645,090 (enzymes); Hunter *et al.*, Nature, 144: 945 (1962); David *et al.*, Biochemistry, 13: 1014-1021 (1974); Pain *et al.*, J. Immunol. Methods, 40: 219-230 (1981); and Nygren, J. Histochem. and Cytochem., 30: 407-412 (1982). Preferred labels herein are enzymes such as horseradish peroxidase and alkaline phosphatase.

The conjugation of such label, including the enzymes, to the antibody is a standard manipulative procedure for one of ordinary skill in immunoassay techniques. See, for example, O'Sullivan *et al.*, "Methods for the Preparation of Enzyme-antibody Conjugates for Use in Enzyme Immunoassay," in Methods in Enzymology, ed. J.J. Langone and H. Van Vunakis, Vol. 73 (Academic Press, New York, New York, 1981), pp. 147-166. Such bonding methods are suitable for use with the antibodies and polypeptides of this invention.

Immobilization of reagents is required for certain assay methods. Immobilization entails separating the binding partner from any analyte that remains free in solution. This conventionally is accomplished by either insolubilizing the binding partner or analyte analogue before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich *et al.*, U.S. 3,720,760), by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the partner or analogue afterward, e.g., by immunoprecipitation.

Other assay methods, known as competitive or sandwich assays, are well established and widely used in the commercial diagnostics industry.

Competitive assays rely on the ability of a tracer analogue to compete with the test sample analyte for a limited number of binding sites on a common binding partner. The binding partner generally is insolubilized before or after the competition and then the tracer and analyte bound to the binding partner are separated from the unbound tracer and analyte. This separation is accomplished by decanting (where the binding partner was preinsolubilized) or by centrifuging (where the binding partner was precipitated after the competitive reaction). The amount of test sample analyte is inversely proportional to the amount of bound tracer as measured by the amount of marker substance. Dose-response curves with known amounts of analyte are prepared and compared with the test results to quantitatively determine the amount of analyte present in the test sample. These assays are called ELISA systems when enzymes are used as the detectable markers.

Another species of competitive assay, called a "homogeneous" assay, does not require a phase separation. Here, a conjugate of an enzyme with the analyte is prepared and used such that when anti-analyte binds to the analyte the presence of the anti-analyte modifies the enzyme activity. In this case, the antigen or its immunologically active fragments are conjugated with a bifunctional organic bridge to an enzyme such as peroxidase. Conjugates are selected for use with antibody so that binding of the antibody inhibits or potentiates the enzyme activity of the label. This method *per se* is widely practiced under the name of EMIT.

Steric conjugates are used in steric hindrance methods for homogeneous assay. These conjugates are synthesized by covalently linking a low-molecular-weight hapten to a small analyte so that antibody to hapten substantially is unable to bind the conjugate at the same time as anti-analyte. Under this assay procedure the analyte present in the test sample will bind anti-analyte, thereby allowing anti-hapten to bind the conjugate, resulting in a change in the character of the conjugate hapten, e.g., a change in fluorescence when the hapten is a fluorophore.

Sandwich assays particularly are useful for the determination of antigen or antibodies. In sequential sandwich assays an immobilized binding partner is used to adsorb test sample analyte, the test sample is removed as by washing, the bound analyte is used to adsorb labeled binding partner, and bound material is then separated from residual tracer. The amount of bound tracer is directly proportional to test sample analyte. In "simultaneous" sandwich assays the test sample is not separated before adding the labeled binding partner. A sequential sandwich assay using an anti-antigen monoclonal antibody as one antibody and a polyclonal anti-antigen antibody as the other is useful in testing samples for particular antigen activity.

The foregoing are merely exemplary diagnostic assays for the import and humanized antibodies of this invention. Other methods now or hereafter developed for the determination of these analytes are included within the scope hereof, including the bioassays described above.

Immunotoxins

This invention is also directed to immunochemical derivatives of the antibodies of this invention such as immunotoxins (conjugates of the antibody and a cytotoxic moiety). Antibodies which carry the appropriate effector functions, such as with their constant domains, are also used to induce lysis through the natural complement process, and to interact with antibody dependent cytotoxic cells normally present.

For example, purified, sterile filtered antibodies are optionally conjugated to a cytotoxin such as ricin for use in AIDS therapy. US Patent Application Serial No. 07/350,895 illustrates methods for making and using immunotoxins for the treatment of HIV infection, and its teachings are specifically incorporated by reference herein. The methods of this invention, for example, are suitable for obtaining humanized antibodies for use as immunotoxins for use in AIDS therapy.

The cytotoxic moiety of the immunotoxin may be a cytotoxic drug or an enzymatically active toxin of bacterial, fungal, plant or animal origin, or an enzymatically active fragment of such a toxin. Enzymatically active toxins and fragments thereof used are diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. In another embodiment, the antibodies are conjugated to small molecule anticancer drugs such as cis-platin or 5FU. Conjugates of the monoclonal antibody and such cytotoxic moieties are made using a variety of bifunctional protein coupling agents. Examples of such reagents are SPDP, IT , bifunctional derivatives of imidoesters such as dimethyl adipimidate HCl, active esters such as disuccinimidyl suberate, aldehydes such as glutaraldehyde, bis-azido compounds such as bis (p-azidobenzoyl) hexanediamine, bis-diazonium derivatives such as bis- (p-diazoniumbenzoyl)- -ethylenediamine, diisocyanates such as tolylene 2,6-diisocyanate and bis-active fluorine compounds such as l,5-difluoro-2,4-dinitrobenzene. The lysing portion of a toxin may be joined to the Fab fragment of the antibodies.

Immunotoxins can be made in a variety of ways, as discussed herein. Commonly known crosslinking reagents can be used to yield stable conjugates.

Advantageously, monoclonal antibodies specifically binding the domain of the antigen which is exposed on the infected cell surface, are conjugated to ricin A chain. Most advantageously the ricin A chain is deglycosylated and produced through recombinant means. An advantageous method of making the ricin immunotoxin is described in Vitetta *et al.*, *Science* 238:1098 (1987) hereby incorporated by reference.

When used to kill infected human cells *in vitro* for diagnostic purposes, the conjugates will typically be added to the cell culture medium at a concentration of at least about 10 nM. The formulation and mode of administration for *in vitro* use are not critical. Aqueous formulations that are compatible with the culture or perfusion medium will normally be used. Cytotoxicity may be read by conventional techniques.

Cytotoxic radiopharmaceuticals for treating infected cells may be made by conjugating radioactive isotopes (e.g. I, Y, Pr) to the antibodies. Advantageously alpha particle-emitting isotopes are used. The term 'cytotoxic moiety" as used herein is intended to include such isotopes.

In a preferred embodiment, ricin A chain is deglycosylated or produced without oligosaccharides, to decrease its clearance by irrelevant clearance mechanisms (e.g., the liver). In another embodiment, whole ricin (A chain plus B chain) is conjugated to antibody if the galactose binding property of B-chain can be blocked ("blocked ricin").

In a further embodiment toxin-conjugates are made with Fab or F(ab')$_2$ fragments. Because of their relatively small size these fragments can better penetrate tissue to reach infected cells.

In another embodiment, fusogenic liposomes are filled with a cytotoxic drug and the liposomes are coated with antibodies specifically binding the particular antigen.

Antibody Dependent Cellular Cytotoxicity

Certain aspects of this invention involve antibodies which are (a) directed against a particular antigen and (b) belong to a subclass or isotype that is capable of mediating the lysis of cells to which the antibody molecule binds. More specifically, these antibodies should belong to a subclass or isotype that, upon complexing with cell surface proteins, activates serum complement and/or mediates antibody dependent cellular cytotoxicity (ADCC) by activating effector cells such as natural killer cells or macrophages.

Biological activity of antibodies is known to be determined, to a large extent, by the constant domains or Fc region of the antibody molecule (Unanue and Benacerraf, *Textbook of Immunology*, 2nd Edition, Williams & Wilkins, p. 218 (1984)). This includes their ability to activate complement and to mediate antibody-dependent cellular cytotoxicity (ADCC) as effected by leukocytes. Antibodies of different classes and subclasses differ in this respect, as do antibodies from the same subclass but different species; according to the present invention, antibodies of those classes having the desired biological activity are prepared. Preparation of these antibodies involves the selection of antibody constant domains are their incorporation in the humanized antibody by known technique. For example, mouse immunoglobulins of the IgG3 and IgG2a class are capable of activating serum complement upon binding to the target cells which express the cognate antigen, and therefore humanized antibodies which incorporate IgG3 and IgG2a effector functions are desirable for certain therapeutic applications.

In general, mouse antibodies of the IgG2a and IgG3 subclass and occasionally IgG1 can mediate ADCC, and antibodies of the IgG3, IgG2a, and IgM subclasses bind and activate serum complement. Complement activation generally requires the binding of at least two IgG molecules in close proximity on the target cell. However, the binding of only one IgM molecule activates serum complement.

The ability of any particular antibody to mediate lysis of the target cell by complement activation and/or ADCC can be assayed. The cells of interest are grown and labeled *in vitro*; the antibody is added to the cell culture in combination with either serum complement or immune cells which may be activated by the antigen antibody complexes. Cytolysis of the target cells is detected by the release of label from the lysed cells. In fact, antibodies can be screened using the patient's own serum as a source of complement and/or immune cells. The antibody that is capable of activating complement or mediating ADCC in the *in vitro* test can then be used therapeutically in that particular patient.

This invention specifically encompasses consensus Fc antibody domains prepared and used according to the teachings of this invention.

Therapeutic and Other Uses of the Antibodies

When used *in vivo* for therapy, the antibodies of the subject invention are administered to the patient in therapeutically effective amounts (i.e. amounts that have desired therapeutic effect). They will normally be administered parenterally. The dose and dosage regimen will depend upon the degree of the infection, the characteristics of the particular antibody or immunotoxin used, e.g., its therapeutic index, the patient, and the patient's history. Advantageously the antibody or immunotoxin is administered continuously over a period of 1-2 weeks, intravenously to treat cells in the vasculature and subcutaneously and intraperitoneally to treat regional lymph nodes. Optionally, the administration is made during the course of adjunct therapy such as combined cycles of radiation, chemotherapeutic treatment, or administration of tumor necrosis factor, interferon or other cytoprotective or immunomodulatory agent.

For parenteral administration the antibodies will be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic, and non-therapeutic. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils and ethyl oleate can also be used. Liposomes may be used as carriers. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The antibodies will typically be formulated in such vehicles at concentrations of about 1 mg/ml to 10 mg/ml.

Use of IgM antibodies may be preferred for certain applications, however IgG molecules by being smaller may be more able than IgM molecules to localize to certain types of infected cells.

There is evidence that complement activation *in vivo* leads to a variety of biological effects, including the induction of an inflammatory response and the activation of macrophages (Unanue and Benecerraf, *Textbook of Immunology*, 2nd Edition, Williams & Wilkins, p. 218 (1984)). The increased vasodilation accompanying inflammation may increase the ability of various agents to localize in infected cells. Therefore, antigen-antibody combinations of the type specified by this invention can be used therapeutically in many ways. Additionally, purified antigens (Hakomori, *Ann. Rev. Immunol.* 2:103 (1984)) or anti-idiotypic antibodies (Nepom *et al.*, *Proc. Natl. Acad. Sci.* 81:2864 (1985); Koprowski *et al.*, *Proc. Natl. Acad. Sci.* 81:216 (1984)) relating to such antigens could be used to induce an active immune response in human patients. Such a response includes the formation of antibodies capable of activating human complement and mediating ADCC and by such mechanisms cause infected cell destruction.

Optionally, the antibodies of this invention are useful in passively immunizing patients, as exemplified by the administration of humanized anti-HIV antibodies.

The antibody compositions used in therapy are formulated and dosages established in a fashion consistent with good medical practice taking into account the disorder to be treated, the condition of the individual patient, the site of delivery of the composition, the method of administration and other factors known to practitioners. The antibody compositions are prepared for administration according to the description of preparation of polypeptides for administration, *infra.*

Deposit of Materials

As described above, cultures of the muMAb4D5 have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC).

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for 30 years from the date of the deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the cultures to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.12 with particular reference to 886 OG 638).

In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)

The assignee of the present application has agreed that if the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the constructs deposited, since the deposited embodiments are intended to illustrate only certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that they represent. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

It is understood that the application of the teachings of the present invention to a specific problem or situation will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of the products of the present invention and representative processes for their isolation, use, and manufacture appear below, but should not be construed to limit the invention. All literature citations herein are expressly incorporated by reference.

EXAMPLES

EXAMPLE 1. HUMANIZATION OF muMAb4D5

Here we report the chimerization of muMAb4D5 (chMAb4D5) and the rapid and simultaneous humanization of heavy (V_H) and light (V_L) chain variable region genes using a novel "gene conversion mutagenesis" strategy. Eight humanized variants (huMAb4D5) were constructed to probe the importance of several FR residues identified by our molecular modeling or previously proposed to be critical to the conformation of particular CDRs (see Chothia, C. & Lesk, A. M., *J. Mol. Biol.* **196**:901-917 (1987); Chothia, C. *et al., Nature* **342**:877-883 (1989); Tramontano, A. *et al., J. Mol. Biol.* **215**:175-182 (1990)). Efficient transient expression of humanized variants in non-myeloma cells allowed us to rapidly investigate the relationship between binding affinity for $p185^{HER2}$ ECD and anti-proliferative activity against $p185^{HER2}$ overexpressing carcinoma cells.

MATERIALS and METHODS

Cloning of Variable Region Genes. The muMAb4D5 V_H and V_L genes were isolated by polymerase chain reaction (PCR) amplification of mRNA from the corresponding hybridoma (Fendly, B. M. *et al., Cancer Res.* 50:1550-1558 (1990)) as described by Orlandi et al. (Orlandi, R. *et al., Proc. Natl. Acad. Sci. USA* 86:3833-3837 (1989)). Amino terminal sequencing of muMAb4D5 V_L and V_H was used to design the sense strand PCR primers, whereas the anti-sense PCR primers were based upon consensus sequences of murine framework residues (Orlandi, R. *et al., Proc. Natl. Acad. Sci. USA* 86:3833-3837 (1989); Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) incorporating restriction sites for directional cloning shown by underlining and listed after the sequences: V_L sense, 5'-TCCGATATCCAGCTGACCCAGTCTCCA-3' (SEQ. ID NO. 7), *Eco*RV; V_L anti-sense, 5'-GTTTGATCTCCAGCTTGGTACCHSCDCCGAA-3' (SEQ. ID NO. 8), *Asp*718; V_H sense, 5'-AGGTSMARCTGCAGSAGTCWGG-3' (SEQ. ID NO. 9), *Pst*I and V_H anti-sense, 5'-TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG-3' (SEQ. ID NO. 10), *Bst*EII; where H = A or C or T, S = C or G, D = A or G or T, M = A or C, R = A or G and W = A or T. The PCR products were cloned into pUC119

(Vieira, J. & Messing, J., *Methods Enzymol.* **153**:3-11 (1987)) and five clones for each variable domain sequenced by the dideoxy method (Sanger, F. *et al.*, *Proc. Natl. Acad. Sci. USA* **74**:5463-5467 (1977)).

Molecular Modelling. Models for muMAb4D5 V_H and V_L domains were constructed separately from consensus coordinates based upon seven Fab structures from the Brookhaven protein data bank (entries 1FB4, 2RHE, 2MCP, 3FAB, 1FBJ, 2HFL and 1REI). The Fab fragment KOL (Marquart, M. *et al.*, *J. Mol. Biol.* **141**:369-391 (1980)) was first chosen as a template for V_L and V_H domains and additional structures were then superimposed upon this structure using their main chain atom coordinates (INSIGHT program, Biosym Technologies). The distance from the template Cα to the analogous Cα in each of the superimposed structures was calculated for each residue position. If all (or nearly all) Cα-Cα distances for a given residue were $\leq$ 1Å, then that position was included in the consensus structure. In most cases the β-sheet framework residues satisfied these criteria whereas the CDR loops did not. For each of these selected residues the average coordinates for individual N, Cα, C, O and Cβ atoms were calculated and then corrected for resultant deviations from non-standard bond geometry by 50 cycles of energy minimization using the DISCOVER program (Biosym Technologies) with the AMBER forcefield (Weiner, S. J. *et al.*, *J. Amer. Chem. Soc.* 106:765-784 (1984)) and Cα coordinates fixed. The side chains of highly conserved residues, such as the disulfide-bridged cysteine residues, were then incorporated into the resultant consensus structure. Next the sequences of muMAb4D5 V_L and V_H were incorporated starting with the CDR residues and using the tabulations of CDR conformations from Chothia et al. (Chothia, C. *et al.*, *Nature* **342**:877-883 (1989)) as a guide. Side-chain conformations were chosen on the basis of Fab crystal structures, rotamer libraries (Ponder, J. W. & Richards, F. M., *J. Mol. Biol.* **193**:775-791 (1987)) and packing considerations. Since V_H-CDR3 could not be assigned a definite backbone conformation from these criteria, two models were created from a search of similar sized loops using the INSIGHT program. A third model was derived using packing and solvent exposure considerations. Each model was then subjected to 5000 cycles of energy minimization.

In humanizing muMAb4D5, consensus human sequences were first derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)), namely V_L κ subgroup I and V_H group III, and a molecular model generated for these sequences using the methods described above. A structure for huMAb4D5 was created by transferring the CDRs from the muMAb4D5 model into the consensus human structure. All huMAb4D5 variants contain human replacements of muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)): V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S. Differences between muMAb4D5 and the human consensus framework residues (Fig. 1) were individually modeled to investigate their possible influence on CDR conformation and/or binding to the $p185^{HER2}$ ECD.

Construction of Chimeric Genes. Genes encoding chMAb4D5 light and heavy chains were separately assembled in previously described phagemid vectors containing the human cytomegalovirus enhancer and promoter, a 5′ intron and SV40 polyadenylation signal (Gorman, C. M. *et al., DNA & Prot. Engin. Tech.* **2**:3-10 (1990)). Briefly, gene segments encoding muMAb4D5 V_L (Fig. 1A) and REI human κ_1 light chain C_L (Palm, W. & Hilschmann, N., *Z. Physiol. Chem.* **356**:167-191 (1975)) were precisely joined as were genes for muMAb4D5 V_H (Fig. 1B) and human $\gamma1$ constant region (Capon, D. J. *et al., Nature* **337**:525-531 (1989)) by simple subcloning (Boyle, A., in *Current Protocols in Molecular Biology*, Chapter 3 (F. A. Ausubel *et al.*, eds., Greene Publishing & Wiley-Interscience, New York, 1990)) and site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The $\gamma1$ isotype was chosen as it has been found to be the preferred human isotype for supporting ADCC and complement dependent cytotoxicity using matched sets of chimeric (Brüggemann, M. *et al., J. Exp. Med.* **166**:1351-1361 (1987)) or humanized antibodies (Riechmann, L. *et al., Nature* **332**:323-327 (1988)). The PCR-generated V_L and V_H fragments (Fig. 1) were subsequently mutagenized so that they faithfully represent the sequence of muMAb4D5 determined at the protein level: V_H Q1E, V_L V104L and T109A (variants are denoted by the amino acid residue and number followed by the replacement amino acid). The human γ1 constant regions are identical to those reported by Ellison et al. (Ellison, J. W. *et al., Nucleic Acids Res.* **13**:4071-4079 (1982)) except for the mutations E359D and M361L (Eu numbering, as in Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) which we installed to convert the antibody from the naturally rare A allotype to the much more common non-A allotype (Tramontano, A. *et al., J. Mol. Biol.* **215**:175-182 (1990)). This was an attempt to reduce the risk of anti-allotype antibodies interfering with therapy.

Construction of Humanized Genes. Genes encoding chMAb4D5 light chain and heavy chain Fd fragment (V_H and C_H1 domains) were subcloned together into pUC119 (Vieira, J. & Messing, J., *Methods Enzymol.* **153**:3-11 (1987)) to create pAK1 and simultaneously humanized in a single step (Fig. 2). Briefly, sets of 6 contiguous oligonucleotides were designed to humanize V_H and V_L (Fig. 1). These oligonucleotides are 28 to 83 nucleotides in length, contain zero to 19 mismatches to the murine antibody template and are constrained to have 8 or 9 perfectly matched residues at each end to promote efficient annealing and ligation of adjacent oligonucleotides. The sets of V_H and V_L humanization oligonucleotides (5 pmol each) were phosphorylated with either ATP or γ-^{32}P-ATP (Carter, P. *Methods Enzymol.* **154**:382-403 (1987)) and separately annealed with 3.7 pmol of pAK1 template in 40 μl 10 mM Tris-HCl (pH 8.0) and 10 mM $MgCl_2$ by cooling from 100 °C to room temperature over ~30 min. The annealed oligonucleotides were joined by incubation with T4 DNA ligase (12 units; New England Biolabs) in the presence of 2 μl 5 mM ATP and 2 μl 0.1 M DTT for 10 min at 14 °C. After electrophoresis on a 6% acrylamide sequencing gel the assembled oligonucleotides were located by autoradiography and recovered by electroelution. The assembled oligonucleotides (~0.3 pmol each) were simultaneously annealed to 0.15 pmol single-stranded deoxyuridine-containing pAK1 prepared according to Kunkel et al. (Kunkel, T. A. *et al., Methods Enzymol.* 154:367-382 (1987)) in 10 μl 40 mM Tris-HCl (pH 7.5) and 16 mM $MgCl_2$ as above. Heteroduplex DNA was constructed by extending the primers with T7 DNA polymerase and transformed into *E. coli* BMH 71-18 *mut*L as previously described (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The resultant phagemid DNA pool was enriched first for huV_L by restriction purification using *Xho*I and then for huV_H by restriction selection using *Stu*I as described in Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991); and in Wells, J. A. *et al., Phil. Trans. R. Soc. Lond. A* 317:415-423 (1986). Resultant clones containing both huV_L and huV_H genes were identified by nucleotide sequencing (Sanger, F. *et al., Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)) and designated pAK2. Additional humanized variants were generated by site-directed mutagenesis (Carter, P., in *Mutagenesis: A Practical Approach*, Chapter 1 (IRL Press, Oxford, UK 1991)). The muMAb4D5 V_L and V_H gene segments in the transient expression vectors described above were then precisely replaced with their humanized versions.

Expression and Purification of MAb4D5 Variants. Appropriate MAb4D5 light and heavy chain cDNA expression vectors were co-transfected into an adenovirus transformed human embryonic kidney cell line, 293 (Graham, F. L. *et al., J. Gen. Virol.* 36:59-72 (1977)) using a high efficiency procedure (Gorman, C. M. *et al., DNA & Prot. Engin. Tech.* 2:3-10 (1990); Gorman, C., in *DNA Cloning*, vol II, pp 143-190 (D. M. Glover, ed., IRL Press, Oxford, UK 1985)). Media were harvested daily for up to 5 days and the cells re-fed with serum free media. Antibodies were recovered from the media and affinity purified on protein A sepharose CL-4B (Pharmacia) as described by the manufacturer. The eluted antibody was buffer-exchanged into phosphate-buffered saline by G25 gel filtration, concentrated by ultrafiltration (Centriprep-30 or Centricon-100, Amicon), sterile-filtered (Millex-GV, Millipore) and stored at 4 °C. The concentration of antibody was determined by using both total immunoglobulin and antigen binding ELISAs. The standard used was huMAb4D5-5, whose concentration had been determined by amino acid composition analysis.

Cell Proliferation Assay. The effect of MAb4D5 variants upon proliferation of the human mammary adenocarcinoma cell line, SK-BR-3, was investigated as previously described (Fendly, B. M. *et al., Cancer Res.* 50:1550-1558 (1990)) using saturating MAb4D5 concentrations.

Affinity Measurements. The antigen binding affinity of MAb4D5 variants was determined using a secreted form of the p185^{HER2} ECD prepared as described in Fendly, B. M. *et al., J. Biol. Resp. Mod.* 9:449-455 (1990). Briefly, antibody and p185^{HER2} ECD were incubated in solution until equilibrium was found to be reached. The concentration of free antibody was then determined by ELISA using immobilized p185^{HER2} ECD and used to calculate affinity (K_d) according to Friguet et al. (Friguet, B. *et al., J. Immunol. Methods* 77:305-319 (1985)).

RESULTS

Humanization of muMAb4D5. The muMAb4D5 V_L and V_H gene segments were first cloned by PCR and sequenced (Fig. 1). The variable genes were then simultaneously humanized by gene conversion mutagenesis using preassembled oligonucleotides (Fig. 2). A 311-mer oligonucleotide containing 39 mismatches to the template directed 24 simultaneous amino acid changes required to humanize muMAb4D5 V_L. Humanization of muMAb4D5 V_H required 32 amino acid changes which were installed with a 361-mer containing 59 mismatches to the muMAb4D5 template. Two out of 8 clones sequenced precisely encode huMAb4D5-5, although one of these clones contained a single nucleotide imperfection. The 6 other clones were essentially humanized but contained a small number of errors: < 3 nucleotide changes and < 1 single nucleotide deletion per kilobase.

Additional humanized variants (Table 1) were constructed by site-directed mutagenesis of huMAb4D5-5.

Expression levels of huMAb4D5 variants were in the range of 7 to 15 μg/ml as judged by ELISA using immobilized p185^{HER2} ECD. Successive harvests of five 10 cm plates allowed 200 μg to 500 mg of each variant to be produced in a week. Antibodies affinity purified on protein A gave a single band on a Coomassie blue stained SDS polyacrylamide gel of mobility consistent with the expected M_r of ~150 kDa. Electrophoresis under reducing conditions gave 2 bands consistent with the expected M_r of free heavy (48 kDa) and light (23 kDa) chains (not shown). Amino terminal sequence analysis (10-cycles) gave the mixed sequence expected (see Fig. 1) from an equimolar combination of light and heavy chains (not shown).

huMAb4D5 Variants. In general, the FR residues were chosen from consensus human sequences (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) and CDR residues from muMAb4D5. Additional variants were constructed by replacing selected human residues in huMAb4D5-1 with their muMAb4D5 counterparts. These are V_H residues 71, 73, 78, 93 plus 102 and V_L residues 55 plus 66 identified by our molecular modeling. V_H residue 71 has previously been proposed by others (Tramontano, A. *et al.*, *J. Mol. Biol.* **215**:175-182 (1990)) to be critical to the conformation of V_H-CDR2. Amino acid sequence differences between huMAb4D5 variant molecules are shown in Table 1, together with their p185^{HER2} ECD binding affinity and maximal anti-proliferative activities against SK-BR-3 cells. Very similar K_d values were obtained for binding of MAb4D5 variants to either SK-BR-3 cells (unpublished data) or to p185^{HER2} ECD (Table 1). However, K_d estimates derived from binding of MAb4D5 variants to p185^{HER2} ECD were more reproducible with smaller standard errors and consumed much smaller quantities of antibody than binding measurements with whole cells.

The most potent humanized variant designed by molecular modeling, huMAb4D5-8, contains 5 FR residues from muMAb4D5. This antibody binds the p185^{HER2} ECD 3-fold *more* tightly than does muMAb4D5 itself (Table 1) and has comparable anti-proliferative activity with SK-BR-3 cells (Fig. 3). In contrast, huMAb4D5-1 is the most humanized but least potent muMAb4D5 variant, created by simply installing the muMAb4D5 CDRs into the consensus human sequences. huMAb4D5-1 binds the p185^{HER2} ECD 80-fold *less* tightly than does the murine antibody and has no detectable anti-proliferative activity at the highest antibody concentration investigated (16 μg/ml).

The anti-proliferative activity of huMAb4D5 variants against p185^{HER2} overexpressing SK-BR-3 cells is not simply correlated with their binding affinity for the p185^{HER2} ECD. For example, installation of three murine residues into the V_H domain of huMAb4D5-2 (D73T, L78A and A93S) to create huMAb4D5-3 does not change the antigen binding affinity but does confer significant anti-proliferative activity (Table 1).

The importance of V_H residue 71 (Tramontano, A. *et al., J. Mol. Biol.* 215:175-182 (1990)) is supported by the observed 5-fold increase in affinity for p185^{HER2} ECD on replacement of R71 in huMAb4D5-1 with the corresponding murine residue, alanine (huMAb4D5-2). In contrast, replacing V_H L78 in huMAb4D5-4 with the murine residue, alanine (huMAb4D5-5), does not significantly change the affinity for the p185^{HER2} ECD or change anti-proliferative activity, suggesting that residue 78 is not of critical functional significance to huMAb4D5 and its ability to interact properly with the extracellular domain of p185^{HER2}.

V_L residue 66 is usually a glycine in human and murine κ chain sequences (Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) but an arginine occupies this position in the muMAb4D5 k light chain. The side chain of residue 66 is likely to affect the conformation of V_L-CDR1 and V_L-CDR2 and the hairpin turn at 68-69 (Fig. 4). Consistent with the importance of this residue, the mutation V_L G66R (huMAb4D5-3 → huMAb4D5-5) increases the affinity for the p185^{HER2} ECD by 4-fold with a concomitant increase in anti-proliferative activity.

From molecular modeling it appears that the tyrosyl side chain of muMAb4D5 V_L residue 55 may either stabilize the conformation of V_H-CDR3 or provide an interaction at the V_L-V_H interface. The latter function may be dependent upon the presence of V_H Y102. In the context of huMAb4D5-5 the mutations V_L E55Y (huMAb4D5-6) and V_H V102Y (huMAb4D5-7) individually increase the affinity for $p185^{HER2}$ ECD by 5-fold and 2-fold respectively, whereas together (huMAb4D5-8) they increase the affinity by 11-fold. This is consistent with either proposed role of V_L Y55 and V_H Y102.

Secondary Immune Function of huMAb4D5-8. MuMAb4D5 inhibits the growth of human breast tumor cells which overexpress $p185^{HER2}$ (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* **9**:1165-1172 (1989)). The antibody, however, does not offer the possibility of direct tumor cytotoxic effects. This possibility does arise in huMAb4D5-8 as a result of its high affinity (K_d = 0.1 μM) and its human IgG_1 subtype. Table 2 compares the ADCC mediated by huMAb4D5-8 with muMAb4D5 on a normal lung epithelial cell line, WI-38, which expresses a low level of $p185^{HER2}$ and on SK-BR-3, which expresses a high level of $p185^{HER2}$. The results demonstrate that: (1) huMAb4D5 has a greatly enhanced ability to carry out ADCC as compared with its murine parent; and (2) that this activity may be selective for cell types which overexpress $p185^{HER2}$.

DISCUSSION

MuMAb4D5 is potentially useful for human therapy since it is cytostatic towards human breast and ovarian tumor lines overexpressing the *HER2*-encoded $p185^{HER2}$ receptor-like tyrosine kinase. Since both breast and ovarian carcinomas are chronic diseases it is anticipated that the optimal MAb4D5 variant molecule for therapy will have low immunogenicity and will be cytotoxic rather than solely cytostatic in effect. Humanization of muMAb4D5 should accomplish these goals. We have identified 5 different huMAb4D5 variants which bind tightly to $p185^{HER2}$ ECD ($K_d \leq 1$ nM) and which have significant anti-proliferative activity (Table 1). Furthermore huMAb4D5-8 but not muMAb4D5 mediates ADCC against human tumor cell lines overexpressing p185^{HER2} in the presence of human effector cells (Table 2) as anticipated for a human γ1 isotype (Brüggemann, M. *et al., J. Exp. Med.* **166**:1351-1361 (1987); Riechmann, L. *et al., Nature* **332**:323-327 (1988)).

Rapid humanization of huMAb4D5 was facilitated by the gene conversion mutagenesis strategy developed here using long preassembled oligonucleotides. This method requires less than half the amount of synthetic DNA as does total gene synthesis and does not require convenient restriction sites in the target DNA. Our method appears to be simpler and more reliable than a variant protocol recently reported (Rostapshov, V. M. *et al., FEBS Lett.* **249**:379-382 (1989)). Transient expression of huMAb4D5 in human embryonic kidney 293 cells permitted the isolation of a few hundred micrograms of huMAb4D5 variants for rapid characterization by growth inhibition and antigen binding affinity assays. Furthermore, different combinations of light and heavy chain were readily tested by co-transfection of corresponding cDNA expression vectors.

The crucial role of molecular modeling in the humanization of muMAb4D5 is illustrated by the designed variant huMAb4D5-8 which binds the p185^{HER2} ECD 250-fold more tightly than the simple CDR loop swap variant, huMAb4D5-1. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based upon molecular modelling (Riechmann, L. *et al., Nature* **332**:323-327 (1988); Queen, C. *et al., Proc. Natl. Acad. Sci. USA* **86**:10029-10033 (1989)). Here we have extended this earlier work by others with a designed humanized antibody which binds its antigen 3-fold *more* tightly ~~than the parent rodent~~ antibody. While this result is gratifying, assessment of the success of the molecular modeling must await the outcome of X-ray structure determination. From analysis of huMAb4D5 variants (Table 1) it is apparent that their anti-proliferative activity is not a simple function of their binding affinity for p185^{HER2} ECD. For example the huMAb4D5-8 variant binds p185^{HER2} 3-fold more tightly than muMAb4D5 but the humanized variant is slightly less potent in blocking the proliferation of SK-BR-3 cells. Additional huMAb4D5 variants are currently being constructed in an attempt to identify residues triggering the anti-proliferative activity and in an attempt to enhance this activity.

In addition to retaining tight receptor binding and the ability to inhibit cell growth, the huMAb4D5-8 also confers a secondary immune function (ADCC). This allows for direct cytotoxic activity of the humanized molecule in the presence of human effector cells. The apparent selectivity of the cytotoxic activity for cell types which overexpress p185^{HER2} allows for the evolution of a straightforward clinic approach to those human cancers characterized by overexpression of the HER2 protooncogene.

Table 1. p185^{HER2} ECD binding affinity and anti-proliferative activities of MAb4D5 variants

MAb4D5 Variant	V_H Residue* 71 FR3	73 FR3	78 FR3	93 FR3	102 CDR3	V_L Residue* 55 CDR2	66 FR3	K_d† nM	Relative cell proliferation‡
huMAb4D5-1	R	D	L	A	V	E	G	102	
huMAb4D5-2	Ala	D	L	A	V	E	G	4.7	101
huMAb4D5-3	Ala	Thr	Ala	Ser	V	E	G	4.4	66
huMAb4D5-4	Ala	Thr	L	Ser	V	E	Arg	0.82	56
huMAb4D5-5	Ala	Thr	Ala	Ser	V	E	Arg	1.1	48
huMAb4D5-6	Ala	Thr	Ala	Ser	V	Tyr	Arg	0.22	51
huMAb4D5-7	Ala	Thr	Ala	Ser	Tyr	E	Arg	0.62	53
huMAb4D5-8	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.10	54
muMAb4D5	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.30	37

* Human and murine residues are shown in one letter and three letter amino acid code respectively.

† K_d values for the p185^{HER2} ECD were determined using the method of Friguet *et al.* (43) and the standard error of each estimate is ≤ ± 10%.

‡ Proliferation of SK-BR-3 cells incubated for 96 hr with MAb4D5 variants shown as a percentage of the untreated control as described (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* 9:1165-1172 (1989)). Data represent the maximal anti-proliferative effect for each variant (see Fig. 3A) calculated as the mean of triplicate determinations at a MAb4D5 concentration

of 8 μg/ml. Data are all taken from the same experiment with an estimated standard error of ≤ ± 15%.

Table 2. Selectivity of antibody dependent tumor cell cytotoxicity mediated by huMAb4D5-8

Effector:Target ratio[†]		WI-38[*]		SK-BR-3	
		muMAb4D5	huMAb4D5-8	muMAb4D5	huMAb4D5-8
A.[‡]	25:1	<1.0	9.3	7.5	40.6
	12.5:1	<1.0	11.1	4.7	36.8
	6.25:1	<1.0	8.9	0.9	35.2
	3.13:1	<1.0	8.5	4.6	19.6
B.	25:1	<1.0	3.1	6.1	33.4
	12.5:1	<1.0	1.7	5.5	26.2
	6.25:1	1.3	2.2	2.0	21.0
	3.13:1	<1.0	0.8	2.4	13.4

[*] Sensitivity to ADCC of two human cell lines (WI-38, normal lung epithelium; and SK-BR-3, human breast tumor cell line) are compared. WI-38 expresses a low level of $p185^{HER2}$ (0.6 pg per μg cell protein) and SK-BR-3 expresses a high level of $p185^{HER2}$ (64 pg $p185^{HER2}$ per μg cell protein), as determined by ELISA (Fendly *et al.*, *J. Biol. Resp. Mod.* 9:449-455 (1990)).

[†] ADCC assays were carried out as described in Brüggemann *et al.*, *J. Exp. Med.* **166**:1351-1361 (1987). Effector to target ratios were of IL-2 activated human peripheral blood lymphocytes to either WI-38 fibroblasts or SK-BR-3 tumor cells in 96-well microtiter plates for 4 hours at 37 °C. Values given represent percent specific cell lysis as determined by ^{51}Cr release. Estimated standard error in these quadruplicate determinations was $\leq \pm 10\%$.

[‡] Monoclonal antibody concentrations used were 0.1 μg/ml (A) and 0.1 μg/ml (B).

EXAMPLE 2. Schematic Method for Humanizing an Antibody Sequence

This example illustrates one stepwise elaboration of the methods for creating a humanized sequence described above. It will be understood that not all of these steps are essential to the claimed invention, and that steps may be taken in different order.

1. ascertain a consensus human variable domain amino acid sequence and prepare from it a consensus structural model.
2. prepare model of import (the non-human domain to be humanized) variable domain sequences and note structural differences with respect to consensus human model.
3. identify CDR sequences in human and in import, both by using Kabat (*supra*, 1987) and crystal structure criteria. If there is any difference in CDR identity from the different criteria, use of crystal structure definition of the CDR, but retain the Kabat residues as important framework residues to import.
4. substitute import CDR sequences for human CDR sequences to obtain initial "humanized" sequence.
5. compare import non-CDR variable domain sequence to the humanized sequence and note divergences.
6. Proceed through the following analysis for each amino acid residue where the import diverges from the humanized.
    a. If the humanized residue represents a residue which is generally highly conserved across all species, use the residue in the humanized sequence. If the residue is not conserved across all species, proceed with the analysis described in 6b.
    b. If the residue is not generally conserved across all species, ask if the residue is generally conserved in humans.
        i. If the residue is generally conserved in humans but the import residue differs, examine the structural models of the

import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs by considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, leave the humanized residue unchanged.

ii. If the residue is also not generally conserved in humans, examine the structural models of the import and human sequences and determine if the import residue would be likely to affect the binding or biological activity of the CDRs be considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, proceed to the next step.

   a) Examine the structural models of the import and human sequences and determine if the residue is exposed on the surface of the domain or is buried within. If the residue is exposed, use the residue in the humanized sequence. If the residue is buried, proceed to the next step.

      (i) Examine the structural models of the import and human sequences and determine if the residue is likely to affect the V_L - V_H interface. Residues involved with the interface include: 34L, 36L, 38L, 43L, 33L, 36L, 85L, 87L, 89L, 91L, 96L, 98L, 35H, 37H, 39H, 43H, 45H, 47H, 60H, 91H, 93H, 95H, 100H, and 103H. If no effect is likely, use the residue in the humanized sequence. If some affect is likely, substitute the import residue.

7. Search the import sequence, the consensus sequence and the humanized sequence for glycosylation sites outside the CDRs, and determine if this glycosylation site is likely to have any affect on antigen binding and/or biological activity. If no effect is likely, use the human sequence at that site; if some affect is likely, eliminate the glycosylation site or use the import sequence at that site.

8. After completing the above analysis, determine the planned humanized sequence and prepare and test a sample. If the sample does not bind well to the target antigen, examine the particular residues listed below, regardless of the question of residue identity between the import and humanized residues.

   a. Examine particular peripheral (non-CDR) variable domain residues that may, due to their position, possibly interact directly with a macromolecular antigen, including the following residues (where the * indicates residues which have been found to interact with antigen based on crystal structures):

      i. Variable light domain: 36, 46, **49***, 63-70

      ii. Variable heavy domain: 2, **47***, 68, 70, 73-76.

   b. Examine particular variable domain residues which could interact with, or otherwise affect, the conformation of variable domain CDRs, including the following (not including CDR residues themselves, since it is assumed that, because the CDRs interact with one another, any residue in one CDR could potentially affect the conformation of another CDR residue) (L= LIGHT, H=HEAVY, residues appearing in **bold** are indicated to be structurally important according the Chothia *et al.*, Nature 342:877 (1989), and residues appearing in *italic* were altered during humanization by Queen *et al.* (PDL), Proc. Natl. Acad. Sci. USA 86:10029 (1989) and Proc. Natl. Acad. Sci. USA 88:2869 (1991).):

      i. Variable light domain:

    - a) CDR-1 (residues 24L-34L): **2L**, 4L, 66L-69L, **71L**
    - b) CDR-2 (residues 50L-56L): 35L, 46L, *47L*, **48L**, *49L*, 58L, 62L, **64L**-66L, 71L, 73L
    - c) CDR-3 (residues 89L-97L): 2L, 4L, 36L, 98L, 37H, 45H, 47H, 58H, 60H
  - ii. Variable heavy domain:
    - a) CDR-1 (residues 26H-35H): 2H, 4H, 24H, 36H, 71H, 73H, 76H, 78H, 92H, ***94H***
    - b) CDR-2 (residues 50H-55H): 49H, 69H, 69H, **71H**, 73H, 78H
    - c) CDR-3 (residues 95H-102H): examine all residues as possible interaction partners with this loop, because this loop varies in size and conformation much more than the other CDRs.

9. If after step 8 the humanized variable domain still is lacking in desired binding, repeat step 8. In addition, re-investigate any buried residues which might affect the V_L - V_H interface (but which would not directly affect CDR conformation). Additionally, evaluate the accessibility of non-CDR residues to solvent.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Carter, Paul J.
Presta, Leonard G.

(ii) TITLE OF INVENTION: Immunoglobulin Variants

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 460 Point San Bruno Blvd
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 14-June-1991
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Adler, Carolyn R.
(B) REGISTRATION NUMBER: 32,324
(C) REFERENCE/DOCKET NUMBER: 709

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415/266-2614
(B) TELEFAX: 415/952-9881
(C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
20 25 30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20 25 30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr

50 55 60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
95 100 105

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
20 25 30

Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                20                  25                  30

Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr
                50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                95                 100                 105

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
               110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
 1               5                  10                  15

Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
                20                  25                  30
```

```
Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
            35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
            50                  55                  60

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
            65                  70                  75

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
            80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
            95                  100                 105

Ile Lys Arg Ala
        109
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
 1          5                   10                  15

Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
            20                  25                  30

Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
            35                  40                  45

Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
            50                  55                  60

Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
            65                  70                  75

Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
            80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
            95                  100                 105
```

```
Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
            110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCGATATCC AGCTGACCCA GTCTCCA 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTGATCTC CAGCTTGGTA CCXXCDCCGA A 31

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGTXXAXCT GCAGXAGTCX GG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

CLAIMS

Sub B1

WE CLAIM:

1. A method for making a humanized antibody comprising amino acid sequence of a non-human, import antibody and a human antibody, comprising the steps of:

   a. obtaining the amino acid sequences of at least a portion of an import variable domain and of a consensus human variable domain;

   b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;

   c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

   d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

   e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

   f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

      1. non-covalently binds antigen directly,
      2. interacts with a CDR; or
      3. participates in the V_L - V_H interface; and

   g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

2. The method of claim 1, having an additional step of determining if

Paul Carter et al
07/715,272
6/14/91

~~any such non-homologous residues are exposed on the surface of the domain or buried within it, and if the residue is exposed, retaining the consensus residue.~~

440 32 Cont

3. ~~The method of claim 1, having the additional steps of searching the import variable domain sequence for glycosylation sites, determining if any such glycosylation site is reasonably expected to affect the antigen binding or affinity of the antibody, and if so, substituting the glycosylation site into the consensus sequence.~~

4. ~~The method of claim 1, having the additional steps of searching the consensus variable domain sequence for glycosylation sites which are not present at the corresponding amino acid in the import sequence, and if the glycosylation site is not present in the import sequence, substituting the import amino acid residues for the amino acid residues comprising the consensus glycosylation site.~~

5. ~~The method of claim 1, having an additional step which comprises aligning import antibody and consensus antibody FR sequences, identifying import antibody FR residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR residue, determining if the corresponding consensus antibody residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.~~

6. The method of claim 1, wherein the corresponding consensus antibody residues are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H,

76H, 78H, 91H, 92H, 93H, and 103H.

7. ~~A method comprising providing at least a portion of an import, non-human antibody variable domain amino acid sequence having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:
4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.~~

8. The method of claim 7, wherein the substituted residue is the residue found at the corresponding location of the non-human antibody.

9. A humanized antibody variable domain having a non-human CDR incorporated into a human antibody variable domain, wherein the improvement comprises substituting an amino acid residue for the human residue at a site selected from the group consisting of:
4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

10. The humanized antibody variable domain of claim 9, wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR was obtained.

11. The humanized antibody variable domain of claim 9, wherein no human FR residue other than those set forth in the group has been substituted.

12. A polypeptide comprising the amino acid sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP
KLLIYSASFLESGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHY
TTPPTFGQGTKVEIKRT

13. A polypeptide comprising the sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE
WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDT
AVYYCSRWGGDGFYAMDVWGQGTLVTVSS

Group 320

101

14. A computer comprising the sequence data of the following amino acid sequence:

a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQ
KPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQ
PEDFATYYCQQYNSLPYTFGQGTKVEIKRT, or

b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVR
QAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKN
TAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQG
TLVTVSS

101

15. A computer representation of the following amino acid sequence:

a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQ
KPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQ
PEDFATYYCQQYNSLPYTFGQGTKVEIKRT, or

b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVR
QAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKN
TAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQG

TLVTVSS

101

16. A method comprising storing a computer representation of the following amino acid sequence:

a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT, or

b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS

Abstract

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

EXPRESS MAIL NO. B59937585
MAILED: 14 JUNE 1991

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

Docket No. 709

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

IMMUNOGLOBULIN VARIANTS

the specification of which (check one) x is attached hereto or _ was filed on as Application Serial No. and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I hereby state that any Sequence Listing submitted with this application is submitted in paper copy and a computer-readable diskette, and that the content of the paper and computer readable copies are the same.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate have a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
			Yes	No
Number	Country	Day/Month/Year Filed		

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Ser. No.	Filing Date	Status: Patented, Pending, Abandoned
Application Ser. No.	Filing Date	Status: Patented, Pending, Abandoned

---

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

301
Carolyn R. Adler - Reg. No. 32,324
Robert H. Benson - Reg. No. 30,446
Walter E. Buting - Reg. No. 23,092
Ginger R. Dreger - Reg. No. 33,055
Debbie Glaister - Reg. No. 33,888
Janet E. Hasak - Reg. No. 28,616

Max D. Hensley - Reg. No. 27,043
Dennis G. Kleid - Reg. No. 32,037
Nancy Olseki - Reg. No. 34,688
Stephen Raines - Reg. No. 25,912
Daryl B. Winter - Reg. No. 32,637

Send correspondence to
601 Genentech, Inc.
602 Attn: Carolyn R. Adler
701 460 Point San Bruno Boulevard
702 South San Francisco, CA 94080
Telephone: (415) 266-2614

I hereby declare that all statements made herein of my own knowledge and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from his foreign patent agent as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Full name of sole or first inventor

Paul J/ Carter 40100

Inventor's signature Date

Residence
2074 18th Avenue
San Francisco, CA 94116

Citizenship
United Kingdom

Post Office Address
460 Point San Bruno Boulevard
South San Francisco, CA 94080

Full name of second joint inventor, if any

Leonard G./ Presta 48200

Second Inventor's signature Date

Residence
1900 Gough Street, #206
San Francisco, CA 94109

Citizenship
United States of America

Post Office Address
460 Point San Bruno Boulevard
South San Francisco, CA 94080

Full name of third joint inventor, if any

Third Inventor's signature Date

Residence

Citizenship

Post Office Address
460 Point San Bruno Boulevard
South San Francisco, CA 94080

BAR CODE LABEL

U.S. PATENT APPLICATION

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT
07/715,272	06/14/91	530	183

APPLICANT

PAUL J. CARTER, SAN FRANCISCO, CA; LEONARD G. PRESTA, SAN FRANCISCO, CA.

CONTINUING DATA********************
VERIFIED

FOREIGN/PCT APPLICATIONS**********
VERIFIED

FOREIGN FILING LICENSE GRANTED 08/03/91

STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	ATTORNEY DOCKET NO.
CA	5	16	8	$1,050.00	709

ADDRESS

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

TITLE

IMMUNOGLOBULIN VARIANTS

This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application as originally filed which is identified above.

By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

Date Certifying Officer

07 715272

PATENT APPLICATION SERIAL NO.______________

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

P 30192 06/27/91 07715272 07-0630 030 101 930.00CH

PTO-1556
(5/87)

PATENT APPLICATION FEE DETERMINATION RECORD

Application or Docket Number

715272

CLAIMS AS FILED - PART I

FOR	NUMBER FILED (Column 1)	NUMBER EXTRA (Column 2)	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
BASIC FEE				$ 315.00	OR		$ 630.00
TOTAL CLAIMS	16 minus 20 =	*	x $10=		OR	x $20 =	
INDEPENDENT CLAIMS	8 minus 3 =	* 5	x 30 =		OR	x 60 =	300
MULTIPLE DEPENDENT CLAIM PRESENT			+ 100 =		OR	+ 200 =	
			TOTAL		OR	TOTAL	930

* If the difference in column 1 is less then zero, enter "0" in column 2

CLAIMS AS AMENDED - PART II

AMENDMENT A

	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	SMALL ENTITY RATE	ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
Total	*	Minus	**	=	x $10=		OR	x $20 =	
Independent	*	Minus	***	=	x 30 =		OR	x 60=	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 100 =		OR	+ 200 =	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	SMALL ENTITY RATE	ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
Total	*	Minus	**	=	x $10 =		OR	x $20 =	
Independent	*	Minus	***	=	x 30 =		OR	x 60 =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 100 =		OR	+ 200 =	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	SMALL ENTITY RATE	ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
Total	*	Minus	**	=	x $10 =		OR	x $20 =	
Independent	*	Minus	***	=	x 30 =		OR	x 60 =	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 100 =		OR	+ 200 =	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

FORM PTO-875
(Rev. 12-90)

For Fees Effective Nov. 5, 1990

Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 709P1	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US92/05126	International filing date *(day/month/year)* 15/06/92	(Earliest) Priority Date *(day/month/year)* 14/06/91
Applicant GENENTECH, INC. et al.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☒ **Certain claims were found unsearchable** (see Box I).

2. ☐ **Unity of invention is lacking** (see Box II).

3. ☐ The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - ☐ filed with the international application.
   - ☐ furnished by the applicant separately from the international application,
     - ☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - ☐ Transcribed by this Authority

4. With regard to the **title**,
   - ☐ the text is approved as submitted by the applicant.
   - ☒ the text has been established by this Authority to read as follows:

METHOD FOR MAKING HUMANIZED ANTIBODIES.

5. With regard to the **abstract**,
   - ☒ the text is approved as submitted by the applicant.
   - ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

Figure No. 2
- ☐ as suggested by the applicant.
- ☐ because the applicant failed to suggest a figure.
- ☐ because this figure better characterizes the invention.

☐ None of the figures.

Form PCT/ISA/210 (first sheet) (July 1992)

INTERNATIONAL S[redacted]CH REPORT

International application No.
PCT/[redacted]92/05126

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 17-18
because they relate to subject matter not required to be searched by this Authority, namely:
see PCT-Rule 39.1(iv)

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

INTERN[illegible]IONAL SEARCH REPORT

International Application No P[illegible]/US 92/05126

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/13; C12P21/08; C07K13/00; C12N5/10
G06F15/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; C12N ; G06F

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	JOURNAL OF MOLECULAR BIOLOGY vol. 215, 1990, ACADEMIC PRESS pages 175 - 182 Tramontano, Anna; Chothia, Cyrus; Lesk, Arthur M. 'Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins' cited in the application See the whole document, especially paragraph 7 ---	1-12,15
Y	WO,A,9 007 861 (PROTEIN DESIGN LABS, INC.) 26 July 1990 See pages 1-6; 9-25 --- -/--	1-12,15

° Special categories of cited documents :[10]

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

2 **IV. CERTIFICATION**

Date of the Actual Completion of the International Search 07 OCTOBER 1992	Date of Mailing of this International Search Report 02. 11. 92
International Searching Authority EUROPEAN PATENT OFFICE	Signature of Authorized Officer NAUCHE S.A.

See notes on accompanying sheet

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9205126
SA 61838

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 07/10/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9007861	26-07-90	AU-A-	5153290	13-08-90
		CA-A-	2006865	28-06-90
		EP-A-	0451216	16-10-91

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

International Application No [illegible]/US 92/05126

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	NATURE. vol. 342, December 1989, LONDON GB pages 877 - 883 Chothia, Cyrus; Lesk, Arthur M.; Tramontano, Anna; Levitt, Michael; Smith-Gill, Sandra J.; Air, Gillian; Sheriff, Steven; Padlan, 'Conformations of immunoglobulin hypervariable region' cited in the application See the whole document, especially 'Discussion' ---	1-12,15
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89, May 1992, WASHINGTON US pages 4285 - 4289 Carter, Paul et al. 'Humanization of an anti-p185HER2 antibody for human cancer therapy.' see the whole document -----	1-15

DocCode - SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: **07715272** Document Date: **06/14/1991**

The presence of this form in the IFW record indicates that the following document type was received in paper and is scanned and stored in the SCORE database.

- Drawings

Images of the original documents are scanned in gray scale or color and stored in SCORE. Bi-tonal images are also stored in IFW. Defects visible in both IFW and SCORE are indicative of defects in the original paper documents.

To access the documents in the SCORE database, refer to instructions developed by SIRA.

At the time of document entry (noted above):
- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: December 8, 2006

NUMBER

FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
06/14/91	530	387	~~183~~ 186	Feisee

APPLICANTS

RTER, SAN FRANCISCO, CA; LEONARD G. PRESTA, SAN FRANCISCO, CA.

CONTINUING DATA***********************
VERIFIED
None

FOREIGN/PCT APPLICATIONS**********
VERIFIED
None

FOREIGN FILING LICENSE GRANTED 08/03/91

	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
Foreign priority claimed ☐ yes [✓ no] 35 USC 119 conditions met ☐ yes [✓ no] Verified and Acknowledged Examiner's Initials	→	CA	5	16	8	$1,050.00	709

ADDRESS

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

TITLE

IMMUNOGLOBULIN VARIANTS

U.S. DEPT. of COMM.-Pat. & TM Office — PTO-436L (rev. 10-78)

ARTS OF APPLICATION
ILED SEPARATELY

NOTICE OF ALLOWANCE MAILED	PREPARED FOR ISSUE		CLAIMS ALLOWED	
	Assistant Examiner	Docket Clerk	Total Claims	Print Claim
ISSUE FEE			DRAWING	
Amount Due	Date Paid	Primary Examiner	Sheets Drwg. / Figs. Drwg.	Print Fig.
Label Area	ISSUE CLASSIFICATION		ISSUE BATCH NUMBER	
	Class	Subclass		

WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.

Form PTO-436
Rev. 9/80

SEARCHED

Class	Sub.	Date	Exmr.
530	387.3	9/29/92	VF
530	350	↓	↓
updated		4/29/93	VF
updated		10/15/93	VF
updated		1/10/94	VF

33. Request For access 6-21-02
34. Request for access
35. Request for access 10-18-02
36. Request for access 11-1-02
37. Request for access 12/18/02
38. Request for Access 5/21/03
39). Request for access 11/3/03
40). Request for access 11-19-03
41 Request for access 12/18/03
42). Request for access 3/9/04
43. Request for access 4-2-04
44). Request for access 5-3
45). Request for access 6-[illegible]-04
46). Request for access 7/9/04
47). Request for access 8-16-04
48). Request for access 11-5-04
49 Request for access 11/29/04
50 Request for access 1-18-05
51. Request for access 1-21-06
58 Request for access 8-22-06
59. Request for access 8/27/06

SEARCH NOTES

	4/29/93	VF
APS updated	10/15/93	VF
updated	1/10/94	VF

52. Request for access 4-15-05

Turn over please

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.

Best Available Copy

POSITION		INIT.	DATE
CLASSIFIER			
EXAMINER		18	6-19-91
TYPIST		416	6/27/[illegible]
VERIFIER			
CORPS CORR.		VD 3C	8-5-[illegible]
SPEC. HAND			
FILE MAINT.			
			1-2-[illegible]

INDEX OF CLAIMS

SYMBOLS
✓ .... Rejected
= .... Allowed
– (Through numeral) Canceled
÷ .... Restricted
N .... Non-elected
I .... Interference
A .... Appeal
O .... Objected

Claim Final	Claim Original	Date 5/11/92	9/28/92	[illegible]	[illegible]	[illegible]	1/11/[illegible]
	10						
	11						
	12						
	13						
	20						
	21						
	22						
	23						
	24						
	25						
	26						
	27						
	28						
	29						
	30						
	31						
	32						
	33						
	34						
	35						
	36						
	37						
	38						
	40						
	41						
	42						
	43						
	45						
	46						
	47						
	48						
	49						

Claim Final	Claim Original	Date
	51	
	52	
	53	
	54	
	55	
	56	
	57	
	58	
	59	
	60	
	61	
	62	
	63	
	64	
	65	
	66	
	67	
	68	
	69	
	70	
	71	
	72	
	73	
	74	
	75	
	76	
	77	
	78	
	79	
	80	
	81	
	82	
	83	
	84	
	85	
	86	
	87	
	88	
	89	
	90	
	91	
	92	
	93	
	94	
	95	
	96	
	97	
	98	
	99	
	100	

Raw Sequence Listing

06/25/91
10:32:10

Patent Application US/07/715,272

#2

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Carter, Paul J.
Presta, Leonard G.

(ii) TITLE OF INVENTION: Immunoglobulin Variants

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 460 Point San Bruno Blvd
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 14-June-1991
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Adler, Carolyn R.
(B) REGISTRATION NUMBER: 32,324
(C) REFERENCE/DOCKET NUMBER: 709

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415/266-2614
(B) TELEFAX: 415/952-9881
(C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
                20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
                50                  55                  60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
                65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                 100                 105

Ile Lys Arg Thr
           109
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
                20                  25                  30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
                50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                95                 100                 105
```

```
Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                 120


(2) INFORMATION FOR SEQ ID NO:3:

   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 109 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
                 20                  25                  30

Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                 65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                 80                  85                  90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
                 95                 100                 105

Ile Lys Arg Thr
            109

(2) INFORMATION FOR SEQ ID NO:4:

   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 120 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                 20                  25                  30
```

```
Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                 65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                 95                 100                 105

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                 120


(2) INFORMATION FOR SEQ ID NO:5:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 109 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
  1               5                  10                  15

Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
                 20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
                 50                  55                  60

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
                 65                  70                  75

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
                 80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
                 95                 100                 105

Ile Lys Arg Ala
            109

(2) INFORMATION FOR SEQ ID NO:6:
```

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
 1               5                  10                  15

Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
                20                  25                  30

Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
                35                  40                  45

Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
                50                  55                  60

Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
                65                  70                  75

Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                95                 100                 105

Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
               110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
TCCGATATCC AGCTGACCCA GTCTCCA 27
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 bases
(B) TYPE: nucleic acid

```
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:


        GTTTGATCTC CAGCTTGGTA CCXXCDCCGA A 31



(2) INFORMATION FOR SEQ ID NO:9:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 22 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:


        AGGTXXAXCT GCAGXAGTCX GG 22



(2) INFORMATION FOR SEQ ID NO:10:

     (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 34 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:


        TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34
```

X's are not valid according to the rule.

```
PAGE: 1                  SEQUENCE VERIFICATION REPORT             DATE: 06/25/91
                         PATENT APPLICATION US/07/715,272         TIME: 10:32:20

LINE ERROR                                         ORIGINAL TEXT

272  Wrong Nucleic Acid Designator                 GTTTGATCTC CAGCTTGGTA CCXXCDCCGA A 31
269  Entered and Calc. Seq. Length differ error due to  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
287  Wrong Nucleic Acid Designator                 AGGTXXAXCT GCAGXAGTCX GG 22
284  Entered and Calc. Seq. Length differ          (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
```

PAGE: 1

SEQUENCE MISSING ITEM REPORT
PATENT APPLICATION US/07/715,272

DATE: 06/25/91
TIME: 10:32:20

MANDATORY IDENTIFIER THAT WAS NOT FOUND

SEQUENCE CORRECTION REPORT
PATENT APPLICATION US/07/715,272

DATE: 06/25/91
TIME: 10:32:20

LINE ORIGINAL TEXT CORRECTED TEXT

Genentech, Inc.
Attn: Carolyn R. Adler
460 Point San Bruno Blvd.
South San Francisco, CA 94080

Paul J. Carter
07/715,272
June 14, 1991

#3

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Mailed: 7·3·91

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR § 1.821(a)(1) and (a)(2). However, this application fails to comply with one or more of the requirements of 37 CFR §§ 1.821 through 1.825 as follows:

☐ 1. This application clearly fails to comply with the collective requirements of §§ 1.821 through 1.825. Applicant's attention is directed to these regulations, a copy of which is attached.

☐ 2. This application does not conform exclusively to the requirements of §§ 1.821 through 1.825. The non-conforming material should be deleted. § 1.821(b).

☐ 3. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing." § 1.821(c).

☐ 4. This application does contain, as a separate part of the disclosure on paper copy, a "Sequence Listing." However, the "Sequence Listing" does not comply with the requirements of §§ 1.821 through 1.825 as follows:

☐ a. The sequence data does not comply with the symbol and format requirements of paragraphs (b) through (p) of § 1.822. Specifically: ____________

☐ b. The "Sequence Listing" does not comply with the location and page requirements of paragraph (a) of § 1.823.

☐ c. The "Sequence Listing" does not comply with the information requirements of paragraph (b) of § 1.823. Specifically: ____________

☐ d. Other: ____________

☐ 5. The description and/or claims of the patent application mention a sequence that is set forth in the "Sequence Listing" but reference is not properly made to the sequence by use of a sequence identifier as required by § 1.821(d).

☐ 6. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by § 1.821(e).

☑ 7. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the computer readable form does not comply with the requirements of § 1.824. Specifically: See attachment

☐ 8. A statement that the content of the paper and computer readable copies are the same has not been submitted as required by § 1.821(f).

☐ 9. The amendment to or replacement of the paper and/or computer readable copies of the "Sequence Listing" does not comply with the requirements of § 1.825(a) through (c).

☐ 10. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable. Applicant must provide a substitute copy of the data in computer readable form accompanied by a statement that the substitute data is identical to that originally
§ 1.825(d). Specifically: ____________

Other: ____________

IVEN ONE MONTH FROM THE DATE OF THIS LETTER WITHIN WHICH
THE ABOVE REQUIREMENTS. Failure to comply with the above require-
ANDONMENT of the application under 37 CFR 1.821(g). Extensions of
v filing a petition accompanied by the extension fee under the provisions of
the response to, and any questions about, this notice to the undersigned. A
be returned with your response.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY DOCKET NO./TITLE
07/715,272	06/14/91	CARTER	P 709

#4

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

000

DATE MAILED: 07/03/91

NOTICE TO FILE MISSING PARTS OF APPLICATION
FILING DATE GRANTED

A filing date has been granted to this application. However, the following parts are missing.

If all missing parts are filed within the period set below, the total amount owed by applicant as a ☑ large entity, ☐ small entity (verified statement filed), is $ 120.00.

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit $________ to complete the basic filing fee and MUST ALSO SUBMIT THE SURCHARGE AS INDICATED BELOW.

2. ☐ Additional claim fees of $________ as a ☐ large entity ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due. NO SURCHARGE IS REQUIRED FOR THIS ITEM.

3. ☐ The oath or declaration:
   - ☐ is missing.
   - ☐ does not cover items omitted at time of execution.

   An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

5. ☑ The signature to the oath or declaration is: ☑ missing; ☐ a reproduction; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW..

6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:
________________. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Receipt Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of $30.00 under 37 CFR 1.17(k), unless this fee has already been paid. NO SURCHARGE IS RERQUIRED FOR THIS ITEM.

8. ☐ A $60.00 processing fee is required for returned checks. (37 CFR 1.21(m)).

9. ☐ Your filing receipt was mailed in error because check was returned without payment.

10. ☐ Other.

   An Application Number and Filing Date have been assigned to this application. The missing parts and fees identified above in items 1 and 3-6 must be timely provided ALONG WITH THE PAYMENT OF A SURCHARGE of $120.00 for large entities or $60.00 for small entities who have filed a verified statement claiming such status. The surchage is set forth in 37 CFR 1.16(e). Applicant is given ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE of this application, WHICHEVER IS LATER, within which to file all missing parts and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Direct the response to, and any questions about, this notice to ATTENTION: Application Division, Special Handling Unit.

A copy of this notice <u>MUST</u> be returned with response.

C M Hayward
For: Manager, Application Division
(703) ~~557-~~ 308-1202

FORM PTO-1533 (REV. 6-90)

OFFICE COPY

MAIL ROOM
JUL 12 1991
PAT. & TRADEMARK OFF.

UNITED STA__ DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

AN

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY DOCKET NO./TITLE
07/715,272	06/14/91	CARTER	P 709

5

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

000

DATE MAILED: 07/03/91

NOTICE TO FILE MISSING PARTS OF APPLICATION
FILING DATE GRANTED

A filing date has been granted to this application. However, the following parts are missing.

If all missing parts are filed within the period set below, the total amount owed by applicant as a

☑ large entity, ☐ small entity (verified statement filed), is $ 120.00.

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit $________ to complete the basic filing fee and MUST ALSO SUBMIT THE SURCHARGE AS INDICATED BELOW.

2. ☐ Additional claim fees of $________ as a ☐ large entity ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due. NO SURCHARGE IS REQUIRED FOR THIS ITEM.

3. ☐ The oath or declaration:
   ☐ is missing.
   ☐ does not cover items omitted at time of execution.

   An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

5. ☑ The signature to the oath or declaration is: ☑ missing; ☐ a reproduction; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration: ________________. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Receipt Date is required. A SURCHARGE MUST ALSO BE SUBMITTED AS INDICATED BELOW.

7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of $30.00 under 37 CFR 1.17(k), unless this fee has already been paid. NO SURCHARGE IS RERQUIRED FOR THIS ITEM.

8. ☐ A $50.00 processing fee is required for returned checks. (37 CFR 1.21(m)).

9. ☐ Your filing receipt was mailed in error because check was returned without payment.

10. ☐ Other.

An Application Number and Filing Date have been assigned to this application. The missing parts and fees identified above in items 1 and 3-6 must be timely provided ALONG WITH THE PAYMENT OF A SURCHARGE of $120.00 for large entities or $60.00 for small entities who have filed a verified statement claiming such status. The surchage is set forth in 37 CFR 1.16(e). Applicant is given ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE of this application, WHICHEVER IS LATER, within which to file all missing parts and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

Direct the response to, and any questions about, this notice to ATTENTION: Application Division, Special Handling Unit.

A copy of this notice <u>MUST</u> be returned with response.

For: Manager, Application Division
(703) 557-

D520157 07/17/91 715272 07 0630 020 105 120.00CH

FORM PTO-1533 (REV. 8-90) COPY TO BE RETURNED WITH RESPONSE

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#5

In re Application of
Paul J. Carter et al.
Serial No. 07/715,272
Filed: 14 June 1991
For: IMMUNOGLOBULIN VARIANTS

Group Art Unit:
Examiner:

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 266-2614

TRANSMITTAL LETTER

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231
Attn: Application Branch

Sir:

Transmitted herewith are the following documents:

1. Declaration duly executed.
2 Copy of PTO-1553.

The Commissioner is hereby authorized to deduct the appropriate surcharge fee of $120 associated with this communication or credit any overpayment to Deposit Account No. 07-0630. A duplicate of this sheet is enclosed.

Respectfully submitted,
GENENTECH, INC.
Carolyn R. Adler
Carolyn R. Adler
Reg. No. 32,324

9 July 1991

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Carol Koehler
Carol Koehler

Date: 9 July 1991

#5

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

Docket No. 709

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

IMMUNOGLOBULIN VARIANTS

the specification of which (check one) __ is attached hereto or x was filed on 14 June 1991 as Application Serial No. 07/715,272 and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I hereby state that any Sequence Listing submitted with this application is submitted in paper copy and a computer-readable diskette, and that the content of the paper and computer readable copies are the same.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate have a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Number	Country	Day/Month/Year Filed	Priority Claimed Yes	Priority Claimed No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Ser. No. Filing Date Status: Patented, Pending, Abandoned

Application Ser. No. Filing Date Status: Patented, Pending, Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Carolyn R. Adler	- Reg. No. 32,324 301	Max D. Hensley	- Reg. No. 27,043
Robert H. Benson	- Reg. No. 30,446	Dennis G. Kleid	- Reg. No. 32,037 302
Walter E. Buting	- Reg. No. 23,092	Nancy Olseki	- Reg. No. 34,688
Ginger R. Dreger	- Reg. No. 33,055	Stephen Raines	- Reg. No. 25,912
Debbie Glaister	- Reg. No. 33,888	Daryl B. Winter	- Reg. No. 32,637
Janet E. Hasak	- Reg. No. 27,043		

Send correspondence to
601 Genentech, Inc.
602 Attn: Carolyn R. Adler
701 460 Point San Bruno Boulevard
702 South San Francisco, CA 94080
Telephone: (415) 266-2614

I hereby declare that all statements made herein of my own knowledge and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from his foreign patent agent as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Full name of sole or first inventor
Paul J. Carter 40100

Inventor's signature
Paul John Carter

Date
18th June 1991.

Residence
2074 18th Avenue
San Francisco, CA 94116

Citizenship
United Kingdom

Post Office Address
460 Point San Bruno Boulevard
South San Francisco, CA 94080

Full name of second joint inventor, if any
Leonard G. Presta 40200

Second Inventor's signature
Leonard G. Presta

Date
6-19-91

Residence
1900 Gough Street, #206
San Francisco, CA 94109

Citizenship
United States of America

Post Office Address
460 Point San Bruno Boulevard
South San Francisco, CA 94080

Full name of third joint inventor, if any

Third Inventor's signature

Date

Residence

Citizenship

Post Office Address
460 Point San Bruno Boulevard
South San Francisco, CA 94080

Best Available Copy

MAIL ROOM
JUL 15 1991

Genentech, Inc.
Attn: Carolyn R. Adler
460 Point San Bruno Blvd.
South San Francisco, CA 94080

RECEIVED
JUL 0 8 1991
Genentech, Inc. Legal Dept.

Paul J. Carter
07/715,272
June 14, 1991

CALENDARED #
3 Aug 91

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Mailed:

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR § 1.821(a)(1) and (a)(2). However, this application fails to comply with one or more of the requirements of 37 CFR §§ 1.821 through 1.825 as follows:

☐ 1. This application clearly fails to comply with the collective requirements of §§ 1.821 through 1.825. Applicant's attention is directed to these regulations, a copy of which is attached.

☐ 2. This application does not conform exclusively to the requirements of §§ 1.821 through 1.825. The non-conforming material should be deleted. § 1.821(b).

☐ 3. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing." § 1.821(c).

☐ 4. This application does contain, as a separate part of the disclosure on paper copy, a "Sequence Listing." However, the "Sequence Listing" does not comply with the requirements of §§ 1.821 through 1.825 as follows:

- ☐ a. The sequence data does not comply with the symbol and format requirements of paragraphs (b) through (p) of § 1.822. Specifically: ______
- ☐ b. The "Sequence Listing" does not comply with the location and page requirements of paragraph (a) of § 1.823.
- ☐ c. The "Sequence Listing" does not comply with the information requirements of paragraph (b) of § 1.823. Specifically: ______
- ☐ d. Other: ______

☐ 5. The description and/or claims of the patent application mention a sequence that is set forth in the "Sequence Listing" but reference is not properly made to the sequence by use of a sequence identifier as required by § 1.821(d).

☐ 6. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by § 1.821(e).

☑ 7. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the computer readable form does not comply with the requirements of § 1.824. Specifically: See attachment

☐ 8. A statement that the content of the paper and computer readable copies are the same has not been submitted as required by § 1.821(f).

☐ 9. The amendment to or replacement of the paper and/or computer readable copies of the "Sequence Listing" does not comply with the requirements of § 1.825(e) through (c).

☐ 10. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable. Applicant must provide a substitute copy of the data in computer readable form accompanied by a statement that the substitute data is identical to that originally filed. § 1.825(d). Specifically: ______

☐ 11. Other: ______

APPLICANT IS GIVEN ONE MONTH FROM THE DATE OF THIS LETTER WITHIN WHICH TO COMPLY WITH THE ABOVE REQUIREMENTS. Failure to comply with the above requirements will result in ABANDONMENT of the application under 37 CFR 1.821(g). Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR § 1.136. Direct the response to, and any questions about, this notice to the undersigned. A copy of this notice MUST be returned with your response.

☑ C McHaywood

Application Processing Division

☐

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Carter, Paul J.
Presta, Leonard G.

(ii) TITLE OF INVENTION: Immunoglobulin Variants

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 460 Point San Bruno Blvd
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 07/715,272
(B) FILING DATE: 14-June-1991
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Adler, Carolyn R.
(B) REGISTRATION NUMBER: 32,324
(C) REFERENCE/DOCKET NUMBER: 709

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415/266-2614
(B) TELEFAX: 415/952-9881
(C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
                20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
                50                  55                  60
```

```
Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
                65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                  100                 105

Ile Lys Arg Thr
            109
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1              5                   10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
                20                  25                  30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
                50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                95                  100                 105

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 1              5                   10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
                20                  25                  30

Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45
```

```
Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                 65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                 80                  85                  90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
                 95                 100                 105

Ile Lys Arg Thr
            109
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1                5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                 20                  25                  30

Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                 65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                 95                 100                 105

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
 1                5                  10                  15

Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
                 20                  25                  30
```

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
50 55 60

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
65 70 75

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
95 100 105

Ile Lys Arg Ala
109

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
20 25 30

Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
35 40 45

Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
50 55 60

Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
65 70 75

Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
95 100 105

Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCGATATCC AGCTGACCCA GTCTCCA 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTGATCTC CAGCTTGGTA CCHSCDCCGA A 31

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGTSMARCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

630 A/N a #7

MAIL ROOM
JUL 15 1991
PAT. & TRADEMARK OFF.

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
)
PAUL J. CARTER ET AL.)
) **Art Unit: to be assigned**
Serial No. 07/715,272)
) **Examiner: to be assigned**
Filed: June 14, 1991)
)
For: IMMUNOGLOBULIN VARIANTS)
)
)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on July 12, 1991
(Date of Deposit)
LOUISE STRASBAUGH
Name of Depositing Party
Louise Strasbaugh
Signature of Depositing Party
July 12, 1991
Date of Signature

RESPONSE AND PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

This is responsive to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide and/or Amino Acid Sequence Disclosures, mailed June 25, 1991. The inventors also take this opportunity to correct two minor grammatical errors in the application, and add no new matter.

Enclosed is an amended sequence listing submitted with a paper copy and a computer-readable diskette. The sequence listing has been corrected to conform exactly to the sequences as recited in the specification as originally filed. I hereby state that the content of this paper and computer readable copies are the same, and that this amendment corrects errors in the previous sequence listing submission without adding new matter.

IN THE SPECIFICATION:

Please make the following amendments:

On page 12, line 1, delete genes and insert --sequences--.

On page 16, line 12, delete intrachain-affecting and insert --interchain--affecting.

Respectfully Submitted,
GENENTECH, INC.

Carolyn R. Adler

Carolyn R. Adler
Reg. No. 32,324

July 12, 1991
460 Point San Bruno Blvd
South San Francisco, CA 94080

#8

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Carter, Paul J.
Presta, Leonard G.

(ii) TITLE OF INVENTION: Immunoglobulin Variants

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 460 Point San Bruno Blvd
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 07/715,272
(B) FILING DATE: 14-June-1991
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Adler, Carolyn R.
(B) REGISTRATION NUMBER: 32,324
(C) REFERENCE/DOCKET NUMBER: 709

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415/266-2614
(B) TELEFAX: 415/952-9881
(C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
                 20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Glu Ser Gly Val Pro Ser
                 50                  55                  60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
                 65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                 80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
                 95                 100                 105

Ile Lys Arg Thr
            109

(2) INFORMATION FOR SEQ ID NO:2:

   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 120 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1               5                  10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
                 20                  25                  30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                 65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                 95                 100                 105
```

```
Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                 120


(2) INFORMATION FOR SEQ ID NO:3:

   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 109 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1             5                   10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser
                20                  25                  30

Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                65                  70                  75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

Tyr Asn Ser Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
                95                  100                 105

Ile Lys Arg Thr
            109

(2) INFORMATION FOR SEQ ID NO:4:

   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 120 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1             5                   10                  15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
                20                  25                  30
```

```
Asp Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                 35                  40                  45

Glu Trp Val Ala Val Ile Ser Glu Asn Gly Gly Tyr Thr Arg Tyr
                 50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
                 65                  70                  75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                 95                 100                 105

Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                110                 115                 120


(2) INFORMATION FOR SEQ ID NO:5:

   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 109 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
  1               5                  10                  15

Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn
                 20                  25                  30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly His Ser Pro Lys
                 35                  40                  45

Leu Leu Ile Tyr Ser Ala Ser Phe Arg Tyr Thr Gly Val Pro Asp
                 50                  55                  60

Arg Phe Thr Gly Asn Arg Ser Gly Thr Asp Phe Thr Phe Thr Ile
                 65                  70                  75

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
                 80                  85                  90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
                 95                 100                 105

Ile Lys Arg Ala
            109

(2) INFORMATION FOR SEQ ID NO:6:
```

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
 1               5                  10                  15

Ala Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys
                20                  25                  30

Asp Thr Tyr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
                35                  40                  45

Glu Trp Ile Gly Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr
                50                  55                  60

Asp Pro Lys Phe Gln Asp Lys Ala Thr Ile Thr Ala Asp Thr Ser
                65                  70                  75

Ser Asn Thr Ala Tyr Leu Gln Val Ser Arg Leu Thr Ser Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                95                 100                 105

Ala Met Asp Tyr Trp Gly Gln Gly Ala Ser Val Thr Val Ser Ser
               110                 115                 120
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCGATATCC AGCTGACCCA GTCTCCA 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 bases
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTGATCTC CAGCTTGGTA CCHSCDCCGA A 31

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGTSMARCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION US/07/715,272A

DATE: 07/19/91
TIME: 16:16:36

LINE	ERROR	ORIGINAL TEXT
27	Wrong application Serial Number	OK (A) APPLICATION NUMBER: 07/715,272

SEQUENCE MISSING ITEM REPORT
PATENT APPLICATION US/07/715,272A

DATE: 07/19/91
TIME: 16:16:36

MANDATORY IDENTIFIER THAT WAS NOT FOUND

PAGE: 1

SEQUENCE CORRECTION REPORT
PATENT APPLICATION US/07/715,272A

DATE: 07/19/91
TIME: 16:16:36

LINE ORIGINAL TEXT

CORRECTED TEXT

18M Feisee

186

PATENT DOCKET 708

#10
5-18-92

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

MAIL ROOM
MAY 4 1992
86
PAT. & TRADEMARK OFC.

RECEIVED
MAY 08 1992
GROUP 180

In re Application of
Paul J. Carter et al.
Serial No. 07/715272
Filed: June 14, 1991
For: Immunoglobulin Variants

Group Art Unit:
Examiner:

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 266-2614

INFORMATION DISCLOSURE STATEMENT

by certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C., 20231 on April 30, 1992
(Date of Deposit)

LOUISE STRASBAUGH
Name of Depositing Party

Louise Strasbaugh
Signature of Depositing Party

April 30, 1992
Date of Signature

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

The following items are supplied to the United States Patent and Trademark Office to advance the prosecution of the subject application.

Chothia *et al.*, *J. Mol. Biol.* 186:651-663 (1985)
Novotny and Haber, *Proc. Natl. Acad. Sci. USA* 82:4592-4596 (1985)
Cabilly *et al.*, U.S. patent No. 4,816,567
Morrison, S. L. *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)
Boulianne, G. L. *et al.*, *Nature* 312:643-646 (1984)
Neuberger, M. S. *et al.*, *Nature* 314:268-270 (1985)
Brüggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987)
Riechmann, L. *et al.*, *Nature* 332:323-327 (1988)
Love *et al.*, *Methods in Enzymology* 178:515-527 (1989)
Bindon *et al.*, *J. Exp. Med.* 168:127-142 (1988)
Jones, P. T. *et al.*, *Nature* 321:522-525 (1986)
Verhoeyen, M. *et al.*, *Science* 239:1534-1536 (1988)
Hale, G. *et al.*, *Lancet* i:1394-1399 (1988)
Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989)
Co *et al.*, *Proc. Natl. Acad. Sci. USA* 88:2869-2873 (1991)
Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4181-4185 (1991)
Daugherty *et al.*, *Nucleic Acids Research* 19(9):2471-2476 (1991)

Brown *et al., Proc. Natl. Acad. Sci. USA* 88:2663-2667 (1991)

Junghans *et al., Cancer Research* 50:1495-1502 (1990)

Davies, D. R. *et al., Ann. Rev. Biochem.* 59:439-473 (1990)

Chothia, C. & Lesk, A. M., *J. Mol. Biol.* 196:901-917 (1987)

Chothia, C. *et al., Nature* 342:877-883 (1989)

Tramontano, A. *et al., J. Mol. Biol.* 215:175-182 (1990)

Margolies *et al., Proc. Natl. Acad. Sci. USA* 72:2180-2184 (1975)

Pluckthun, *Biotechnology* 9:545-51 (1991)

Spiegelberg *et al., Biochemistry* 9:4217-4223 (1970)

Wallick *et al., J. Exp. Med.* 168:1099-1109 (1988)

Sox *et al., Proc. Natl. Acad. Sci. USA* 66:975-982 (1970)

Margni *et al., Ann. Rev. Immunol.*6:535-554 (1988)

Fendly, B. M. *et al., Cancer Res.* 50:1550-1558 (1990)

Neuberger *et al., Nature* 312:604-608 (1984)

Takeda *et al., Nature* 314:452-454 (1985)

Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267-279, Alan R. Liss, Inc. pubs. (1986)

Cheetham, J., *Protein Engineering*, 2(3): 170-172 (1988)

WO 91/09967, pub. 07/11/91, Adair *et al.*

One copy of each item cited above is supplied, along with a completed Form PTO-1449. The Examiner is requested to make the citations of record.

This submission is understood to complement the results of the Examiner's own independent search. The submission of this Disclosure Statement should not be construed as a representation that a search was made, or that the cited itms are inclusive of all the relevant and amterial citations that may be available publicly.

The citation of any item is not an admission that the item is prior art. The right is reserved to antedate any item in adherence with standard procedures.

Respectfully submitted,
GENENTECH, INC.
Carolyn R. Adler
Carolyn R. Adler
Reg. No. 32,324

Dated: April 30, 1992

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR		ATTORNEY DOCKET NO.
07/715,272	06/14/91	CARTER	P	709

	EXAMINER
FETSEE,J	

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

ART UNIT	PAPER NUMBER
1806	9

DATE MAILED: 05/12/92

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined restricted ☒ Responsive to communication filed on 7/15/91 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 0 month(s), 30 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☒ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ ______

Part II SUMMARY OF ACTION

1. ☒ Claims 1-16 are pending in the application.
   Of the above, claims ______ are withdrawn from consideration.
2. ☐ Claims ______ have been cancelled.
3. ☐ Claims ______ are allowed.
4. ☐ Claims ______ are rejected.
5. ☐ Claims ______ are objected to.
6. ☒ Claims 1-16 are subject to restriction or election requirement.
7. ☒ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on ______. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on ______ has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed ______, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. ______ ; filed on ______.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-13, drawn to a method of making an antibody and an antibody comprising a polypeptide, classified in Class 435, 530 subclass 69.1, 350.

II. Claims 14-16, drawn to computer representations, classified in Class 364, subclass 282.1+.

The inventions are distinct, each from the other because of the following reasons:

The two Groups are drawn to two different products, Group I being a biological molecule and Group II being a machine. These constitute two different statutory classes of invention and are therefore patentably distinct one from the other.

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification and divergent subject matter, and because the searches for the individual Groups are not coextensive, restriction for examination purposes as indicated is proper.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. 1.48(b) and by the fee required under 37 C.F.R. 1.17(h).

A telephone call was made to Carolyn Adler, on 12/9/91, to request an oral election to the above restriction requirement, but did not result in an election being made and a written restriction was requested.

Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed. (37 C.F.R. 1.143).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lila Feisee whose telephone number is (703) 308-2731.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO FAX Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 FAX

Serial No. 715272

Art Unit 1806

Center number is (703) 308-4227. The hours of operation of the Center are 8:45 am - 4:45 pm, Monday - Friday.

Feisee/lf
May 11, 1992

JOHN J. DOLL
SUPERVISORY PATENT EXAMINER
GROUP 180

PTO FORM 948
(REV. 5-90)

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

ATTACHMENT TO PAPER NUMBER 9

APPLICATION NUMBER 715222

NOTICE OF DRAFTSMAN'S PATENT DRAWING REVIEW

THE PTO DRAFTSMEN REVIEW ALL ORIGINALLY FILED DRAWINGS REGARDLESS OF WHETHER THEY WERE DESIGNATED AS INFORMAL OR FORMAL.

The drawings filed 6/14/91

A. ☐ are approved.

B. ☒ are objected to under 37 CFR 1.84 for the reason(s) checked below. The examiner will require submission of new, corrected drawings at the appropriate time. Corrected drawings must be submitted according to the instructions listed on the back of this Notice.

1. Paper and ink. 37 CFR 1.84(a)

☐ Sheet(s)__________Poor.

2. Size of Sheet and Margins. 37 CFR 1.84(b)

Acceptable Paper Sizes and Margins

	Paper Size		
Margin	8 1/2 by 14 inches	8 1/2 by 13 inches	DIN size A4 21 by 29.7 cm.
Top	2 inches	1 inch	2.5 cm.
Left	1/4 inch	1/4 inch	2.5 cm.
Right	1/4 inch	1/4 inch	1.5 cm.
Bottom	1/4 inch	1/4 inch	1.0 cm.

☑ Proper Size Paper Required.
All Sheets Must be Same Size.
Sheet(s) Fig 1-4

☐ Proper Margins Required.
Sheet(s) __________

☐ TOP ☐ RIGHT
☐ LEFT ☐ BOTTOM

3. Character of Lines. 37 CFR 1.84(c)

☐ Lines Pale or Rough and Blurred.
Fig(s)__________

☐ Solid Black Shading Not Allowed.
Fig(s)__________

4. Hatching and Shading. 37 CFR 1.84(d)

☐ Shade Lines are Required.
Fig(s) __________

☐ Criss-Cross Hatching Not Allowed.
Fig(s) __________

☐ Double Line Hatching Not Allowed.
Fig(s) __________

☐ Parts in Section Must be Hatched.
Fig(s) __________

5. Reference Characters. 37 CFR 1.84(f)

☑ Reference Characters Poor or Incorrectly Sized.
Fig(s) 2-4

☐ Reference Characters Placed Incorrectly.
Fig(s) __________

6. Views. 37 CFR 1.84(i) & (j)

☐ Figures Must be Numbered Properly.
____

☐ Figures Must Not be Connected.
Fig(s) __________

7. ☑ Photographs Not Approved.
Fig 4

8. ☐ Other.

Telephone inquires concerning this review should be directed to the Chief Draftsman at telephone number (703) 557-6404.

Reviewing Draftsman

8/6
Date

MAIL ROOM
JUL 13 1992
82
PAT. & T.M. OFF.

Elec
#12
Srs
7/24/92

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Paul J. Carter et al.
Serial No. 07/715,272
Filed: 14 June 1991
For: Immunoglobulin Variants

Group Art Unit: 1806
Examiner: L. Feisee

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-2614

Response

JUL 22 1992

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

This is responseive to the Restriction Requirement mailed 12 May 1992. A request for a one-month extension of time to respond is submitted herewith, bringing the due date for this response to 11 July 1992. This response is timely filed.

The inventors hereby elect to prosecute Group 1, claims 1-13.

Respectfully submitted,
GENENTECH, INC.

Carolyn R. Adler

Carolyn R. Adler
Reg. No. 32,324

10 July 1992

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 10 July 1992.

Carolyn R. Adler

Carolyn R. Adler

Dated: 10 July 1992

184

180 feis

PATENT DOCKET 709

#11
SM
7/24/92

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
Paul J. Carter et al.)
Serial No. 07/715,272)
Filed: 14 June 1991)
For: Immunoglobulin Variants)

Group Art Unit: 1806
Examiner: L. Feisee

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-2614

PETITION AND FEE FOR EXTENSION OF TIME (37 CFR 1.136(a))

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office action dated 12 May 1992 for one month(s) from 11 June 1992 to 11 July 1992. The extended time for response does not exceed the statutory period.

Please charge Deposit Account Number 07-0630 in the amount of $110 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

Respectfully submitted,
GENENTECH, INC.

Carolyn R. Adler

Carolyn R. Adler
Reg. No. 32,324

Date: 10 July 1992

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Carolyn R. Adler

Carolyn R. Adler

Date: 10 July 1992

LA10099 07/17/92 07715272 07-0630 010 115 110.00CH

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

ONLINE SEARCH REQUEST FORM

* *

USER [redacted] Feisee SERIAL NUMBER 715272

ART UNIT 1806 PHONE 2731 DATE 9/11/92.

Please give a detailed statement of requirements. Describe as specifically as possible the subject matter to be searched. Define any terms that may have special meaning. Give examples or relevant citations, authors, or keywords, if known.

You may include a copy of the broadest and or relevant claim(s).

Please search.
Making Humanized Antibodies
by CDR Grafting. ~~and~~

See claims 1-13
especially!!

138
17hr

RECEIVED
STIC
BIOTECH/CHEMICAL
LIBRARY
92 SEP 10 PM 3:01
U.S. PAT. & TM. OFF.

STAFF USE ONLY *

9/15/92

Feise
715272

```
show files
File 155:MEDLINE_1966-1992/NOV (9211W1)
File   5:BIOSIS PREVIEWS_69-92/OCT BA9407:BARRM4307
         (C. BIOSIS 1992)
File  73:EMBASE (EXCERPTA MEDICA)_74-92/ISS37
         (COPR. ESP BV/EM 1992)
File 399:CA SEARCH 1967-1992 UD=11710
         (Copr. 1992 by the Amer. Chem. Soc.)
?ds

Set     Items   Description
S1         16   HUMANIZED()ANTIBODIES/TI
S2     332298   ANTIBODIES! FROM 155
S3       2253   IMMUNOGLOBULIN VARIABLE REGION! FROM 155   synonyn for CDR
S4       2253   S2 AND S3
S5        862   HUMANIZ?
S6       2005   HUMANIS?
S7         16   S4 AND (HUMANIZ? OR HUMANIS?)
S8     636823   ANTIBOD? FROM 5,73,399
S9     165469   IMMUNOGLOBULIN
S10     41830   IG
S11    113462   VARIABLE
S12    392448   REGION
S13       862   (IMMUNOGLOBULIN OR IG)(W)VARIABLE(W)REGION
S14       604   CDR
S15     67991   COMPLEMENTARY
S16    112646   DETERMINING
S17        63   COMPLEMENTARY(W)DETERMINING
S18      1904   HYPERVARIABLE
S19    392448   REGION
S20       747   (COMPLEMENTARY(W)DETERMINING OR HYPERVARIABLE)(W)REGION
S21    428778   ANTIBODY
S22   1469126   RELATED
S23    623755   BINDING
S24    544344   SITE? ?
S25         0   ANTIBODY(W)RELATED(W)BINDING(W)SITE? ?
S26      2161   (IMMUNOGLOBULIN OR IG)()VARIABLE()REGION OR CDR OR (COMPLE-
             MENTARY()DETERMINING OR HYPERVARIABLE)()REGION OR ANTIBODY()R-
             ELATED()BINDING()SITE? ? FROM 5,73,399   synonyms for CDR
S27       897   8 AND 26
S28        18   27 AND (5 OR 6)
S29        34   28 OR 7
S30        21   RD (unique items)
S31        21   Sort S30/ALL/PY,D
?t31/7/1-21

 31/7/1      (Item 1 from file: 5)
9568885      BIOSIS Number: 94073885
  *HUMANIZED* OKT3 *ANTIBODIES* SUCCESSFUL TRANSFER OF IMMUNE MODULATING
PROPERTIES AND IDIOTYPE EXPRESSION
  WOODLE E S; THISTLEWAITE J R; JOLLIFFE L K; ZIVIN R A; COLLINS A; ADAIR J
A; BODMER M; ATHWAL D; ALEGRE M-L; BLUESTONE J A
  SECT. ORGAN TRANSPLANTATION, DEP. SURGERY, WASH. UNIV. SCH. MED., ONE
BARNES HOSP. PLAZA, QUEENY TOWER, SUITE 6107, ST. LOUIS, MO. 63110.
  J IMMUNOL 148 (9). 1992. 2756-2763.    CODEN: JOIMA
  Full Journal Title: Journal of Immunology
  Language: ENGLISH
```

Antibodies that possess the Ag-binding regions of OKT3 within the context of a human framework (Hu-OKT3 Ab) offer distinct advantages for optimizing anti-CD3 mAb therapy. First, manipulation of Ab genes to produce *humanized*. Ab that retain Ag-binding activity may circumvent antigenicity problems. Second, Ab gene engineering provides a means for modifying functional properties, including T cell activation and immune suppression. The purpose of this study was to determine the functional properties of Hu-OKT3 Ab and to compare the functional properties and idiotypes of Hu-OKT3 Ab to those of maurine OKT3. Three Hu-OKT3 IgG4 aAb, a chimeric OKT3 *antibody* (cOKT3-1) (grafted sequences comprising all OKT3 VH and VL regions) and two complementarity determining region (*CDR*)-grafted *antibodies* , gOKT3-5 and gOKT3-6 (grafted sequences comprising only OKT3 VH and VL *CDR* and some framework amino acids, were analyzed. Initial studies demonstrated that the cOKT3 and gOKT3-5 Ab bound selectively to T cells and competitively inhibited OKT3-FITC binding with avidities similar to that of murine OKT3. binding avidity of the gOKT3-6 Ab was markedly less than that of the other Hu-OKT3 Ab. Serologic analysis suggested that cOKT3 and gOKT3-5 Ab possess idiotypes (combining sites) similar to murine OKT3. C cell activation potency of all three Hu-OKT3 Ab was assessed by proliferation, induction of activation marker expression (IL-2R and Leu 23), and lymphokine production (TNF-.alpha. and IFN-.gamma.). The cOKT3 and gOKT3-5 Ab demonstrated T cell activation potencies similar to murine OKT3 as assessed by each parameter. CD3 coating and modulation by these two Ab was effective but somewhat less potent than that observed with OKT3. Finally, cOKT3 and gOKT3-5 Ab both inhibited CTL activity comparably to murine OKT3. In conclusion, these studies indicate that gOKT3-5 and cOKT3 Ab possess immune modulating properties similar to murine OKT3 and thus offer attractive alternatives to murine OKT3 for in vivo therapy.

31/7/2 (Item 2 from file: 155)
08124424 92262424
Humanization of an anti-p185HER2 antibody for human cancer therapy.
Carter P; Presta L; Gorman CM; Ridgway JB; Henner D; Wong WL; Rowland AM; Kotts C; Carver ME; Shepard HM
Department of Protein Engineering, Genentech Inc., South San Francisco, CA 94080.
Proc Natl Acad Sci U S A (UNITED STATES) May 15 1992, 89 (10) p4285-9, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The murine monoclonal antibody mumAb4D5, directed against human epidermal growth factor receptor 2 (p185HER2), specifically inhibits proliferation of human tumor cells overexpressing p185HER2. However, the efficacy of mumAb4D5 in human cancer therapy is likely to be limited by a human anti-mouse antibody response and lack of effector functions. A "*humanized* " antibody, humAb4D5-1, containing only the antigen binding loops from mumAb4D5 and human variable region framework residues plus IgG1 constant domains was constructed. Light- and heavy-chain variable regions were simultaneously *humanized* in one step by "gene conversion mutagenesis" using 311-mer and 361-mer preassembled oligonucleotides, respectively. The humAb4D5-1 variant does not block the proliferation of human breast carcinoma SK-BR-3 cells, which overexpress p185HER2, despite tight antigen binding (Kd = 25 nM). One of seven additional *humanized* variants designed by molecular modeling (humAb4D5-8) binds the p185HER2 antigen 250-fold and 3-fold more tightly than humAb4D5-1 and mumAb4D5, respectively. In addition, humAb4D5-8 has potency comparable to the murine antibody in blocking SK-BR-3 cell proliferation. Furthermore, humAb4D5-8 is much more efficient in supporting antibody-dependent cellular cytotoxicity against SK-BR-3 cells than mumAb4D5, but it does not efficiently kill WI-38 cells, which express p185HER2 at lower levels.

31/7/3 (Item 3 from file: 155)
08081267 92219267

Antibody framework residues affecting the conformation of the hypervariable loops.

Foote J; Winter G

MRC Laboratory of Molecular Biology, Cambridge, England.

J Mol Biol (ENGLAND) Mar 20 1992, 224 (2) p487-99, ISSN 0022-2836 Journal Code: J6V

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Rodent monoclonal antibodies have been "*humanized*" or "reshaped" for therapy by transplanting the antigen-binding loops from their variable domains onto the beta-sheet framework regions of human antibodies. However, additional substitutions in the human framework regions are sometimes required for high affinity antigen binding. Here we describe antigen binding by a reshaped antibody derived from the mouse anti-lysozyme antibody D1.3, and several variants in which point mutations had been introduced into framework positions to improve its affinity. The affinities were determined from the relaxation kinetics of reactant mixtures using quenching of fluorescence that occurs upon formation of the antibody-antigen complex. The dissociation constant of lysozyme ranged from 3.7 nM (for D1.3) to 260 nM. Measurement of antibody-antigen association kinetics using stopped-flow showed that D1.3 and most of the reshaped antibodies had bimolecular rate constants of 1.4 x 10(6) s-1 M-1, indicating that differences in equilibrium constant were predominantly due to different rates of dissociation of lysozyme from immune complexes. Mutations in a triad of heavy chain residues, 27, 29 and 71, contributed 0.9 kcal/mol in antigen binding free energy, and a Phe to Tyr substitution of light chain residue 71 contributed an additional 0.8 kcal/mol. The combined effect of all these mutations brought the affinity of the reshaped antibody to within a factor of 4 of D1.3. All of these substitutions were in the beta-sheet framework closely underlying the complementarity-determining regions, and do not participate in a direct interaction with antigen. The informed selection of residues in such positions may prove essential for the success of loop transplants in antibodies. Variation of these sites may also have a role in shaping the diversity of structures found in the primary repertoire, and in affinity maturation.

31/7/4 (Item 4 from file: 155)
08010135 92148135

Chimeric and *humanized* antibodies with specificity for the CD33 antigen.

Co MS; Avdalovic NM; Caron PC; Avdalovic MV; Scheinberg DA; Queen C

Protein Design Labs, Inc., Mountain View, CA 94043.

J Immunol (UNITED STATES) Feb 15 1992, 148 (4) p1149-54, ISSN 0022-1767 Journal Code: IFB

Contract/Grant No.: NIH CA55349

Languages: ENGLISH

Document type: JOURNAL ARTICLE

L and H chain cDNAs of M195, a murine mAb that binds to the CD33 Ag on normal and leukemic myeloid cells, were cloned. The cDNAs were used in the construction of mouse/human IgG1 and IgG3 chimeric antibodies. In addition, *humanized* antibodies were constructed which combined the complementarity-determining regions of the M195 antibody with human framework and constant regions. The human framework was chosen to maximize homology with the M195 V domain sequence. Moreover, a computer model of M195 was used to identify several framework amino acids that are likely to interact with the complementarity-determining regions, and these residues were also retained in the *humanized* antibodies. Unexpectedly, the *humanized* IgG1 and IgG3 M195 antibodies, which have reshaped V regions, have higher apparent binding affinity for the CD33 Ag than the chimeric or mouse antibodies.

31/7/5 (Item 5 from file: 155)
07996790 92134790
Gene conversion of immunoglobulin variable regions in mutagenesis cassettes by replacement PCR mutagenesis.
Near RI
Cellular and Molecular Research Laboratory, Massachusetts General Hospital, Boston 02144.
Biotechniques (UNITED STATES) Jan 1992, 12 (1) p88-97, ISSN 0736-6205
Journal Code: AN3
Contract/Grant No.: HL-19259
Languages: ENGLISH
Document type: JOURNAL ARTICLE

A technique, Replacement PCR Mutagenesis, was developed to replace one immunoglobulin variable region (V) in a M13 phage cassette with a different, homologous V. This allows the use of the same mutagenesis and subsequent expression vectors for many V regions or V segments. The method combines PCR of V fragments and in vitro mutagenesis. Primers homologous to 3' and 5' ends of both V regions initiate PCR synthesis of the V DNA fragment (donor) that will replace the V region (recipient) in M13. Donor V PCR DNA may originate from mRNA, cloned V genes or genomic templates. The donor V PCR DNA is denatured and annealed to the M13 cassette containing the recipient V to be supplanted. The second strand is synthesized, transfected into bacteria and mutant plaques selected by hybridization. Since restriction sites in primers are not required, altered primer-encoded amino acids are avoided. Further, the PCR donor piece can be of any length if it shares homology with the recipient gene. This allows construction and expression of complete gene replacements and chimeras. This method is also applicable to V "*humanization* " and studying sets of homologous genes containing polymorphic or evolutionary disparities. The potential uses of the technique are discussed.

31/7/6 (Item 6 from file: 5)
8779979 BIOSIS Number: 42004979
IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE *CDR*-GRAFTED *HUMANIZED* MONOCLONAL *ANTIBODY* BW 431-26 HUMAB PRECLINICAL STUDY
MASCHEK W; BOSSLET K
INST. NUCLEARMED., LINZ BEHRING RES. LABS, MARBURG, FRG.
EUROPEAN ASSOCIATION OF NUCLEAR MEDICINE CONGRESS, VIENNA, AUSTRIA, SEPTEMBER 1-5, 1991. EUR J NUCL MED 18 (8). 1991. 546. CODEN: EJNMD
Language: ENGLISH

31/7/7 (Item 7 from file: 5)
8563624 BIOSIS Number: 92028624
POLYMERASE CHAIN REACTION FACILITATES THE CLONING *CDR*-GRAFTING AND RAPID EXPRESSION OF A MURINE MONOCLONAL *ANTIBODY* DIRECTED AGAINST THE CD18 COMPONENT OF LEUKOCYTE INTEGRINS
DAUGHERTY B L; DEMARTINO J A; LAW M-F; KAWKA D W; SINGER I I; MARK G E
DEP. CELL. MOL. BIOL., MERCK SHARP DOHME RES. LAB., RAHWAY, N.J. 07065, USA.
NUCLEIC ACIDS RES 19 (9). 1991. 2471-2476. CODEN: NARHA
Full Journal Title: Nucleic Acids Research
Language: ENGLISH

Two novel approaches of recombinant PCR technology were employed to graft the complementarity determining regions from a murine monoclonal *antibody* (mAb) onto human *antibody* frameworks. One approach relied on the availability of cloned human variable region templates, whereas the other strategy was dependent only on human variable region protein sequence data. The transient expression of recombinant *humanized* *antibody* was driven by the adenovirus major late promoter and was detected 48 hrs post-transfection into non-lymphoid mammalian cells. The application of these new approaches enables the expression of a recombinant *humanized* *antibody* just 6 weeks after initiating the cDNA cloning of the murine mAB.

31/7/8 (Item 8 from file: 155)
08049594 92187594
Humanization of a mouse monoclonal antibody by CDR-grafting: the importance of framework residues on loop conformation.
Kettleborough CA; Saldanha J; Heath VJ; Morrison CJ; Bendig MM
Medical Research Council Collaborative Centre, London, UK.
Protein Eng (ENGLAND) Oct 1991, 4 (7) p773-83, ISSN 0269-2139
Journal Code: PR1
Languages: ENGLISH
Document type: JOURNAL ARTICLE

A mouse monoclonal antibody (mAb 425) with therapeutic potential was ' *humanized* ' in two ways. Firstly the mouse variable regions from mAb 425 were spliced onto human constant regions to create a chimeric 425 antibody. Secondly, the mouse complementarity-determining regions (CDRs) from mAb 425 were grafted into human variable regions, which were then joined to human constant regions, to create a reshaped human 425 antibody. Using a molecular model of the mouse mAb 425 variable regions, framework residues (FRs) that might be critical for antigen-binding were identified. To test the importance of these residues, nine versions of the reshaped human 425 heavy chain variable (VH) regions and two versions of the reshaped human 425 light chain variable (VL) regions were designed and constructed. The recombinant DNAs coding for the chimeric and reshaped human light and heavy chains were co-expressed transiently in COS cells. In antigen-binding assays and competition-binding assays, the reshaped human antibodies were compared with mouse 425 antibody and to chimeric 425 antibody. The different versions of 425-reshaped human antibody showed a wide range of avidities for antigen, indicating that substitutions at certain positions in the human FRs significantly influenced binding to antigen. Why certain individual FR residues influence antigen-binding is discussed. One version of reshaped human 425 antibody bound to antigen with an avidity approaching that of the mouse 425 antibody.

31/7/9 (Item 9 from file: 155)
07969093 92107093
Humanization of monoclonal antibodies.
Gussow D; Seemann G
Methods Enzymol (UNITED STATES) 1991, 203 p99-121, ISSN 0076-6879
Journal Code: MVA
Languages: ENGLISH
Document type: JOURNAL ARTICLE

31/7/10 (Item 10 from file: 155)
07953750 92091750
Construction, expression and characterization of *humanized* antibodies directed against the human alpha/beta T cell receptor.
Shearman CW; Pollock D; White G; Hehir K; Moore GP; Kanzy EJ; Kurrle R
Genzyme Corporation, Framingham, MA 01701.
J Immunol (UNITED STATES) Dec 15 1991, 147 (12) p4366-73, ISSN 0022-1767 Journal Code: IFB
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Completely *humanized* antibodies with specificity for the human alpha/beta TCR have been produced by genetic engineering. The L and H chain V region exons encoding the murine mAb BMA 031 CD regions and human EU framework regions were synthesized and replaced into previously isolated genomic fragments. These fragments were inserted into mammalian expression vectors containing the human kappa and gamma 1 C region exons. Two variants were constructed each containing selected BMA 031 amino acids within the human frameworks. The *humanized* genes were transfected into Sp2/0 hybridoma cells by electroporation and transfectomas secreting *humanized* antibody were isolated. Levels of antibody expression up to 7 pg/cell/24 h were obtained. The *humanized* antibody, BMA 031-EUCIV2, competed poorly with murine BMA 031 for binding to T cells. BMA 031-EUCIV3, however, bound specifically to T cells and competed effectively with both the murine BMA 031 antibody and a previously constructed chimeric BMA 031 antibody for binding to these cells. The relative affinity of BMA 031-EUCIV3 was about 2.5 times lower than BMA 031. The ability to promote antibody dependent cell-mediated cytolysis was significantly enhanced with the engineered antibodies as compared to murine BMA 031. *Humanized* BMA 031 is a clinically relevant, genetically engineered antibody with potential uses in transplantation, graft vs host disease, and autoimmunity.

31/7/11 (Item 11 from file: 155)
07909485 92047485
Antigenicity of mouse monoclonal antibodies. A study on the variable region of the heavy chain.
Olsson PG; Hammarstrom L; Smith CI
Department of Clinical Immunology, Karolinska Institute, Huddinge University Hospital, Sweden.
J Theor Biol (ENGLAND) Jul 7 1991, 151 (1) p111-22, ISSN 0022-5193
Journal Code: K8N
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Mouse monoclonal antibodies (Mabs) against human tumour antigens are currently used in therapy, but up to 50% of the patients receiving treatment form anti-Mab antibodies thus reducing the efficiency of the treatment. One attempt to minimize the immunogenicity of the mouse Mabs is to "*humanize* " them by replacing the constant part of the molecule with the human equivalent by genetic engineering. However, this does not reduce the immunogenicity of the variable part of the antibody. Some variable regions may be expected to be less antigenic than others. We therefore compared consensus sequences for the 11 mouse VH families with the human VH sequences published so far. Theoretical antigenicity predictions (hydrophilicity, flexibility, surface accessibility and relative antigenicity) were made and two families; VH I(J558) and VH XI (CP5 B5-3) were predicted to be immunogenic by all four methods. One family, VH X (MRL-DNA4), was not predicted to be immunogenic by any of the four methods. The residues predicted to form antigenic epitopes in the two families VH II (Q52) and VH III (36-60) are predicted not to be exposed on the surface of the antibody molecule and may therefore not be immunogenic.

31/7/12 (Item 12 from file: 5)
7905670 BIOSIS Number: 40106670

CHIMERIC MOUSE-HUMAN AND *CDR*-GRAFTED *ANTIBODIES* TO HUMAN IL2 RECEPTOR
WEIDLE U H; RUSSMANN E; LENZ H; KALUZA B
BOEHRINGER MANNHEIM GMBH, NONNENWALD 2, D-8122 PENZBERG, FRG.
MEETING ON MOLECULAR BIOLOGY AND THE IMMUNOPATHOGENESIS OF RHEUMATOID ARTHRITIS HELD AT THE 20TH ANNUAL MEETING OF THE KEYSTONE SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, LAKE TAHOE, CALIFORNIA, USA, MARCH 15-21, 1991. J CELL BIOCHEM SUPPL 15 (PART E). 1991. 186. CODEN: JCBSD
Language: ENGLISH

31/7/13 (Item 13 from file: 155)
07899816 92037816

A *humanized* monovalent CD3 antibody which can activate homologous complement.

Routledge EG; Lloyd I; Gorman SD; Clark M; Waldmann H

Department of Pathology, Cambridge University.

Eur J Immunol (GERMANY) Nov 1991, 21 (11) p2717-25, ISSN 0014-2980
Journal Code: EN5

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The rat monoclonal antibody (mAb) YTH12.5, specific for the CD3 antigen complex on human T cells has been modified in order to improve its efficacy in human therapy. With the aim of rendering it less immunogenic, it has been *humanized* using the method of framework grafting. During this process sequence analysis of the YTH12.5 VL gene indicated that it was of the lambda subclass, however, it was markedly dissimilar from previously published rat and mouse V lambda gene sequences and may represent a new V lambda gene family. The *humanization* of this light chain represents the first successful reshaping of a lambda light chain V region. To improve the effector function of the antibody we have created a monovalent form (1 Fab, 1 Fc) using a novel method involving the introduction of an N-terminally truncated human IgG1 heavy chain gene into cells producing the *humanized* CD3 mAb. Comparison of the mono- and bivalent *humanized* mAb in a complement-mediated cell lysis assay revealed that the monovalent antibody mediated lysis of human T cell blasts whereas the bivalent form did not. The availability of a *humanized*, complement-fixing CD3 mAb may improve opportunities for human therapy, in the management of organ rejection, autoimmunity and the treatment of T cell lymphoma.

31/7/14 (Item 14 from file: 155)
07768736 91287736

A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties.

Get this

Padlan EA

Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.

Mol Immunol Apr-May 1991, 28 (4-5) p489-98, ISSN 0161-5890
Journal Code: NG1

Languages: ENGLISH

Document type: JOURNAL ARTICLE

It is proposed to reduce the immunogenicity of allogeneic antibody variable domains, while preserving ligand-binding properties, by reducing their antigenicity through replacement of the exposed residues in the framework regions which differ from those usually found in host antibodies. The results of a comparison of representative murine antibody sequences with those of human origin suggest that the number of residues that need to be replaced to "*humanize*" those antibodies could be small.

Get this

31/7/15 (Item 15 from file: 155)
07757287 91276287

Immunoglobulin complementarity-determining region grafting by recombinant polymerase chain reaction to generate *humanised* monoclonal antibodies.

Lewis AP; Crowe JS

Department of Cell Biology, Wellcome Research Laboratories, Beckenham, Kent, U.K.

Gene May 30 1991, 101 (2) p297-302, ISSN 0378-1119 Journal Code: FOP

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We describe an approach to rapidly generate *humanised* monoclonal antibodies by grafting rodent complementarity-determining regions onto human immunoglobulin frameworks using recombinant polymerase chain reaction (PCR) methodology. The approach was applied to grafting a rat complementarily-determining region onto a human framework and amplifying the entire *humanised* heavy chain. The terminal oligodeoxyribonucleotide primers incorporated restriction sites to allow forced cloning into plasmid vectors for sequencing and expression. No nucleotide errors were introduced into the 1463-bp sequence even after sequential applications of PCR.

31/7/16 (Item 16 from file: 155)
07668893 91187893
Humanized antibodies for antiviral therapy.
Co MS; Deschamps M; Whitley RJ; Queen C
Protein Design Labs, Inc., Mountain View, CA 94043.
Proc Natl Acad Sci U S A Apr 1 1991, 88 (7) p2869-73, ISSN 0027-8424
Journal Code: PV3
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Got this

Antibody therapy holds great promise for the treatment of cancer, autoimmune disorders, and viral infections. Murine monoclonal antibodies are relatively easy to produce but are severely restricted for therapeutic use by their immunogenicity in humans. Production of human monoclonal antibodies has been problematic. *Humanized* antibodies can be generated by introducing the six hypervariable regions from the heavy and light chains of a murine antibody into a human framework sequence and combining it with human constant regions. We *humanized*, with the aid of computer modeling, two murine monoclonal antibodies against herpes simplex virus gB and gD glycoproteins. The binding, virus neutralization, and cell protection results all indicate that both *humanized* antibodies have retained the binding activities and the biological properties of the murine monoclonal antibodies.

31/7/17 (Item 17 from file: 399)
117024688 CA: 117(3)24688r PATENT
Humanized complementarily-determing region (CDR)-grafted antibodies to intercellular adhesion molecule-1 (ICAM-1), methods of preparation and usage thereof
INVENTOR(AUTHOR): Adair, John Robert; Athwal, Diljeet Singh; Rothlein, Robert A.
LOCATION: UK,
ASSIGNEE: Celltech Ltd.; Boehringer Ingelheim Pharmaceuticals, Inc.
PATENT: PCT International ; WO 9116927 A1 DATE: 911114
APPLICATION: WO 91US2942 (910429) *GB 909549 (900427)
PAGES: 81 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: A61K-039/395A; C07K-015/28B DESIGNATED COUNTRIES: AT; AU; BB; BG; BR; CA; CH; DE; DK; ES; FI; GB; HU; JP; KP; KR; LK; LU; MC; MG; MW; NL; NO; PL; RO; SD; SE; SU; US
DESIGNATED REGIONAL: AT; BE; BF; BJ; CF; CG; CH; CM; DE; DK; ES; FR; GA; GB; GR; IT; LU; ML; MR; NL; SE; SN; TD; TG
SECTION:
CA215003 Immunochemistry
CA201XXX Pharmacology
CA203XXX Biochemical Genetics
IDENTIFIERS: humanized antibody intercellular adhesion mol 1, inflammation inhibitor humanized antibody ICAM1, asthma inhibitor humanized antibody ICAM1, AIDS virus humanized antibody ICAM1, virucide humanized antibody ICAM1, diagnosis humanized antibody ICAM1
DESCRIPTORS:
Dermatitis...

acute, treatment of, with humanized antibody to intercellular adhesion mol.-1

Immunosuppressants...
and humanized antibody to intercellular adhesion mol.-1, pharmaceutical compn. contg.

Rodent...
anti-intercellular adhesion mol.-1 antibody variable region complementary detg. region of, in humanized antibody prodn.

Integrins,antigens LFA-1...
antibody to, and humanized antibody to intercellular adhesion mol.-1, for inflammation treatment

Neoplasm inhibitors,metastasis...
chimeric antibody to intercellular adhesion mol.-1, for hemopoietic cell tumors

Toxicity...
cytokine-induced, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for

Inflammation...
diagnosis of, with chimeric antibody binding to cell expressing intercellular adhesion mol.-1

Deoxyribonucleic acids...
for antibody heavy and light chains, in humanized antibody to intercellular adhesion mol.-1 prodn.

Deoxyribonucleic acid sequences...
for monoclonal antibody R6-5-D6 heavy and light chain components for humanized antiintercellular adhesion mol.-1 antibody

Leukocyte...
human immunodificiency virus infection of, inhibition of, with humanized antibody to intercellular adhesion mol.-1

Bronchodilators,antiasthmatics... Inflammation inhibitors... Inflammation inhibitors,antirheumatics... Therapeutics... Virucides and Virustats...
humanized antibody to intercellular adhesion mol.-1

Toxins...
humanized antibody to intercellular adhesion mol.-1 derivatized with, for inhibition of intercellular adhesion mol.-1-expressing tumor cell

Diagnosis...
humanized antibody to intercellular adhesion mol.-1 for

Inflammation inhibitors,antiarthritics...
humanized antibody to intercellular adhesion mol.-1, for reaction arthritis

Glycoproteins,specific or class, ICAM-1 (intercellular adhesion mol. 1)...
humanized recombinant antibody to

Antibodies...
humanized recombinant, to intercellular adhesion mol.-1

Thyroid gland,disease, autoimmune thyroiditis...
inflammation in, treatment of, with humanized antibody to intercellular adhesion mol.-1

Nervous system,central...
inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for

Autoimmune disease... Blood vessel,disease, Raynaud's phenomenon... Brain,disease, stroke... Dialysis,hemo-... Encephalomyelitis... Intestine,disease, Crohn's... Intestine,disease, pseudomembranous enterocolitis... Intestine,disease, ulcerative colitis... Kidney,disease, acute glomerulonephritis... Leukapheresis... Lupus erythematosus... Multiple sclerosis... Psoriasis... Respiratory distress syndrome,adult...
inflammation of, treatment of, with humanized antibody to intercellular adhesion mol.-1

Neoplasm,composition...
intercellular adhesion mol.-1-expressing, diagnosis of, with humanized

antibody to intercellular adhesion mol.-1
Mouse...
monoclonal antibody R6-5-D6 of, in humanized antibody to intercellular adhesion mol.-1 prodn.
Sepsis and Septicemia...
multiple organ injury syndrome secondary to, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for
Protein sequences...
of monoclonal antibody R6-5-D6 heavy and light chain components for humanized antiintercellular adhesion mol.-1 antibody
Plasmid and Episome...
pAL5, in grafted humanized antibody to intercellular adhesion mol.-1 prodn.
Plasmid and Episome...
pAL6, in grafted humanized antibody to intercellular adhesion mol.-1 prodn.
Plasmid and Episome...
pBJ1, in grafted humanized antibody to intercellular adhesion mol.-1 prodn.
Kidney,transplant... Organ,transplant... Transplant and Transplantation...
rejection of, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for
Antibodies,monoclonal...
R6-5-D6, of mouse, in humanized antibody to intercellular adhesion mol.-1 prodn.
Organ,disease, multiple organ failure...
secondary to septicemia or trauma, treatment of, humanized antibody to intercellular adhesion mol.-1 for
Temperature effects,biological...
thermal injury, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for
Perfusion,re-...
tissue injury from, treatment of, humanized antibody to intercellular adhesion mol.-1 for
Lymphokines and Cytokines...
toxicity induced by, inflammation of, treatment of, humanized antibody to intercellular adhesion mol.-1 for
Neoplasm inhibitors...
toxin-derivatized humanized antibody to intercellular adhesion mol.-1, for intercellular adhesion mol.-1-expressing tumor cell
Leukocyte,granulocyte...
transfusion-assocd. syndrome, treatment of, humanized antibody to intercellular adhesion mol.-1 for
Allergy,delayed hypersensitivity...
treatment of, humanized antibody to intercellular adhesion mol.-1 for
Picornaviridae... Virus,animal, Coxsackie A... Virus,animal, human immunodeficiency... Virus,animal, human immunodeficiency 1... Virus,animal, Mengo... Virus,animal, rhino-...
treatment of infection with, with humanized antibody to intercellular adhesion mol.-1
Hematopoietic precursor cell...
tumorous, metastasis of, inhibition of, chimeric antibody to intercellular adhesion mol.-1
Genetic vectors...
with DNA for antibody heavy and light chains, in humanized antibody to intercellular adhesion mol.-1 prodn.
CAS REGISTRY NUMBERS:
142007-78-1 142007-79-2 142007-80-5 142007-81-6 142007-82-7
142007-83-8 142007-85-0 amino acid sequence of
142007-84-9 amino acid sequence of, humanized antibody to intercellular

adhesion mol.-1 in relation to
140876-28-4 140876-29-5 142007-86-1 142007-87-2 amino acid sequence of, humanized antibody to intercellular adhesion mol.-1 prodn. in relation to
140857-88-1 142008-94-4 nucleotide sequence of, humanized antibody to intercellular adhesion mol.-1 prodn. in relation to
140857-89-2 142008-93-3 nucleotide sequence of, humanized antibody to intercellular adhesion mol.01 prodn. in relation to

31/7/18 (Item 18 from file: 155)
07449972 90356972
Immunoglobulin V regions of a bactericidal anti-Neisseria meningitidis outer membrane protein monoclonal antibody.
Larrick JW; Coloma MJ; del Valle J; Fernandez ME; Fry KE; Gavilondo-Cowley JV
Genelabs Inc., Redwood City, California.
Scand J Immunol Aug 1990, 32 (2) p121-8, ISSN 0300-9475
Journal Code: UCW
Languages: ENGLISH
Document type: JOURNAL ARTICLE

C6 is a potentially therapeutic murine monoclonal antibody that recognizes the class 1 outer membrane protein of Neisseria meningitidis. C6 specifically immunoblots this antigen and augments in vitro killing of N. meningitidis bacteria. We describe a general method of obtaining the heavy and light chain variable-region sequence from immunoglobulin-secreting cells. The method uses mixed polymerase chain reaction (PCR) primers designed from the 5′ end of the framework 1 (FR1) sequences of the heavy and light chains, and 3′-end primers for constant-region conserved sequences. The method has been applied to the cloning and sequencing of the variable region of C6 to construct a *humanized* monoclonal antibody. Rapid amplification and sequencing of variable regions by this general method have multiple applications in the study of the immune response to infectious diseases.

31/7/19 (Item 19 from file: 155)
07292738 90199738
Cloning of the genes for T84.66, an antibody that has a high specificity and affinity for carcinoembryonic antigen, and expression of chimeric human/mouse T84.66 genes in myeloma and Chinese hamster ovary cells.
Neumaier M; Shively L; Chen FS; Gaida FJ; Ilgen C; Paxton RJ; Shively JE; Riggs AD
Division of Biology, Beckman Research Institute of the City of Hope, Duarte, California 91010.
Cancer Res Apr 1 1990, 50 (7) p2128-34, ISSN 0008-5472
Journal Code: CNF
Contract/Grant No.: CA 43904
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Carcinoembryonic antigen (CEA) is one of the best characterized tumor-associated antigens and is extensively used in the in vitro immunodiagnosis of human colon adenocarcinomas. Among a number of anti-CEA monoclonal antibodies, the murine monoclonal antibody T84.66 shows the highest specificity and affinity for CEA and has been used successfully for in vivo tumor imaging in mice and humans. We report here the cloning and sequencing of the genes coding for monoclonal antibody T84.66 and the amino acid sequence of the variable regions for the heavy and light chains. We also report the construction of mouse/human chimeric IgG1 antibody genes using T84.66 variable region genes and human constant region genes. The resulting chimeric gene constructs were transfected into murine myeloma

```
cells  (Sp2/0)  by  electroporation and into Chinese hamster ovary cells by
lipofection.   The   chimeric   antibodies   obtained   exhibited   the   same
specificity  and  affinity  for  CEA  as  that of the T84.66 immunoglobulin
produced  by  the  murine  hybridoma  cell line. Antibody concentrations in
culture  medium  supernatants  were  clonally  variable but similar (15-480
ng/ml)  for both Sp2/0 and Chinese hamster ovary transfectants; the average
production  by  Chinese  hamster ovary transfectants was only 3-5-fold less
than  Sp2/0  transfectants.  Ascites  production  of Sp2/0 transfectants is
sufficiently  high  (900  micrograms/ml)  for  initial in vivo studies with
*humanized* T84.66.

 31/7/20     (Item 20 from file: 155)
07192290    90099290
  A *humanized* antibody that binds to the interleukin 2 receptor.
  Queen  C;  Schneider  WP;  Selick  HE;  Payne PW; Landolfi NF; Duncan JF;
Avdalovic NM; Levitt M; Junghans RP; Waldmann TA
  Protein Design Labs, Palo Alto, CA 94304.
  Proc Natl Acad Sci U S A   Dec 1989,  86 (24) p10029-33,  ISSN 0027-8424
Journal Code: PV3
```

Get this.

```
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  The anti-Tac monoclonal antibody is known to bind to the p55 chain of the
human  interleukin  2  receptor  and to inhibit proliferation of T cells by
blocking  interleukin  2  binding.  However,  use  of  anti-Tac  as  an
immunosuppressant  drug  would  be  impaired  by  the human immune response
against this murine antibody. We have therefore constructed a "*humanized*"
antibody by combining the complementarity-determining regions (CDRs) of the
anti-Tac  antibody  with  human  framework  and constant regions. The human
framework  regions  were  chosen  to  maximize  homology  with the anti-Tac
antibody  sequence.  In  addition,  a computer model of murine anti-Tac was
used  to  identify  several  amino acids which, while outside the CDRs, are
likely  to  interact with the CDRs or antigen. These mouse amino acids were
also  retained  in  the  *humanized*  antibody.  The  *humanized*  anti-Tac
antibody has an affinity for p55 of 3 x 10(9) M-1, about 1/3 that of murine
anti-Tac.

 31/7/21     (Item 21 from file: 155)
06533056    88178056
  Reshaping human antibodies: grafting an antilysozyme activity.
  Verhoeyen M; Milstein C; Winter G
  Medical  Research  Council  Laboratory  of  Molecular Biology, Cambridge,
England.
  Science   Mar 25 1988,  239 (4847) p1534-6,  ISSN 0036-8075
Journal Code: UJ7
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  The  production  of  therapeutic human monoclonal antibodies by hybridoma
technology  has  proved difficult, and this has prompted the "*humanizing*"
of  mouse monoclonal antibodies by recombinant DNA techniques. It was shown
previously  that  the binding site for a small hapten could be grafted from
the  heavy-chain  variable  domain  of  a mouse antibody to that of a human
myeloma  protein  by transplanting the hypervariable loops. It is now shown
that  a  large  binding  site  for a protein antigen (lysozyme) can also be
transplanted  from  mouse  to  human  heavy  chain.  The  success  of  such
constructions may be facilitated by an induced-fit mechanism.
?save temp

Temp SearchSave "TD101" stored
?b351,350
```

```
     15sep92 09:23:49 User209197 Session D126.3

SYSTEM:OS   - DIALOG OneSearch
  File 351:Derwent World Patents Index Latest
          1981+;DW=9227,UA=9214,UM=9143
 **FILE351: Formats 32,33,35,37 & 39 display the new 'Expanded' Patent
  Family table for UD=9216 and greater.  For more info. type ?NEWS351
  File 350:Derwent World Patents Index
          1963-1980, EQUIVALENTS THRU DW=9227
 **FILE350: Formats 32,33,35,37 & 39 display the new 'Expanded' Patent
  Family table for UD=9219 and greater.  For more info. type ?NEWS350

     Set  Items  Description
     ---  -----  -----------
?exs

Executing TD101
HILIGHT set on as '*'
                0  HUMANIZED/TI
             2945  ANTIBODIES/TI
     S1         0  HUMANIZED()ANTIBODIES/TI
>>>File 155 is not open
>>>No valid files specified in FROM
>>>File 155 is not open
>>>No valid files specified in FROM
>>>Set "S2" does not exist
>>>"S4" does not exist
     S2         0  S4
     S3         1  HUMANIZ?
     S4        26  HUMANIS?
     S5         0  S4 AND (HUMANIZ? OR HUMANIS?)
HILIGHT set on as '*'
>>>File 5 is not open
>>>File 73 is not open
>>>File 399 is not open
>>>No valid files specified in FROM
>>>File 5 is not open
>>>File 73 is not open
>>>File 399 is not open
>>>No valid files specified in FROM
>>>Set "S8" does not exist
>>>Set "S27" does not exist
>>>Set "S28" does not exist
>>>Duplicate detection is not supported for File 351.
>>>Duplicate detection is not supported for File 350.
>>>All specified files are unsupported, command ignored.
>>>Set '30' has not yet been created.
COST        = OFF.
?ss antibod? and (s3 or s4)

     S6   13936  ANTIBOD?
              1  S3
             26  S4
     S7      22  ANTIBOD? AND (S3 OR S4)
?ss cdr or (ig or immunoglobulin)()variable()region or (complementary()determing

Processing
Processing
     S8      31  CDR
     S9     786  IG
```

```
    S10      1576  IMMUNOGLOBULIN
    S11    108404  VARIABLE
    S12    108131  REGION
    S13         4  (IG OR IMMUNOGLOBULIN)(W)VARIABLE(W)REGION
    S14     23564  COMPLEMENTARY
    S15       501  DETERMING
    S16         0  COMPLEMENTARY(W)DETERMING
    S17        23  HYPERVARIABLE
    S18    108131  REGION
    S19        12  (COMPLEMENTARY(W)DETERMING OR HYPERVARIABLE)(W)REGION
    S20     11218  ANTIBODY
    S21     43127  RELATED
    S22     28329  BINDING
    S23     29492  SITE? ?
    S24         0  ANTIBODY(W)RELATED(W)BINDING(W)SITE? ?
    S25        45  CDR OR (IG OR IMMUNOGLOBULIN)()VARIABLE()REGION OR
                   (COMPLEMENTARY()DETERMING OR HYPERVARIABLE)()REGION OR
                   ANTIBODY()RELATED()BINDING()SITE? ?
?c 7 and 25

                22  7
                45  25
    S26          8  7 AND 25
?t26/7/1-8


 26/7/1     (Item 1 from file: 351)
009040436  WPI Acc No: 92-167794/21
XRAM Acc No: C92-077239
    New *humanised* *antibody* specific for interleukin-2 receptor - with
    complementarity determn. regions and framework from different
    immunoglobulin(s), is non immunogenic and used to treat T-cell
Patent Assignee: (PROT-) PROTEIN DESIGN LABS INC
Author (Inventor): QUEEN C L; SELICK H E
Number of Patents: 001
Number of Countries: 001
Patent Family:
    CC Number     Kind     Date       Week
    DD 296964      A5      911219     9221    (Basic)

Priority Data (CC No Date): DD 337159 (900117)
Abstract (Basic): DD 296964    A
        Compsn. comprises a practically pure human-type immunoglobulin
    (Ig) that reacts specifically with p55-Tac protein and/or inhibits
    binding of human interleukin-2 (Il-2) to its specific receptor.
        Also new are (1) human-type Ig having 2 pairs of light
    chain/heavy chain dimers and able to react specifically with an epitope
    of human IL-2 receptor with affinity at least 10 power 8 M-1, in which
    the complementarity determining regions (*CDR*) and human-type frame
    work regions are from different Ig molecules; (2) *humanised* Ig able
    to bind to IL-2 receptors with one or more *CDR* from anti-Tac
    *antibody* in a human framework, where the framework includes includes
    at least one amino acid (AA) from anti-Tac; (3) nucleic acid encoding a
    human Ig framework and murine *CDR* which, when expressed, produces an
    Ig specifically reactive with p55-Tac protein and can block binding of
    IL-2 to its receptor; (4) cells transformed with this nucleic acid.
        USE/ADVANTAGES - These Ig are used to treat humans with
    T-cell related diseases (e.g. transplant rejection; T-cell leukaemia or
    autoimmune diseases such as diabetes, multiple sclerosis, etc.). They
    are specific for the IL-2 receptors; are engineered to be
```

non-immunising and can be produced by recombinant DNA method. The new Ig are admin. in usual parenteral formulation e.g. in doses of 150 mg for therapy or 0.5-2.5 mg for prophylaxis. Ig can also be used, opt. labelled, for diagnosis; T-cell typing; specific receptor isolation or vaccine prodn. 0/10

Derwent Class: B04; D16;
Int Pat Class: A61K-039/395; C12N-015/13

26/7/2 (Item 2 from file: 351)
009039793 WPI Acc No: 92-167155/20
XRAM Acc No: C92-076891

Prepn. of chimeric *humanised* *antibodies* - using a new polymerase chain reaction technique; PCR

Patent Assignee: (WELL) WELLCOME FOUND LTD
Author (Inventor): CROWE J S; LEWIS A P
Number of Patents: 001
Number of Countries: 015
Patent Family:

CC Number	Kind	Date	Week	
WO 9207075	A1	920430	9220	(Basic)

Priority Data (CC No Date): GB 9022011 (901010)
Applications (CC,No,Date): WO 91GB1744 (911008)
Language: English
EP and/or WO Cited Patents: 4.Jnl.Ref; WO 9007861
Designated States
(National): JP; US
(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE
Abstract (Basic): WO 9207075 A

Prodn. of ds or ss DNA of formula: 5' F1-M-F2 3' encoding an *antibody* (Ab) chain or fragment in which at least one of the complementarily determining regions (CDRs) of the variable region is derived from a first mammalian Ab and the framework of the variable region is derived from a second different mammalian Ab, where M is DNA encoding a *CDR* of the second Ab and F1 and F2 resp. encode 5' and 3' sequences flanking M, by: (a) prepg. a ss or ds DNA template of formula: 5' f1-H-f2 3' where H is DNA encoding a *CDR* of a different specificity from M, and f1 and f2 are homologous to F1 and F2, resp.; (b) obtaining DNA oligonucleotide primers A, B, C and D, where: A comprises the sequence a1 with a 5' end corresp. to the 5' and of F1 and which is identical to the corresp. length of F1 and is oriented in a 5' to 3' direction towards H; B has of the sequence 5' b1-b2 3', where b1 comprises a sequence complementary to a corresp. length of M and has a 3' end complementary to the 5' end of M, and b2 is complementary to a sequence of corresp. length in F1 and has a 5' end which starts at the nucleotide complementary to the 3' end of F1, C has of the sequence 5' c1-c2 3' where c1 comprises a sequence identical to the corresp. length of M and has a 3'end corresp. to the 3' end of M, and c2 is identical to a sequence of corresp. length in F2 and has a 5' end which starts at the nucleotide corresp. to the 5' end of F2, and D comprises a sequence d1 which has a 5' end complementary to the 3' end of F2 and which is complementary to a corresp. length of F2 and is oriented in a 5' to 3' direction towards H, where b1 and c1 overlap by a sufficient length to permit annealing of their 5' ends under conditions which allow PCR to be performed; (c) performing, in any desired order, PCR reactions with primer pairs A, B and C, D on the template prepd. in (a), and (d) mixing the prods. of (c) and performing PCR using primers A and D.

USE/ADVANTAGE - The method allows the prepn. of chimeric, esp. *humanised* Abs. The resulting Ab retains the antigen binding

capability of the non-human Ab from which the *CDR*(s) are derived.
0/4
Derwent Class: B04; D16;
Int Pat Class: C12N-005/10; C12N-015/12; C12N-015/69; C12P-021/08

26/7/3 (Item 3 from file: 351)
008937440 WPI Acc No: 92-064709/08
XRAM Acc No: C92-029621
New multivalent anti-cytokine immunoglobulins - for treating disorders associated with elevated cytokine levels, e.g. septic and endotoxic shock, AIDS, allergies, etc.; ACQUIRE IMMUNE DEFICIENT SYNDROME
Patent Assignee: (CLLT) CELLTECH LTD; (CELL-) CELLTECH LTD
Author (Inventor): ALLEN R A; MORGAN S A
Number of Patents: 002
Number of Countries: 035
Patent Family:

CC Number	Kind	Date	Week	
WO 9201472	A	920206	9208	(Basic)
AU 9182381	A	920218	9222	

Priority Data (CC No Date): GB 9015908 (900719)
Applications (CC,No,Date): AU 9182381 (910719); WO 91GB1216 (910719)
Language: English
EP and/or WO Cited Patents: 2.Jnl.Ref; EP 347057; EP 355067; WO 9006371; WO 9007118; WO 9106305
Designated States
(National): AT; AU; BB; BG; BR; CA; CH; CS; DE; DK; ES; FI; GB; HU; JP; KP ; KR; LK; LU; MC; MG; MW; NL; NO; PL; RO; SD; SE; SU; US
(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; OA; SE
Filing Details: AU9182381 Based on WO 9201472
Abstract (Basic): WO 9201472

New multivalent immunoglobulin (I) has at least 3 linked antigen-binding domains (ABD's) each being specific for a complementary site on a cytokine.

The combining interactions between ABD and cytokine sites are neutralising. (I) is specific for tumour necrosis factor (TNF) alpha or beta; an interleukin, an interferon or a colony-stimulating factor, and it contains 4-20 ABD.

ABD are all of class IgG (most pref.) or all of class IgM (but must be different from a native IgM molecule) and can be linked by covalent crosslinking (e.g. 2-iminothiolane/ maleimide system) or by non-covalent interaction (e.g. using an *antibody* reactive with sites on Ig other than those involved in antigen binding; or the biotin-avidin system). (I) are made by joining together appropriate immunoglobulin molecules or fragments esp *CDR*-grafted or *humanised* chimaeric Ig. USE/ADVANTAGE- (I) are used to treat or prevent diseases assciated with elevated cytokine levels, e.g. immuno regulatory and inflammatory disease, sepsis, endotoxic or cardiovascular shock, AIDS, psoriasis, organ transplant rejection or excessive TNF generation induced cancer therapy etc., Compared with monomeric Ig, (I) have much greater neutralising activity. @(43pp)@

Derwent Class: B04; D16;
Int Pat Class: A61K-039/39; A61K-039/395; C07K-015/28; C12P-021/08

26/7/4 (Item 4 from file: 351)
008929605 WPI Acc No: 92-056874/07
Related WPI Accession(s): 91-222915
XRAM Acc No: C92-025713
New *cdr*-grafted anti carcinoembryonic antigen *antibodies* - useful in therapy and diagnosis of carcinoma

```
Patent Assignee: (CELL-) CELLTECH LTD
Author (Inventor): ADAIR J R; BODMER M W; MOUNTAIN A; OWENS R J
Number of Patents: 001
Patent Family:
    CC Number     Kind     Date      Week
    WO 9201059     A      920123     9207   (Basic)
Priority Data (CC No Date): WO 91GB1108 (910705); GB 9014932 (900705); WO
    90GB2017 (901221)
Language: English
EP and/or WO Cited Patents: WO 8910140; WO 8901783; EP 323806; 6.Jnl.REF
Designated States
 (National): AT; AU; BB; BG; BR; CA; CH; CS; DE; DK; ES; FI; GB; HU; JP; KP
    ; KR; LK; LU; MC; MG; MW; NL; NO; PL; RO; SD; SE; SU; US
 (Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE; OA
Abstract (Basic): WO 9201059
        New *humanised* *antibody* molecule (HAM) is specific for
    carcino-embryonic antigen (CEA) and has an antigen binding site in
    which at least one of the complementarity determining regions (*CDR*'s)
    of the variable domain is derived from the mouse monoclonal *antibody*
    (MAb) A5B7. The remaining Ig-derived parts of HAM are of human origin.
        HAM is a chimeric or *CDR*-grafted *humanised* *antibody*, prepd.
    by recombinant DNA techniques. It can be a complete *antibody* or an
    Fab, Fab', (Fab')2 or Fv fragment, or a single-chain fragment. It may
    have a reporter or effector molecule attached to it.
        USE/ADVANTAGE - HAM are useful in therapy or diagnosis (including
    imaging) of carcinomas which produce CEA, e.g., when coupled to a toxin
    such as ricin. @(70pp Dwg.No.0/19
Derwent Class: B04; D16;
Int Pat Class: A61K-039/39; C07K-015/28; C12N-015/13; C12P-021/08

 26/7/5     (Item 5 from file: 351)
008849515  WPI Acc No: 91-353533/48
XRAM Acc No: C91-152448
    New *humanised* *CDR*-grafted anti-ICAM *antibodies* - used to treat
    and prevent inflammation (e.g. psoriasis) tumours, viral infections and
    asthma and in diagnosis; INTER CELLULAR ADHESIVE MOLECULAR
Patent Assignee: (CELL-) CELLTECH LTD; (BOEH ) BOEHRINGER INGELHEIM PHA
Author (Inventor): ADAIR J R; ATHWAL D S; ROTHLEIN R A
Number of Patents: 002
Patent Family:
    CC Number     Kind     Date      Week
    WO 9116927     A      911114     9148   (Basic)
    AU 9179001     A      911127     9210
Priority Data (CC No Date): GB 909549 (900427)
Applications (CC,No,Date): WO 91US2942 (910429)
Language: English
EP and/or WO Cited Patents: US 4816567; WO 8901783; 7.Jnl.REF
Designated States
 (National): AT; AU; BB; BG; BR; CA; CH; DE; DK; ES; FI; GB; HU; JP; KP; KR
    ; LK; MC; MG; MW; NL; NO; PL; RO; SD; SE; SU; US
 (Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE; OA
Abstract (Basic): WO 9116927
        A recombinant *antibody* molecule comprising antigen binding
    regions derived from the heavy and/or light chain variable regions of
    an anti-intracellular adhesion molecule-1 (anti-ICAM-1) *antibody* is
    claimed. The Ab is *CDR*-grafted and comprises several non-human
    residues. Also claimed are DNA encoding an Ab heavy or light chain, a
    vector comprising the DNA, host cells transformed with the vector and a
    method for producing the anti-ICAM-1 grafted Ab.
        USE/ADVANTAGE - The Abs are used to treat - and prevent
```

inflammation in e.g. delayed type hypersensitivity, psoriasis, an autoimmune disease e.g. Reynaud7s syndrome, autoimmune thyroiditis, EAE, multiple sclerosis, rheumatoid arthritis and lupus erythematosus, tissue or organ transplant or graft rejection. They are also used to treat and prevent tumours, viral infections (e.g. rhinoviruses of the major serotype within the genus Picornavididae, group A coxsackievirus, a Mengo virus and HIV); asthma and non-specific defence system response, e.g. adult respiratory distress syndrome, CNS inflammatory disorder, multiple organ injury syndrome secondary to septicaemia or trauma, ulcerative colitis and Crohn's disease. Administration can be enteral, parenteral, topical, intranasal or by inhalation. The Abs are also used to diagnose an ICAM-1-expressing tumour cell and inflammation. @(68pp Dwg.No.0/4

Derwent Class: B04; D16;
Int Pat Class: A61K-039/39; C07K-015/28

26/7/6 (Item 6 from file: 351)
008718897 WPI Acc No: 91-222916/30
XRAM Acc No: C91-096865
CD3 specific *humanised* recombinant *antibody* - is chimeric or *cdr* grafted for immunotherapy and diagnosis; COMPLEMENTARY DETERMINE REGION
Patent Assignee: (CELL-) CELLTECH LTD
Author (Inventor): JOLLIFFE L K; ZIVIN R A; ADAIR J R; ATHWAL D S
Number of Patents: 003
Patent Family:

CC Number	Kind	Date	Week	
WO 9109968	A	910711	9130	(Basic)
AU 9170330	A	910724	9143	
GB 2246781	A	920212	9207	

Priority Data (CC No Date): WO 90GB2018 (901221); GB 8928874 (891221); GB 9117611 (910815)
Applications (CC,No,Date): GB 9017611 (901221)
Language: English
EP and/or WO Cited Patents: EP 403156; EP 328404
Designated States
(National): AT; AU; BB; BG; BR; CA; CH; DE; DK; ES; FI; GB; GR; HU; JP; KR ; LK; LU; MC; MG; MW; NL; NO; RO; SD; SE; SU; US
(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE; OA
Filing Details: GB2246781 Based on WO9109968 (E) (1251CH)
Abstract (Basic): WO 9109968

A recombinant *antibody* (RAM) comprising antigen binding regions derived from the heavy and or light chain variable regions of a donor anti- CD3 *antibody*. The *antibody* preferably has binding affinity similar to that of OKT3. The RAM comprises antigen binding regions from suitable anti-CD3 *antibodies* such as rodent e.g. mouse or rat anti-CD3 MAb. The RAM may comprises only the variable region (VH and/or VL) or one or more CDRs of such a MAb.

The RAM is preferably a *humanised* *antibody* molecule specific for CD3 having an antigen binding site where at least one of the CDRs of the variable domain and usually two more of the CDRs are derviced from non human anti-CD3 *antibody*. The RAM may be a chimeric or *CDR* grafted *antibody*. Usually, the donor and acceptor *antibodies* are derived from different species. Typically the donor anti CD3 *antibody* is non-human (e.g. rodent) and the acceptor *antibody* is human. A *CDR* grafted *antibody* heavy chain comprising variable region with acceptor and donor CD3 binding comprising donor residues at one or more of positions 6, 37, 48 and 94. The *CDR* grafted light chain is also claimed.

DNA coding these *antibodies* and their production by recombinant DNA technology is claimed.

USE/ADVANTAGE - The *antibodies* may be used for treatment or diagnosis of human or veterinary conditions. The *humanised* *antibodies* do not have the immunologic complications associated with administration of non human *antibodies* to human subjects. @(81pp Dwg.No.0/13)@

Derwent Class: B04; D16;

Int Pat Class: A61K-039/39; A61K-049/00; C07K-015/06; C12N-005/10; C12N-015/13; C12P-021/08

26/7/7 (Item 7 from file: 351)

008718896 WPI Acc No: 91-222915/30

Related WPI Accession(s): 92-056874

XRAM Acc No: C92-025713

New *humanised* *antibodies* comprising *CDR* grafted *antibody* - with heavy and light chains, for use in vivo therapy and diagnosis; COMPLEMENTARY DETERMINE REGION

Patent Assignee: (CLLT) CELLTECH LTD; (CELL-) CELLTECH LTD

Author (Inventor): ADAIR J R; BODMER M W; MOUNTAIN A; OWENS R J; ATHWAL D S ; EMTAGE J S

Number of Patents: 005

Number of Countries: 035

June 8/

Patent Family:

CC Number	Kind	Date	Week	
WO 9109967	A	910711	9130	(Basic)
AU 9169740	A	910724	9143	
GB 2246570	A	920205	9206	
WO 9201059	A	920123	9207	
AU 9182005	A	920204	9220	

Priority Data (CC No Date): GB 8928874 (891221); WO 90GB20174 (901221); GB 9014932 (900705)

Applications (CC,No,Date): AU 9182005 (910705); WO 91GB1108 (910705); GB 9017612 (901221)

Language: English

EP and/or WO Cited Patents: EP 239400; EP 323806; EP 328404; EP 403156; 6.Jnl.Ref; WO 8901783; WO 8910140

Designated States

(National): AT; AU; BB; BG; BR; CH; DE; DK; FI; GB; HU; JP; KP; KR; LK; LU ; MC; MG; MW; NL; NO; RO; SD; SE; SU; US; CA; CS; ES; PL

(Regional): AT; BE; CH; DE; FR; GB; GR; IT; LU; NL; OA; SE; DK; ES

Filing Details: AU9182005 Based on WO 9201059

Abstract (Basic): WO 9109967

A *CDR* grafted *antibody* heavy chain is claimed having a variable region comprising acceptor frame-work and donor antigen binding regions in at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. Preferably, the heavy chain framework also comprises donor residues at positions 6, 37, 48 and 94. Also claimed is a *CDR*-grafted *antibody* light chain having a variable region domain comprising acceptor framework and donor antigen binding regions comprising donor residues in at least one of positions 1 and/or 3 and preferably at positions 46 and/or 47. A *CDR* grafted *antibody* molecule is also claimed comprising at least one *CDR* grafted heavy chain and light chain. DNA encoding the *CDR* grafted heavy and light chains is also claimed. The heavy or light chains may have an effector or reporter molecule attached e.g. a macrocycle for chelating a metal atom or a toxin such as ricin. The *CDR* grafted *antibodies* preferably have non-human e.g. rodent donor and human acceptor frameworkers.

USE/ADVANTAGE - For use in treatment and diagnosis of human and veterinary conditions. @(91pp Dwg.No.0/13

Derwent Class: B04; D16;
Int Pat Class: A61K-039/39; A61K-039/395; C07K-015/06; C07K-015/28; C12N-005/10; C12N-015/13; C12P-021/08; C12R-001/91

26/7/8 (Item 8 from file: 351)
008366799 WPI Acc No: 90-253800/33
XRAM Acc No: C90-109897
Chimaeric immunoglobulin(s) blocking IL-2 binding to receptors - comprising human framework and murine complementary determining regions, less immunogenic than murine *antibodies*
Patent Assignee: (PROT-) PROTEIN DESIGN LABS INC; (PROT-) PROTEIN DESIGN LABS
Author (Inventor): QUEEN C L; SELICK H E
Number of Patents: 010
Number of Countries: 034
Patent Family:

CC Number	Kind	Date	Week	
WO 9007861	A	900726	9033	(Basic)
PT 92758	A	900629	9033	
CA 2006865	A	900628	9037	
AU 9051532	A	900813	9044	
ZA 8909956	A	901031	9048	
CN 1043875	A	900718	9115	
FI 9102436	A	910520	9133	
NO 9102385	A	910619	9142	
DK 9101191	A	910619	9143	
JP 4502408	W	920507	9225	

Priority Data (CC No Date): US 290975 (881228); US 310252 (890213)
Applications (CC,No,Date): WO 89US5857 (891228); JP 90503677 (891228); ZA 899956 (891228)
Language: English; German
EP and/or WO Cited Patents: 7.Jnl.Ref; EP 239400; GB 2188941; US 4816567; WO 8901783
Designated States
(National): AT; AU; BB; BG; BR; CH; DE; DK; FI; GB; HU; JP; KP; KR; LK; LU ; MC; MG; MW; NL; NO; RO; SD; SE; SU
(Regional): AT; BE; CH; DE; ES; FR; GB; IT; LU; NL; OA; SE
Filing Details: JP04502408 Based on WO 9007861
Abstract (Basic): WO 9007861

Compsn. comprises a pure human-like immunoglobulin (Ig) which (a) reacts specifically with p55 Tac protein and/or (b) inhibits binding of human interleukin-2 (IL-2) to its receptor. Also new are (1) human-like Ig having 2 pairs of light/heavy chains and able to react specifically with an epitope of a human IL-2 receptor with affinity at least 10 power 8 per mole, the chains including complementarily determg. regions (*CDR*'s) and human-like framework regions (FR's), the *CDR*'s being from different Ig molecules than FR's; (2) *humanised* Ig (hIg) which can bind to IL-2 receptors and contain at least one *CDR* from anti-Tac *antibody* in a human-like FR contg. at least one amino acid from the anti-Tac *antibody*; (3) nucleic acid encoding for human-like FR and at least one murine *CDR*, and (4) cells transfected with nucleic acid.

USE/ADVANTAGE - hIG are not significantly immunogenic in humans; are easily and economically produced, and have a longer half-life in vivo than mouse *antibodies*. They are useful (opt. when attached to a cytotoxic agent, for treatment of T-cell mediated disorders, e.g. graft or transplant rejection, and autoimmune diseases. LIG can also be used in vitro for T-cell typing; isolation of IL-2 receptor bearing cells, vaccine prodn., etc. @(52pp Dwg.No.0/10)@

Abstract (EP): 9142 EP 451216

Compsn. comprises a pure human-like immunoglobulin (Ig) which (a) reacts specifically with p55 Tac protein and/or (b) inhibits binding of human interleukin-2 (IL-2) to its receptor. Also new are (1) human like Ig having 2 pairs of light/heavy chains and able to react specifically with an epitope of a human IL-2 receptor with affinity at least 10 power 8 per mole, the chains including complementarily determg. regions (*CDR*'s) and human-like framework regions (FR's) the *CDR*'s being from different Ig molecules than FR's. (2) *humanised* IG (hIg) which can bind to IL-2 receptors and contain at least one *CDR* from anti-Tac *antibody* in a numan-like FR contg. at lesdt one amino acid from the anti-Tac *antibody*, (3) nucleic acid encoding for human-like FR and at least one murine *CDR*, and (4) cells transfected with nucleic acid.

USE/ADVANTAGE - hIG are not significantly immunogenic in humans, are easily and economically produced, and have a longer half-life in vivo than mouse *antibodies*. They are useful (opt. when attached to a cytotoxic agent, for treatment of T-cell mediated disorders, e.g. graft or transplant rejection, and autoimmune diseases, LIG can also be used in vitro for T-cell typing, isolation of IL-2 receptor bearing cells, vaccine prodn etc.

Derwent Class: B04; D16;

Int Pat Class: A61K-039/39; C07K-007/10; C07K-013/00; C07K-015/14; C12N-005/10; C12N-007/01; C12N-015/00; C12P-021/08

?

```
set cost off

COST        = OFF.
?set hi *

HILIGHT set on as '*'
Hilight option is not available in file(s) 399.
?show files

File 155:MEDLINE 1966-1992/NOV (9211W1)
File   5:BIOSIS PREVIEWS_69-92/OCT BA9407:BARRM4307
         (C. BIOSIS 1992)
File  73:EMBASE (EXCERPTA MEDICA)_74-92/ISS37
         (COPR. ESP BV/EM 1992)
File 399:CA SEARCH_1967-1992 UD=11710
         (Copr. 1992 by the Amer. Chem. Soc.)
?ds


Set      Items   Description
S1          16   HUMANIZED()ANTIBODIES/TI
S2      332298   ANTIBODIES! FROM 155
S3        2253   IMMUNOGLOBULIN VARIABLE REGION! FROM 155
S4        2253   S2 AND S3
S5         862   HUMANIZ?
S6        2005   HUMANIS?
S7          16   S4 AND (HUMANIZ? OR HUMANIS?)
S8      636823   ANTIBOD? FROM 5,73,399
S9      165469   IMMUNOGLOBULIN
S10      41830   IG
S11     113462   VARIABLE
S12     392448   REGION
S13        862   (IMMUNOGLOBULIN OR IG)(W)VARIABLE(W)REGION
S14        604   CDR
S15      67991   COMPLEMENTARY
S16     112646   DETERMINING
S17         63   COMPLEMENTARY(W)DETERMINING
S18       1904   HYPERVARIABLE
S19     392448   REGION
S20        747   (COMPLEMENTARY(W)DETERMINING OR HYPERVARIABLE)(W)REGION
S21     428778   ANTIBODY
S22    1469126   RELATED
S23     623755   BINDING
S24     544344   SITE? ?
S25          0   ANTIBODY(W)RELATED(W)BINDING(W)SITE? ?
S26       2161   (IMMUNOGLOBULIN OR IG)()VARIABLE()REGION OR CDR OR (COMPLE-
              MENTARY()DETERMINING OR HYPERVARIABLE)()REGION OR ANTIBODY()R-
              ELATED()BINDING()SITE? ? FROM 5,73,399
S27        897   S8 AND S26
S28         18   S27 AND (S5 OR S6)
S29         34   S28 OR S7
S30         21   RD (unique items)
S31         21   Sort S30/ALL/PY,D
S32       3165   COMPLEMENTARITY
S33    2005813   DETERMIN?
S34     524927   REGION
S35        358   COMPLEMENTARITY(W)DETERMIN?(W)REGION
S36         12   COMPLEMENTARITY()DETERMIN?()REGION AND (S5 OR S6) AND S8
S37         28   7 OR 36
S38          8   (37 OR 29) NOT 29
```

used complementary rather than complementarity in previous search.

Picked a few more references here.

```
S39         6   RD (unique items)
S40         6   Sort S39/ALL/PY,D
?t40/7/1-6
```

40/7/1 (Item 1 from file: 5)
9081780 BIOSIS Number: 93066780
DEVELOPMENT OF *HUMANIZED* BISPECIFIC *ANTIBODIES* REACTIVE WITH CYTOTOXIC LYMPHOCYTES AND TUMOR CELLS OVEREXPRESSING THE HER2 PROTOONCOGENE
SHALABY M R; SHEPARD H M; PRESTA L; RODRIGUES M L; BEVERLEY P C L; FELDMANN M; CARTER P
DEP. CELL BIOL., GENENTECH, INC., 460 POINT SAN BRUNO BOULEVARD, SOUTH SAN FRANCISCO, CALIF. 94080.
J EXP MED 175 (1). 1992. 217-226. CODEN: JEMEA
Full Journal Title: Journal of Experimental Medicine
Language: ENGLISH

The HER2 protooncogene encodes a 185-kD transmembrane phosphoglycoprotein, human epidermal growth factor receptor 2 (p185HER2), whose amplified expression on the cell surface can lead to malignant transformation. Overexpression of HER2/p185HER2 is strongly correlated with progression of human ovarian and breast carcinomas. Recent studies have shown that human T cells can be targeted with bispecific *antibody* to react against human tumor cells in vitro. We have developed a bispecific F(ab')2 *antibody* molecule consisting of a *humanized* arm with a specificity to 185HER2 linked to another arm derived from a murine anti-CD3 monoclonal *antibody* that we have cloned from UCHT1 hybridoma. The antigen-binding loops for the anti-CD3 were installed in the context of human variable region framework residues, thus forming a fully *humanized* BsF(ab')2 fragment. Additional variants were produced by replacement of amino acid residues located in light chain *complementarity* *determining* *region* 2 and heavy chain framework region 3 of the *humanized* anti-CD3 arm. Flow cytometry analysis showed that the bispecific F(ab')2 molecules can bind specifically to cells overexpressing p185HER2 and to normal human peripheral blood mononuclear cells bearing the CD3 surface marker. In additional experiments, the presence of bispecific F(ab')2 caused up to fourfold enhancement in the cytotoxic activities of human T cells against tumor cells overexpressing p185HER2 as determined by a 51Cr release assay. These bispecific molecules have a potential use as therapeutic agents for the treatment of cancer.

40/7/2 (Item 2 from file: 399)
117068366 CA: 117(7)68366p PATENT
Chimeric and complementarity-determining region-grafted anti-carcinoembryonic antigen antibodies and their production
INVENTOR(AUTHOR): Adair, John Robert; Bodmer, Mark William; Mountain, Andrew; Owens, Raymond John
LOCATION: UK,
ASSIGNEE: Celltech Ltd.
PATENT: PCT International ; WO 9201059 A1 DATE: 920123
APPLICATION: WO 91GB1108 (910705) *GB 9014932 (900705) *WO 90GB2017 (901221)
PAGES: 70 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12P-021/08A; A61K-039/395B; C12N-015/13B; C07K-015/28B DESIGNATED COUNTRIES: AT; AU; BB ; BG; BR; CA; CH; CS; DE; DK; ES; FI; GB; HU; JP; KP; KR; LK; LU; MC; MG; MN; MW; NL; NO; PL; RO; SD; SE; SU; US DESIGNATED REGIONAL: AT; BE; BF; BJ ; CF; CG; CH; CI; CM; DE; DK; ES; FR; GA; GB; GN; GR; IT; LU; ML; MR; NL; SE; SN; TD; TG
SECTION:
CA215003 Immunochemistry
IDENTIFIERS: carcinoembryonic antigen humanized chimeric antibody,

complementarity detg region grafted antibody CEA, cloning DNA humanized antibody CEA
  DESCRIPTORS:
Antibodies,monoclonal...
    A5B7 murine, to carcinoembryonic antigen, in humanized antibody prodn.
Animal cell line...
    CHO L761 h, humanized anti-carcinoembryonic antigen antibody
    recombinant prodn. in
Deoxyribonucleic acid sequences...
    for antibody variable regions in humanized anti-carcinoembryonic
    antigen antibody prodn.
Genetic vectors... Molecular cloning...
    for humanized anti-carcinoembryonic antigen antibody prodn.
Diagnosis... Therapeutics...
    humanized anti-carcinoembryonic antigen antibodies for
Escherichia coli...
    humanized anti-carcinoembryonic antigen antibody fragment recombinant
    prodn. in
Animal cell line,CHO-K1... Animal cell line,COS-1... Bacteria...
    humanized anti-carcinoembryonic antigen antibody recombinant prodn. in
Mammal...
    humanized anti-carcinoembryonic antigen antibody recombinant prodn. in
    cells of
Immunoglobulins,fusion products...
    humanized, prodn. of
Antibodies...
    humanized, to carcinoembryonic antigen
Immunoglobulins...
    in humanized anti-carcinoembryonic antigen antibody prodn.
Protein sequences...
    of antibody variable regions in humanized anti-carcinoembryonic antigen
    antibody prodn.
Plasmid and Eiisme...
    pAL43, for humanized anti-carcinoembryonic antigen antibody prodn.
Plasmid and Episome...
    pAL44, for humanized anti-carcinoembryonic antigen antibody prodn.
Plasmid and Episome...
    pAL45, for humanized anti-carcinoembryonic antigen antibody prodn.
Plasmid and Episome...
    pAL46, for humanized anti-carcinoembryonic antigen antibody prodn.
Plasmid and Episome...
    pAL53, for humanized anti-carcinoembryonic antigen antibody prodn.
Plasmid and Episome...
    pAL54, for humanized anti-carcinoembryonic antigen antibody prodn.
Genetic vectors...
    pEE6hCMV gpt, for humanized anti-carcinoembryonic antigen antibody
    prodn.
Plasmid and Episome...
    pHMC19, for humanized anti-carcinoembryonic antigen antibody prodn.
Plasmid and Episome...
    pHMC30, for humanized anti-carcinoembryonic antigen antibody prodn.
Plasmid and Episome...
    pHMC31, for humanized anti-carcinoembryonic antigen antibody prodn.
Plasmid and Episome...
    pHMC43, for humanized anti-carcinoembryonic antigen antibody prodn.
Plasmid and Episome...
    pHMC44, for humanized anti-carcinoembryonic antigen antibody prodn.
Genetic vectors...
    pMRR028, for humanized anti-carcinoembryonic antigen antibody fragment
    prodn.

Genetic vectors...
pMRR045, for humanized anti-carcinoembryonic antigen antibody fragment prodn.
CAS REGISTRY NUMBERS:
142661-53-8 142661-54-9 142661-55-0 142661-56-1 142661-57-2
142661-58-3 amino acid sequence of, humanized anti-carcinoembryonic antigen antibody prodn. in relation to
142662-69-9 142662-70-2 142662-71-3 142662-72-4 142662-81-5
142662-82-6 nucleotide sequence of, humanized anti-carcinoembryonic antigen antibody prodn. in relation to

40/7/3 (Item 3 from file: 5)
8599131 BIOSIS Number: 92064131
IMMUNOGLOBULIN *COMPLEMENTARITY*-*DETERMINING* *REGION* GRAFTING BY RECOMBINANT POLYMERASE CHAIN REACTION TO GENERATE *HUMANIZED* MONOCLONAL *ANTIBODIES*
LEWIS A P; CROWE J S
DEP. CELL BIOLOGY, WELLCOME RES. LAB., LANGLEY COURT, BECKENHAM, KENT, BR3 3BS UK.
GENE (AMST) 101 (2). 1991. 297-302. CODEN: GENED
Full Journal Title: GENE (Amsterdam)
Language: ENGLISH

We describe an approach to rapidly generate *humanised* monoclonal *antibodies* by grafting rodent complementarity-determining regions into human immunoglobulin frameworks using recombinant polymerase chain reaction (PCR) methodology. The approach was applied to grafting a rat *complementarity*-*determining* *region* onto a human framework and amplifying the entire *humanised* heavy chain. The terminal oligodeoxyribonucleotide primers incorporated restriction sites to allow forced clonign into plasmid vectors for sequencing and expression. No nucleotide errors were introduced into the 1463-bp sequence even after sequential applications of PCR.

40/7/4 (Item 4 from file: 5)
7912269 BIOSIS Number: 40113269
CONSTRUCTION OF *HUMANIZED* *ANTIBODIES* AND TESTING IN PRIMATES
QUEEN C; CO M S; DESCHAMPS M; WHITLEY R; BENJAMIN W; HAKIMI J
PROTEIN DESIGN LAB. INC., 2375 GARCIA AVE., MOUNTAIN VIEW, CALIF. 94043.
MEETING ON MONOCLONAL ANTIBODIES HELD AT THE 20TH ANNUAL MEETING OF THE KEYSTONE SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, DENVER, COLORADO, USA, MARCH 10-16, 1991. J CELL BIOCHEM SUPPL 15 (PART E). 1991. 137. CODEN: JCBSD
Language: ENGLISH

40/7/5 (Item 5 from file: 5)
7400987 BIOSIS Number: 89052006
A *HUMANIZED* *ANTIBODY* THAT BINDS TO THE INTERLEUKIN 2 RECEPTOR
QUEEN C; SCHNEIDER W P; SELICK H E; PAYNE P W; LANDOLFI N F; DUNCAN J F; AVDALOVIC N M; LEVITT M; JUNGHANS R P; WALDMANN T A
PROTEIN DESIGN LABS., 3181 PORTER DRIVE, PALO ALTO, CALIF. 94304.
PROC NATL ACAD SCI U S A 86 (24). 1989. 10029-10033. CODEN: PNASA
Full Journal Title: Proceedings of the National Academy of Sciences of the United States of America
Language: ENGLISH

The anti-Tac monoclonal *antibody* is known to bind to the p55 chain of the human interleukin 2 receptor and to inhibit proliferation of T cells by blocking interleukin 2 binding. However, use of anti-Tac as an immunosuppressant drug would be impaired by the human immune response against this murine *antibody*. We have therefore constructed a " *humanized*" *antibody* by combining the complementarity-determining regions (CDRs) of the anti-Tac *antibody* with human framework and constant regions. The human framework regions were chosen to maximize homology with the anti-Tac *antibody* sequence. In addition, a computer model of murine anti-Tac was used to identify several amino acids which, while outside the CDRs, are likely to interact with the CDRs or antigen. These mouse amino acids were also retained in the *humanized* *antibody*. The *humanized* anti-Tac *antibody* has an affinity for p55 of 3 .times. 109 M-1, about 1/3 that of murine anti-Tac.

40/7/6 (Item 6 from file: 399)
113170316 CA: 113(19)170316b PATENT
Recombinant antibodies to Campath-1 antigen, containing foreign complementarity determining region(s), and their use in immunosuppression and cancer therapy
INVENTOR(AUTHOR): Waldmann, Herman; Clark, Michael Ronald; Winter, Gregory Paul; Riechmann, Lutz
LOCATION: UK,
ASSIGNEE: Medical Research Council
PATENT: PCT International ; WO 8907452 A1 DATE: 890824
APPLICATION: WO 89GB113 (890210) *GB 883228 (880212) *GB 884464 (880225)
PAGES: 61 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: A61K-039/395A; C12N-015/00B DESIGNATED COUNTRIES: AU; DK; JP; US
SECTION:
CA215003 Immunochemistry
CA201XXX Pharmacology
CA203XXX Biochemical Genetics
IDENTIFIERS: chimeric antibody Campath 1 antigen, lymphoma neoplasm inhibitor Campath 1H antibody
DESCRIPTORS:
Rat...
complementarity detg. regions of, in recombinant antibody to Campath-1 antigen
Immunoglobulins,G2... Immunoglobulins,G3... Immunoglobulins,G4...
const. domains of human, in recombinant antibody contg. complementarity detg. regions to Campath-1 antigen
Lymphocyte...
depletion of, in human, by recombinant human antibody contg. foreign complementarity detg. regions to Campath-1 antigen
Gene and Genetic element,animal, synthetic...
for humanized light chain variable region, construction of, in prodn. of recombinant human antibody contg. rat complementarity detg. regions to Campath-1 antigen
Protein sequences...
of IgG2a YTH 34.5 HL heavy and light chain variable domains, of rat
Deoxyribonucleic acid sequences,IgG2a-specifying...
of rat
Antigens,CAMPATH-1...
recombinant antibodies to, foreign complementarity detg. regions in
Immunosuppressants... Neoplasm inhibitors... Neoplasm inhibitors,lymphoma ...
recombinant antibody contg. foreign complementarity detg. regions to Campath-1 antigen as
Gene and Genetic element,animal...
recombinant, for anti-Campath-1 antigen antibody of human, sequences encoding rat complementary detg. regions in
Immunoglobulins,G2a...
recombinant human antibody to Campath-1 antigen contg. complementary detg. regions of rat

```
Leukemia,B-cell...
    recombinant human antibody to Campath-1 antigen killing leukemia cells
    of
Antibodies...
    recombinant, to Campath-1 antigen, foreign complementarity detg.
    regions in
Immunoglobulins,G1... Immunoglobulins,G... Immunoglobulins,M...
    recombinant, to Campath-1 antigen, foreign complementary detg. regions
    in
  CAS REGISTRY NUMBERS:
129711-40-6  amino acid sequence encoded by HuVLLYS gene
129711-41-7  amino acid sequence encoded by synthetic HuVLLYS.degree. gene
129711-01-9  129711-02-0  cloning and nucleotide sequence of, of human and
    rat
129711-19-9  129711-20-2  cloning and nucleotide sequence of, of rat
128096-06-0  128096-07-1  128096-08-2  128096-09-3  128096-10-6
    128096-11-7  complementarity detg. region of rat YTH 34.5 HL, human
    recombinant antibody contg., Campath-1 antigen binding by
129711-56-4  heavy chain variable region of human contg. rat
    complementarity detg. regions, recombinant antibody contg., Campath-1
    antigen binding by
129711-60-0  heavy chain variable region of rat YTH 34.5 HL, recombinant
    antibody contg., Campath-1 antigen binding by
129710-86-7P  HuVLLYS gene, prepn. of, in prepn. of recombinant human
    antibody contg. rat complementarity detg. regions to Campath-1 antigen
129711-59-7  light chain variable region of human contg. rat
    complementarity detg. regions, recombinant antibody contg., Campath-1
    antigen binding by
129711-61-1  light chain variable region of rat YTH 34.5 HL, recombinant
    antibody contg., Campath-1 antigen binding by
127859-21-6P  127859-23-8P  127859-24-9P  127859-26-1P  127859-62-5P
    127859-70-5P  127859-72-7P  127859-79-4P  127859-82-9P  127859-92-1P
    127859-93-2P  127859-94-3P  127859-99-8P  127860-01-9P  127860-02-0P
    127860-03-1P  127860-04-2P  129924-57-8P  129924-59-0P  prepn. of, in
    gene synthesis for recombinant human antibody contg. rat
    complementarity detg. regions to Campath-1 antigen
129711-57-5  129711-58-6  recombinant human antibody contg., Campath-1
    antigen binding by
129710-91-4P  synthetic gene HuVLLYS.degree., prepn. of, in prepn. of
    recombinant human antibody contg. rat complementary detg. regions to
    Campath-1 antigen

Copyright 1992 by the American Chemical Society
?b351,350

      15sep92 10:26:26 User209197 Session D127.2

SYSTEM:OS  - DIALOG OneSearch
  File 351:Derwent World Patents Index Latest
         1981+;DW=9227,UA=9214,UM=9143
 **FILE351: Formats 32,33,35,37 & 39 display the new 'Expanded' Patent
  Family table for UD=9216 and greater.  For more info. type ?NEWS351
  File 350:Derwent World Patents Index
         1963-1980, EQUIVALENTS THRU DW=9227
 **FILE350: Formats 32,33,35,37 & 39 display the new 'Expanded' Patent
  Family table for UD=9219 and greater.  For more info. type ?NEWS350

      Set  Items  Description
      ---  -----  -----------
?ds
```

```
Set     Items   Description
S1         22   ANTIBOD? AND (HUMANIS? OR HUMANIZ?)
S2          8   S1 AND (CDR OR (IG OR IMMUNOGLOBULIN)()VARIABLE()REGION OR
             HYPERVARIABLE()REGION)
S3          0   S1 AND COMPLEMENTARITY()DETERMIN?()REGION
S4          3   S1 AND COMPLEMENT?()DETERMIN?()REGION
S5          1   (2 OR 4) NOT 2
?t5/7/1


 5/7/1     (Item 1 from file: 351)
007820291  WPI Acc No: 89-085403/11
XRAM Acc No: C89-037905
    Recombinant *humanised* *antibody* specific for TAG-72 - having
    complementarity determining regions of variable domains from mouse
    *antibody* and the remainder from human immunoglobulin
Patent Assignee: (CELL-) CELLTECH LTD
Author (Inventor): BODMER M W; ADAIR J R; WHITTLE N R
Number of Patents: 001
Patent Family:
    CC Number     Kind     Date      Week
    WO 8901783     A      890309     8911   (Basic)
Priority Data (CC No Date): WO 88GB731 (880905); GB 8720833 (870904)
Language: English
EP and/or WO Cited Patents: No.SR.Pub; 4.Jnl.REF
Designated States
 (National): AU; DK; FI; HU; JP; KR; NO; RO; SU; US
 (Regional): AT; BE; CH; DE; FR; GB; IT; LU; NL; SE
Abstract (Basic): WO 8901783
        A *humanised* *antibody* molecule (HAM) is claimed having
    specificity for the TAG-72 antigen and having an antigen binding site
    in which at least the *complementary* *determining* *region* (CDRs) of
    the variable domains are derived from the mouse monoclonal *antibodies*
    (MAb) B72.3 and the remaining immunoglobulin-derived parts of the HAM
    are derived from a human immunoglobulin.
        USE/ADVANTAGE - *Humanising* the B72.3 MAb does not adversely
    affect its binding activity and this produces a HAM which is useful in
    both therapy and diagnosis of certain carcinomas, e.g. solid tumours
    expressing TAG-72. @(49pp Dwg.No.0/13)@
Derwent Class: B04; D16;
Int Pat Class: A61K-039/39; C12N-015/00; C12P-021/00
?s complement?()determin?(w)region? ?

Processing
Processing
Processing
            27431  COMPLEMENT?
           234285  DETERMIN?
           124968  REGION? ?
      S6       23  COMPLEMENT?()DETERMIN?(W)REGION? ?
?c 1 and 6

               22  1
               23  6
      S7       10  1 AND 6
?

?c 7 not (2 or 4)
```

```
               10   7
                8   2
                3   4
      S8        3   7 NOT (2 OR 4)
?t8/7/1-3


 8/7/1     (Item 1 from file: 351)
009004842  WPI Acc No: 92-132139/16
XRAM Acc No: C92-061892
    *Humanisation* of *antibodies* binding to human CD4 antigen - by
    mutation of framework-encoding regions of DNA encoding variable domain
    of rat or mouse *antibody* chain
Patent Assignee: (GORM/) GORMAN S D
Author (Inventor): CLARK M R; COBBOLD S P; GORMAN S D; WALDMANN H
Number of Patents: 001
Number of Countries: 018
Patent Family:
    CC Number    Kind     Date      Week
    WO 9205274     A      920402    9216   (Basic)

Priority Data (CC No Date): GB 9020282 (900917)
Applications (CC,No,Date): WO 91GB1578 (910916)
Language: English
EP and/or WO Cited Patents: 7.Jnl.Ref; EP 328404; EP 365209; EP 403156; WO
    9007861; WO 9107492; WO 9109966; WO 9109967
Designated States
 (National): AU; CA; JP; KR; US
 (Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LU; NL; SE
Abstract (Basic): WO 9205274  A
        *Complementarity* *determining* *regions* (CDRs) of the variable
    domain of the *antibody* chain are derived from a first mammalian
    species and the framework of the variable domain and any constant
    domains of the Ab chain are derived from a second different mammalian
    species; comprising (a) mutating the framework-encoding regions of DNA
    encoding a variable domain of the first mammalian Ab chain such that it
    encodes the framework derived from the second species; and (b)
    expressing the Ab chain using this mutated DNA.
             The process specifically comprises: (i) determining
    nucleotide and predicted aminoacid sequence of a variable domain of a
    selected Ab chain of the first species; (ii) determining the Ab
    framework to which the framework of this domain is to be altered; (iii)
    mutating framework-encoding regions of DNA encoding this variable
    domain such that the mutated region encodes the framework determined in
    (ii); (iv) linking mutated DNA to DNA encoding a constant domain of the
    second species and cloning the DNA into an expression vector; and (v)
    introducing expression vector into a compatible host cell and culturing
    it to express Ab chain.
             USE/ADVANTAGE - Altered Abs is prepd., used to *humanise* an
    Ab, typically a monoclonal Ab and, e.g. a rat or mouse Ab. The
    resulting Ab retains the antigen binding capabilities of the Ab from
    which it is derived. Reshaped CD4 Ab is used to induce tolerance
    against an antigen. Used to alleviate autoimmune diseases e.g.
    rheumatoid arthritis, and to prevent graft rejection.  0/13
Derwent Class: B04; D16;
Int Pat Class: A61K-039/39; C12N-015/13; C12P-021/08

 8/7/2     (Item 2 from file: 351)
008712964  WPI Acc No: 91-216983/30
```

XRAM Acc No: C91-094177

Prodn. of *humanised* recombinant immunoglobulin - including polymerase chain reaction amplification of murine *antibody* light and heavy chain variable portions

Patent Assignee: (MERI) MERCK & CO INC
Author (Inventor): LAW M F; MARK G E; WILLIAMSON A R
Number of Patents: 002
Patent Family:

CC Number	Kind	Date	Week	
EP 438310	A	910724	9130	(Basic)
CA 2034553	A	910720	9139	

Priority Data (CC No Date): US 627423 (901220); US 467700 (900119)
Applications (CC,No,Date): EP 91300362 (910117)
Language: English
EP and/or WO Cited Patents: EP 239400; WO 8901783; 1.Jnl.REF
Designated States
(Regional): CH; DE; FR; GB; IT; LI; NL
Abstract (Basic): EP 438310

Method for producing a *humanised* recombinant immunoglobulin comprises: (a) prepg. polymerase chain reaction (PCR) primers to amplify the variable portion of the light and heavy chain of a murine *antibody* which binds to a predefined antigen; (b) using the primers to amplify the variable portions of both heavy and light chains and sequencing the resulting nucleotide chains; (c) determining the murine *complementary* *determining* *regions* of the heavy and light chains; (d) selecting human variable heavy and light chain frameworks which show a high degree of amino acid similarity with the variable heavy and light chain framework of the murine immunoglobulin; (e) selecting human constant heavy and light chain frameworks; (f) grafting the murine *complementary* *determining* *regions* of (c) to the human framework regions of (e); (g) incorporating the complete DNA sequence for the *humanised* recombinant immunoglobulin into an appropriate expression vector; (h) transfecting host cells with the vector; (i) growing the transfected cells in an environment in which the *humanised* recombinant immunoglobulin is expressed; and (j) collecting the immunoglobulin.

A PCR method for the simultaneous synthesis and assembly of at least 4 deoxyoligonucleotides is also claimed.

USE/ADVANTAGE - The *humanised* recombinant immunoglobulins are weakly immunogenic or non-immunogenic when admin. to humans, and may be used as therapeutic agents. Recombinant human anti-CD18 *antibodies* or active fragments which bind to the CD18 antigen of leukocytes can be used to inhibit influx of the leukocytes into a site of inflammation or tissue liable to become inflamed following influx. @(78pp Dwg.No.0/38)@

Derwent Class: B04; D16;
Int Pat Class: C12N-015/13; C12P-021/08; C12Q-001/68

8/7/3 (Item 3 from file: 351)
007275804 WPI Acc No: 87-272811/39
XRAM Acc No: C87-115825

Recombinant altered *antibodies* - having *complementarity* *determining* *regions* replaced with those from *antibody* of different specificity

Patent Assignee: (WINT/) WINTER G P
Author (Inventor): WINTER G P
Number of Patents: 004
Patent Family:

CC Number	Kind	Date	Week	
EP 239400	A	870930	8739	(Basic)
GB 2188638	A	871007	8740	

```
  JP 62296890      A      871224      8806
  GB 2188638       B      900523      9021
Priority Data (CC No Date): GB 867679 (860327); GB 877252 (870326)
Applications (CC,No,Date): EP 87302620 (870326); JP 8773980 (870327)
Language: English
EP and/or WO Cited Patents: A3...8914; 3.Jnl.REF
Designated States
 (Regional): AT; BE; CH; DE; ES; FR; GB; GR; IT; LI; LU; NL; SE
Abstract (Basic): EP 239400
        An altered *antibody* in which at least parts of the
    *complementary* *determining* *regions* (CDRs) in the light or heavy
    chain variable domains have been replaced by analogous parts of CDRs
    from an *antibody* of different specificity is new.
        The altered *antibody* can be produced by (a) prepg. a first
    replicable expression vector including a suitable promoter operably
    linked to a DNA sequence which encodes at least a variable domain of an
    Ig heavy or light chain, the variable domain comprising framework
    regions from a first *antibody* and CDRs comprising at least parts of
    the CDRs from a second *antibody* of different specificity, (b) if
    necessary, prepg. a second replicable expression vector including a
    suitable promoter operably linked to a DNA sequence which encodes at
    least the variable domain of a complementary Ig light or heavy chain,
    (c) transforming a cell line with the first or both prepd. vectors and
    (d) culturirg the transformed cell line to produce the altered
    *antibody*.
        USE/ADVANTAGE - The method is used for ''*humanising*'' non-human
    monoclonal *antibodies* (MAbs) e.g. CDRs from mouse MAb can be
    partially or totally grafted into the framework regions of a human MAb,
    which is then produced in quantity by a suitable cell line. Only the
    CDRs of the *antibody* will be foreign to the body and this should
    minimise side effects if used for human therapy. @(41pp Dwg.No.0/8)@
Derwent Class: B04; D16;
Int Pat Class: C12N-015/00; C12P-021/02; C07K-015/00; A61K-039/39;
    C12N-005/00; C12R-001/91
?ds

Set     Items   Description
S1         22   ANTIBOD? AND (HUMANIS? OR HUMANIZ?)
S2          8   S1 AND (CDR OR (IG OR IMMUNOGLOBULIN)()VARIABLE()REGION OR
             HYPERVARIABLE()REGION)
S3          0   S1 AND COMPLEMENTARITY()DETERMIN?()REGION
S4          3   S1 AND COMPLEMENT?()DETERMIN?()REGION
S5          1   (2 OR 4) NOT 2
S6         23   COMPLEMENT?()DETERMIN?(W)REGION? ?
S7         10   1 AND 6
S8          3   7 NOT (2 OR 4)
S9          5   S1 AND CDRS
S10         0   (9 OR 7 OR 2 OR 4) NOT (7 OR 2 OR 4)
?
```

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/715,273	06/14/91		[illegible]

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

EXAMINER
1806

ART UNIT	PAPER NUMBER
	13

DATE MAILED:

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 7/13/92 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☒ Notice of Art Cited by Applicant, PTO-1449. 3 pages
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ ______

Part II SUMMARY OF ACTION

1. ☒ Claims 1 – 16 are pending in the application.

   Of the above, claims 14 – 16 are withdrawn from consideration.

2. ☐ Claims ______ have been cancelled.
3. ☐ Claims ______ are allowed.
4. ☒ Claims 1-13 are rejected.
5. ☐ Claims ______ are objected to.
6. ☐ Claims ______ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on ______. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on ______ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on ______, has been ☐ approved. ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. ______ ; filed on ______
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

PTOL-326 (Rev. 9-89)

EXAMINER'S ACTION

Applicant's election of Group 1, in Paper No. 12, is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse. See M.P.E.P. 818.03(a).

Claims 1-10 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject, matter which applicant regards as the invention. Claims 1, 3, 4, 5 and 7 are indefinite in the use of the language "import antibody" in that it is not clear what constitutes an important antibody, ie. what the determines what is to be an import antibody. Claim 1 step a) is indefinite in that it is not clear what is meant by a "consensus human variable domain". Claim 1 step d) is indefinite in that it is not clear what is actually taking place when one aligns the amino acid sequences of the FR, ie. is this a physical or mental step? Claim 1 step e) is unclear in what type of homology is indicated, ie. are conservative amino acids considered as homologs or should their be identical amino acid residues at the indicated portion of the framework. Claim 1 step f), 3 is indefinite in the use of the language "participates" in that the nature of participation is unclear. Claim 1 step f) is indefinite in that it is not clear how one of ordinary skill can determine the effects which are listed in steps 1-3, ie. through antigen binding, through hybridization? Claim 1 step g) is indefinite in that it is not clear what effects are reasonably expected to occur. Claim 2 is indefinite in that the antecedent basis for "the domain" is unclear. Claim 3 is indefinite in that it is not clear when in the process of making the antibody one would search for the glycosylation sites. Claim 4 is indefinite for the same reason that claim 3 is indefinite. Claim 5 is indefinite in that it is believed that the claims up to this point were directed to making a "humanized antibody", and it is unclear how "preparing a humanized antibody" in claim 5 differs from the preparation of the antibody up to this point. Furthermore, it is not clear what is intended in the preparation of the antibody of claim 5. Claim 6 is vague in that it is not clear what the numbers are meant to designate. It is suggested that applicant clarify the nature of the numbers or point to a figure. Claim 7 is indefinite in that it is not clear what the method is drawn to. it is suggested that the language "a method of making a humanized antibody" be inserted within the claim.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> **The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.**

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to adequately describe the invention and failing to adequately teach how to make and or use the invention, ie. failing to provide an enabling disclosure. The following terms lack enablement in the specification:.

Claims 1 and 7 lack enablement in the language "at least a portion of an import variable domain". Applicant has only indicated specific residues which may be transferred, but they are claiming an antibody wherein the a portion of the import antibody are to be transferred. There is no guidance in the specification which would enable one of skill in the art to make antibodies with transferred variable domains other than CDRs. Applicant is aware that a portion of the variable domain can be any one of the CDRs as well as the framework regions. However, this language also reads on small amino acid sequences which are incomplete regions of the variable region of the antibody. There is no support in the specification for linking the variable region of the antibody to any or all of the myriad "portions" which are encompassed within this language. One of skill in the art would neither expect nor predict the appropriate functioning of the antibody as broadly as is claimed. It is suggested that the specific portion of the human variable region which is described in the specification be recited within the claim or this language be removed completely in order to obviate this rejection.

Claim 1 step c) lacks enablement in that it is not clear how one would determine which amino acids are to be substituted. There is no specific recitation of what characteristics of the amino acids are necessary for deciding whether it is to be replaced or not. Without this description one of skill in the art would not be able to choose the appropriate amino acid residues without hindering the function of the antibody.

Claim 1 step f), lacks enablement in that the protocol for determining whether the amino acid residues in the import amino acid sequence are reasonably expected to interact with the antigen is not described anywhere in the specification. There is no explicit step which enables one of ordinary skill in the art to determine the effects which are recited. It would require undue experimentation of one of ordinary skill in the art to make the variations which may be made in order to test the effects of the mutant antibodies.

W/drawn

Claim 2 lacks enablement in that there is no description in the specification of how to determine which residues are exposed on the surface or which residues are buried within the domain, is this through computer modeling or through x-ray crystallography or other methods?

Maintain

Claim 3 lacks enablement in that there is no guidance in the specification on how one would determine which glycosylation site affects antigen binding, or what comprises "reasonable expectation".

Claims 6, 7 and 9 lack enablement in that it would appear that these amino acids are relevant to IgG and not to other isotypes. There is no indication that one of skill in the art would extrapolate the use of these amino acids to all or other isotypes of immunoglobulins. Furthermore, there is insufficient description and guidance in the specification with regards to the properties of these amino acids which would enable one of ordinary skill in the art to make humanized antibodies with other isotypes using these amino acid sequences.

Applicant has not shown that antibodies which have been modified as that which is claimed are capable of functioning as that which is being disclosed, ie. maintaining the binding affinity of the parent antibody. Protein chemistry is probably one of the most unpredictable areas of biotechnology. For example, replacement of a single lysine residue at position 118 of acidic fibroblast growth factor by glutamic acid led to the substantial loss of heparin binding, receptor binding and biological activity of the protein. Burgess et. al. Journal of Cell biology, 111: 2129-2138 (1990). In transforming growth factor alpha, replacement of aspartic acid at position 47 with alanine or asparagine did not affect biological activity while replacement with serine or glutamic acid sharply reduced the biological activity of the mitogen. Lazar et. al. Molecular and Cellular Biology, 8:1247-1252 (1988). Similarly it has been shown that aglycosylation of antibodies reduces the resistance of the antibodies to proteolytic degradation, while CH2 deletions increase the binding affinity of the antibodies. See Tao et. al. The Journal of Immunology, Vol. 143, No. 8. 2595-2601 (1989) and Gillies et. al. Human Antibodies and Hybridomas, Vol 1, no. 1, 47-54 (1990). These references demonstrate that even a single amino acid substitution or what appears to be an inconsequential chemical modification, will often dramatically affect the biological activity and characteristic of a protein. Therefore, without sufficient guidance in the specification to support the use of the above terms and for the reasons mentioned above one of ordinary skill in the art would forced into undue experimentation in order to practice the invention as is claimed.

Claims 1-11 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

35 U.S.C. § 101 reads as follows:

> **Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title.**

Claims 1-4, 6-8 are rejected under 35 U.S.C. § 101 because the claimed invention is directed to non-statutory subject matter. The above claims are drawn to a method of preparing an antibody, however, there is no indication within the claims that actual physical steps are taking place. For example, there is no step which includes isolating an antibody, rather obtaining an amino acid sequence. All of the steps which are listed in the claims can be done on paper as mental steps or on a computer terminal.

The specification is objected to under 35 U.S.C. § 112, first paragraph, and claims 9-13 are rejected under 35 U.S.C. § 112, first paragraph and 35 U.S.C. § 101 as the specification fails to adequately teach how to use the claimed monoclonal antibodies in the manner in which they are disclosed ie. for the therapeutic purposes. Applicants claims are supported only by in vitro data showing the ability of muMab4D5, which is a humanized anti-p185 antibody which reacts with breast and ovarian cancers, to react with different cell lines (see page 88-90 of the specification). Applicant has made no showing that these data correlate with utility for in vivo therapy in humans of the complex array of diseases encompassed by the claims. In general, effective treatment of human cancers has not been routinely achieved in the art using monoclonal antibodies. Further, in vitro data such as that reported in the specification and animal model studies frequently do not correlate with clinical utility in in vivo trials in patients. Based on the evidence of record, the alleged utility of the claimed composition for the treatment of cancer would not be believable on its face to the person of skill in the art in view of the contemporary knowledge in the art. Applicant has not provided any showing of therapeutic utility of the subject monoclonal antibodies which would lead one of skill in the art to believe that the antibodies are broadly applicable for the treatment of all types of autoimmune diseases. Applicant is required to provide evidence commensurate with the scope of the claims, which would be convincing to those skilled in the art that the claimed compositions have utility for the treatment of malignant and autoimmune diseases in humans. See MPEP 608.01(p).

Waldmann, in a recent review of the literature pertaining to clinical applications of monoclonal antibodies for diagnosis and therapy of human disease, teaches that effective therapy using monoclonal antibodies has been elusive and indicates that hopes for antibody-based treatment methods engendered by in vitro studies have not correlated well with in vivo clinical trial results in patients with cancer. It does not appear that the exemplary material provided in the specification in support of the assertions that the claimed antibodies have therapeutic utility would be viewed by those skilled in the art as being predictive of their utility for treating humans. Applicant has not exemplified how to use the claimed antibodies in vivo and has not shown that the antibodies would be effective in vivo. It appears that undue experimentation would be required of one skilled in the art to practice the claimed invention for the single utility disclosed in the specification.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office Action:

> **A person shall be entitled to a patent unless--**
>
> **(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.**
>
> **(b) the invention was patented or described in a printed publication in this country or a**

> foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 1, 2, 5-10 are rejected under 35 U.S.C. § 102(b) as being anticipated by Queen et. al.. The above claims are drawn to a method of producing a humanized antibody wherein the amino acid sequences of an import antibody and a consensus antibody are compared, wherein the CDRs of the import antibody are substituted for the antibody of the consensus antibody, and wherein certain framework residues which are responsible for the binding of antigen, interaction with CDR, or participating in the Vl-Vh interaction are also imported to the consensus antibody. In essence, residues of the framework region are also transferred with the CDRs in order to retain the antigen binding affinity of the parent antibody.

Queen et. al. describe the production of humanized antibodies wherein the murine antibody is compared to human antibodies and the most homologous human antibody is chosen as the acceptor molecule. The CDRs of the murine antibody are then substituted for the CDRs of the human antibody and certain framework residues are also changed. Queen et. al. describe computer modeling and sequence comparison in order to determine the amino acid residues which are to be substituted (see page 10031-10033). Although the steps of the methods are not in exactly the same order, all of the claimed elements are present with in the reference.

Claims 1,2 and 5-10 are rejected under 35 U.S.C. § 102(a) as being anticipated by Co et. al.. See above discussion.

Co et. al. show the production of humanized anti-HSV using the general concept of Queen et. al. (see Results and Table 1).

Claims 3 and 4 are rejected under 35 U.S.C. § 103 as being unpatentable over Queen et. al. or Co et. al. in view of Wallick et. al.

The above claims are drawn to a method of making a humanized antibody wherein the CDRs of an import antibody are transferred to a consensus human antibody along with certain residues of the framework. Furthermore, the claims require that the glycosylation sites, if any, of the import amino acid also be imported with the CDRs and framework regions if these sites have an affect on the binding of antigen.

Queen et. al. and Co et. al. both describe the production of humanized antibodies by transferring the CDRs and certain framework regions of the donor antibody to the human consensus antibody (see Queen et. al. pages 10031-10033 and Co et. al. page 2871). They further state that any residue which might have an affect on the antigen binding of the antibody should be changed substituted in order to maintain the binding affinity of the parent antibody (see page 10033 of Queen et. al. at the last paragraph on the page). They do not however, specifically discuss the glycosylation sites as potential targets for transfer. Wallick et. al. teach the importance of carbohydrate interaction with antigen for maintaining or increasing antigen binding affinity (see pages 1107-1108). It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to make humanized antibodies using the method of Queen et. al. or Co et. al. and further incorporating the concept taught by Wallick et. al.. One of ordinary skill in the art would have been motivated to combine the teachings of the two references in view of the teaching of Queen that retaining high antigen binding affinity is desirable in the production of humanized antibodies. Knowing the role of carbohydrates in antigen antibody interaction as was pointed out by

Wallick et. al. one of ordinary skill would have had the means and the motivation to make humanized antibodies using both of the teachings of the primary and secondary references.

Claim 11 is rejected under 35 U.S.C. § 103 as being unpatentable over Queen et. al. or Co et. al. in view of Reichmann et. al.

The above claim is drawn to a humanized antibody wherein only one amino acid (listed in claim 9) in the framework and the CDRs have been substituted in the consensus antibody.

Queen et. al. and Co et. al. both teach the production of humanized antibodies by transferring the CDRs of a murine antibody along with specific residues of the framework region to the acceptor antibody molecule. They do not however teach only substituting one of the framework residues among those listed in claim 9. Queen et. al. introduce the general concept of a scaffold wherein certain amino acid residues of the framework must be present and certain are dispensable. Reichmann et. al. teach that a single amino acid substitution in an antibody is sufficient to retain the antigen binding specificity of the parent antibody (see final paragraph). It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to make only a single substitution in the antibody of Queen et. al. or Co et. al. in positions among those listed in claim 9. It would have been obvious to one of ordinary skill to complete the invention in light of the success of Reichmann et. al. in only mutating one amino acid of the framework. Knowing that each antibody varies slightly in the non-conserved region, and given the computer modelling protocol set forth by Queen et. al. one of ordinary skill would have been motivated to make a single mutation in the variable region with the expectation of obtaining a functional antibody.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lila Feisee whose telephone number is (703) 308-2731.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Feisee/lf OP
September 29, 1992

DAVID L. LACEY
SUPERVISORY PATENT EXAMINER
GROUP 180
9/30/92

TO SEPARATE, H TOP AND BOTTOM EDGES, SNAP-APART AN DISCARD CARBON

FORM PTO-892 (REV. 3-78) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO. 715272	GROUP ART UNIT 1806	ATTACHMENT TO PAPER NUMBER	13
NOTICE OF REFERENCES CITED	APPLICANT(S) Paul Carter et al.			

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

R	Lazar et al. Molecular and cellular Biology 8: 1247 1988
S	Burgess et al. The Journal of cell Biology 111: 2129 1990
T	Tao et al. The Journal of Immunology 143(8) 2595 1989
U	

EXAMINER	DATE 9/29/92

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

Sheet 1 of 3

FORM PTO-1449

U.S. Dept. of Commerce
Patent and Trademark Office

Atty Docket No. 709

Serial No. 07/715,272

Applicant Paul J. Carter et al.

#12 attach

Filing Date June 14, 1991

Group 1806

LIST OF PRIOR ART CITED BY APPLICANT

(Use several sheets if necessary)

*Examiner Initials		OTHER PRIOR ART (Including Author, Title, Date, Pertinent Pages, Etc.)
	A	Chothia et al., J. Mol. Biol. 186:651-663 (1985)
	B	Novotny and Haber, Proc. Natl. Acad. Sci. USA 82:4592-4596 (1985)
	C	Morrison, S. L. et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)
	D	Boulianne, G. L. et al., Nature 312:643-646 (1984)
	E	Neuberger, M. S. et al., Nature 314:268-270 (1985)
	F	Brüggemann, M. et al., J. Exp. Med. 166:1351-1361 (1987)
	G	Riechmann, L. et al., Nature 332:323-327 (1988)
	H	Love et al., Methods in Enzymology 178:515-527 (1989)
	I	Bindon et al., J. Exp. Med. 168:127-142 (1988)
	J	Jones, P. T. et al., Nature 321:522-525 (1986)
	K	Verhoeyen, M. et al., Science 239:1534-1536 (1988)
	L	Hale, G. et al., Lancet i:1394-1399 (1988)
	M	Queen, C. et al., Proc. Natl. Acad. Sci. USA 86:10029-10033 (1989)
	N	Co et al., Proc. Natl. Acad. Sci. USA 88:2869-2873 (1991)
	O	Gorman et al., Proc. Natl. Acad. Sci. USA 88:4181-4185 (1991)
	P	Daugherty et al., Nucleic Acids Research 19(9):2471-2476 (1991)
	Q	Brown et al., Proc. Natl. Acad. Sci. USA 88:2663-2667 (1991)
	R	Junghans et al., Cancer Research 50:1495-1502 (1990)
	S	Davies, D. R. et al., Ann. Rev. Biochem. 59:439-473 (1990)
	T	Chothia, C. & Lesk, A. M., J. Mol. Biol. 196:901-917 (1987)

Examiner

Date Considered 9/29/92

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

USCOMM-DC 80-398

Sheet 2 of 3

FORM PTO-1449	U.S. Dept. of Commerce Patent and Trademark Office	Atty Docket No. 709
		Serial No. 07/715272
LIST OF PRIOR ART CITED BY APPLICANT (Use several sheets if necessary)		Applicant Paul J. Carter et al.
		Filing Date June 14, 1991
		Group 1806

U.S. PATENT DOCUMENTS

*Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
	AA	4,816,567	3/28/89	Cabilly et al.	530	387.1	
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
	AL							
	AM							
	AN							
	AO							
	AP							

OTHER PRIOR ART (Including Author, Title, Date, Pertinent Pages, Etc.)

	AR	Chothia, C. et al., Nature 342:877-883 (1989)
	AS	Tramontano, A. et al., J. Mol. Biol. 215:175-182 (1990)
	AT	Margolies et al., Proc. Natl. Acad. Sci. USA 72:2180-2184 (1975)
	AU	Pluckthun, Biotechnology 9:545-51 (1991)
	AV	Spiegelberg et al., Biochemistry 9:4217-4223 (1970)
	AW	Wallick et al., J. Exp. Med. 168:1099-1109 (1988)
	AX	Sox et al., Proc. Natl. Acad. Sci. USA 66:975-982 (1970)

Examiner	Date Considered 9/29/92

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

USCOMM-DC 80-398.

Sheet 3 of 3

FORM PTO-1449	U.S. Dept. of Commerce Patent and Trademark Office	Atty Docket No. 709
		Serial No. 07/715272
LIST OF PRIOR ART CITED BY APPLICANT (Use several sheets if necessary)		Applicant Paul J. Carter et al.
		Filing Date June 14, 1991
		Group 806

U.S. PATENT DOCUMENTS

*Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
	BA						
	BB						
	BC						
	BD						
	BE						
	BF						
	BG						
	BH						
	BI						
	BJ						
	BK						

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
	BL	WO 91/09967	7/11/91	PCT				
	BM							
	BN							
	BO							
	BP							

OTHER PRIOR ART (Including Author, Title, Date, Pertinent Pages, Etc.)

	BR	Margni *et al.*, *Ann. Rev. Immunol.*6:535-554 (1988)
	BS	Fendly, B. M. *et al.*, *Cancer Res.* 50:1550-1558 (1990)
	BT	Neuberger *et al.*, *Nature* 312:604-608 (1984)
	BU	Takeda *et al.*, *Nature* 314:452-454 (1985)
	BV	Snow and Amzel, *Protein: Structure, Function, and Genetics* 1:267-279, Alan R. Liss, Inc. pubs. (1986)
	BW	Cheetham, J., *Protein Engineering*, 2(3): 170-172 (1988)
	BX	

Examiner	Date Considered 9/29/92

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

USCOMM-DC 80-398.

18C 1.16.9 #14 Cf-
1-25-93

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

MAIL ROOM
JAN 4 1993
PAT. & TRADEMARK OFF.

RECEIVED
JAN 21 1993
GROUP 180

In re Application of)
Paul J. Carter et al.)
Serial No. 07/715,272) Art Unit: 1806
Filed: 14 JUNE 1991) Examiner: L.FEISEE
For: IMMUNOGLOBULIN VARIANTS)

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

The attached materials were received in connection with the prosecution of a foreign patent application corresponding to the captioned case. These materials contain at least two reference citations, the relevance of which is apparent from the communication from the foreign patent office that is also enclosed.

A PTO Form 1449 is submitted herewith to facilitate citation to the record of all references contained in these materials.

Respectfully submitted,
GENENTECH, INC.
Carolyn R. Adler
Carolyn R. Adler
Reg. No. 32,324

December 30, 1992
460 Point San Bruno Boulevard
South San Francisco, CA 94080
415-225-2614

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Louise Strasbaugh
Louise Strasbaugh

Date: December 30, 1992

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Paul J. Carter et al.
Serial No. 07/715,272
Filed: June 14, 1991
For: Immunoglobulin Variants

Group Art Unit: 1806
Examiner: L. FEISEE

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-2614

CERTIFICATION UNDER 37 C.F.R. § 1.97(e)

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I hereby certify that each item of information contained in this information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this statement. Pursuant to §1.97, this information disclosure has been filed in a timely fashion and no fees are required.

Respectfully submitted,

GENENTECH, INC.
Carolyn R. Adler
Carolyn R. Adler
Reg. No. 32,324

Dated: December 30, 1992

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Louise Strasbaugh
Louise Strasbaugh

Date: December 30, 1992

7e. 1806

MAIL ROOM
FEB 13 1993
PAT. & TRADEMARK OFC.

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Paul J. Carter et al.
Serial No. 07/715,272
Filed: 14 June 1991
For: Immunoglobulin Variants

Group Art Unit: 1806

#16

Examiner: L. Feisee

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-2614

Amendment and Response

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Responsive to the Office Action mailed 5 October 1992, please amend the claims as follows:

Suggest different language

B

1. (Amended) A method for making at least a portion of a humanized antibody variable domain comprising amino acid sequence of a non-human[, import] antibody which is desired to be humanized ~~(import antibody)~~ and a human antibody, comprising the steps of:

   a. obtaining the amino acid sequences of [at least a portion of] an import variable domain and of a consensus human variable domain;
   b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;
   c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
   d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
   e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
   f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:
      1. non-covalently binds antigen directly,

Mention

c124.u

SC13194 02/17/93 07715272 07-0630 130 102 148.00CH

    2. interacts with a CDR; or
    3. participates in the V_L - V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another; [and]

g. for any non-homologous import antibody amino acid residue which is [reasonably] expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence; and

h. preparing a humanized antibody variable domain having amino acid sequences determined in steps a-g.

2. (Amended) The method of claim 1, having an additional step of determining if any such non-homologous residues are exposed on the surface of the consensus human antibody variable domain or buried within it, and if the residue is exposed, retaining the consensus residue.

3. (Amended) The method of claim 1, having the additional steps, which may be taken between any two steps in the method of claim 1, of searching the import antibody variable domain amino acid sequence for glycosylation sites, determining if any such glycosylation site is reasonably expected to affect the antigen binding or affinity of the antibody, and if so, substituting the glycosylation site into the consensus amino acid sequence.

4. (Amended) The method of claim 1, having the additional steps, which may be taken between any two steps in the method of claim 1, of searching the consensus variable domain amino acid sequence for glycosylation sites which are not present at the corresponding amino acid in the import antibody amino acid sequence, and if the glycosylation site is not present in the import sequence, substituting the import amino acid residues for the amino acid residues comprising the consensus glycosylation site.

5. (Amended) The method of claim 1, having an additional step which comprises aligning import antibody and consensus antibody FR amino acid sequences, identifying import antibody FR amino acid residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR amino acid residue, determining if the corresponding consensus antibody amino acid residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

7. (Amended) A method for making a humanized antibody comprising providing [at least a portion of] a non-human antibody variable domain amino acid sequence which is desired to be humanized (import antibody) having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

Please add the following new claims 17-21:

--17. A method of using a consensus human antibody variable domain amino acid sequence in the preparation of a humanized antibody.-- 112/2nd no method steps.

--18. In a method for making a humanized antibody variable domain, the improvement consisting of using consensus human antibody variable domain amino acid sequence.--

--19. A method for making an improved antibody, comprising amino acid sequence from a non-human (import) antibody and a human antibody, comprising the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human antibody variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or
3. participates in the V_L - V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another;

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence; and

h. preparing an improved, humanized antibody having amino acid sequences determined in steps a-g; and

i. evaluating the antigen binding or immunogenicity of the improved, humanized antibody with respect to the parental antibody.--

--20. A method comprising, following the identification of an antibody by the method of any one of claims 1, 7, or 17-19, the manufacture of the antibody.--

--21. A method comprising, following the identification of an antibody by the method of any one of claims 1, 7, or 17-19, the expression of nucleic acid encoding the antibody.--

---

Remarks

Claims 1-13, and 17-21 are presented herein for examination. Reconsideration of the outstanding rejections is respectfully requested for the reasons that follow. A request for a one-month extension of time to respond is submitted herewith, bringing the due date for this response to 5 February 1993. This response is timely filed.

Amendments

Claims 1, 3, 4, 5 and 7 have been amended to indicate that an import antibody is a non-human antibody which is desired to be humanized. Support for this language is found in the specification at page 6, line 27 to page 7, line 3.

Claim 1, step (f) has been amended to clarify that the word "participates" in the V_L - V_H interface means to affect the proximity or orientation of the V_L and V_H regions with respect to one another. Support for this amendment is found on page 15, lines 30-32. New step (h) has been added to claim 1, directed to the physical step of preparation of a humanized antibody variable domain. Support for this step appear throughout the specification.

Claims 3 and 4 have been amended to provide that the additional steps may be taken between any two steps in the method of claim 1. Claims 2-7 have been amended to clarify that the residues or sequences referred to relate to amino acids.

New claims 17 - 21 have been added. These claims are alternate approaches to claiming the subject matter claimed in claim 1. Additional support for claims 20-21 is found in Example 1.

It is believed that these amendments introduce no new matter. The inventors respectfully request entry of these amendments.

The rejection under 35 U.S.C. § 112, second paragraph

Claims 1-10 were rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. Claims 1, 3, 4, 5 and 7 were rejected for use of the term "import antibody". These claims have been amended to indicate that the import antibody is a non-human antibody which is desired to be humanized.

Claim 1 step (a) was rejected because of the term "consensus human variable domain". The terms "consensus sequence", "consensus antibody" and "consensus human variable domain" are defined at specification page 16, line 29 to page 17, line 17:

> The terms "consensus sequence" and "consensus antibody" as used herein refers to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins of any particular subclass. In preferred embodiments, the consensus human variable domain sequences are derived from the most abundant subclasses in the sequence compilation of Kabat *et al., Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda MD (1987), namely V_L κ subgroup I and V_H group III....

As described in the specification, a "consensus human variable domain" would have an amino acid sequence comprising, amino acid residue by residue, the most frequently occurring amino acid residue gathered from a group of human immunoglobulins. The identity of each amino acid residue making up the consensus sequence is determined separately, requiring merely routine tabulation of the amino acids present in each member of a particular immunoglobulin subclass. To expedite the routine tabulation of the most commonly occurring amino acids, workers in the field are referred to the Kabat *et al.* publication cited in the quoted material above, which presents such tabulations.

Claim 1 step (d) was rejected as indefinite as to whether the alignment of the amino acid sequences is a physical or mental step. This rejection is somewhat confusing. The inventors intend claim 1, step (d) to refer to a maximal homology alignment of representations of amino acid sequences, as described in the specification at page 17, lines 18-27. Preparing such a homology alignment typically combines physical and mental actions. This connotation for the phrase "alignment of sequences" is common in the art to which this invention pertains. Step (d) of claim 1 does not require manipulation of the actual, tangible amino acids, merely manipulation of symbolic representations of the actual amino acids.

Wdraw

Claim 1 step (e) was rejected because of the term "homology"; the Examiner questioned whether conservative amino acids are to be considered as homologs. Identity or homology with respect to a specified amino acid sequence of this invention is defined on page 17, lines 18-27. At lines 22-23, the specification indicates that this invention does "not consider[ing] any conservative substitutions as part of the sequence identity". Conservative substitutions are therefore not considered as homologs.

Wdraw

Claim 1 step (f) was rejected for use of the language "participates". Step (f) of claim 1 refers to an amino acid residue which "participates in the in the V_L - V_H interface". This step has been amended to clarify that immunoglobulin residues which so participate are those that affect the proximity or orientation of the V_L and V_H regions with respect to one another.

Claim 1 step (f) was also rejected as indefinite as to how one of ordinary skill can determine the effects listed in steps 1-3. Steps 1-3 presently list the following effects an import amino acid residue might have:

1. non-covalently binds antigen directly,
2. interacts with a CDR; or
3. participates in the V_L - V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another.

The specification discusses, at pages 13-16, the interactions of amino acid residues within an immunoglobulin and describes at least two methods for evaluating the role of any particular amino acid residue: three dimensional models and assays. As stated at page 14, lines 2-9:

?

> "Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen."

The specification provides detailed information how to evaluate the three-dimensional models to determine the various potential effects of amino acid residue changes.

The specification also suggests an alternate method for evaluating the effect of an amino acid residue change. On page 16, lines 14-18, the specification teaches:

> "Since it is not entirely possible to predict in advance what the exact impact of a given substitution will be it may be necessary to make the substitution and assay the candidate antibody for the desired characteristic. These steps, however, are *per se*

"routine and well within the ordinary skill of the art."

The inventors submit that methods for determining the effects of amino acid changes are known in the art, and that those skilled in the art would understand what is claimed in step (f).

W/draw

Claim 1 step (g) was rejected as being indefinite as to what effects are reasonably expected to occur. The word "reasonably" has been deleted from the claim.

Claim 2 was rejected as lacking antecedent basis for "the domain". This claim has been amended to clarify that the intended domain is the consensus human antibody variable domain.

Claims 3 and 4 were rejected as indefinite for not specifying when in the process one would search for the glycosylation sites. These claims have been amended to indicate that one would search for glycosylation sites between any two steps in the method of claim 1.

W/draw

Claim 5 was rejected as unclear in the use of the phrase "preparing a humanized antibody"; this phrase has now been added by amendment as the last step of claim 1. The phrase is intended to mean the physical making of a humanized antibody, methods for which are described in the specification, including *in vitro* mutagenesis and recombinant engineering. The Examiner also seems to be questioning how claim 5 differs from the previous claims. Claim 5 adds an additional step of determining if a particular amino acid residue in the consensus human variable domain--which differs from the import antibody amino acid residue at that site--also appears at that site in antibodies of other species at that particular site (is conserved). If the particular amino acid residue is conserved across species at that site, than that residue is retained in the humanized antibody, and not substituted by the import antibody amino acid residue at that site, and without requiring evaluation of the impact of such a change on the antibody's characteristics.

W/draw

Claim 6 was rejected as vague for unclear use of numbers. These numbers refer to particular amino acids in the light (L) and heavy (H) chains of immunoglobulins. By convention, workers in this field generally utilize the immunoglobulin numbering system set forth in Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)), as described in the specification at page 8, lines 19-21. The Examiner's attention is drawn to Queen *et al.*, already of record in this case, especially at page 10032 column 1 first paragraph (and reference 38 therein) where antibody amino acid residues are referred to with numbers representing certain positions. It is submitted that workers in the field will understand clearly what is claimed in claim 5.

W/draw

Claim 7 was rejected as indefinite as to what the method is drawn, and has been amended according to the Examiner's suggestion.

According to the CAFC, a decision as to whether a claim is invalid for indefiniteness "requires a determination whether those skilled in the art would understand what is claimed", Amgen v. Chugai, 18 USPQ2d 1116, 1030 (CAFC 1991). The presently pending claims use terminology with clear meanings in the field, especially in light of the definitions provided in the specification. The wordings of the claims comply with the requirements of 35 USC § 112, and this rejection should be reconsidered and withdrawn.

The rejection under 35 U.S.C. § 112, first paragraph

Claims 1-11 were rejected under 35 U.S.C. § 112, first paragraph as lacking enablement.

Claims 1 and 7 were rejected as lacking enablement in the language "at least a portion of an import variable domain". These terms have been deleted from the claims.

Claim 1 step (c) was rejected for being unclear as to how one would determine which amino acids are to be substituted. This step recites "substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence". The identification of the Complementarity Determining Region (CDR) amino acid sequence of the import and the human amino variable domain sequences is made in previous step (b). To accomplish step (c), therefore, one substitutes the amino acids identified in step (b).

Methods for identifying CDRs and distinguishing them from Framework Residues (FRs) are known in the art. As the specification describes on page two, antibody variable domains of natural light and heavy chains have the same general structure, and each domain comprises four framework (FR) regions, whose sequences are somewhat conserved, connected by three hyper-variable or complementarity determining regions (CDRs) (see Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, MD, (1987)). The four framework regions largely adopt a β-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the β-sheet structure, The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site. The CDR may be identified following three-dimensional modeling of the antibody. The CDRs may also be identified based on comparison of the an antibody amino acid sequence with a known antibody.

Attached as Exhibit A for the Examiner's convenience are pages from the Introduction to Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest, Fifth Edition*, National Institutes of Health, Bethesda, MD, (1991). This work, along with the earlier Kabat compendiums referred to in the specification and other references, guide the practitioner in the numbering of antibody amino acid sequences, and the assignment of particular amino acids to one of the FR or CDR regions. The Examiner's attention is drawn the sections beginning on page xv, the section entitled "Variable Region Sequence" and especially to Table I, page xvi. Table I presents the amino acid residues associated with FRs and CDRs of the variable domains of immunoglobulin light and heavy chains. See also Figure 1. page xviii, which shows a schematic view of an immunoglobulin; please note the mention in that figure description to the use of a maximum homology alignment to determine the proper numbering of the amino acids (as referred to in the response to the previous § 112 rejection). The inventors submit that the identification of immunoglobulin amino acid residues as belonging to a CDR or to the framework is routine in the art, requiring no undue experimentation.

The specification teaches, in detail, several ways to substitute amino acid residues, including mutagenesis and the construction of nucleic acid encoding the desired sequence. Alanine scanning mutagenesis is described at page 36, line 20 to page 37, line 3. Oligonucleotide-mediated mutagenesis, PCT mutagenesis and cassette mutagenesis are described in the specification at page 39, line 10 through page 44, line 10. The inventors submit that steps (b) and (c) of claim 1 are fully enabled by the specification.

Claim 1 step (f) was rejected as lacking enablement for determining which amino acid residues may be expected to interact with the antigen. At page 29, lines 4-10, the specification teaches that:

> "Differences between the non-human import and the human consensus framework residues are individually investigated to determine their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is desirably performed through modeling, by examination of the characteristics of the amino acids at particular locations, or determined experimentally through evaluating the effects of substitution or mutagenesis of particular amino acids."

Techniques for molecular modeling are described on pages 27-28. Experimental evaluation of the role of particular amino acids will utilize assays tailored to the activities of the antibody to be humanized.

More detailed teaching on identifying residues that influence antigen binding is contained in the specification at page 14, line 10 through page 15, line 6, where it is stated:

> "A residue that noncovalently directly binds to antigen is one that, by three dimensional analysis, is reasonably expected to noncovalently directly bind to antigen. Typically, it is necessary to impute the position of antigen from the spatial location of neighboring CDRs and the dimensions and structure of the target antigen. In general, only those humanized antibody residues that are capable of forming salt bridges, hydrogen bonds, or hydrophobic interactions are likely to be involved in non-covalent antigen binding, however residues which are separated spatially by 3.2 Angstroms or less may also non-covalently interact. Such residues typically are the relatively larger amino acids, such as tyrosine, arginine, and lysine. Antigen-binding FR residues also typically will

> have side chains that are oriented into an envelope surrounding the solvent oriented face of a CDR which extends about 7 Angstroms into the solvent from the CDR domain and about 7 Angstroms on either side of the CDR domain, again as visualized by three dimensional modeling.

The inventors submit that determining whether a residue may be expected to influence antigen binding is routine in the art, in light of the detailed teachings of the specification.

Claim 2 was rejected as lacking enablement for determining which residues are exposed on the surface or buried within the domain. As indicated in the specification, for example at page 91, lines 18-21, the worker in this field would examine the structural models of the import and human sequences to determine if an amino acid residue is exposed on the surface of the domain or is buried within. Evaluation of structural models, preparation of which are described in the specification, to determine whether a residue is exposed or buried is routine and within the ordinary skill in the art.

Claim 3 was rejected as lacking enablement for how one would determine which glycosylation site affects antigen binding, or what comprises "reasonable expectation". The specification teaches, at page 8, lines 22-32, teaches that determining if the glycosylation is reasonably expected to be important for the desired antigen binding and biological activity of the antibody involves determining if the glycosylation site binds to antigen or changes a side chain of an amino acid residue that binds to antigen, or if the glycosylation enhances or weakens antigen binding, or is important for maintaining antibody affinity. As with other aspects of this invention, evaluation of the impact of glycosylation typically is performed by evaluation of molecular models, or experimental evaluation of a modified polypeptide. Such evaluation is routine within the field.

Claims 6, 7 and 9 were rejected as being enabled only with respect to IgG and not other antibody isotypes. The specification, at page 13 lines 14-22, states:

> "The humanized antibody will be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG_1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art."

The Examples presented in the specification involve the use of a IgG_1 constant domain. As noted in the specification, specific method steps and illustrative reagents for the use of IgG_1 are taught, as well as their applicability to other antibody isotypes. The inventors respectfully reminds the Examiner that working examples are not a required component of a patent application. As stated in MPEP § 608.01(h), "There is no statutory requirement for the disclosure of a specific example." Thus, the absence of a working example describing particular embodiments of the invention cannot negative the patentability of the invention. The examples included in the specification, which illustrate the preparation of IgG_1 antibodies, are representative of the manner in which the invention may be practiced. From reading these examples and the detailed description of the invention, the ordinarily skilled artisan would immediately deduce the applicability of the methods described in the specification to other immunoglobulin isotypes.

The Examiner has not made a prima facie case for the § 112, first paragraph rejections, supplying no basis for her skepticism about the scope of the claims. The burden is on the Examiner to provide evidence to support rejections of this sort. "Mere broad generalizations and allegations are insufficient for holding of non-enablement," Ex parte Goeddel, 5 U.S.P.Q. 1449, 1450 (TTAB 1987).

If the Examiner is only prepared to allow claims to exemplified embodiments, what incentive exists for an inventor to disclose the invention to the public? Trade secret protection obviously would be superior to the following circumstances foreseen by the CCPA in In re Goffe, 191 USPQ 429, 431 (CCPA 1976):

> For all practical purposes, the board would limit appellant to claims involving the specific materials disclosed in the examples, so that a competitor seeking to avoid [literally] infringing the claims would merely have to follow the disclosure in the subsequently-issued patent to find a substitute. However, to provide effective incentives, claims must adequately protect inventors. To demand that the first to disclose shall limit his claims to what he has found will work or to materials which meet the guidelines specified for 'preferred' materials in a process such as the one herein involved would not serve the constitutional purpose of promoting progress in the useful arts. See In re Fuetterer, 50 CCPA 1453, 1462, 319 F.2d 259, 265, 138 USPQ 217, 223 (1963).

For a similar case, see In re Strahilevitz, 212 USPQ 561 (P.O.B.A. 1982), where the Board was reversed for rejecting as non-enabling an application that was devoid of even a single working example.

The first paragraph of 35 U.S.C. § 112 requires nothing more than objective enablement. Whether this is achieved by the use of illustrative examples or by broad terminology is of no importance, In re Marzocchi et al., 169 USPQ 267 (CCPA 1971). Further, an assertion by the Examiner that the enabling disclosure is not commensurate with the protection being sought must be supported by reasons for doubting the truth or accuracy of any statement in the presumptively accurate supporting disclosure. It is also incumbent upon the Examiner to back up such assertions with acceptable evidence or reasoning to substantiate the doubts so expressed, In re Armbruster, 185

USPQ 152 (CCPA 1975), In re Strahilevitz, *op cit*.

Se also In re Smith, *supra*, wherein the CCPA reversed an Office ruling that the description in the specification of two categories of prepolymers was not sufficient to support the broad claim for all polymers having a certain desired property. In this case, the court even acknowledged that the specification did not contain language that was precisely identical to the language of the claims. However, the tenor of the specification was that the applicant had made a generic invention rather than one limited to two categories of polymers.

In the present situation, the Examiner has provided no evidence to support the assertion that the invention is not enabled for the preparation of humanized antibodies. Broad claims should be allowed if there is adequate disclosure and where, as in the present situation, there is no pertinent art to prevent such claims. As stated in In re Sus and Schaefer, 134 USPQ 301, 304 (CCPA 1962) (emphasis added):

> The public purpose on which the patent law rests required the granting of claims commensurate in scope with the invention disclosed. This requires as much the granting of broad claims on broad inventions as it does the granting of more specific claims on more specific inventions. It is neither contemplated by the public purpose of the patent laws nor required by the statute that an inventor shall be forced to accept claims narrower than his invention in order to secure allowance of his patent.

The inventors submit that in view of the detailed information provided in the specification as discussed above, the specification adequately teaches how to practice the claimed invention. The rejections under 35 USC § 112, first paragraph, should be reconsidered and withdrawn, as they are not statutorily based, are inconsistent with court and Patent Office decisions on the subject, and are contrary to public policy.

The rejection under 35 U.S.C. § 101

Wdraw

Claims 1-4, and 6-8 were rejected under 35 U.S.C. § 101 as being directed to non-statutory subject matter. It is believed that the amendments to the claims made above render moot this ground of rejection.

The rejection under 35 U.S.C. § 112, first paragraph and under 35 U.S.C. § 101

Wdraw

Claims 9-13 were rejected under 35 U.S.C. § 112, first paragraph and under 35 U.S.C. § 101 as lacking utility for the treatment of malignant and autoimmune diseases in humans. The inventors request clarification of this rejection, because none of claims 9-13 are directed to methods of treatment. These claims are directed to humanized antibody variable domains and the polypeptides of recited sequences. These polypeptides are useful as probes, and in diagnostic assays, as described in the specification at pages 65-66, and need not rely on therapeutic utility.

The rejections under 35 U.S.C. § 102(b) and § 102(a)

Claims 1, 2, and 5-10 were rejected under 35 U.S.C. § 102(b) as being anticipated by Queen *et al.*, and under 35 U.S.C. § 102(a) as being anticipated by Co *et al.*. The inventors respectfully traverse these rejections.

To constitute anticipation, all material elements of a claim must be found in one prior art source. In re Marshall, 198 USPQ 344 (CCPA 1978); In re Kalm, 154 USPQ 10 (CCPA 1967). The inventors will show that neither Queen nor Coe contains all the material elements of these claims, particularly the limitation regarding the use of a consensus sequence.

The rejected claims are directed to the humanization an antibody, namely the combination of amino acid sequence from a non-human antibody desired to be humanized, and from a consensus human variable domain. Methods for preparing such a consensus sequence are fully described in the specification and are discussed above. The inventors believe that the use of a such a consensus sequence achieve a superior result, or a "better" humanized antibody.

The cited prior art utilizes a different approach, which approach had apparently been taken by all other workers in the field prior to the present invention. These workers did not prepare a consensus human antibody to combine with their non-human antibody. Instead, they selected only one human antibody for use, based on the similarity of that human antibody to their non-human antibody. Queen *et al.* state this objective explicitly, at page 10031, column 2 of their paper:

> "In selecting a human antibody to provide the variable region framework for the humanized anti-Tac antibody, we reasoned that the more homologous the human antibody was to the original anti-Tac antibody, the less likely would combining the anti-Tac CDRs with the human framework be to introduce distortions into the CDRs."

Queen continues to describe selecting a human heavy chain V region which was 57% identical to their non-human antibody, after dismissing all other candidate as between 30-52% identical to their non-human. They selected the human light chain V region from the same human antibody for their use.

Co *et al.* are equally explicit describing their similar reasoning. At page 2871, column 1 they state:

> "First, a human antibody variable region with maximal homology to the mouse antibody is selected to provide the framework sequence for humanization of the mouse antibody. Normally the heavy chain and light chain from the same human antibody are

chosen so as to reduce the possibility of incompatibility in the assembly of the two chains. Based on a sequence homology search against the NBRF protein sequence data base, the antibody Pom was chosen to provide the framework sequences for humanization of Fd79."

The approach of the present invention is quite distinct, in its use of a consensus human variable domain sequence. This consensus sequence might or might not have a high degree of homology with the non-human antibody. Neither Queen *et al.* or Coe *et al.* supply this teaching, and therefore do not anticipate the claimed invention. This rejection should be reconsidered and withdrawn.

The rejection under 35 U.S.C. § 103

Claims 3 and 4 were rejected under 35 U.S.C. § 103 as being obvious over Queen *et al.* or Co *et al.* in view of Wallick *et al.*. Claim 11 was rejected under 35 U.S.C. § 103 as being unpatentable over Queen *et al.* or Co *et al.* in view of Reichmann *et al.*

None of the cited references teaches or suggests the claimed invention, which involves the preparation humanized antibodies using a consensus human antibody variable domain. Such a method is not suggested in any of the prior references, and absent such a teaching there was no motivation to try the methods described in the present specification.

The Obviousness Rejections Do Not Meet the Test of Graham v. Deere

The proper context for determining the issue of obviousness is provided in the seminal decision of Graham v. John Deere, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). In that case, the U.S. Supreme Court set forth the following considerations for deciding this issue:

(1) The scope and the content of the prior art;

(2) The difference between the prior art and the claims at issue;

(3) The level of ordinary skill in the pertinent art; and

(4) Secondary considerations such as commercial success, long-felt and unresolved needs, failure of others, etc.

a. Scope and Content of the Prior Art.

1. Queen *et al.* teach the humanization of an anti-Tac antibody. They do not teach the use of a human consensus variable domain to provide the framework for their non-human CDRs.

2. Co *et al.* teach the humanization of an anti-HSV antibody. They do not teach the use of a human consensus variable domain to provide the framework for their non-human CDRs.

3. Wallick *et al.* teach the importance of glycosylation for maintaining the affinity of a monoclonal antibody for its antigen. They do not teach methods for humanization of antibodies, nor teach the creation of a human antibody variable domain consensus sequence.

4. Reichmann *et al.* teach the humanization of an anti-CAMPATH-1 antibody. They do not teach the creation of a human antibody variable domain consensus sequence, or suggest that such might be desirable to provide the framework for their non-human antibody CDRs.

b. The Differences Between the Prior Art and the Claims at Issue

The Examiner has chosen various pieces of prior art and concludes that the combination of these references would have rendered the invention obvious.

The prior art shows that it was known as of the filing date to produce antibody fragments comprising sequence from a non-human antibody and from a human antibody. Prior to the present filing date, however, methods were not known which included the use of a consensus human variable domain for mounting the non-human CDRs. There would have been no impetus on the part of the skilled artisan at the filing date to attempt to produce such a consensus sequence or use it in antibody humanization, in view of the teachings of the prior art literature. The cited references do not teach or suggest the claimed invention, alone or in any combination, nor would there have been any reason from these references to practice the claimed methods. The absence of a suggestion of the claimed invention in the art of record precludes the Patent Office from satisfying its initial burden of showing prima facie obviousness.

c. Level of Ordinary Skill in the Art.

The Graham inquiries point to a conclusion of non-obviousness of the present claims regardless of the presumed level of skill in the art. However, absent evidence to the contrary, a person of ordinary skill in the art is presumed to be one who essentially follows conventional wisdom and does not undertake to innovate. As stated by the Federal Circuit in Standard Co. v. American Cyanamid Co., 227 U.S.P.Q. 293, 298 (Fed. Cir. 1985):

> A person of ordinary skill in the art is also presumed to be one who thinks along the line of convention wisdom in the art and is not one who undertakes to innovate, whether by patient, and often expensive, systematic research or by extraordinary insights, it makes no difference which.

The inventors submit that one who followed the conventional wisdom would not have extrapolated from the teachings of the cited references methods for using a consensus human antibody variable domain for humanizing a non-human antibody. Such an extension of the prior art teachings is based entirely upon hindsight analysis of the inventors' methods. The teachings of this invention should not be considered sufficient to support a conclusion of obviousness in this regard.

The inventors submit that in light of the foregoing amendments and remarks the subject matter defined by the pending claims is useful, enabled, and patentable over the references relied upon by the Examiner, which in no way teach or suggest the invention. The inventors believe the claims are now in condition for allowance and earnestly solicit a Notice to that effect. If the Examiner has any questions, she should feel free to contact the undersigned attorney at the telephone number indicated above.

Respectfully Submitted,
GENENTECH, INC.
Carolyn R. Adler
Carolyn R. Adler
Reg. No. 32,324

29 January 1993

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on 29 January 1993.

Dated: 29 January 1993

Carolyn R. Adler
Carolyn R. Adler

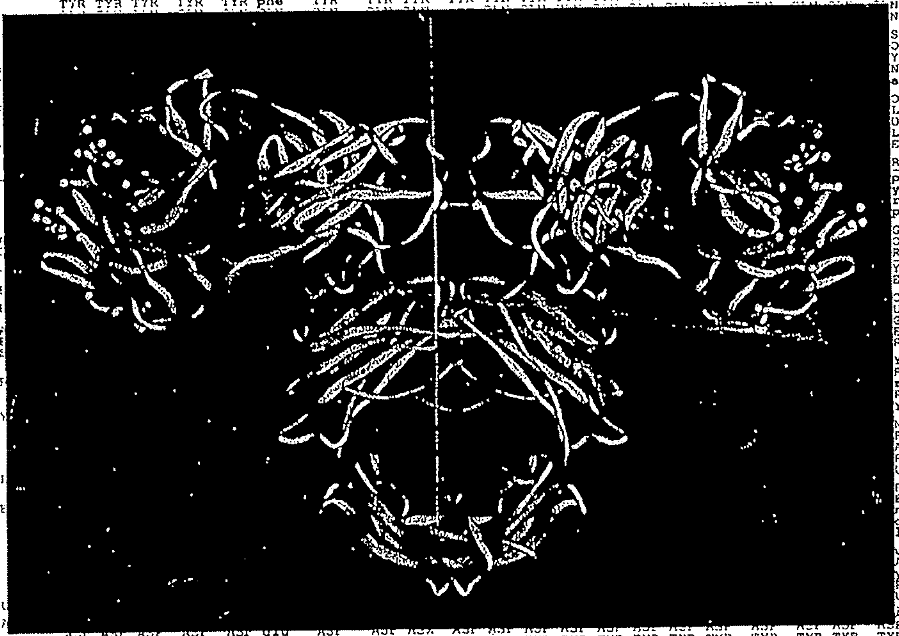
c124.u

Carter

SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

VOL. I

FIFTH EDITION



1991

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

EXHIBIT A

SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

FIFTH EDITION

Tabulation and Analysis of
Amino Acid and Nucleic Acid Sequences of Precursors,
V-Regions, C-Regions, J-Chain, T-Cell Receptors for Antigen,
T-Cell Surface Antigens, β_2-Microglobulins,
Major Histocompatibility Antigens, Thy-1, Complement,
C-Reactive Protein, Thymopoietin, Integrins, Post-gamma Globulin,
α_2-Macroglobulins, and Other Related Proteins

1991

Elvin A. Kabat[*], *Tai Te Wu*[+], *Harold M. Perry*[†],
Kay S. Gottesman[‡], *and Carl Foeller*[†]

[*] Depts. of Microbiology, Genetics and Development, and Neurology, Cancer Center/Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, NY 10032 and the National Institute of Allergy and Infectious Diseases, and the Office of the Director National Institutes of Health, Bethesda, MD 20892.

[+] Depts. of Biochemistry, Molecular Biology, and Cell Biology, and Engineering Sciences and Applied Mathematics and Biomedical Engineering, Northwestern University, Evanston, IL 60208 and the Cancer Center, Northwestern University Medical School, Chicago, IL 60611

[†] BBN Systems and Technologies, 10 Moulton Street, Cambridge, MA 02138

[‡] Formerly with BBN. Present address Laboratory for Applied Research in Academic Information, William H. Welch Medical Library, The Johns Hopkins University, Baltimore, Md 21205

The collection and maintenance of this data base is sponsored under grant 5R01 AI-125616 to E.A. Kabat of Columbia University by the following components of the National Institutes of Health, Bethesda, MD 20892:

- Office of the Director
- National Center for Research Resources
- National Cancer Institute
- National Institute of Allergy and Infectious Diseases
- National Institute of Diabetes, Digestive and Kidney Diseases
- National Institute of General Medical Sciences
- National Library of Medicine

Work with the PROPHET software package is supported by a subcontract from Columbia University to BBN Systems and Techologies, Cambridge, MA 02138

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES

Public Health Service
National Institutes of Health

NIH Publication No. 91-3242

INTRODUCTION

Our earlier "Variable Regions of Immunoglobulin Chains" (1), the second edition "Sequences of Immunoglobulin Chains" (2) and the third edition "Sequences of Proteins of Immunological Interest" (3) have been further exixpanded in the Fourth Edition (4) and now in the Fifth Edition to include amino acid and nucleotide sequences of precursors, variable regions, constant regions, J-chains of immunoglobulins, β2-microglobulins, antigens of the major histocompatibility complex (HLA, H-2, Ia, DR) as well as of Thy-1, complement, T-lymphocyte receptors for antigens, other T-cell antigens of the immunoglobulin superfamily, interleukins, integrins and various other proteins related to immune functions. The identification and sequencing of clones obtained using recombinant DNA techniques has yielded nucleotide sequences of signal, variable, and constant regions of immunoglobulins (5,6), and these nucleotide sequences have been translated into amino acid sequences. The latter have been included in the tables of amino acid sequences with those determined earlier directly by amino acid sequencing and are indicated by an apostrophe followed by CL after the name of the clone. We have continued to use the PROPHET Software Package of the National Center for Research Resources, National Institutes of Health (7,8) to tabulate the sequences.

In compiling the data for this Fifth Edition we have tried to be as up-to-date as possible and have included only sequences which have been published or which have been accepted for publication. Residues which have not been definitely determined have been excluded. It should be remembered that sequences are often published in review articles without detailed documentary evidence. These have often been revised. We have listed such revisions in the notes in many instances; others can readily be found by comparison with sequences in previous editions. We have compiled sequences determined directly as amino acids and have merged with them those translated from the nucleotide sequences thus making all comparable data available. When antibody activities were known, they have been listed after the amino acid and nucleotide sequence tables and are included in the indexes.

When doubts arise as to the validity of any residue in a sequence, the original reference should be examined to ascertain whether definitive evidence for the sequence has been provided. In earlier editions, we have sent the amino acid and nucleotide sequences as stored in the computer to the original authors for verification. If so verified, this was denoted by "checked by author" at the end of each reference and except for the earliest sequences, the date on which the checked sequence was returned to us is given. Whenever possible, nucleotide sequences from GenBank (9) have been used. Programs for converting a GenBank sequence to the codon format of our tables have been developed. The correctness of the table sequence has been verified by converting back into the linear form and comparing with GenBank. When this has been done the sequence is listed as "from GenBank". Recently we have developed newer programs that automatically process a GenBank entry completely - e.g.: extract the relevant feature, determine the appropriate table, and perform alignment. In such cases, the reference will end with "processed automatically from GenBank:" followed by a list of the GenBank accession numbers from which the data was obtained. Some nucleotide sequences were transmitted to us by electronic mail, and they are indicated by "received from authors through e-mail." If the sequences were entered by us from the literature and then checked with GenBank, this is indicated by "checked with GenBank". We have entered many nucleotide sequences which were not then available from GenBank. In general, we have not included stretches of sequence such as enhancers, switch regions and introns. Much information about such sequences may be found in references (10-13). We have also had access to the Protein Information Resource (14) and to the European Molecular Biology Laboratories Data Base (15).

It is also possible, by examining the numbers of sequences at the end of each table and the summary tables, to evaluate the probability that a given amino acid at a given position may not be correct. This is most readily done for the framework residues of the V-region and for the C-region; in the complementarity-determining regions this is more difficult because of the high variability.

AMINO ACID SEQUENCES

The first column in each table gives the residue number. Except for complement, T-cell surface antigens, integrins and miscellaneous proteins, the second column is a tabulation of invariant residues. Since exceptions to invariance are found, the frequency, if less than 1.0 and greater than or equal to 0.95, is indicated alongside the residue listed as invariant; when only a single sequence is available, this is not given. These rows are shaded in grey. Each sequence is tabulated in each subsequent column. Three dashes (---) indicate that no amino acid is present at that position and that the sequence continues. In all instances residues considered uncertain by the authors have not been included in the table. In some instances the symbol # is used to indicate that several amino acid residues were found in one position, and these residues are listed in the notes. The four columns at the end of each table give:

1. the number of residues sequenced at that position,
2. the number of different amino acids found at that position,
3. the number of times the most common amino acid occurred and that amino acid in parentheses, and
4. the variability.

These columns are included only in tables with more than five sequences. Miscellaneous tables have only columns corresponding to the first two above.

Variability is calculated (16) as:

$$\text{Variability} = \frac{\text{Number of different amino acids occurring at a given position}}{\text{Frequency of the most common amino acid at that position}}$$

An invariant position would have a variability of one; if 20 amino acids occurred with equal frequency, the variability would be 20 divided by 0.05 equals 400. If, for example, four different amino acids Ser, Asp, Pro, and Thr occurred at a given position, and of 100 sequences available at that position, Ser occurred 80 times, the variability would be 4/0.8 = 5. When any of the amino acid residues, sequenced directly as amino acids, were not identified completely and are listed as Glx (or Asx), two values, separated by a comma, are given in the last three columns. The first value in each of these columns is calculated assuming that only one of the two possibilities, e.g., Glu or Gln (or Asp or Asn) occurred, while the second considers that both were present and maximizes the variability. In the variability plots, the horizontal bars indicate the two values.

When two or more amino acids are most common and occur with equal frequency, they are tabulated as a note, and the symbol + is used in the next to last column. If no sequence data have been reported for any position, there are no entries in the last four columns. Variability is not calculated for insertions or if only a single sequence is known. When the translated sequence of a clone corresponds to a previously listed sequence of a plasmacytoma from

---

which it was prepa
variability computa
If a given sequence
is indicated by an
antibody specificit
constants if avail
rabbit heavy chain
domain of the rabb
sequence is given;
usually the most ne
included, especial
Notes are of two t
the symbol #, and s

Signal Sequences
The signal (precu
chains are listed
light chains, for
total of nine precu
sequencing of sign
sequences from DNA
acid residues in
Genomic DNA clones
the coding sequenc
-4, and in rare ca
leader peptide to
for positions -4 t

The signal amino
antigens, β2-mic
proteins, complem
proteins are liste

By conformational
Leu-Leu-Leu-Trp-V
alpha helical
conformations in t
four amino termin
(20).

Variable Region S
The variable regi
contain hypervari
(27-30) chains,
labeled with hap
segments of lig
examination of se
chains aligned
These and the thr
were hypothesize
regions or segme
contact with vari
high resolution x
been verified by
all antibodies
hypervariable re
antibody combinin
the framework (
framework segme
complementarity-
the three CDRs
Figures 3-47 have
comments are gi
bibliography. Th
Table I.

which it was prepared, only one sequence is listed so that the variability computations are not affected, and a note is included. If a given sequence is associated with any antibody activity, this is indicated by an asterisk alongside the protein heading, and the antibody specificities are given in a separate list with binding constants if available. The notes list the a-allotypes for the rabbit heavy chain V-region and the b-allotypes for the constant domain of the rabbit kappa light chain. A key reference to the sequence is given; generally the most recent reference since it is usually the most nearly complete, but often several references are included, especially when revisions of a sequence have been made. Notes are of two types: general notes about a table indicated by the symbol #, and specific notes indicated by the sequence number.

Signal Sequences

The signal (precursor) amino acid sequences of immunoglobulin chains are listed as human, mouse, and miscellaneous for kappa light chains, for lambda light chains, and for heavy chains for a total of nine precursor tables. They were obtained either by direct sequencing of signal proteins (17-19) or by translating nucleotide sequences from DNA clones. Signal segments range from 17-29 amino acid residues in length and are thus numbered from -29 to -1. Genomic DNA clones contain introns of varying length that interrupt the coding sequence of the precursor within the codon for position -4, and in rare cases for position -6. Thus, the L-gene encodes the leader peptide to position -4 and the 5′ end of the V-gene codes for positions -4 to -1.

The signal amino acid sequences of the T-cell receptors for antigens, β2-microglobulins, major histocompatibility complex proteins, complement components, integrins, and other related proteins are listed in separate tables.

By conformational energy calculations, the core V_κ hydrophobic Leu-Leu-Leu-Trp-Val-Leu-Leu-Leu (MOPC321, MOPC63) exists in an alpha helical conformation, terminated by chain reversal conformations in the four C-terminal residues Trp-Val-Pro-Gly; the four amino terminal residues are compatible with the alpha helix (20).

Variable Region Sequences

The variable regions (21) of immunoglobulins have been shown to contain hypervariable segments in their light (16,22-26) and heavy (27-30) chains, of which certain residues have been affinity labeled with haptenic determinants (31-44). Three hypervariable segments of light chain were delineated from a statistical examination of sequences of human V_κ, human V_λ, and mouse V_κ light chains aligned for maximum sequence similarity (16,23,24,27). These and the three corresponding segments of the heavy chains (27) were hypothesized (16,27) to be the complementarity-determining regions or segments (CDR) containing the residues which make contact with various antigenic determinants, several years before high resolution x-ray structures were determined, and this has now been verified by X-ray diffraction studies at high resolution for all antibodies examined Figures 3-47. The proposed fourth hypervariable region (cf. 30) of heavy chains is not part of the antibody combining site (27). The rest of the V-region constitutes the framework (16,27,45-54). It is convenient to identify the framework segments (FR1, FR2, FR3, and FR4) and the complementarity-determining segments (CDR1, CDR2, and CDR3) with the three CDRs separating the four FRs. The CDRs in the stereo Figures 3-47 have solid circles for each residue. References and comments are given with each figure and are not listed in the bibliography. The residue numbers for these segments are given in Table I.

TABLE I

Amino Acid Residues Associated with Framework (FR) and Complementarity Determining Regions (CDR) of the Variable Domains of Immunoglobulin Light (V_L) and Heavy (V_H) Chains

Segment	Light Chain	Heavy Chain
FR1	1-23 (with an occasional residue at 0, and a deletion at 10 in V_λ chains)	1-30 (with an occasional residue at 0)
CDR1	24-34 (with possible insertions numbered as 27A,B,C,D,E,F)	31-35 (with possible insertions numbered as 35A,B)
FR2[a]	35-49[a]	36-49
CDR2	50-56	50-65 (with possible insertions numbered as 52A,B,C)[b]
FR3	57-88	66-94 (with possible insertions numbered as 82A,B,C)
CDR3	89-97 (with possible insertions numbered as 95A,B,C,D,E,F)	95-102 (with possible insertions numbered as 100A,B,C,D,E,F,G,H,I,J,K)
FR4	98-107 (with a possible insertion numbered as 106A)	103-113

[a] Five Basilea rabbits (λ) immunized with type II pneumococci and which produced anti-type II pneumococcal polysaccharide had Met at position 48 and an insertion of four amino acid residues between positions 48 and 49; in four of the five the sequence was Glu, Leu, Lys, Ser and the fifth was Trp, Leu, Arg, Lys (53,54,63,64); the others were not sequenced at these positions (for references see table of rabbit λ amino acid sequences.)

[b] In the rabbit, Mage et al. (65) consider position 65 in V_H to be in FR3, since it is allotype related.

The V-genes for the
and the J-minigenes
kappa light chains.
by recombination and
by the J-minigene.
occur at different
residues may result
of the inserted resi
for better alignment
the V-gene region.
times more frequentl

The V-genes for the
and are followed
extensive variation
ability to be read
boundary between D a
acid position. In ad
sequences vary by a
of D-J joining appea
between V and D and
and correlates with
B cells (60). The
has therefore been
evidence suggesting
perhaps a minigene
nucleotides. Light
V_L-J_L junction (62),
probably results fro
in fetal and neonata
and 17/146 RNA sequ
lower than in adults
regulated both in T
diversity but are te

In the tables of
horizontal lines for
chain, MPC 11, has
between position 1
have internal deleti

The V-genes for the light chains code to amino acid position 95, and the J-minigenes from position 97 to 107 for lambda and 108 for kappa light chains. Position 96 is usually the site of V-J joining by recombination and may be coded partly by the V-gene and partly by the J-minigene. Because the site of V-J recombination could occur at different positions within a codon, different amino acid residues may result at this position. We have changed the location of the inserted residues from 97A-F (2) to 95A-F, since it makes for better alignment by confining chains of different lengths to the V-gene region. In mouse V_κ chains, J1 and J2 were used 5 to 10 times more frequently than J4 and J5 (55).

The V-genes for the heavy chains code up to amino acid position 94 and are followed by the D- and J-minigenes. Because of the extensive variation in the lengths of D-minigenes, and their ability to be read in different reading frames (56), the exact boundary between D and J is not always located at the same amino acid position. In addition, the lengths of the J encoded amino acid sequences vary by a few amino acid residues. Moreover, the process of D-J joining appears to involve insertions of extra nucleotides between V and D and between D and J, termed the N region (57-61) and correlates with the appearance of terminal deoxytransferase in B cells (60). The original numbering system for the heavy chains has therefore been retained. Wysocki et al. (61) have provided some evidence suggesting a non-random origin for the V_H-D_H junction, perhaps a minigene, rather than random addition of the N nucleotides. Light chains do not appear to have N sequences at the V_L-J_L junction (62), but show an additional residue 95A which probably results from V_L-J_L joining. N sequences are generally rare in fetal and neonatal mouse V_H-D-J_H junctions (62), only 1/87 DNA and 17/146 RNA sequences contained N regions, an incidence much lower than in adults indicating that N insertion is developmentally regulated both in T and B cells. P elements also contribute to diversity but are templated (62a).

In the tables of V-regions, the FR and CDR are separated by horizontal lines for convenience in reading. One mouse kappa light chain, MPC 11, has an extra segment of 12 amino acid residues between position 1 and the signal sequence (66). Several chains have internal deletions.

Figure 1 (50) shows the domain structure for IgG1 protein EU. Numbering on the left half indicates the CDR for the light and heavy chains (50), while that on the right half gives the EU numbering (67).

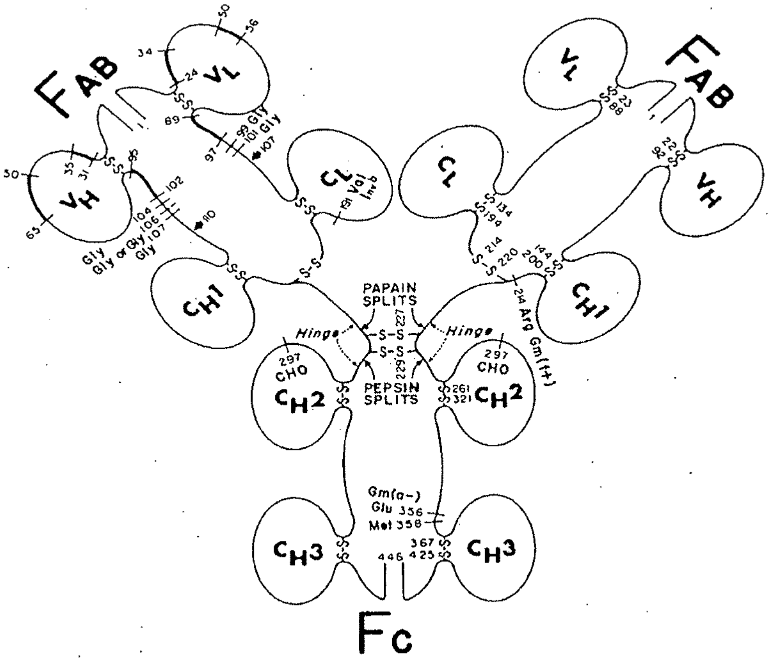


FIG. 1. Schematic view of four-chain structure of human $\mathrm{IgG1}_{\kappa}$ molecule. *Numbers on right side:* actual residue numbers in protein EU [Edelman *et al.* (67)]; *Numbers* of Fab fragment on *left side* aligned for maximum homology; light chains numbered as in Wu and Kabat (16) and heavy chains as in Kabat and Wu (27). Heavy chains of EU have residue 52A, three residues 82A,B,C, and lack residues termed 100A,B,C,D,E,F,G,H,I,J,K, and 35A,B. Thus residue 110 (end of variable region) is 114 in actual sequence. Hypervariable regions, complementarity-determining segments or regions (CDR): *heavier lines*. V_L and V_H: light and heavy chain variable region; C_H1, C_H2, C_H3: domains of constant region of heavy chain; C_L: constant region of light chain. *Hinge region* in which two heavy chains are linked by disulfide bonds is indicated approximately. Attachment of carbohydrate is at residue 297. *Arrows* at residues 107 and 110 denote transition from variable to constant regions. Sites of action of papain and pepsin and locations of a number of genetic factors are given. Modified from 50.

Critical understandin
sites and the geneti
antibody complementar
evaluation of a large
and especially of the c
and heavy chains of i
to locate residues i
determinants (68,69) a
combining sites will d
and scope V_H and V_L cha
must be resolved.
immunochemical data in
in addition to other
resolution X-ray cryst

Through the generous c
been provided with the
Fab molecules, V_H dime
Drs. Eduardo Padlan
shown. Legends and k
model.

Critical understanding of the architecture of antibody combining sites and the genetics of the generation of diversity and of antibody complementarity depends to a great extent on the evaluation of a large number of sequences of the variable regions and especially of the complementarity-determining segments of light and heavy chains of immunoglobulins of different species. Ability to locate residues in the site making contact with antigenic determinants (68,69) and to predict (70) the structures of antibody combining sites will depend heavily upon such sequences. The role and scope V_H and V_L chains in contributing to binding of the epitope must be resolved. This can be often accomplished by use of immunochemical data in defining antibody combining sites (68,70-73) in addition to other methodologies such as 2D-NMR (71,51) or high resolution X-ray crystallography.

Through the generous cooperation of X-ray crystallographers we have been provided with the α-carbon coordinates of almost all available Fab molecules, V_H dimers and antigen-antibody complexes from which Drs. Eduardo Padlan and Chantal Abergal made the stereo models shown. Legends and key references for each are listed with the model.

E. Long, R. Mage, C.
and L. Steiner for
ier editions.

of Fab and antigen
α-carbon coordinates
the text where these
n and Chantal Abergel
stereodiagrams from
indebted to them. Dr.
the x-ray structure of
ed to Drs. Rose Mage
introduction and to
tions to organizing the
Margulies made helpful
text. Dr. Lawrence A.
e V_H and V_κ families as
and V_κ sequences into

REFERENCES TO INTRODUCTION

1. Kabat, E.A., Wu, T.T., and Bilofsky, H. (1976) Variable Regions of Immunoglobulin Chains. Medical Computer Systems, Bolt Beranek and Newman Inc., Cambridge, MA.

2. Kabat, E.A., Wu, T.T., and Bilofsky, H. (1979) Sequences of Immunoglobulin Chains. National Institutes of Health, NIH Publication 80-2008.

3. Kabat, E.A., Wu, T.T., Bilofsky, H., Reid-Miller, M., and Perry, H. (1983) Sequences of Proteins of Immunological Interest. National Institutes of Health.

4. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., and Gottesman, K.S. Sequences of Proteins of Immunological Interest. (1987) U.S. Dept. of Health and Human Services, Public Health Service National Institutes of Health.

5. Tonegawa, S., Maxam, A.M., Tizard, R., Bernard, O., and Gilbert, W. (1978) Sequence of a mouse germ-line gene for a variable region of an immunoglobulin light chain. Proc. Natl. Acad. Sci. U.S.A. 5:1485-1489.

6. Seidman, J.G., Leder, A., Edgell, M.H., Plosky, F., Tilghman, S.M., Tiemeier, D.C., and Leder, P. (1978) Multiple related immunoglobulin variable region genes identified by cloning and sequence analysis. Proc. Natl. Acad. Sci. U.S.A. 75:3881-3885.

7. Raub, W.F. (1974) The PROPHET system and resource sharing. Federation Proceedings 33:2390-2392.

8. Hollister, C. (1988) A national computational resource for life science research. Nucleic Acids Research 16:1873.

9. Burks, C., Fickett, J.W., Goad, W.B., Kanehisa, M., Lewitter, F.I., Rindone, W.P., Swindell, C.D., Tung, C.-S., and Bilofsky, H. (1985) The GenBank nucleic acid sequence database. CABIOS 1:225-233.

10. Trifonov, E.N. and Brendel, V. (1986) GNOMIC, A Dictionary of Genetic Codes. Balaban Publishers; Rehovot; Philadelphia.

11. Nussinov, R. (1986) Some guidelines for identification of recognition sequences: regulatory sequences frequently contain (T)GTG/CAC(A), TGA/TCA and (T)CTC/GAG(A). Biochimica et Biophysica Acta 866:93-108.

12. Ghosh, D. (1990) A relational data base of transcription factors. Nucleic Acids Res. 18:1749-1756.

13. Wu, T.T., Reidmiller, M., Perry, H.M., and Kabat, E.A. (1984) Long identical repeats in the mouse $\gamma 2b$ switch region and their implications for the mechanism of class switching. EMBO J. 3:2033-2040.

14. Protein Information Resource. National Biomedical Research Foundation, Georgetown University Medical Center, Washington, DC 20007.

15. European Molecular Biology Organization (1990) EMBL File Server. See issues of Nucleic Acid Research for recent sequences added to their data bank.

16. Wu, T.T. and Kabat, E.A. (1970) An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light [illegible] implications for antibody complementarity. J.

17. Milstein, C., Brownlee, G.G., Harrison, T.M., and Mathews, M.B. (1972) A possible precursor of immunoglobulin light chains. Nature New Biol. 239:117-120.

18. Schechter, I. and Burstein, Y. (1976) Partial evidence of the precursors of immunoglobulin light chains of different subgroups: Evidence that the immunoglobulin variable-region gene is larger than hitherto known. Biochem. Biophys. Res. Comm. 68:489-496.

19. Rose, S.M., Kuehl, W.M., and Smith, G.P. (1977) Cloned MPC11 myeloma cells express two kappa genes: a gene for a complete light chain and a gene for a constant region polypeptide. Cell 12:453-462.

20. Pincus, M.R. and Klausner, R.D. (1982) Prediction of the three dimensional structure of the leader sequence of the pre-light chain, a hexadecapeptide. Proc. Natl. Acad. Sci. U.S.A. 79:3413-3417.

21. Hilschmann, N. and Craig, L.C. (1965) Amino acid sequence studies with Bence Jones proteins. Proc. Natl. Acad. Sci. U.S.A. 53:1403-1409.

22. Milstein, C. (1967) Linked groups of residues in immunoglobulin chains. Nature 216:330-332.

23. Kabat, E.A. (1967) Unique features of the variable regions of Bence Jones proteins and their possible relation to antibody complementarity. Proc. Natl. Acad. Sci. U.S.A. 59:613-619.

24. Kabat, E.A. (1970) Heterogeneity and structure of antibody combining sites. Landsteiner Centennial, Dec. 5,6,7, 1968. Ann. N.Y. Acad. Sci. 169:43-54.

25. Franêk, F. (1969) The character of variable sequences in immunoglobulins and its evolutionary origin. In Developmental Aspects of Antibody Formation and Structure. Academia, Czechoslovak Academy of Sciences, Prague, pp. 311-313.

26. Kabat, E.A. (1969) Discussion in Developmental Aspects of Antibody Formation and Structure. Academia, Czechoslovak Academy of Sciences, Prague, pp. 391-393.

27. Kabat, E.A. and Wu, T.T. (1971) Attempts to locate complementarity determining residues in the variable positions of light and heavy chains. Ann. N.Y. Acad. Sci. 190:382-393.

28. Milstein, C. and Pink, J.L.R. (1970) Structure and evolution of immunoglobulins. Prog. Biophys. Mol. Biol. 21:209-263.

29. Capra, J.D., Kehoe, J.M., Winchester, R.J., and Kunkel, H.G. (1971) Structure-function relationships among anti-gamma globulin antibodies. Ann. N.Y. Acad. Sci. 190:371-381.

30. Capra, J.D. and Kehoe, J.M. (1975) Hypervariable regions, idiotypy and the antibody combining site. Advances in Immunology. Academic Press, New York 20:1-40.

31. Thorpe, N.O. and Singer, S.J. (1969) The affinity-labeled residues in antibody active sites. II. Nearest-neighbor analyses. Biochemistry 8:4523-4534.

32. Goetzl, E.J. and Metzger, H. (1970) Affinity labeling of a mouse myeloma protein which binds nitrophenyl ligands. Kinetics of labeling and isolation of a labeled peptide. Biochemistry 9:1267-1278.

33. Franêk, F. (1971) Affinity labeling by m-nitrobenzenediazonium fluoroborate of porcine anti-dinitrophenyl antibodies. Position of labeled tyrosine in the λ-chains. Eur. J. Biochem. 19:176-183.

---

34. Fleet, G.W.J., Kn
antibody binding site.
photoprecursor of an a

35. Haimovich, J., Eis
Localization of affini
chains of two myelo
Biochemistry 11:2389-2

36. Ray, A. and Cebra,
residues in the prima
raised in strain 13 gu

37. Yoshioka, M., L
Armstrong, M.Y.K., Kor
Studies on the combi
immunoglobulin which bi
of two types of photo
12:4679-4685.

38. Fisher, C.E. and P
binding site of rabbit
the hypervariable re
139:135-149.

39. Koo, P.H., and Ce
distinctive lysyl resid
γ2 chain of guinea pi
Biochemistry 13:184-195

40. Chesebro, B., Hadl
labeling studies on
phosphorylcholine. In
and Cells. 3rd Int. C
Karger, Basel, pp. 205-

41. Givol, D. (1974) Aff
combining site. Essays

42. Roholt, O.A., Fried
(1973) A light chain ty
rabbit antibenzoate anti

43. Cebra, J.J., Koo,
antibodies: primary stru
186:263-265.

44. Richards, F.F., Li
Konigsberg, W.H. (1974)
region of myeloma prot
labeling patterns. Bioc

45. Padlan, E.A. (1977)
antigen-antibody reacti
diversification of anti
Biophysics 10:35-65.

46. Davies, D.R. and
antibody function. Ann.

47. Kabat, E.A. (1983)
Immunology V, 67-85. Fi
Eds. Yamamura and T. Tad

48. Novotny, J., Bruccole
and Karplus, M. (1983) M
site. J. Biol. Chem. 23:

34. Fleet, G.W.J., Knowles, J.R., and Porter, R.R. (1972) The antibody binding site. Labeling of a specific antibody against the photoprecursor of an aryl nitrene. Biochem. J. 128:499-508.

35. Haimovich, J., Eisen, H.N., Hurwitz, E., and Givol, D. (1972) Localization of affinity-labeled residues on the heavy and light chains of two myeloma proteins with anti-hapten activity. Biochemistry 11:2389-2398.

36. Ray, A. and Cebra, J.J. (1972) Localization of affinity-labeled residues in the primary structure of antidinitrophenyl antibody raised in strain 13 guinea pigs. Biochemistry 11:3647-3656.

37. Yoshioka, M., Lifter, J., Hew, C.-L., Converse, C.A., Armstrong, M.Y.K., Konigsberg, W.H., and Richards, F.F. (1973) Studies on the combining region of protein 460, a mouse γA immunoglobulin which binds several haptens. Binding and reactivity of two types of photoaffinity labeling reagents. Biochemistry 12:4679-4685.

38. Fisher, C.E. and Press, E.M. (1974) Affinity labeling of the binding site of rabbit antibody. Evidence for the involvement of the hypervariable regions of the heavy chain. Biochem J. 139:135-149.

39. Koo, P.H. and Cebra, J.J. (1974) Affinity labeling of a distinctive lysyl residue within the second hypervariable region of γ2 chain of guinea pig anti-p-azobenzenearsonate antibody. Biochemistry 13:184-195.

40. Chesebro, B., Hadler, N., and Metzger, H. (1973) Affinity labeling studies on five mouse myeloma proteins which bind phosphorylcholine. In Specific Receptors of Antibodies, Antigens and Cells. 3rd Int. Convoc. Immunol., 1972, Buffalo, N.Y., S. Karger, Basel, pp. 205-217.

41. Givol, D. (1974) Affinity labeling and topology of the antibody combining site. Essays in Biochemistry 10:1-31.

42. Roholt, O.A., Friedenson, B., Radzimski, G., and Pressman, D. (1973) A light chain tyrosyl sequence in the antibody site of a rabbit antibenzoate antibody. J. Immunol. 111:1367-1375.

43. Cebra, J.J., Koo, P.H., and Ray, A. (1974) Specificity of antibodies: primary structural basis of antibody binding. Science 186:263-265.

44. Richards, F.F., Lifter, J., Hew, C.-L., Yoshioka, M., and Konigsberg, W.H. (1974) Photo- affinity labeling of the combining region of myeloma protein 460. II. An interpretation of the labeling patterns. Biochemistry 13:3572-3574.

45. Padlan, E.A. (1977) Structural basis for the specificity of antigen-antibody reactions and structural mechanisms for the diversification of antigen-binding specificities. Quart. Rev. Biophysics 10:35-65.

46. Davies, D.R. and Metzger, H. (1983) Structural basis of antibody function. Ann. Rev. Immunol. 1:87-117.

47. Kabat, E.A. (1983) The antibody combining site. Progress in Immunology V, 67-85. Fifth International Congress of Immunology. Eds. Yamamura and T. Tada. Academic Press, Tokyo.

48. Novotny, J., Bruccoleri, R.,Newell, J., Murphy, D., Haber, E., and Karplus, M. (1983) Molecular anatomy of the antibody binding site. J. Biol. Chem. 23: 14433-14437.

49. Kabat, E.A., Wu, T.T., and Bilofsky, H. (1977) Unusual distributions of amino acids in complementarity-determining (hypervariable) segments of heavy and light chains of immunoglobulins and their possible roles in specificity of antibody-combining sites. J. Biol. Chem. 252:6609-6616.

50. Kabat, E.A. (1978) The structural basis of antibody complementarity. Adv. Protein Chem. 32:1-75.

51. Anglister, J. and Zilber, B. (1990) Antibodies against a peptide of cholera toxin differing in cross reactivity with the toxin differ in their specific interactions with the peptide as observed by 1H NMR spectroscopy. Biochemistry 29:921-928.

52. Kabat, E.A., Wu, T.T., and Bilofsky, H. (1978) Variable region genes for the immunoglobulin framework are assembled from small segments of DNA -- A hypothesis. Proc. Natl. Acad. Sci. U.S.A. 75:2429-2433.

53. Hayzer, D.J. and Jaton, J.-C. (1987) Nucleotide Sequence of a cDNA clone encoding a rabbit immunoglobulin-λ light chain: The V_λ region differs markedly from that of other species. J. Immunol. 138:2316-2322.

54. Hayzer, D.J. and Jaton, J.-C. (1989) Cloning and sequencing of two functional rabbit germ-line immunoglobulin V_λ genes. Gene 80:185-191.

55. Wood, D. and Coleclough, C. (1984) Different joining region J elements of the murine K immunoglobulin light chain locus are used at markedly different frequencies. Proc. Natl. Acad. Sci. U.S.A. 81:4756-4762.

56. Kaartinen, M. and Mäkela, O. (1985) Reading of D genes in variable frames as a source of antibody diversity. Immunology Today 6:324-327.

57. Alt, F.W. and Baltimore, D. (1982) Joining of immunoglobulin heavy chain gene segments: Implications from a chromosome with evidence of three D-J_H fusions. Proc. Natl. Acad. Sci. U.S.A. 79:4118-4122.

58. Perlmutter, R.M., Crews, J.T., Douglas, R., Sorensen, G., Johnson, N., Nivera, N., Gearhart, P.J., and Hood, L. (1984) The generation of diversity in phoshorylcholine-binding antibodies. Adv. in Immunol. Academic Press, New York 35:1-37.

59. Manser, T., Huang, S.Y., and Gefter, M. (1984) Influence of clonal selection on the expression of variable region genes. Science 226:1283-1288.

60. Desiderio, S.V., Yancopoulos, G.D., Paskind, M., Thomas, E., Boss, M.A., Landau, N., Alt, F.W. and Baltimore, D. (1984) Insertion of N regions into heavy-chain genes is correlated with expression of terminal deoxytransferase in B cells. Nature 311:752-755.

61. Wysocki, L., Manser, T., Gridley, T. and Gefter, M.L. (1986) Molecular limitations on variable-gene junctional diversity. J. Immunol. 137:3699-3701.

62. Feeney, A.J. (1990) Lack of N regions in fetal and neonatal mouse immunoglobulin V-D-J junctional sequences. J. Exp. Med. 172:1377-1390.

62a. Lafaille, J.J., DeCloux, A., Bonneville, M., Takagaki, Y., and Tonegawa, S. (1989) Junctional sequences of T cell receptor γδ T cell lineage and for a novel intermediate of VDJ joining. Cell 59:859-870.

---

63. Garcia, I. and Jato
II and type VII pneumoco
light chains. Immunoc
studies of the light ch

64. Duvoisin, R.M., Ko
Jaton, J.C. (1984) Nuc
constant region of a ra
Eur. J. Immunol. 14:379-

65. Mage, R.G., Bernste
C.B., Young-Cooper, G.O.
structural and genetic
allotypes of the rabbit.

66. Smith, G.P. (1978)
kappa-chain of mouse mye

67. Edelman, G.M. Cunn
Rutishauser, U., and Wax
an entire γG immunoglob
63:78-85.

68. Kabat, E.A., Wu, T.
locate residues in compl
combining sites which m
Proc. Natl. Acad. Sci. U

69. Padlan, E.A., Davies
C. (1976) Model buildin
hapten-binding site of M
Biol. 41:627-637.

70. Kabat, E.A. (1982
complementarity. Pharma

71. Dwek, R.A., Wain-Ho
B., Perkins, S.J., and
combining site by magnet

72. Feldman, R.J., Pott
hypothetical space-filli
binding myeloma J539. M

73. Givol, D. (1979) The
Recognition II (E. Lenno
11B, 23:71-125.

74. Padlan, E.A. and K
combining sites. in Mol
and Applications. Meth
Abelson and M.I. Simon,

75. Kabat, E.A. and Wu,
and segments of sequences
Relative contributions o
binding of antibody comb

76. Gibbs, M.R., Moody,
structure of the as
chloramphenicol acetyltr
consequences of disrupti
29:11261-11265.

77. Kriegler, M. (1990)
Press, New York, pp.1-24

78. Horwitz, A.H., Chang,
Secretion of functional a
Proc. Natl. Acad. Sci. U

63. Garcia, I. and Jaton, J.C. (1979) The Immune response to type II and type VII pneumococcal vaccines in Basilea rabbits lacking κ light chains. Immunochemical and partial amino acid sequence studies of the light chains. Mol. Immunol. 16:1063-1071.

64. Duvoisin, R.M., Kocher, H.P., Garcia, I., Rougeon, F. and Jaton, J.C. (1984) Nucleotide sequence of a cDNA encoding the constant region of a rabbit immunoglobulin light chain of λ type. Eur. J. Immunol. 14:379-382.

65. Mage, R.G., Bernstein, K.E., McCartney-Francis, N., Alexander, C.B., Young-Cooper, G.O., Padlan, E.A., and Cohen, G.H. (1984) The structural and genetic basis for expression of normal and latent allotypes of the rabbit. Mol. Immunol. 21:1067-1081.

66. Smith, G.P. (1978) Sequence of the full-length immunoglobulin kappa-chain of mouse myeloma MPC 11. Biochem. J. 171:337-347.

67. Edelman, G.M. Cunningham, B.A. Gall, W.E., Gottlieb, P.D., Rutishauser, U., and Waxdal, M.J. (1969) The covalent structure of an entire γG immunoglobulin molecule. Proc. Natl. Acad. Sci. USA 63:78-85.

68. Kabat, E.A., Wu, T.T., and Bilofsky, H. (1976) Attempts to locate residues in complementarity determining regions of antibody combining sites which make contact with antigenic determinants. Proc. Natl. Acad. Sci. U.S.A. 73:617-619.

69. Padlan, E.A., Davies, D.R., Pecht, I., Givol. D., and Wright, C. (1976) Model building studies of antigen-binding sites: the hapten-binding site of MOPC 315. Cold Spring Harbor Symp. Quant. Biol. 41:627-637.

70. Kabat, E.A. (1982) Antibody diversity versus antibody complementarity. Pharmacol. Reviews 34:23-38.

71. Dwek, R.A., Wain-Hobson, S., Dower, S., Gettins, P., Sutton, B., Perkins, S.J., and Givol, D. (1977) Structure of an antibody combining site by magnetic resonance. Nature 266:31-37.

72. Feldman, R.J., Potter, M., and Glaudemans, C.P.J. (1981) A hypothetical space-filling model of the V-regions of the galactan binding myeloma J539. Mol. Immunol. 18:683-698.

73. Givol, D. (1979) The antibody combining site. In Defense and Recognition II (E. Lennox, Ed.) Univ. Park Press, Baltimore, MD, 11B, 23:71-125.

74. Padlan, E.A. and Kabat, E.A. (1991) Modelling of antibody combining sites. in Molecular Design and Modelling. in Concepts and Applications. Methods in Enzymology Academic Press. J.N. Abelson and M.I. Simon, Editors in chief.

75. Kabat, E.A. and Wu, T.T. (1991) Identical V-region sequences and segments of sequences in antibodies of different specificities- Relative contributions of V_H and V_L genes, minigenes and CDRs to binding of antibody combining sites. J. Immunol. (in press).

76. Gibbs, M.R., Moody, P.C.E., and Leslie, A.G.W. (1990) Crystal structure of the aspartic acid-199→asparagine mutant of chloramphenicol acetyltransferase to 2.35-Å resolution: structural consequences of disruption of a buried salt bridge. Biochemistry 29:11261-11265.

77. Kriegler, M. (1990) Gene Transfer and Expression. Stockton Press, New York, pp.1-240.

78. Horwitz, A.H., Chang, C.P., Better, M. and Hellstrom, K. (1988) Secretion of functional antibody and Fab fragment from yeast cells. Proc. Natl. Acad. Sci. USA 8678-8682.

MAIL ROOM
FEB 3 1993
PAT. & TRADEMARK OFF.

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Paul J. Carter et al.
Serial No. 07/715,272
Filed: 14 June 1991
For:Immunoglobulin Variants

Group Art Unit: 1806
Examiner: L. Feisee

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-2614

PETITION AND FEE FOR EXTENSION OF TIME (37 CFR 1.136(a))

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office action dated 05 October 1992 for one month(s) from 5 January 1993 to 5 February 1993. The extended time for response does not exceed the statutory period.

Please charge Deposit Account Number 07-0630 in the amount of $110. to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

Respectfully submitted,
GENENTECH, INC.
Carolyn R. Adler
Carolyn R. Adler
Reg. No. 32,324

Date: 29 January 1993

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Carolyn R. Adler
Carolyn R. Adler

Date: 29 January 1993

c124.u

GENENTECH, INC.
460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-2614

#15
2.22.93

MAIL ROOM
FEB 3 1993
PAT. & TRADEMARK OFF.

Attorney Docket No. 709
Examiner: L. Feisee
Group Art Unit 1806

In re Application of: Paul J. Carter et al.
Serial No.: 07/715,272
Filed: 14 June 1991
For: Immunoglobulin Variants

18

ECEIVED
FEB 19 1993
GROUP 180

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

The fee has been calculated as shown below.

	(Col. 1) Claims Remaining After Amendment		(Col. 2) Highest No. Previously Paid For	(Col. 3) Present Extra	Rate	Addit. Fee
Total	* 21	Minus	** 21	= 0	x 20=	$ 0
Indep.	* 10	Minus	*** 8	= 2	x 72=	$ 144
____	First Presentation of Multiple Dep. Claim				+ 220=	$ 0
					TOTAL . . .	$ 144.

*If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.
**If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, write "20" in this space.
***If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.
The "Highest Number Previously Paid For" (Total or Independent is the highest number found from the equivalent box in col. 1 of a prior amendment or the number of claims originally filed.)

1. ___ No additional fee is required.
2. x Please charge any additional fees, including any fees necessary for extensions of time, or credit overpayment to Deposit Account No. 07-0630. A duplicate copy of this sheet is enclosed.
   - x Any additional filing fees required under 37 CFR 1.16.
   - x Any patent application processing fees under 37 CFR 1.17.

Dated: 29 January 1993

Carolyn R. Adler
(Attorney of Record)

Carolyn R. Adler
Registration No. 32,324

SC13193 02/17/93 07715272 07-0630 130 115 110.00CH

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Service on the date below as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Dated: 29 January 1993

Carolyn R. Adler
Carolyn R. Adler

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/715,272	06/14/91	CARTER	P 709

EXAMINER
FEISEE, L

18M2

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

ART UNIT	PAPER NUMBER
1805	17

DATE MAILED: 05/19/93

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 2/3/93 ☒ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☒ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ ______

Part II SUMMARY OF ACTION

1. ☒ Claims 1-21 are pending in the application.
   Of the above, claims 14-16 are withdrawn from consideration.
2. ☐ Claims ______ have been cancelled.
3. ☒ Claims 12 and 13 are allowed.
4. ☒ Claims 1-11, 17-21 are rejected.
5. ☐ Claims ______ are objected to.
6. ☐ Claims ______ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on ______. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on ______ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on ______, has been ☐ approved. ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. ______; filed on ______.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Some of the rejections under 35 USC 112 second paragraph have been obviated in view of the amendments to the claims. However, the following rejection still remain. The language "consensus human variable domain" is still unclear despite the description in the specification. It is unclear whether the consensus human variable domain is a culmination of different variable domains or a single universal variable domain which is homologous to other human variable domains.

With regards to the langauge "import amino acid", it is suggested the import amino acid be described in the following manner: "an import antibody comprising the amino acid sequence of a non-human antibody which binds to ...". The language "reasonably expected" is unclear since it is not known what criteria determines what is "reasonable".

W/draw
W/draw.

Claim 1 remains rejected and new claims 19-21 are rejected under 35 USC 112 first paragraph as lacking enablement for the language "at least a portion" for the same reasons as set forth in pages 3 and 4 of paper #13.

W/draw

Applicant states that this language has been deleted from claim 1, but, this is not the case. This language has been moved to the beginning of the claim and the claim contains the same objectionalble language, therefore, the rejection set forth previously still applies.

The rejection of claims 1-4, 6-8 under 35 USC 101 is withdrawn in view of the amendment to the claims.

The rejection of claims 9-13 as lacking utility is withdrawn in view of the argument set forth in the letter of 2/3/93.

The objection to the specification and the rejection of claims 1-11 under 35 USC 112 first paragraph is maintained and newly added claims 17-21 are rejected for the reasons of record.

The language "at least a portion" still remains in claim 1 and newly added claims 19-21. Therefore, the rejection set forth previously on pages 3-4 of paper #13 still applies. With regards to substituting an import CDR in place of the human CDR, the rejection still applies, since there is no clear guidance in the specification to enable one of ordinary skill in the art to make the human "consensus variable region" which is to contain the claimed substitution. It is true that once the amino acid sequences are known, it is routine to determine the CDRs according to Kabat, and substitute the rodent CDRs in place of the human CDRs. However, the only guidance presented in the specification with regards to the substitutions is the amino acid sequences of SEQ ID NO: 3 and 4, which are specific variable regions. The specification vaguely alludes to variable domain sequences which are derived from the most abundant subclasses but shows no way of making such variable domains. The fact remains that applicant has not clearly taught how to determine which amino acids are the ones to be substituted since there is only a single example of the appropriate variable region which is to support the substitutions.

The rejection of claim 2 with regards to determining which residues are surface or buried residues is withdrawn in view of the argument presented explaining that computer modeling is well known in the art to determine the position of various amino acid residues.

The rejection of claims 1 and 3 with regards to the language "reasonably" and newly added claim 19 is maintained, since there is no set standard for determining what is reasonable interaction, or interfacing or what amount of glycosylation reasonably affects binding.

W/drawn

The rejection of claims 6,7 and 9 based on the specific amino acids sequences which are only relevant to IgG is maintained. Applicant argues that he is not required to exemplify every embodiment, however, if the claim requires the presence of a certain sequence which does not exist in a particular isotype, than clearly there is a lack of enablement for making that particular embodiment of the claim.

The rejections of claims 1,2,5-10 under 35 USC 102(a) and 102(b) is maintained and newly added claims 17-21 are rejected under 35 USC 102(a) and 35 USC 102(b) as being anticipated by

Queen et. al. or Co et. al. for the same reasons as set forth in the previous Office action.

Applicant argues that the distinction between the prior art and the instant invention is that the framework amino acids are chosen from a consensus human variable region. However, as previously mentioned there is no clear indication of what is meant by consensus variable regions and as it is stated by applicant on page 14 of the response the chosen amino acids in the references may indeed be the same as what applicant calls consensus variable domain sequences.

The rejection of claims 3 and 4 under 35 USC 103 is maintained for the same reasons as set forth in the previous Office action. Applicant again argues that the use of "consensus region variable domains" is different from the prior art methods, however, as previously mentioned, the consensus amino acids may be the same as the most homologous murine antibodies of the references. The lack of clarity of the language "consensus" amino acid region" is what allows this particular interpretation of the claims.

Claims 17,18, 20 and 21 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject, matter which applicant regards as the invention. New claims 17,18,20 and 21 are indefinite in that there are no discrete method steps.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. § 1.136(a).

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 C.F.R. § 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lila Feisee whose telephone number is (703) 308-2731.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Feisee/em
May 18, 1993

DAVID L. LACEY
SUPERVISORY PATENT EXAMINER
GROUP 180
5/19/93

Sheet 1 of 1

FORM PTO-1449	U.S. Dept. of Commerce Patent and Trademark Office	Atty Docket No. 709
		Serial No. 07/715,272
		Applicant Paul J. Carter et al.
		Filing Date June 14, 1991
		Group 1806

#14

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

MA 86 JAN 4 1993 PAT. & TRADE

U.S. PATENT DOCUMENTS

*Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
CP	AL	WO 90/07861	7/26/90	PCT				
	AM							
	AN							
	AO							
	AP							

OTHER DISCLOSURES (Including Author, Title, Date, Pertinent Pages, Etc.)

CP	AR	Carter et al., Proc. Natl. Acad. Sci., 89: 4285-4289 (1992)
	AS	
	AT	
	AU	
	AV	
	AW	
	AX	

Examiner	Date Considered 4/29/93

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

MAIL DATE CANCELED

MAIL ROOM
25 SEP 22
PAT. & TRADEMARK OFF.

GENENTECH, INC.
460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-2614

MAIL ROOM
25 SEP 23 1993
PAT. & TRADEMARK OFF.

Corres. and Mail
BOX AF

Attorney Docket No. 709
Examiner: L. FEISEE
Group Art Unit 1806

In re Application of: Paul J. Carter et al.
Serial No.: 07/715272
Filed: June 14, 1991
For: Immunoglobulin Variants

RECEIVED
SEP 29 1993
GROUP 1800

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment in the above-identified application.

The fee has been calculated as shown below.

	(Col. 1) Claims Remaining After Amendment		(Col. 2) Highest No. Previously Paid For	(Col. 3) Present Extra	Rate	Addit. Fee
Total	* 17	Minus	** 21	= 0	x 22=	$ 0
Indep.	* 6	Minus	*** 10	= 0	x 74=	$ 0
______ First Presentation of Multiple Dep. Claim					+ 230=	$
					TOTAL . . .	$ 0

*If the entry in Col. 1 is less than the entry in Col. 2, write "0" in Col. 3.
**If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, write "20" in this space.
***If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, write "3" in this space.
The "Highest Number Previously Paid For" (Total or Independent is the highest number found from the equivalent box in col. 1 of a prior amendment or the number of claims originally filed.)

1. x No additional fee is required.
2. x Please charge any additional fees, including any fees necessary for extensions of time, or credit overpayment to Deposit Account No. 07-0630. A duplicate copy of this sheet is enclosed.
   - x Any additional filing fees required under 37 CFR 1.16.
   - x Any patent application processing fees under 37 CFR 1.17.

Dated: September 20, 1993

Janet E. Hasak
(Attorney of Record)

Janet E. Hasak
Registration No. 28,616

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Service on the date below as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Dated: 20 Sept 1993

Louise Strasbaugh
Louise Strasbaugh

Corres. and Mail

BOX AF

81806

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#18
[illegible]
9-30-93

In re Application of
[illegible]rter et al.
Serial No. 07/715272
Filed: June 14, 1991
For: Immunoglobulin Variants

Group Art Unit: 1806
Examiner: L. FEISEE

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-1896

PETITION AND FEE FOR EXTENSION OF TIME (37 CFR 1.136(a))

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office action dated 19 May 1993 for one month(s) from 19 August 1993 to 19 September 1993. The extended time for response does not exceed the statutory period.

Please charge Deposit Account Number 07-0630 in the amount of $110 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

Respectfully submitted,
GENENTECH, INC.

Janet E. Hasak

Janet E. Hasak
Reg. No. 28,616

Date: September 20, 1993

CS14005 09/24/93 07715272 07-0630 140 115 110.00CH

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Louise Strasbaugh
Louise Strasbaugh

Date: September 20, 1993

Amendment under 37 CFR 1.116
Expedited Procedure
Examining Group 1806

PATENT DOCKET 709

19(a)(ne)
[illegible]
9-30-93

MAIL ROOM
25 SEP 23 1993
PAT. & TRADEMARK OFF.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	Group Art Unit: 1806
Paul J. Carter et al.	Examiner: L. FEISEE
Serial No. 07/715272	
Filed: June 14, 1991	
For: Immunoglobulin Variants	460 Point San Bruno Boulevard South San Francisco, CA 94080 (415) 225-1896

Do not enter

AMENDMENT AFTER FINAL REJECTION PURSUANT TO 37 CFR § 1.116

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

This is responsive to the Office Action mailed May 19, 1993, which is a final rejection of claims 1 to 11 and 17 to 21. Claims 12 & 13 have been allowed. A request for a one-month extension of time to respond is submitted herewith, bringing the due date for this response to September 20, 1993 as September 19 is a Sunday. This response is timely filed.

IN THE SPECIFICATION:

On page 19, line 3, please delete "effect or" and insert --effector--.

On page 87, please amend Table 1 as follows:

line 6, please amend the heading of the second to last column to read --$K_d{}^{+}$--;

lines 6 & 7, please amend the heading of the last column to read --Relative cell proliferation‡--;

line 8, please delete "proliferation‡";

line 10, please amend the second to last column to read --25-- and the last column to read --102--.

IN THE CLAIMS:

Please cancel claims 14-16 and 18 from the application, without prejudice. Please amend claims 1, 3, 7, 17, 19, 20 and 21 as follows:

1. (Twice amended) A method for making [at least a portion of] a humanized antibody variable domain comprising amino acid sequences of an import antibody comprising a non-human antibody which is desired to be humanized [(import antibody)] and a human antibody, comprising the steps of:

   a. obtaining the amino acid sequences of an import variable domain and of a consensus human variable domain of a human immunoglobulin subgroup; New
   b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;
   c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;
   d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;
   e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;
   f. determining if the non-homologous import amino acid residue is [reasonably] expected to have at least one of the following effects:
      1. non-covalently binds antigen directly,
      2. interacts with a CDR; or
      3. participates in the V_L - V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another;

g. for any non-homologous import antibody amino acid residue which is expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence; and

h. preparing a humanized antibody variable domain having amino acid sequences determined in steps a-g.

In claim 3, line 4, please delete "reasonably".

7. (Twice amended) A method for making a humanized antibody comprising providing an import antibody comprising a non-human antibody variable domain amino acid sequence which is desired to be humanized [(import antibody)] having a CDR and a FR, obtaining the amino acid sequence of [at least a portion of] a consensus human antibody variable domain of a human immunoglobulin subgroup, having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:
4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

17. (Amended) A method of making a humanized antibody variable domain comprising the step of substituting Complementary Determining Region (CDR) amino acid residues of a variable domain of a non-human antibody for the corresponding CDR amino acid residues of [using] a consensus human antibody variable domain amino acid sequence of a human immunoglobulin subgroup [in the preparation of a humanized antibody].

19. (Amended) A method for making an improved antibody, comprising amino acid sequences from an import antibody comprising a non-human [(import)] antibody and a human antibody, comprising the steps of:

a. obtaining the amino acid sequences of [at least a portion of] an import antibody variable domain and of a consensus human antibody variable domain of a human immunoglobulin subgroup;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human [amino] variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is [reasonably] expected to have at least one of the following effects:
   1. non-covalently binds antigen directly,
   2. interacts with a CDR; or
   3. participates in the V_L - V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another;

g. for any non-homologous import antibody amino acid residue which is [reasonably] expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence[; and] ; and

h. preparing an improved, humanized antibody having amino acid sequences determined in steps a-g; and

i. evaluating the antigen binding or immunogenicity of the improved, humanized antibody with respect to the parental antibody.

20. A method of making a humanized antibody comprising the step of making the antibody identified [, following the identification of an antibody] by the method of any one of claims [1,] 7[,] or 17 [-19, the manufacture of the antibody].

21. A method of making a humanized antibody comprising the step of expressing nucleic acid encoding the antibody identified [, following the identification of an antibody] by the method of any one of claims 1, 7, [or] 17, [-] or 19 [, the expression of nucleic acid encoding the antibody].

REMARKS

The claims pending in this application are claims 1 to 13, 17 and 19 to 21. Applicants have canceled claims 14 to 16 and 18, without prejudice to file divisional applications directed thereto.

The proposed amendments to the claims are purely in response to the rejections of the Final Action. No new matter has been introduced by the claim amendments. These amendments should be considered under Rule 116 because they do not introduce issues not already fully joined in this case and because they are believed to place the claims in better condition for appeal. Further, they are offered in a good faith effort to place this case in condition for allowance.

I. Amendments

The specification has been amended to correct obvious typographical errors. With respect to the amendment to Table 1 on page 87, a copy of Carter *et al., Proc. Natl. Acad. Sci.*, 89, (1992) is attached, which is a publication of the experimental data disclosed in the above application, and was published after the filing date thereof. It is clear that the last two column headings of Table 1 were inadvertently superimposed and the amendment to the specification serves merely to correct these errors. It would have been obvious from the information provided on page 87 of the specification, that the last two headings were intended to be "Kd nM", and "Relative cell proliferation", respectively, as the key under Table 1 discloses what the headings indicated by † and ‡ are. Also, it is clear that the figures in the last two columns of the first line of data in Table 1 were intended to be 25 and 102 respectively, and were inadvertently superimposed. Applicants respectfully request that the specification be amended to correct the obvious typographical errors discussed above.

Claims 1, 7, 17 and 19 have been amended to refer to the consensus human variable domain "of a human immunoglobulin subgroup", with support for the amendment found on at least page 16, lines 29-32 and page 17, line 4. Claim 17, 19, and 20 have been amended to recite a preamble and a positive step, which steps are clear from at least the original set of claims filed.

II. Rejections under 35 U.S.C. § 112, second paragraph

Most of the rejections under 35 U.S.C. § 112, second paragraph, which were raised in the earlier Office Action dated October 5, 1992 have been withdrawn. Applicants thank the Examiner for withdrawing these rejections.

The Examiner has, however, maintained some of the rejections under 35 U.S.C. § 112, second paragraph, which relate to claims 1, 3-5 and 7. The separate sets of rejections are addressed separately below.

A. The Examiner has maintained the rejection of claim 1 with respect to the phrase "consensus human variable domain" because it is allegedly not clear whether the consensus domain is a culmination of different variable domains or a single universal variable domain which is homologous to other human variable domains.

In the interests of expediting examination, claims 1, 7, 17 and 19 have been amended to recite that the consensus human variable domain is "of a human immunoglobulin subgroup". Information concerning the amino acid sequences of the *variable* domains of antibodies belonging to various human immunoglobulin subgroups was compiled by Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fourth Edition, U.S. Dept. of Health & Human Services, pubs., (1987), a copy of which is attached to the enclosed Kelley Declaration as Exhibit "B". Kabat *et al.* grouped various heavy and light chain variable domains according to their amino acid sequence identity to form several human immunoglobulin "subgroups" i.e. human kappa light chains subgroups I to IV, human lambda light chains subgroups I to VI and human heavy chains subgroups I to III (see pages 41-76 and 160-167 of Kabat *et al.*). The "occurrences of most common amino acids" at each position of the variable domain are provided in the second to last column for each immunoglobulin subgroup in Kabat *et al.* The consensus human variable domain claimed in the above application is an amino acid sequence comprising the most commonly occurring amino acid residues at each position of the variable domain for a particular human immunoglobulin subgroup as defined by Kabat *et al.* It would have been readily apparent, to the ordinarily skilled biochemist, what constitutes a consensus human variable domain of a human immunoglobulin subgroup upon reading the above application.

Applicants respectfully request the withdrawal of the rejection of claim 1 as indefinite in light of the above submissions.

B. The Examiner has suggested that the "import amino acid" be described as "an import antibody comprising the amino acid sequence of a non-human antibody which binds to ...". Applicants understand that the Examiner considers that inclusion of the wording "import antibody" in parentheses is unclear and that the rejection relates to claims 1, 3, 4, 5 and 7. In order to overcome the rejection, claims 1, 7 and 19 have been amended to recite "an import antibody comprising a non-human antibody...". The non-human, import antibody may be the muMAb4D5 disclosed in Example 1 of the application, for example. Claims 3-5 depend on claim 1 and because there is clear antecedence basis for the phrases "import antibody variable domain amino acid sequence", "import sequence" and "import antibody" in claim 1, the rejection of these claims is also rendered moot.

C. The Examiner has maintained the rejection of claim 1 under 35 U.S.C. §112, second paragraph, with respect to the wording "reasonably expected" on the grounds that it is not known what criteria determines what is "reasonable". In order to obviate the rejection, Applicants have deleted the word "reasonably" from claims 1, 3 and 19. Applicants respectfully submit that the amendment to the claims renders the rejection moot.

Applicants respectfully request that the maintained rejections of claims 1, 3-5 and 7 under 35 U.S.C. § 112, second paragraph, be withdrawn in light of the amendments to the claims and the submissions under paragraphs A to C above.

III. Objection and Rejections under 35 U.S.C. § 112, first paragraph

The Examiner has maintained the objection to the specification and the rejection of claims 1 to 11 under 35 U.S.C. § 112, first paragraph as lacking enablement. New claims 17 to 21 have also been rejected under 35 U.S.C. § 112, first paragraph as lacking enablement. The various sets of rejections are addressed separately below.

A. The Examiner has maintained the rejection of claim 1 and has rejected claims 19 to 21 for including the language "at least a portion". In the interests of expediting examination, claims 1, 7 and 19 have been amended by deleting the wording "at least a portion of" therefrom. Applicants submit that the amendment of the claims renders the rejection of claims 1 and 19-20 under 35 U.S.C. § 112, first paragraph, moot and respectfully request the withdrawal thereof.

B. The Examiner has maintained the rejection that step c) of claim 1 (i.e. the step of substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence) is not enabled by the specification. The Examiner asserts that there is no clear guidance in the specification to enable one of ordinary skill in the art to make the human "consensus variable domain". The Examiner further asserts that the only guidance presented in the specification with regards to the substitutions is the amino acid sequences of SEQ ID NO: 3 and 4. Applicants understand that the basis for the Examiner's rejection is that the information provided in the specification would not have enabled the ordinarily skilled biochemist to carry out the methods claimed in order to produce a humanized antibody.

Applicants respectfully traverse this rejection on the grounds that the specification is enabling for the method claimed. In support of the above position, a Declaration pursuant to 37 C.F.R. § 1.132 by Robert Kelley is attached. See specifically his opinion in paragraph 3 and the bases for this opinion set forth in paragraphs 4 to 7.

This Declaration was not earlier submitted because it was believed, in good faith, that the rejection would be overcome without the need for a Declaration. Applicants respectfully request the entry of this Declaration in the above application pursuant to Rule 116, because it does not introduce issues not already fully joined in this case. The Declaration is offered in a good faith effort to place this case in condition for allowance.

As discussed under section II (A) above and in paragraph 4 of the Kelley Declaration, the consensus human variable domain constitutes an amino acid sequence comprising the most commonly occurring amino acids at each position in the variable domain of a particular human immunoglobulin subgroup as defined by Kabat *et al.* The immunoglobulin subgroups referred to in Kabat *et al.* were grouped according to the amino acid sequence homology between human immunoglobulin *variable* domains, and the most commonly occurring amino acids at each position in the variable domain for each subgroup were identified (i.e. the "consensus human variable domain"). The skilled biochemist could have used the consensus human variable domains of the light chain and heavy chain subgroups having the greatest number of sequences therein (i.e. light chains kappa subgroup I and heavy chains subgroup III) as disclosed in Kabat *et al.* (see page 17, first paragraph of the specification) to humanize the non-human antibody of interest. Alternatively, the skilled biochemist could have chosen the consensus human variable domain of another human immunoglobulin subgroup as defined in Kabat *et al.*

i.e. the consensus human variable domain for human kappa light chains subgroups II to IV, human lambda light chains subgroups I to VI, or human heavy chains subgroups I or II (see pages 41-76 and 160-167 of Kabat *et al.*). Therefore, the skilled biochemist could have elected to use a consensus human variable domain other than those defined as SEQ ID NO: 3 & 4 on page 17 of the above application, as the consensus human variable domains for other subgroups were compiled in Kabat *et al.* Page ix of Kabat *et al.* identifies the residues forming the CDR regions of heavy and light chain variable domains tabulated from human and mouse variable domains. Kabat *et al.* have adopted standardized numbering for each of the residue locations. Accordingly, the skilled biochemist could have identified the CDR regions of the consensus human variable domain and the import variable domain using the teachings of Kabat *et al.* Alternatively, the structural definition of Chothia *et al.*, *J. Mol. Biol.*, **196:** 901-917 (1987) (see page 16, third paragraph of the specification) could have been adopted to identify the CDR regions of the consensus and import variable domains. See paragraph 4 of the Kelley Declaration. The above submissions show that steps a & b of claim 1 were enabled by the specification as filed.

Also, step c of claim 1 could have been carried out by the ordinarily skilled biochemist using the information provided in the specification and techniques such as manual tabulation of amino acid sequences or a computer program which was known in the art prior to June 14, 1991. See paragraph 5 of the Kelley Declaration.

Steps d to g of claim 1 would similarly have been straightforward to perform. These steps of claim 1 relate to the identification of Framework Region (FR) residues in the consensus human variable domain which are non-homologous to the corresponding import FR residues and replacement of such non-homologous human residues with corresponding import residues, if the residues are expected to have any one of the effects specified in step f. The locations of FR residues in human and mouse variable domains are indicated in Kabat *et al.* (see page ix) and the structural definition of the FR's was available (see Chothia *et al.*) Hence, it would have been straightforward for the skilled immunologist to identify the FR residues in the consensus human variable domain and the import sequence. Using computer programs available before June 14, 1991, the skilled biochemist would have been able to study the 3-dimensional structure of the antibody in order to establish whether a particular non-homologous import amino acid residue is likely to have one of the effects discussed in section f of claim 1. Information is provided on pages 14 to 16 of the specification which would have enabled the skilled biochemist to determine whether any non-homologous residue(s) would be expected to have the effects claimed. The techniques claimed in steps d to g of claim 1 could have been carried out routinely by a person versed in the relevant art, prior to June 14, 1991. See paragraph 6 of the Declaration.

As discussed in paragraph 7 of the Declaration, once the primary amino acid sequence of the antibody had been characterized, it would have been routine to make the protein using recombinant techniques or a peptide synthesizer, which techniques were well known in the art prior to the filing date of the above application.

Applicants conclude that, contrary to the Examiner's assertions, the ordinarily skilled biochemist would have been able to carry out the method claimed in the above application, using the information provided in the specification and techniques which were well known in the relevant art, prior to June 14, 1991.

Accordingly, Applicants request that the rejection of claim 1 under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn in light of the above submissions and the Declaration.

C. The Examiner has maintained the rejection of claims 1 and 3, and has rejected claim 19 under 35 U.S.C. § 112, first paragraph, with respect to the wording "reasonably" therein. In order to obviate the rejection, the wording "reasonably" has been deleted from claims 1, 3 and 19.

Accordingly, Applicants request that the rejection of claims 1, 3 and 19 under 35 U.S.C. § 112, first paragraph, be withdrawn.

D. The Examiner has maintained the rejection of claims 6, 7 and 9 as lacking enablement under 35 U.S.C. § 112, first paragraph, the Examiner's position being that the amino acids are relevant to IgG only and not to other isotypes. Applicants respectfully traverse this rejection on the basis that the immunoglobulin *sites* claimed would have been relevant with respect to antibodies, other than IgG antibodies. Applicants refer the Examiner to paragraphs 8 & 9 of the Kelley Declaration which support this position. The Examiner appears to suggest that the rejected claims cover *sequences* which would not be found in immunoglobulin isotypes, other than IgG isotypes. However, as pointed out in paragraph 9 of the Kelley Declaration, the claims refer to *positions* or *sites* of the variable domain, not specific amino acid residues. These sites relate to the position of a residue in the 3-D structure of the variable domain. Kabat *et al.* have used universal numbering for the amino acid residue locations of the variable domains for each of the immunoglobulin subgroups mentioned therein. The FR residue sites indicated may be occupied by an amino acid residue which is non-homologous to the corresponding consensus human variable domain residue, and which residue is likely to have at least one of the effects discussed in step f of claim 1. The residue at the particular site can be any amino acid residue, depending on the antibody in which it is located. These residue locations or sites are applicable *across species* (see page 16, line 8). Accordingly, it is likely that an amino acid residue located at one of the sites indicated in claims 6, 7 and 9 will have one of the effects of claim 1 (step f) regardless of the antibody in which it is located. It is apparent that the particular sites claimed are applicable to immunoglobulins other than IgG.

Accordingly, Applicants submit that the rejection of claims 6, 7 & 9 under 35 U.S.C. § 112, first paragraph, should be reconsidered and withdrawn in light of the above submissions and Declaration.

In light of the submissions presented in paragraphs A to D above, Applicants respectfully request that the objection to the specification and the rejection of claims 1-11 and 17-21 under 35 U.S.C. §112, first paragraph, be withdrawn.

Applicants thank the Examiner for withdrawing the rejections which were raised under 35 U.S.C. § 101 in the earlier Office Action dated October 5, 1992 .

IV. Rejection of claims 1, 2 and 5-10 under 35 U.S.C. 102 (a) and 102(b)

The rejection of claims 1, 2 and 5-10 under 35 U.S.C. § 102(a) and 102(b) has been maintained and newly added claims 17-21 have been rejected under 35 U.S.C. § 102(a) and 102(b) as being anticipated by Queen *et al., Proc. Natl. Acad. Sci.*, 86:10029-10033 (1989) and Co *et al., Proc. Natl. Acad. Sci.*, 88:2869-2873 (1991). The basis for the rejection is that there is allegedly no clear indication as to what is meant by the consensus human variable domain claimed in the above application.

To constitute anticipation, all material elements of a claim must be found in one prior art source. *In re Marshall*, 198 USPQ 344 (CCPA 1978), *In re Kalm*, 154 USPQ 10 (CCPA 1967). Applicants will show that Queen *et al.* and Co *et al.* do not contain all material elements of claims 1, 2, 5-10 and 17-21.

The nature of the "consensus human variable domain of a human immunoglobulin subgroup" as defined in the claims as amended has been discussed above under Section II(A) of this response and in paragraph 4 of the Kelley Declaration, those discussions being incorporated herein. Applicants submit that the meaning of the phrase consensus human variable domain of a human immunoglobulin subgroup would have been clearly understood by those skilled in the art upon reading the specification. The prior art relied upon in the Office Action fails to disclose a method of making a humanized antibody using a consensus human variable domain to "humanize" a non-human antibody. The Declaration by Kelley supports this position. In particular, Applicants direct the Office's attention to paragraphs 11-13 of the attached Declaration. It is apparent from the information given in Table 1 of Exhibit C and in the Figures of Exhibits D and E of the Kelley Declaration (see paragraphs 12 & 13 thereof), that the variable domains of the human immunoglobulin sequences used by Queen *et al.* and Co *et al.* are not a consensus human variable domain of any human immunoglobulin subgroup as set forth in the claims of the above application.

Since, as shown above, Queen *et al.* and Co *et al.* do not teach all the material elements of the instant claims as required under *Marshall* and *Kalm, supra*, Applicants respectfully submit that the rejection of claims 1, 2, 5-10 and 17-21 under 35 U.S.C. § 102(a) and (b) can not be upheld and therefore request that the rejections be withdrawn.

V. Rejection of claims 3 and 4 under 35 U.S.C. § 103

The rejection of claims 3 and 4 as unpatentable under 35 U.S.C. § 103 over Queen *et al.* or Co *et al., supra*, in view of Wallick *et al., J. Exp. Med.*, **168** (1988) has been maintained. The basis for the rejection relates to the alleged lack of clarity of the language "consensus human variable domain" in the claims of the above application. The consensus human variable domain as defined in the above application would have been readily understood by the ordinarily skilled biochemist (see paragraph 4 of the Kelley Declaration). Claim 1 of the above application relates to a method of using a consensus human variable domain to "humanize" a non-human antibody (e.g. muMAb4D5). As established in section IV above, use of a consensus human variable domain from a human immunoglobulin subgroup is not disclosed in Queen *et al.* or Co *et al.*

The publication by Wallick *et al.* does not compensate for the deficiencies in the primary references. Wallick *et al.* refer to the importance of glycosylation for maintaining antigen binding affinity of monoclonal antibodies. Wallick *et al.* fail to disclose or suggest a method of humanizing a non-human antibody, much less a method of humanizing a non-human antibody using a consensus human variable domain of a immunoglobulin subgroup. The skilled biochemist would have had no motivation to use a consensus human variable domain based on the prior art referred to in the Office Action, because the prior art techniques had all relied upon using a human variable domain sequence which has the closest sequence homology to the non-human variable sequence (to be humanized) in order to reduce the likelihood of introducing distortions into the CDR's (see column 2 on page 10031 of Queen *et al.*) and "to retain high binding affinity in the humanized antibody" (see column 1 on page 2871 of Co *et al.*). The method claimed in the above application does not rely on a high degree of homology between the variable domain of the non-human sequence and the consensus variable domain which is used to humanize the non-human sequence.

Also, as supported by paragraph 15 of the Kelley Declaration, the invention claimed in the above application resulted in an unexpected result which could not have been reasonably predicted from the prior art. It was surprising that a consensus variable domain of a selected immunoglobulin subgroup could be used to humanize a non-human antibody, regardless of the degree of homology between the human and non-human amino acid sequences. It was also surprising that the humanized antibody so formed retained, and in some instances, had increased antigen binding affinity compared to the non-human antibody from which it was derived. The above application shows that the huMAb4D5-8 variant actually binds the $p185^{HER2}$ ECD 3-fold more tightly than muMAb4D5 (see page 82 lines 31 & 32 to page 83, line 1 of the specification) which could not have been predicted by the ordinarily skilled biochemist. See paragraph 15 of the Kelley Declaration. The evidence of unexpected results in Applicants' application is sufficient to support a conclusion of nonobviousness. *Ralston Purina Co. Far-Mar-Co., Inc.*, 222 USPQ 863 (DC KS, 1984).

It is apparent that the invention claimed in claim 1 was novel and nonobvious over the citations because the combination of the prior art failed to disclose, or suggest, the invention claimed in claim 1 and, moreover, the method resulted in a new and unexpected result which could not have been reasonably predicted from the art.

Claims 3 & 4 depend on claim 1 which, as established above, is novel and nonobvious over the citations. Claim 3 refers to the step of finding any glycosylation site which is likely to affect the antigen binding or affinity in the import antibody and substituting the glycosylation site *into* the *consensus* amino acid sequence. Claim 4 refers to the step of *replacing* glycosylation sites of the consensus domain with the corresponding import amino acid residues if such glycosylation sites are not present in the import sequence. These claims would not have been obvious over the prior art of record because the prior art failed to disclose the use of a human consensus variable domain to humanize the non-human antibody. Accordingly, the skilled biochemist would have had no motivation to replace or insert glycosylation sites into a consensus amino acid sequence, as claimed in claims 3 and 4 of the application. See paragraph 15 of the Kelley Declaration.

The law is clear that obviousness cannot be established by combining the teachings of the references to produce the claimed invention, absent some teaching, suggestion, or incentive supporting the combination. *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 221 USPQ 929, 933 (Fed. Cir. 1984). The above discussion shows that the cited references, alone or in combination, lack the requisite teaching of the use of a consensus human variable domain to humanize a non-human antibody. In this case, the combined art would not have reasonably enabled or motivated the skilled practitioner to use a human consensus variable domain in this manner, which provides a method of making improved humanized antibodies. Accordingly, it is clear that the invention claimed in claims 3 & 4 is novel and nonobvious over the prior art of record.

Applicants submit that the rejection of claims 3 and 4 under 35 U.S.C. § 103 should be reconsidered and withdrawn in light of the above submissions and the Declaration.

VI. Rejection of claims 17,18, 20 and 21 under 35 U.S.C. §112, second paragraph.

Claims 17, 18, 20 and 21 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite in that there are allegedly no discrete method steps. In order to obviate the rejection, claims 17, 20 and 21 have been amended to each recite a definite method step and claim 18 has been deleted.

Applicants respectfully request the withdrawal of the rejection of claims 17, 20, and 21 under 35 U.S.C. § 112, second paragraph, in light of the amendments to the claims.

As all objections and rejections have been addressed and overcome, Applicants believe that the claims are now in condition for allowance. Notice to that effect is respectfully requested. If the Examiner has any questions concerning the response, she should feel free to call the undersigned attorney at the number indicated above.

Respectfully submitted,
GENENTECH, INC.

Janet E. Hasak

Janet E. Hasak
Reg. No. 28,616

Date: September 20, 1993

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Louise Strasbaugh
Louise Strasbaugh

Date: September 20, 1993

MAIL ROOM
25 SEP 23 1993
PAT. & TRADEMARK OFF.

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#20
Supp
9-20-93

In re Application of

Paul J. Carter et al.

Serial No. 07/715272

Filed: June 14, 1991

For: Immunoglobulin Variants

Group Art Unit: 1806

Examiner: L. FEISEE

460 Point San Bruno Boulevard
South San Francisco, CA 94080

DECLARATION OF ROBERT F. KELLEY PURSUANT TO 37 CFR §1.132

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, ROBERT F. KELLEY, do hereby declare as follows:

1. I received my Ph.D. in Biochemistry in 1984 from the University of Iowa. Following my Ph.D, I was a NIH postdoctoral fellow in the Department of Molecular Biophysics & Biochemistry at Yale University from July 1984 to December 1985. In 1986, I joined the Biocatalysis Department at Genentech, Inc. as an Associate Scientist. In September 1988, I was promoted to Scientist and I am employed in that capacity at present. (The Biocatalysis Department has been renamed "Protein Engineering"). I am the author or co-author of 22 publications relating to the 3-D structures and folding of various proteins. A copy of my curriculum vitae is attached as Exhibit "A".

2. I understand that the Patent Office has rejected the above application on the basis that the application as filed does not provide sufficient disclosure to enable a skilled biochemist to carry out the method of claim 1 because the Examiner believes no clear guidance exists in the specification to allow a skilled biochemist to make the "consensus human variable domain" and substitute an import (i.e. non-human) Complementary Determining Region (CDR) amino acid sequence for the corresponding human CDR amino acid sequence, as set forth in claim 1. I further understand that the Office considers that

the only guidance in the specification with regards to the substitutions is the amino acid sequences of SEQ ID NO: 3 and 4.

3. I have read the above application, the Office Action date May 19, 1992 (Paper # 17) rejecting the claims of the application, and the proposed amendment of the claims in response to the rejection. In my opinion, the skilled biochemist could have readily carried out the method of claim 1 in order to make a humanized antibody, using the general knowledge available in the field on and before June 14, 1991, and the information given in the above application. The bases for my opinion are given in paragraphs 4 to 7 below.

4. Claim 1 relates to a method of making a humanized antibody. Steps a and b of claim 1, as amended, discuss identification of the CDR amino acid sequences of a non-human import antibody (to be humanized) and a consensus human variable domain of a human immunoglobulin subgroup. The consensus human variable domain constitutes an amino acid sequence comprising the most commonly occurring amino acids at each position in the variable domain of a particular human immunoglobulin subgroup as defined by Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fourth Edition, U.S. Dept. of Health & Human Services, pubs., (1987), a copy of which is attached as Exhibit "B". The immunoglobulin subgroups referred to in Kabat *et al.* were grouped according to the amino acid sequence homology between human immunoglobulin *variable* domains, and the most commonly occurring amino acids at each position in the variable domain for each subgroup were identified (i.e. the "consensus human variable domain"). The skilled biochemist could have used the consensus human variable domains of the light chain and heavy chain subgroups having the greatest number of sequences (i.e. light chains kappa subgroup I and heavy chains subgroup III) as disclosed in Kabat *et al.* (see page 17, first paragraph of the specification) to humanize the non-human antibody of interest. Alternatively, the skilled biochemist could have chosen the consensus human variable domain of another human immunoglobulin subgroup as defined in Kabat *et al.* (i.e. the consensus human variable domain for human kappa light chains subgroups II to IV, human lambda light chains subgroups I to VI, or human

heavy chains subgroups I or II [see pages 41-76 and 160-167 of Kabat *et al.*]). Therefore, the skilled biochemist could have elected to use a consensus human variable domain other than those defined as SEQ ID NO: 3 & 4 on page 17 of the above application, as the consensus human variable domains for other subgroups were compiled in Kabat *et al.* Page ix of Kabat *et al.* identifies the residues forming the CDR regions of heavy and light chain variable domains tabulated from human and mouse variable domains. Kabat *et al.* have adopted standardized numbering for each of the residue locations. Accordingly, the skilled biochemist could have identified the CDR regions of the consensus human variable domain and the import variable domain using the teachings of Kabat *et al.* Alternatively, the structural definition of Chothia *et al.*, *J. Mol. Biol.*, **196**: 901-917 (1987) (see page 16, third paragraph of the specification) could have been adopted to identify the CDR regions of the consensus and import variable domains. Hence, it would have been straightforward for the skilled biochemist to carry out steps a and b of claim 1 using the information provided in the specification.

5. Step c of claim 1 discloses the step of replacing the corresponding human CDR sequence with the import CDR amino acid sequence. This step could have been carried out routinely by the skilled biochemist by manual tabulation or using a computer program such as the ALIGN program, (Dayhoff *et al.*, *Meth. Enzymol.*, **91**:524-545 [1983]) which was available prior to June 14, 1991. Steps a to c of claim 1 would have resulted in the characterization of a primary amino acid sequence encoding a humanized variable domain with import (non-human) CDR regions.

6. Steps d to g of claim 1 relate to the identification of Framework Region (FR) residues in the consensus human variable domain which are non-homologous to the corresponding import FR residues and replacement of such non-homologous human residues with corresponding import residues, if the residues are expected to have any one of the effects specified in step f. The locations of FR residues in human and mouse variable domains are indicated in Kabat *et al.* (see page ix) and the structural definition of the FR's was available (see Chothia *et al.*) Hence, it would have been straightforward for the skilled immunologist to identify the FR residues in the consensus human variable domain and the

import sequence. Using computer programs (such as the INSIGHT program [Biosym Technologies], available before June 14, 1991), the skilled biochemist would have been able to study the 3-dimensional structure of an antibody in order to establish whether a particular non-homologous import amino acid residue is likely to have one of the effects discussed in section f of claim 1. Information is provided on pages 14 to 16 of the specification which would have enabled the skilled biochemist to determine whether any non-homologous residue(s) would be expected to have the effects claimed. The techniques claimed in steps d to g of claim 1 could have been carried out routinely by a person versed in the relevant art, prior to June 14, 1991.

7. Steps a to g of claim 1 would have lead to the characterization of an amino acid sequence of a humanized antibody having non-human CDR amino acid residues and, optionally, having one or more non-human FR residues. In order to prepare the humanized antibody as claimed in claim 1, step h, the skilled biochemist could have synthesized the antibody using a peptide synthesizer which was commercially available before June 14, 1991. Alternatively, the antibody could have been made in recombinant cell culture (see page 26, last paragraph of the specification). Preparation of the antibody would have been straightforward to perform by the person skilled in the art, once the amino acid sequence of the humanized antibody had been characterized.

8. I understand that the Patent Office has rejected the above application on the basis that the sites in the variable domain referred to in claims 6, 7, and 9 are relevant to IgG antibodies only. It is my opinion that the sites referred to in claims 6, 7, and 9 would be relevant to other immunoglobulins. The basis for my opinion is given in paragraph 9 below.

9. The sites referred to in claims 6, 7, and 9 are the residue locations, or sites, of the FR residues in the heavy or light chain forming the variable domain of immunoglobulins. The residue sites referred to in claims 6, 7 & 9 relate to the position of a residue in the 3-D structure of the variable domain. Kabat *et al.* have used universal numbering for the amino acid residue locations of the variable domains for each of the immunoglobulin subgroups mentioned in the reference. The FR residue sites

indicated may be occupied by an amino acid residue which is non-homologous to the corresponding consensus human variable domain residue, and which is likely to have at least one of the effects discussed in step f of claim 1. These residue locations or sites are applicable *across species* (see page 16, line 8 of the specification). Accordingly, it is likely that an amino acid residue located at one of the sites indicated in claims 6, 7 and 9 will have one of the effects of claim 1 (step f), regardless of the antibody in which it is located, because it will be in the same position in the 3-D structure of the antibody variable domain as the residue sites referred to in the rejected claims. Accordingly, the examples of residue locations to be substituted in the variable domains would be applicable to antibodies, other than IgG antibodies.

10. I understand that the Patent Office has rejected the above application on the grounds that the invention as claimed is disclosed in Queen *et al.*, *Proc. Natl. Acad. Sci.*, 86:10029-10033 (1989) or Co *et al.*, *Proc. Natl. Acad. Sci.*, 88:2869-2873 (1991) and that the Office has suggested that the human variable domains disclosed in these references may have the same amino acid sequences as one of the consensus human variable domains disclosed in Kabat *et al.*

11. The above statements regarding the state of knowledge as of June 14, 1991, do not establish that the invention claimed in this application was known, or would have been obvious, to the skilled biochemist at the time the invention was made. To the contrary, after having read the citations relied upon by the Patent Office, it is my judgement that these documents would not have disclosed, nor suggested, the methods claimed. The basis for my opinion is given below.

12. The invention of the above application can be distinguished on the basis that a *consensus human variable domain* is used to "humanize" a non-human antibody of interest. The Queen *et al.* and Co *et al.* publications fail to disclose a consensus human variable domain. Instead, these publications refer to the use of a human variable domain having the closest sequence homology to the variable domain of the non-human antibody to be humanized. Queen *et al.* used the Eu human variable domain sequence (see Fig 2 thereof) and Co *et al.* used the variable domains of the Pom or Eu human

antibodies (see Fig 1 thereof). The sequences used in Queen *et al.* and Co *et al.* do not constitute a consensus human variable domain of a human immunoglobulin subgroup. The sequence identity between the amino acid sequences of the FR residues of the variable domains of the Pom or Eu heavy or light chains compared to the FR residues of the consensus human variable domains of each of the human immunoglobulin subgroups as defined by Kabat *et al.* is illustrated in Table 1 (see Exhibit "C", attached hereto). The CDR residues were not used in the comparison because of the large number of differences between these residues for variable domains of different antibodies. The Pom and Eu variable domain sequences were taken from Kabat *et al.* The consensus human variable domains of the V_L lambda subgroups IV and V were not compared, as these subgroups have too few members. While the variable domain of Eu is classified in subgroups V_L kappa I and V_H I, and the variable domain of Pom is classified in subgroups V_L kappa III and V_H III, it is apparent that the Eu and Pom variable domain amino acid sequences are not consensus human variable domains of any immunoglobulin subgroup. This is further demonstrated in the following paragraph.

13. Exhibits "D" and "C" attached hereto, show the differences in the amino acid sequences of the Pom and Eu heavy and light chain variable domains compared to the consensus human variable domain of the subgroup in which they are classified. Exhibit D illustrates an alignment of the amino acid sequences of the light chain variable domains of Eu, Pom and the consensus variable domain of the V_L kappa subgroup I (in which the light chain variable domain of Eu is classified). Exhibit E illustrates an alignment of the amino acid sequences of the heavy chain variable domains of Eu, Pom and the consensus variable domain of the V_H subgroup III (in which the heavy chain variable domain of Pom is classified). Even though Eu is classified in V_L kappa I, it has seven framework residues which are different from the framework residues of the kappa I consensus sequence. Furthermore, while Pom is classified in the V_H III subgroup, eight of its framework residues differ from the corresponding framework residues of the V_H III consensus sequence. There are, of course, many differences between the CDR residues of the consensus sequences and the corresponding CDR residues of Pom and Eu.

It is clear from the information in Exhibits C, D, & E that the Queen *et al.* and Co *et al.* publications fail to disclose a method wherein a non-human import antibody is humanized using a consensus human variable domain of an immunoglobulin subgroup.

14. I understand the Patent Office has rejected the above application on the basis that the invention claimed in claims 3 & 4 would have been obvious in light of Queen *et al.*, or Co *et al.*, when read in conjunction with Wallick *et al., J. Exp. Med.*, **168** (1988). After reading these references, it is my opinion that the invention claimed in claims 3 and 4 is novel and would not have been obvious in light of the citations. The basis for my opinion is given in the following paragraph.

15. Claim 1 of the above application relates to a method of using a consensus human variable domain to "humanize" a non-human antibody (e.g. muMAb4D5). Use of a consensus human variable domain from a human immunoglobulin subgroup to humanize a non-human antibody is not disclosed in Queen *et al.*, Co *et al.* or Wallick *et al.* Wallick *et al.* does not relate to a method of humanizing a non-human antibody, much less a method of humanizing a non-human antibody using a consensus human variable domain of a human immunoglobulin subgroup. The skilled biochemist would have had no motivation at the filing date of this application to use a consensus human variable domain to humanize a non-human antibody, because the techniques in the prior literature had all relied upon using a human variable domain sequence which has the closest sequence homology to the non-human variable sequence (to be humanized) in order to reduce the likelihood of introducing distortions into the CDR's (see column 2 on page 10031 of Queen *et al.*) or to "retain high binding affinity in the humanized antibodies" (see column 1 on page 2871 of Co *et al.*). The method claimed in the above application does not rely on a high degree of homology between the variable domain of the non-human sequence and the consensus variable domain which is used to humanize the non-human sequence. It was surprising that a consensus variable domain of a selected immunoglobulin subgroup could be used to humanize a non-human antibody, regardless of the degree of homology between the human and non-human amino acid sequences. It was also surprising that the humanized antibody so formed retained,

and in some instances, had increased antigen binding affinity compared to the non-human antibody from which it was derived. The above application shows that the huMAb4D5-8 variant actually binds the p185^{HER2} ECD 3-fold more tightly than muMAb4D5 (see page 82 lines 31 & 32 to page 83, line 1 of the specification), which could not have been predicted by the ordinarily skilled biochemist at the time the specification was filed. Claim 3 refers to the step of finding any glycosylation site which is likely to affect the antigen binding or affinity in the import antibody and substituting the glycosylation site *into* the *consensus* amino acid sequence. Claim 4 refers to the step of *replacing* glycosylation sites of the consensus domain with the corresponding import amino acid residues if such glycosylation sites are not present in the import sequence. In my opinion, these claims would not have been obvious over the prior literature because the reference failed to disclose the use of a human consensus variable domain to humanize the non-human antibody. Accordingly, the skilled biochemist would have had no motivation to replace or insert glycosylation sites into a consensus amino acid sequence, as claimed in claims 3 and 4 of the application.

16. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 9/20/93

Signed: Robert F. Kelley
ROBERT F. KELLEY

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on September 20, 1993.

Dated: September 20, 1993

Louise Strasbaugh
Louise Strasbaugh

w012.u

XHIBIT A

Robert F. Kelley **CURRICULUM VITAE** **September, 1993**

Work address:

Genentech, Inc.
Protein Engineering Dept.
South San Francisco, CA 94080

Home address:

1029 San Felipe Ave.
San Bruno, CA 94066

Personal information:

Birthdate: November 7, 1957
Married, Wife's name: Wendy L. Kelley
One child: Brian F. Kelley

Education:

B.S., Biochemistry, Michigan State University, 1979
Ph. D., Biochemistry, University of Iowa, 1984

Employment positions:

Teaching assistant, Chemistry Dept., Michigan State Univ., 1978-1979
Teaching assistant, Biochemistry Dept., University of Iowa, 1979-1981
Graduate research assistant, Biochemistry Dept., University of Iowa, 1982-April 1984
Postdoctoral associate, Biochemistry Dept., University of Iowa, May 1984-June 1984
NIH postdoctoral fellow, Dept. of Molecular Biophysics & Biochemistry, Yale University, July 1984-Dec. 1985
Associate Scientist, Biocatalysis Dept., Genentech, Inc., January 1986-September 1988
Scientist, Biomolecular Chemistry Dept., Genentech, Inc., September 1988-present

Awards and membership in professional organizations:

Biophysical Society, 1983-present
American Chemical Society, 1991-present

Scientific publications

Kelley, R.F., & Stellwagen, E. (1984) "Conformational transitions of thioredoxin in guanidine hydrochloride", Biochemistry 23, 5095-5102.

Kelley, R.F., Wilson, J., Bryant, C., & Stellwagen, E. (1986)"Effects of guanidine hydrochloride on the refolding kinetics of denatured thioredoxin", Biochemistry 25, 728-732.

Wilson, J., Kelley, R.F., Shalongo, W., Lowery, D., & Stellwagen, E. (1986) "Equilibrium and kinetic measurements of the conformational transitionof thioredoxin in urea", Biochemistry 25, 7560-7566.

Kelley, R., Wilson, J., Bryant, C., Shalongo, W., Ledger, R., Lowery, D., & Stellwagen, E. (1986) "Folding of E. coli thioredoxin into its native conformation" in Thioredoxin and Glutaredoxin Systems: Structure & Function (Holmgren, A., Branden, C.-I., Jornvall, H., & Sjoberg, B.-M., Eds.) pp 77-85, Raven Press, New York.

Kelley, R., Ledger, R., Shalongo, W., & Stellwagen, E. (1986)"Spectral and hydrodynamic methods for identification of intermediates in protein folding", UCLA Symp. Mol. Chem. Biol., New Ser. 39, 269-281.

Kelley, R.F., Shalongo, W., Jagannadham, M.V., & Stellwagen, E. (1987) "Equilibrium and kinetic measurements of the conformational transition of reduced thioredoxin", Biochemistry 26, 1406-1411.

Kelley, R.F., & Richards, F.M. (1987) "Replacement of proline 76 with alanine eliminates the slowest kinetic phase in thioredoxin folding",Biochemistry 26, 6765-6774.

Cleary, S., Mulkerrin, M.G., & Kelley, R.F. (1989) "Purification and characterization of tissue plasminogen activator kringle-2 domain expressed in E. coli", Biochemistry, 28, 1884-1891.

Kelley, R.F., & Cleary, S. (1989) "Effect of residue 65 substitutions on thermal stability of t-PA kringle-2 domain", Biochemistry 28, 4047-4054.

EXHIBIT C

TABLE 1
SEQUENCE IDENTITY - (%)

CONSENSUS VARIABLE DOMAIN SUBGROUP	EU	POM
V_L kappa I	92	76
V_L kappa II	61	71
V_L kappa III	72	85
V_L kappa IV	73	78
V_L lambda I	61	59
V_L lambda II	57	54
V_L lambda III	59	56
V_L lambda VI	52	49
V_H I	83	64
V_H II	53	62
V_H III	61	91

EXHIBIT D

Variable Light Domain

```
                        10        20        30        40
EU              DIQMTQSPSTLSASVGDRVTITCRASQ-SINTWLAWYQQKPGKAPKLLMY
                         *                  @ @@@               *
Kappa-I         DIQMTQSPSSLSASVGDRVTITCRASQ--ISNYLAWYQQKPGKAPKLLIY
                * *     **  * * * * **     @@@@@         *** *
POM             EIVMTQSPVTLSVSPGERATLSCRASQSISNSYLAWYQQKPSGSPRLLIY
                                       -----------
                                          CDR-L1

                50       60        70        80        90       100
EU              KASSLESGVPSRFIGSGSGTEFTLTISSLQPDDFATYYCQQYNSDSKMFGQ
                @            *      *          *            @@@@
Kappa-I         AASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPWTFGQ
                @  @@@@ * *         *         *    *       @@ @
POM             GASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPPTFGQ
                -------                                ----------
                CDR-L2                                   CDR-L3

EU              GTKVEVKGT
                     * *
Kappa-I         GTKVEIKRT
                  *
POM             GTRVEIKR
```

KEY: * = differences in FR residues

@ = differences in CDR residues

EXHIBIT E

Variable Heavy Domain

```
                    10        20        30        40
EU          QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRSAIIWVRQAPGQGLEWMG
            *    *  *****  * ***  *   *   @@ @@       *    **
human-III   EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVS
                *                          @                *
POM         EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSAMSWVRQAPGKGLEWVA
                                          -----
                                          CDR-H1

           50  a      60        70        80  abc      90
EU          GIVPMFGPPNYAQKFQGRVTITADESTNTAYMELSSLRSEDTAFYFCAG
            @ @@@@ @@@  @@@@  *  ** * *  * ****   *    * *  *
human-III   VISGDGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
            @@@@@ @@@@     @       **       *    *     *
POM         WKYENGNDKHYADSVNGRFTISRNDSKNTLYLLMNSLQAEDTALYYCAR
            -----------------
               CDR-H2

                              110
EU          GYGIYSPE-----EYNGGLVTVSS
             @ @@@@@     *** *
human-III   GRGGGSDY-----WGQGTLVTVSS
            @@ @@@@@     *
POM         DAGPYVSPTFFAHYGQGTLVT
            -------------
               CDR-H3
```

KEY: * = differences in FR residues

@ = differences in CDR residues

Byeon, I.-J.L., Kelley, R.F., & Llinas, M. (1989) "^{1}H-NMR structural characterization of a recombinant kringle-2 domain from human tissue-type plasminogen activator", Biochemistry 28, 9350-9360.

Kelley, R.F., & Winkler, M.E. (1990) "Folding of eukaryotic proteins produced in *Escherichia coli*" Genetic Engineering (ed. J.K. Setlow) 12, 1-19.

Byeon, I.-J., Kelley, R.F., & Llinas, M. (1991) "Kringle-2 domain of the tissue-type plasminogen activator: ^{1}H-NMR assignments and secondary structure" Eur. J. Biochem. 197, 155-165.

Kelley, R.F., DeVos, A.M., & Cleary, S. (1991) "Thermodynamics of ligand binding and denaturation for His64 mutants of tissue plasminogen activator kringle-2 domain" Proteins: Structure, Function and Genetics 11, 35-44.

Menhart, N., Sehl, L.C., Kelley, R.F., & Castellino, F.J. (1991) "Construction, expression and purification of recombinant kringle 1 of human plasminogen and analysis of its interaction with ω-amino acids" Biochemistry 30, 1948-1957.

deVos, A.M., Ultsch, M.H., Kelley, R.F., Padmanabhan, K., Tulinsky, A., Westbrook, M.L., & Kossiakoff, A.A. (1991) "Crystal structure of the kringle-2 domain of tissue plasminogen activator at 2.4 Å resolution" Biochemistry, 31, 270-279.

Garrard, L.J., Yang, M., O'Connell, M.P., Kelley, R.F., & Henner, D.J. (1991) "Fab assembly and enrichment in a monvalent phage display system" Bio/Technology, 9, 1373-1377.

Carter, P., Kelley, R.F., Rodrigues, M.L., Snedecor, B.R., Covarrubias, M., Velligan, M.D., Wong, W.-L. T, Rowland, A.M., Kotts, C.E., Carver, M.E., Yang, M., Bourell, J.H., Shepard, H.M., & Henner, D.J. (1992) "High level Escherichia coli expression and production of a bivalent humanized antibody fragment" Bio/Technology, 10, 163-167.

Kelley, R.F., O'Connell, M.P., Carter, P., Presta L., Eigenbrot, C., Covarrubias, M., Snedecor, B., Bourell, J.H., & Vetterlein, D. (1992) "Antigen binding thermodynamics and anti-proliferative effects of chimeric and humanized anti-$p185^{HER2}$ antibody Fab fragments" Biochemistry, 31, 5434-5441.

Kelley, R.F., O'Connell, M.P., Carter, P., Presta, L., Eigenbrot, C., Covarrubias, M., Snedecor, B., Speckart, R., Blank, G., Vetterlein, D., & Kotts, C. (1993) "Characterization of humanized anti-$p185^{HER2}$ antibody Fab fragments produced in *E. coli*" ACS Symposium Series No. 526, *Protein Folding: In Vivo and In Vitro*, J.L. Cleland & J. King, eds., pp. 218-239.

Kelley, R.F., & O'Connell, M.P. (1993) "Thermodynamic analysis of an antibody functional epitope" Biochemistry, 32, 6828-6835.

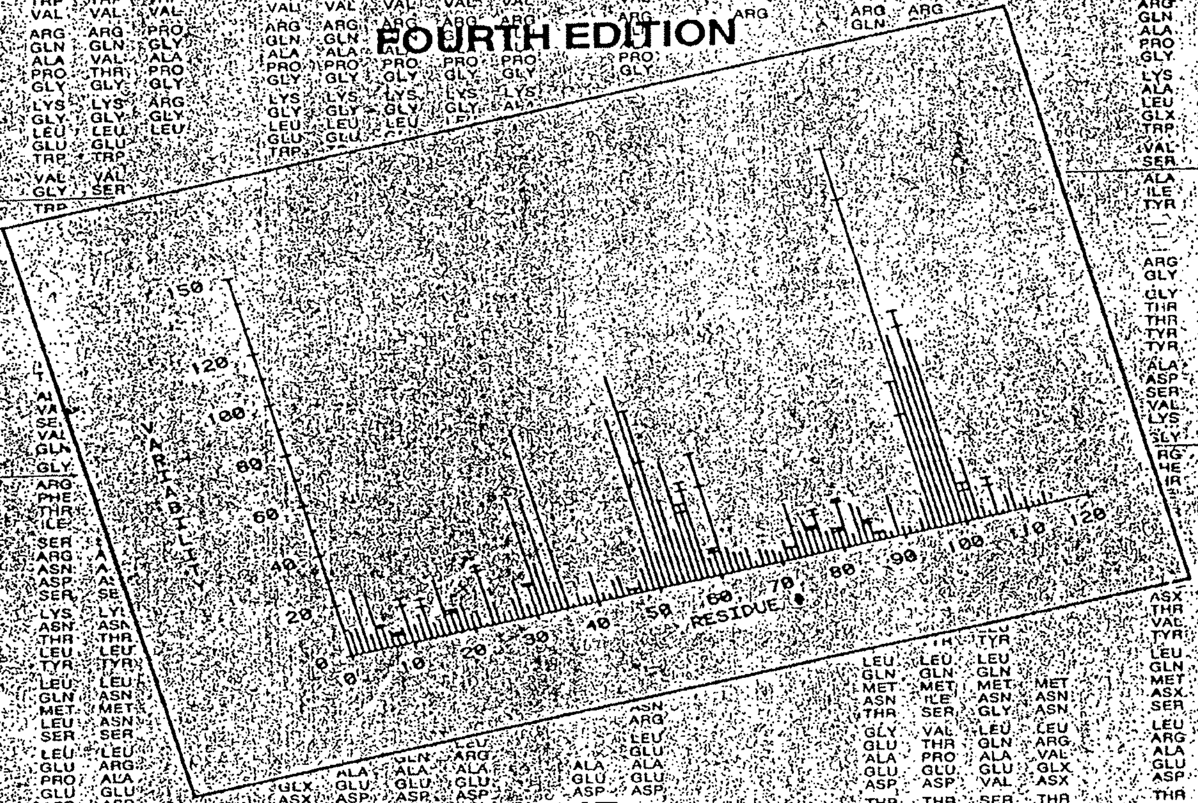
Huber, A.H., Kelley, R.F., Gastinel, L.N, & Bjorkman, P.J. (1993) "Crystallization and stoichiometry of binding of a complex between a rat intestinal Fc receptor and Fc" J. Mol. Biol., 230, 1077-1083.

Kelley, R.F. (1993) "Engineering Therapeutic Antibodies", in Protein Engineering: Principles & Practice, eds. J.L. Cleland & C. Craik, Hanser, in press.

Exhibit B

SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

FOURTH EDITION



1987

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST

FOURTH EDITION

Tabulation and Analysis of
Amino Acid and Nucleic Acid Sequences of
Precursors, V-Regions, C-Regions, J-Chain,
T-Cell Receptor for Antigen, T-Cell Surface Antigens,
β_2-Microglobulins, Major Histocompatibility Antigens,
Thy-1, Complement, C-Reactive Protein, Thymopoietin,
Post-gamma Globulin, and α_2-Macroglobulin

1987

*Elvin A. Kabat**, *Tai Te Wu* †, *Margaret Reid-Miller* ‡,
Harold M. Perry ‡, and *Kay S. Gottesman* ‡

* Depts. of Microbiology, Genetics and Development, and Neurology, Cancer Center/Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, NY 10032 and the National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

† Depts. of Biochemistry, Molecular Biology, and Cell Biology, and Engineering Sciences and Applied Mathematics and Biomedical Engineering, Northwestern University, Evanston, IL 60201 and the Cancer Center, Northwestern University Medical School, Chicago, IL 60611

‡ Bolt Beranek and Newman Inc., Cambridge, MA 02238

The collection and maintenance of this data base is sponsored through Contract N01-RR-8-2158 by the following components of the National Institutes of Health, Bethesda, MD 20892:

Division of Research Resources
National Cancer Institute
National Institute of Allergy and Infectious Diseases
National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases
National Institute of General Medical Sciences

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health
(1987)

TABLE OF CONTENTS

INTRODUCTION . . . vii
REFERENCES TO INTRODUCTION . . . xxix

SIGNAL SEQUENCES

SIGNAL PEPTIDES OF KAPPA LIGHT CHAINS . . . 1
SIGNAL PEPTIDES OF LAMBDA LIGHT CHAINS . . . 8
SIGNAL PEPTIDES OF HEAVY CHAINS . . . 11
SIGNAL PEPTIDES OF BETA-2-MICROGLOBULINS . . . 19
SIGNAL PEPTIDES OF MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I . . . 21
SIGNAL PEPTIDES OF I REGION ANTIGENS CLASS II . . . 24
SIGNAL PEPTIDES OF COMPLEMENT . . . 28
SIGNAL PEPTIDES OF T-LYMPHOCYTE RECEPTOR FOR ANTIGEN . . . 30
SIGNAL PEPTIDES OF T-CELL SURFACE ANTIGENS . . . 36
SIGNAL PEPTIDES OF OTHER PROTEINS . . . 38

VARIABLE REGION LIGHT CHAIN SEQUENCES

HUMAN KAPPA LIGHT CHAINS SUBGROUP I . . . 41
HUMAN KAPPA LIGHT CHAINS SUBGROUP II . . . 50
HUMAN KAPPA LIGHT CHAINS SUBGROUP III . . . 53
HUMAN KAPPA LIGHT CHAINS SUBGROUP IV . . . 60

HUMAN LAMBDA LIGHT CHAINS SUBGROUP I . . . 63
HUMAN LAMBDA LIGHT CHAINS SUBGROUP II . . . 66
HUMAN LAMBDA LIGHT CHAINS SUBGROUP III . . . 69
HUMAN LAMBDA LIGHT CHAINS SUBGROUP IV . . . 72
HUMAN LAMBDA LIGHT CHAINS SUBGROUP V . . . 74
HUMAN LAMBDA LIGHT CHAINS SUBGROUP VI . . . 76

MOUSE KAPPA LIGHT CHAINS I . . . 78
MOUSE KAPPA LIGHT CHAINS II . . . 85
MOUSE KAPPA LIGHT CHAINS III . . . 95
MOUSE KAPPA LIGHT CHAINS IV . . . 102
MOUSE KAPPA LIGHT CHAINS V . . . 105
MOUSE KAPPA LIGHT CHAINS VI . . . 117
MOUSE KAPPA LIGHT CHAINS VII . . . 124
MOUSE KAPPA LIGHT CHAINS MISCELLANEOUS . . . 126

MOUSE LAMBDA LIGHT CHAINS . . . 129

RAT KAPPA LIGHT CHAINS . . . 134

RABBIT KAPPA LIGHT CHAINS . . . 136
RABBIT LAMBDA LIGHT CHAINS . . . 149

OTHER KAPPA LIGHT CHAINS . . . 151
OTHER LAMBDA LIGHT CHAINS . . . 154
MISCELLANEOUS LIGHT CHAINS . . . 157

VARIABLE REGION HEAVY CHAIN SEQUENCES

HUMAN HEAVY CHAINS SUBGROUP I . . . 160
HUMAN HEAVY CHAINS SUBGROUP II . . . 164
HUMAN HEAVY CHAINS SUBGROUP III . . . 167

MOUSE HEAVY CHAINS SUBGROUP I (A) . . . 176
MOUSE HEAVY CHAINS SUBGROUP I (B) . . . 181
MOUSE HEAVY CHAINS SUBGROUP II (A) . . . 186
MOUSE HEAVY CHAINS SUBGROUP II (B) . . . 192
MOUSE HEAVY CHAINS SUBGROUP II (C) . . . 200
MOUSE HEAVY CHAINS SUBGROUP III (A) . . . 204
MOUSE HEAVY CHAINS SUBGROUP III (B) . . . 214
MOUSE HEAVY CHAINS SUBGROUP III (C) . . . 219
MOUSE HEAVY CHAINS SUBGROUP III (D) . . . 222
MOUSE HEAVY CHAINS SUBGROUP V (A) . . . 226
MOUSE HEAVY CHAINS SUBGROUP V (B) . . . 232
MOUSE HEAVY CHAINS SUBGROUP MISCELLANEOUS . . . 235

RAT HEAVY CHAINS . . . 241

RABBIT HEAVY CHAINS . . . 243

GUINEA PIG HEAVY CHAINS . . . 248
CAT HEAVY CHAINS . . . 250
DOG HEAVY CHAINS . . . 252
CHICKEN HEAVY CHAINS . . . 254
SHARK HEAVY CHAINS . . . 256
MISCELLANEOUS HEAVY CHAINS . . . 259

T-LYMPHOCYTE RECEPTOR FOR ANTIGEN VARIABLE REGION (ALPHA CHAINS) . . . 262
T-LYMPHOCYTE RECEPTOR FOR ANTIGEN VARIABLE REGION (BETA CHAINS SUBGROUP I) . . . 266
T-LYMPHOCYTE RECEPTOR FOR ANTIGEN VARIABLE REGION (BETA CHAINS SUBGROUP II) . . . 274
T-LYMPHOCYTE RECEPTOR FOR ANTIGEN VARIABLE REGION (GAMMA CHAINS) . . . 279

CONSTANT REGION SEQUENCES

KAPPA LIGHT CONSTANT CHAINS . . . 282
LAMBDA LIGHT CONSTANT CHAINS . . . 287

HEAVY CONSTANT CHAINS CH1 REGION . . . 293
HEAVY CONSTANT CHAINS HINGE REGION . . . 300
HEAVY CONSTANT CHAINS CH2 REGION . . . 307
HEAVY CONSTANT CHAINS CH3 REGION . . . 314
HEAVY CONSTANT CHAINS EXTRA LONG CH3 REGION . . . 321
HEAVY CONSTANT CHAINS CH4 REGION . . . 323
HEAVY CONSTANT CHAINS EXTRA LONG CH4 AND MEMBRANE BOUND REGIONS . . . 325

SEQUENCES OF RELATED PROTEINS

J CHAIN . . . 332

BETA-2-MICROGLOBULINS . . . 334

MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I N REGION . . . 337
MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I C1 REGION . . . 341
MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I C2 REGION . . . 345
MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I MEMBRANE REGION . . . 349
MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I CYTOPLASMIC REGION . . . 353

I REGION GENE PRODUCTS CLASS II A-CHAIN A1 REGION . . . 359
I REGION GENE PRODUCTS CLASS II A-CHAIN A2 REGION . . . 362
I REGION GENE PRODUCTS CLASS II A-CHAIN MEMBRANE BOUND REGION . . . 365

I REGION GENE PRODUCTS CLASS II B-CHAIN B1 REGION . . . 370

I REGION GENE PRODUCTS CLASS II B-CHAIN B2 REGION 375
I REGION GENE PRODUCTS CLASS II B-CHAIN MEMBRANE BOUND REGION 380

THY-1 ANTIGENS 387

HUMAN COMPLEMENT COMPONENTS 389
OTHER COMPLEMENT COMPONENTS 406

T-LYMPHOCYTE RECEPTOR FOR ANTIGEN CONSTANT REGION 421

T-CELL SURFACE ANTIGENS 429

MISCELLANEOUS PROTEINS OF THE IMMUNOGLOBULIN SUPERFAMILY 434
MISCELLANEOUS PROTEINS ASSOCIATED WITH THE IMMUNE SYSTEM 439

CODONS OF NUCLEIC ACID SEQUENCES

CODONS OF SIGNAL PEPTIDES OF KAPPA LIGHT CHAINS 452
CODONS OF SIGNAL PEPTIDES OF LAMBDA LIGHT CHAINS 458
CODONS OF SIGNAL PEPTIDES OF HEAVY CHAINS 460
CODONS OF SIGNAL PEPTIDES OF BETA-2MICROGLOBULINS 467
CODONS OF SIGNAL PEPTIDES OF MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I 469
CODONS OF SIGNAL PEPTIDES OF I REGION ANTIGENS CLASS II 471
CODONS OF SIGNAL PEPTIDES OF COMPLEMENT 476
CODONS OF SIGNAL PEPTIDES OF T-LYMPHOCYTE RECEPTOR FOR ANTIGEN 478
CODONS OF SIGNAL PEPTIDES OF T-CELL SURFACE ANTIGENS 489
CODONS OF SIGNAL PEPTIDES OF OTHER PROTEINS 491

CODONS OF VARIABLE REGION KAPPA LIGHT CHAINS 494
CODONS OF VARIABLE REGION LAMBDA LIGHT CHAINS 506
CODONS OF VARIABLE REGION HEAVY CHAINS 508

CODONS OF KAPPA LIGHT CONSTANT CHAINS 526
CODONS OF LAMBDA LIGHT CONSTANT CHAINS 528

CODONS OF HEAVY CONSTANT CHAINS CH1 REGION 530
CODONS OF HEAVY CONSTANT CHAINS HINGE REGION 533
CODONS OF HEAVY CONSTANT CHAINS CH2 REGION 536
CODONS OF HEAVY CONSTANT CHAINS CH3 REGION 539
CODONS OF HEAVY CONSTANT EXTRA LONG CH3 REGION 542
CODONS OF HEAVY CONSTANT CHAINS CH4 REGION 545
CODONS OF HEAVY CONSTANT EXTRA LONG CH4 AND MEMBRANE BOUND REGIONS 548

CODONS OF BETA-2-MICROGLOBULINS 553

CODONS OF MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I N REGION 555
CODONS OF MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I C1 REGION 557
CODONS OF MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I C2 REGION 559
CODONS OF MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I MEMBRANE REGION 561
CODONS OF MAJOR HISTOCOMPATIBILITY ANTIGENS CLASS I CYTOPLASMIC REGION 563

CODONS OF I REGION GENE PRODUCTS CLASS II A-CHAIN A1 REGION 566
CODONS OF I REGION GENE PRODUCTS CLASS II A-CHAIN A2 REGION 568
CODONS OF I REGION GENE PRODUCTS CLASS II A-CHAIN MEMBRANE REGION 570

CODONS OF I REGION GENE PRODUCTS CLASS II B-CHAIN B1 REGION 573
CODONS OF I REGION GENE PRODUCTS CLASS II B-CHAIN B2 REGION 575
CODONS OF I REGION GENE PRODUCTS CLASS II B-CHAIN MEMBRANE BOUND REGION 577

CODONS OF HUMAN COMPLEMENT COMPONENTS 580
CODONS OF OTHER COMPLEMENT COMPONENTS 595

CODONS OF T-LYMPHOCYTE RECEPTOR FOR ANTIGEN VARIABLE REGION (ALPHA CHAINS) 610
CODONS OF T-LYMPHOCYTE RECEPTOR FOR ANTIGEN VARIABLE REGION (BETA CHAINS SUBGROUP I) 616
CODONS OF T-LYMPHOCYTE RECEPTOR FOR ANTIGEN VARIABLE REGION (BETA CHAINS SUBGROUP II) 623
CODONS OF T-LYMPHOCYTE RECEPTOR FOR ANTIGEN VARIABLE REGION (GAMMA CHAINS) 628

CODONS OF T-LYMPHOCYTE RECEPTOR FOR ANTIGEN CONSTANT REGION 630

CODONS OF T-CELL SURFACE ANTIGENS 637

CODONS OF MISCELLANEOUS PROTEINS OF THE IMMUNOGLOBULIN SUPERFAMILY 641
CODONS OF MISCELLANEOUS PROTEINS ASSOCIATED WITH THE IMMUNE SYSTEM 644

CODONS OF HUMAN D-MINIGENES 656
CODONS OF MOUSE D-MINIGENES 659

CODONS OF HUMAN T-LYMPHOCYTE RECEPTOR FOR ANTIGEN D-MINIGENES 663
CODONS OF MOUSE T-LYMPHOCYTE RECEPTOR FOR ANTIGEN D-MINIGENES 664

CODONS OF HUMAN KAPPA J-MINIGENES 665
CODONS OF HUMAN LAMBDA J-MINIGENES 666

CODONS OF MOUSE KAPPA J-MINIGENES 667
CODONS OF MOUSE LAMBDA J-MINIGENES 668

CODONS OF RAT KAPPA J-MINIGENES 669

CODONS OF RABBIT KAPPA J-MINIGENES 671

CODONS OF HUMAN HEAVY J-MINIGENES 674
CODONS OF MOUSE HEAVY J-MINIGENES 675

CODONS OF HUMAN T-LYMPHOCYTE RECEPTOR FOR ANTIGEN ALPHA CHAIN J-MINIGENES 677
CODONS OF HUMAN T-LYMPHOCYTE RECEPTOR FOR ANTIGEN BETA CHAIN J-MINIGENES 680
CODONS OF HUMAN T-LYMPHOCYTE RECEPTOR FOR ANTIGEN GAMMA CHAIN J-MINIGENES 683

CODONS OF MOUSE T-LYMPHOCYTE RECEPTOR FOR ANTIGEN ALPHA CHAIN J-MINIGENES 684
CODONS OF MOUSE T-LYMPHOCYTE RECEPTOR FOR ANTIGEN BETA CHAIN J-MINIGENES 685

PSEUDOGENES OF IMMUNOGLOBULIN VARIABLE REGIONS 687
PSEUDOGENES OF T-LYMPHOCTYE RECEPTOR FOR ANTIGEN VARIABLE REGIONS 699
PSEUDOGENES OF IMMUNOGLOBULIN CONSTANT REGIONS 705
PSEUDOGENES OF OTHER PROTEINS 711

SUMMARY TABLES

AMINO ACID DISTRIBUTION IN ALL VARIABLE REGION LIGHT CHAINS 716
AMINO ACID DISTRIBUTION IN ALL VARIABLE REGION HEAVY CHAINS 718

CODON DISTRIBUTION IN ALL VARIABLE REGION KAPPA LIGHT CHAINS 720
CODON DISTRIBUTION IN ALL VARIABLE REGION LAMBDA LIGHT CHAINS 723
CODON DISTRIBUTION IN ALL VARIABLE REGION HEAVY CHAINS 726

VARIABILITY PLOTS

LIGHT CHAINS 730
HEAVY CHAINS 730

KAPPA LIGHT CHAINS 731
LAMBDA LIGHT CHAINS 731

HUMAN LIGHT CHAINS 732
HUMAN HEAVY CHAINS 732

HUMAN KAPPA LIGHT CHAINS 733
HUMAN LAMBDA LIGHT CHAINS 733

HUMAN KAPPA LIGHT CHAINS SUBGROUP I 734
HUMAN KAPPA LIGHT CHAINS SUBGROUP II 734
HUMAN KAPPA LIGHT CHAINS SUBGROUP III 734

HUMAN HEAVY CHAINS SUBGROUP I 735
HUMAN HEAVY CHAINS SUBGROUP II 735
HUMAN HEAVY CHAINS SUBGROUP III 736

HUMAN LAMBDA LIGHT CHAINS SUBGROUP I 737
HUMAN LAMBDA LIGHT CHAINS SUBGROUP II 737
HUMAN LAMBDA LIGHT CHAINS SUBGROUP III 737

MOUSE KAPPA LIGHT CHAINS 738

RABBIT KAPPA LIGHT CHAINS 738

MOUSE KAPPA LIGHT CHAINS SUBGROUP II 739
MOUSE KAPPA LIGHT CHAINS SUBGROUP III 739
MOUSE KAPPA LIGHT CHAINS SUBGROUP V 739
MOUSE KAPPA LIGHT CHAINS SUBGROUP VI 739

MOUSE HEAVY CHAINS SUBGROUP IA 740
MOUSE HEAVY CHAINS SUBGROUP IB 740
MOUSE HEAVY CHAINS SUBGROUP IIA 741
MOUSE HEAVY CHAINS SUBGROUP IIB 741
MOUSE HEAVY CHAINS SUBGROUP IIC 741
MOUSE HEAVY CHAINS SUBGROUP IIIIA 742
MOUSE HEAVY CHAINS SUBGROUP IIIIB 742

T-LYMPHOCYTE RECEPTOR FOR ANTIGEN ALPHA CHAINS 743
T-LYMPHOCYTE RECEPTOR FOR ANTIGEN BETA CHAINS 743
T-LYMPHOCYTE RECEPTOR FOR ANTIGEN BETA CHAINS SUBGROUP I 744
T-LYMPHOCYTE RECEPTOR FOR ANTIGEN BETA CHAINS SUBGROUP II 744

CYTOCHROMES C 745

HUMAN HLA DR BETA CHAIN CLASS II 746
HUMAN HLA DC BETA CHAIN CLASS II 746

MOUSE MHC IA ALPHA CHAIN CLASS II 746
MOUSE MHC IE BETA CHAIN CLASS II 746

INDICES

INDEX OF PROTEINS 747
INDEX OF ANTIBODY SPECIFICITIES 795

considered uncertain by the authors have not been included in the table. In some instances the symbol # is used to indicate that several amino acid residues were found in one position, and these residues are listed in the notes. The four columns at the end of each table give:

1. the number of residues sequenced at that position,
2. the number of different amino acids found at that position,
3. the number of times the most common amino acid occurred and that amino acid in parentheses, and
4. the variability.

Variability is calculated (11) as:

$$\text{Variability} = \frac{\text{Number of different amino acids occurring at a given position}}{\text{Frequency of the most common amino acid at that position}}$$

An invariant position would have a variability of one; if 20 amino acids occurred with equal frequency, the variability would be 20 divided by 0.05 equals 400. If, for example, four different amino acids Ser, Asp, Pro, and Thr occurred at a given position, and of 100 sequences available at that position, Ser occurred 80 times, the variability would be 4/0.8 = 5. When any of the amino acid residues sequenced were not identified completely and are listed as Glx (or Asx), two values, separated by a colon, are given in the last three columns. The first value in each of these columns is calculated assuming that only one of the two possibilities, e.g., Glu or Gln (or Asp or Asn) occurred, while the second considers that both were present and maximizes variability. In the variability plots, the horizontal bars indicate the two values.

When two or more amino acids are most common and occur with equal frequency, they are tabulated as a note, and the symbol + is used in the next to last column. If no sequence data have been reported for any position, there are no entries in the last four columns. Variability is not calculated for insertions or if only a single sequence is known. When the translated sequence of a clone corresponds to a previously listed sequence of a plasmacytoma from which it was prepared, only one sequence is listed so that the variability computations are not affected, and a note is included.

If a given sequence is associated with any antibody activity, this is indicated by an asterisk alongside the protein heading, and the antibody specificities are given in a separate list with binding constants if available. The notes list the a-allotypes for the rabbit heavy chain V-region and the b-allotypes for the constant domain of the rabbit kappa light chain. A key reference to the sequence is given; generally the most recent reference since it is usually the most nearly complete, but often several references are included, especially when revisions of a sequence have been made. Notes are now of two types; general notes about a table indicated by the symbol #, and specific notes indicated by the sequence number.

Signal Sequences

The signal (precursor) amino acid sequences of immunoglobulin chains are listed in three tables: one for kappa light chains, one for lambda light chains, and one for heavy chains. They were obtained either by direct sequencing of signal proteins (12-14) or by translating nucleotide sequences from DNA clones. Signal segments range from 17-29 amino acid residues in length and are thus numbered from -29 to -1. Genomic DNA clones contain introns of varying length that interrupt the coding sequence of the precursor within the codon for position -4, and in rare cases for position -6. Thus, the L-gene encodes the leader peptide to position -4 and the 5′ end of the V-gene codes for positions -4 to -1.

The signal amino acid sequences of the T-cell receptors for antigens, β_2-microglobulins, major histocompatibility complex proteins, and complement components are listed in separate tables.

By conformational energy calculations, the core V_κ hydrophobic Leu-Leu-Leu-Trp-Val-Leu-Leu-Leu (MOPC321, MOPC63) exists in an alpha helical conformation, terminated by chain reversal conformations in the four C-terminal residues Trp-Val-Pro-Gly; the four amino terminal residues are compatible with the alpha helix (15).

Variable Region Sequences

The variable regions (16) of immunoglobulins have been shown to contain hypervariable segments in their light (11,17-23) and heavy (22,24-27) chains, of which certain residues have been affinity labeled (28-41). Three hypervariable segments of light chain were delineated from a statistical examination of sequences of human V_κ, human V_λ, and mouse V_κ light chains aligned for maximum homology (11,22). These and the three corresponding segments of the heavy chains (22,26,27) were hypothesized (11,22) to be the complementarity-determining regions or segments (CDR) containing the residues which make contact with various antigenic determinants, and this has been verified by X-ray diffraction studies at high resolution (42-67). The rest of the V-region constitutes the framework (11,22,66-68). It is convenient to identify the framework segments (FR1, FR2, FR3, and FR4) and the complementarity-determining segments (CDR1, CDR2, and CDR3) with the three CDRs separating the four FRs. The residue numbers for these segments are as follows:

Segment	*Light Chain*	*Heavy Chain*
FR1	1-23 (with an occasional residue at 0, and a deletion at 10 in V_λ chains)	1-30 (with an occasional residue at 0)
CDR1	24-34 (with possible insertions numbered as 27A,B,C,D,E,F)	31-35 (with possible insertions numbered as 35A,B)
FR2	35-49	36-49
CDR2	50-56	50-65 (with possible insertions numbered as 52A,B,C)[a]
FR3	57-88	66-94 (with possible insertions numbered as 82A,B,C)
CDR3	89-97 (with possible insertions numbered as 95A,B,C,D,E,F)	95-102 (with possible insertions numbered as 100A,B,C,D,E,F,G,H,I,J,K)
FR4	98-107 (with a possible insertion numbered as 106A)	103-113

[a] In the rabbit, Mage *et al.* (69) consider position 65 in V_H to be in FR3, since it is allotype related.

In the tables of V-regions, the FR and CDR are separated by horizontal lines for convenience in reading. One mouse kappa light chain, MPC11, has an extra segment of 12 amino acid residues between position 1 and the signal sequence (70). Several chains have internal deletions.

In the tables, the V-genes for the light chains code to amino acid position 95, and the J-minigenes from position 97 to 107 for lambda and 108 for kappa light chains. Position 96 is usually the site of V-J joining by recombination and may be coded partly by the V-gene and partly by the J-minigene. Because the site of V-J recombination could occur at different positions within a codon, different amino acid residues may result at this position. We have changed the location of the inserted residues from 97A-F (2) to 95A-F, since it makes for better alignment by confining chains of different lengths to the V-gene region. In V_κ chains, J1 and J2 were used 5 to 10 times more frequently than J4 and J5 (71).

The V-genes for the heavy chains code up to amino acid position 94 and are followed by the D- and J-minigenes. Because of the extensive variation in the lengths of D-minigenes, the exact boundary between D and J is not always located at the same amino acid position. In addition, the lengths of the J encoded amino acid sequences vary by a few amino acid residues. Moreover, the process of D-J joining appears to involve insertions of extra nucleotides between V and D and between D and J, termed the N region (72-76) and correlates with the appearance of terminal deoxytransferase in B cells (75). The original numbering system for the heavy chains has therefore been retained. Wysocki *et al.* (76) have provided some evidence suggesting a non-random origin for the V_H-D_H junction, perhaps a minigene, rather than random addition of the N nucleotides.

It has become evident that a critical understanding of the architecture of antibody combining sites and the genetics of the generation of diversity and of antibody complementarity will depend to a great extent on the evaluation of a large number of sequences of the variable regions and especially of the complementarity-determining segments of light and heavy chains of immunoglobulins of different species. Ability to locate residues in the site making contact with antigenic determinants (77) and to predict (67,78-82) the structures of antibody combining sites will depend heavily upon such sequences.

Figures 1 and 2 are stereoviews of the α-carbon skeletons of the four Fv regions for which high resolution X-ray structures have been determined, NEWM (44), KOL (62), MCPC603 (47, 48, 63), and J539 (64). The residues in the CDRs are shown as solid circles. In Fig. 1 the combining site is at the

HUMAN KAPPA LIGHT CHAINS SUBGROUP I

		INVARIANT RESIDUES	1 ROY	2 AU	3 REI	4 HAU	5 HK101 'CL #	6 SCW	7 AG #	8* WEA	9 HK 137 'CL #	10 HK 134 'CL #	11 DAUDI 'CL	12 WALKER 'CL	13 HF3-16/6	14 HF2-1/13B	15 HF2-18/2	16 HF2-1/17	17 BJ 26 #	18 RFZ	19 PSM	20 HOM	21 ESM IGG	22 ESM IGM	23 WAT
FR 1	0		---	---	---	---	#	---	---	---	---	#	---	---	---	---	---	---	---	---	---	---	---	---	---
	1		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	2		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	3		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	4		MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET
	5	THR(.98)	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	7	SER(.95)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO(.98)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	10		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	11		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	12	SER(.96)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	14		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	15	VAL(.96)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY(.99)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	18		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
	19	VAL(.97)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	20	THR(.96)	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	21	ILE(.95)	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	22		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
CDR 1	24		GLN	GLN	GLN	ARG	ARG	GLN	GLN	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	GLN	GLN	GLN	GLN	ARG	ARG	GLX
	25	ALA(.95)	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	26		SER	SER	SER	SER	ARG	SER	SER	SER	SER	SER	GLY	SER	SER	SER	SER	SER	SER	ASN	SER	ARG	SER	SER	SER
	27		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	HIS	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	HIS	GLX	GLX	GLN
	27A		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	27B		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	27C		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	27D		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	27E		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	27F		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	28		ASP	ASP	ASP	SER	GLY	ASP	ASP	GLY	GLY	GLY	ASN	SER	GLY	GLY	GLY	GLY	SER	VAL	ASP	ASP	SER	SER	ASP
	29		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	30		SER	SER	ILE	SER	SER	ARG	ASN	ARG	SER	SER	THR	SER	ARG	ARG	ARG	ARG	ASN	SER	ARG	SER	SER	SER	SER
	31		ILE	ASP	LYS	SER	SER	LYS	HIS	ASN	ASN	SER	ASN	ASN	ASN	ASN	ASN	ASN	LYS	LYS	SER	ASN	SER	SER	ASP
	32		PHE	TYR	TYR	TYR	TRP	HIS	TYR	ASP	TYR	TRP	PHE	TYR	ASP	ASP	ASP	ASP	TYR	SER	TYR	TYR	TYR	TYR	TYR
	33		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	ALA	LEU	LEU	LEU	LEU	LEU	VAL
	34		ASN	ASN	ASN	SER	ALA	ASN	ASN	THR	ALA	ALA	SER	ASN	GLY	GLY	GLY	GLY	ASN	ASN	ASN	ASN	ASX	ASX	ASN
FR 2	35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP
	36		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	PHE	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	PHE	TYR	TYR	
	37		GLN	GLN	GLN	GLN	GLN	ASP	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLU	GLN	GLN	GLN	GLX	GLX	
	38		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN			
	39		LYS	LYS	THR	LYS	LYS	LYS	#	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	#	ARG	LYS	LYS			
	40	PRO(.95)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	GLN	PRO			
	41		GLY	GLY	GLY	GLY	GLU	GLY	#	GLY	GLY	GLU	GLY	GLY					#	GLY	GLY	GLY			
	42		LYS	LYS	LYS	LYS	LYS	LYS	LYS	THR	LYS	LYS	LYS	LYS					LYS	GLN	LYS				
	43		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA					ALA	ALA	ALA				
	44	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO					PRO	PRO	PRO				
	45		LYS	LYS	LYS	GLN	LYS	ARG	LYS	LYS	LYS	LYS	THR	LYS					LYS	LYS	LYS				
	46		LEU	LEU	LEU	VAL	SER	LEU	ILE	ARG	SER	SER	LEU	LEU					LEU	LEU	LEU				
	47	LEU(.98)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU						LEU	LEU				
	48	ILE(.98)	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE						ILE	ILE				
	49		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR						TYR	TYR				
CDR 2	50		ASP	ASP	GLU	ALA	ALA	GLY	ASP	GLY	ALA	ALA	ALA	ALA						ASP	ASP				
	51		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	VAL	ALA					ASP	ALA	ALA				
	52		SER	SER	SER	SER	SER	SER	SER	THR	SER	SER	SER	SER					SER	ALA	SER				
	53		LYS	ASN	ASN	SER	SER	THR	ASN	SER	SER	SER	ASN	SER					ARG	ASX					
	54	LEU(.98)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU					LEU	LEU	LEU				
	55		GLU	GLU	GLN	PRO	GLN	GLU	GLU	GLN	GLN	GLN	GLN	GLN					GLU	GLU	GLU				
	56		ALA	SER	ALA	SER	SER	THR	THR	SER	SER	SER	VAL	SER					THR						
FR 3	57	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY					GLY	GLY					
	58	VAL(.98)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL					ASX	VAL					
	59		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	THR					PRO	PRO					
	60	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER					SER						
	61	ARG(.95)	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG					LYS	ARG					
	62	PHE(.95)	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE					VAL	PHE					
	63		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER					SER	THR					
	64	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY					GLY	GLY					
	65		THR	GLY	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER					SER						
	66	GLY(.95)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY					GLY	GLY					
	67		SER	SER	SER	SER	SER	SER	PHE	SER	SER	SER	SER	SER					SER						
	68		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY					GLU						
	69		THR	ALA	THR	THR	THR	THR	THR	THR	THR	THR	ALA	THR					THR						
	70		ASP	HIS	ASP	ASP	ASP	ASP	ASP	GLU	ASP	ASP	GLU	ASP					ASP						
	71		PHE	PHE	TYR	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE					VAL						
	72		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR					THR						
	73		PHE	PHE	PHE	LEU	LEU	LEU	PHE	LEU	LEU	LEU	LEU	LEU					VAL						
	74		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR					ASX						
	75	ILE(.95)	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE					GLX						
	76	SER(.95)	SER	SER	SER	SER	SER	SER	SER	ASN	SER	SER	SER	SER					SER						
	77		GLY	SER	SER	SER	SER	THR	GLY	SER	SER	SER	SER	SER					SER						
	78		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU					LEU						
	79		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN					GLN						
	80		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO					PRO						
	81		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU					GLU						
	82		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP					ASP						
	83		ILE	ILE	ILE	PHE	PHE	ILE	ILE	PHE	PHE	PHE	PHE	SER					ILE						
	84	ALA(.98)	ALA	ALA	ALA	ALA	ALA	GLY	ALA	ALA	ALA	ALA	ALA	ALA					ALA						
	85		THR	THR	THR	THR	THR	ASN	THR	THR	THR	THR	THR	THR					PRO						
	86	TYR(.98)	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR					LYS						
	87	TYR(.98)	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR											
	88	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS											
CDR 3	89		GLN	GLN	GLN	GLN	GLN	GLN	GLN	LEU	GLN	GLN	GLN	GLN					GLN						
	90		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN					GLN						
	91		PHE	TYR	TYR	ASN	TYR	TYR	TYR	TYR	TYR	TYR	ASN	SER					ARG						
	92		ASP	ASP	GLN	TYR	ASN	ASP	ASP	SER	ASN	ASN	TYR	TYR					ASP						
	93		ASN	TYR	SER	ILE	SER	ASN	THR	SER	SER	SER	ASN	SER					ASP						
	94		LEU	LEU	LEU	THR	TYR	VAL	LEU	PHE	TYR	TYR	PHE	THR					LEU						
	95		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	SER	LEU					PRO						
	95A		---	---	---	---	#	---	---	---	---	#	---	---					---						
	95B		---	---	---	---	---	---	---	---	---	---	---	---					---						
	95C		---	---	---	---	---	---	---	---	---	---	---	---					---						
	95D		---	---	---	---	---	---	---	---	---	---	---	---					---						
	95E		---	---	---	---	---	---	---	---	---	---	---	---					---						
	95F		---	---	---	---	---	---	---	---	---	---	---	---					---						
	96		LEU	TRP	TYR	THR	---	ILE	ARG	TRP	---	---	PHE	ILE					TYR						
	97		THR	THR	THR	SER	---	THR	THR	THR	---	---	THR	THR					THR						
FR 4	98		PHE	PHE	PHE	PHE	---	PHE	PHE	PHE	---	---	PHE	PHE					LEU						
	99	GLY	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	---	---	GLY	GLY					GLY						
	100		GLY	GLN	GLN	GLN	---	GLN	GLN	GLN	---	---	GLY	GLN					GLY						
	101	GLY	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	---	---	GLY	GLY					GLY						
	102	THR(.97)	THR	THR	THR	THR	---	THR	THR	THR	---	---	THR	THR					THR						
	103		LYS	LYS	LYS	ARG	---	ARG	LYS	LYS	---	---	LYS	ARG					LYS						
	104		VAL	VAL	LEU	VAL	---	VAL	LEU	VAL	---	---	VAL	LEU					VAL						
	105		ASP	GLU	GLN	GLU	---	GLU	GLU	GLU	---	---	ASP	GLU					GLU						
	106		PHE	ILE	ILE	ILE	---	ASN	ILE	VAL	---	---	ASN	ILE					MET						
	106A		---	---	---	---	---	---	---	---	---	---	---	---					---						
	107		LYS	LYS	THR	LYS	---	LYS	LYS	LYS	---	---	LYS	LYS					LYS						
	108		ARG	ARG	ARG	ARG	---	GLY	ARG	ARG	---	---	---	---											
	109	THR	THR	THR	THR	THR	---	THR	THR	THR	---	---	---	---											

HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		24 AMYLOID VIII-B	25* LOW	26 DIE	27 CAR A	28 TEI	29 BJ 48	30 CON	31 TRA	32 AMYLOID LEP	33 F-GUI #	34 OU (IOC)	35 DEE	36 GAL (I)	37 JOH	38 KER	39* LAY	40 BRA	41 WES	42 Vb 'CL	43 Vb' 'CL	44 HK102 'CL #	45 EU	46 DEN	47 PAU
FR 1	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	#	---	---	---
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	3	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	4	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	10	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	thr	thr	thr
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	val	val	LEU	LEU	LEU	LEU
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	val	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	15	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASX	ASP	ASP	ASP	ASP	ASP	glx	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	18	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
	19	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	ile	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	20	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	21	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	22	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR	ile	ser	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	23	CYS	CYS	CYS	CYS	CYS	CYS				CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
CDR 1	24	GLN	ALA									ARG	ARG	ARG		GLN	GLN		ARG	ARG	ARG	ARG	ARG	ARG	ARG
	25	ALA	ALA								ALA	ALA	ALA	ALA		ALA	ALA		ALA	ALA	ALA	ALA	ALA	THR	ALA
	26	SER	SER									SER	GLY	SER		SER	SER		SER	SER	SER	SER	SER	SER	SER
	27	GLN	GLN									GLX	GLN	GLN		GLN	GLN		GLN	GLN	GLN	GLN	GLN	GLN	GLN
	27A	---	---									---	---	---		---	---		---	---	---	---	---	---	---
	27B	---	---									---	---	---		---	---		---	---	---	---	---	---	---
	27C	---	---									---	---	---		---	---		---	---	---	---	---	---	---
	27D	---	---									---	---	---		---	---		---	---	---	---	---	---	---
	27E	---	---									---	---	---		---	---		---	---	---	---	---	---	---
	27F	---	---									---	---	---		---	---		---	---	---	---	---	---	---
	28	ASX	ASX									THR	SER	GLY		ASP	ASN		ASP	GLY	GLY	SER	SER	SER	SER
	29	ILE	ILE									ILE	VAL	ILE		ILE	VAL		ILE	ILE	ILE	ILE	ILE	ILE	ILE
	30	GLY	SER									SER	ASN	ARG		LYS	ASN		SER	SER	SER	SER	ASN	SER	SER
	31											SER	LYS	ASN			ALA		HIS	SER	SER	SER	THR	ARG	SER
	32	TYR										TYR	TYR	ASP			TYR		TRP	TRP	TRP	TRP	TRP	TRP	SER
	33	LEU										LEU	LEU	LEU			LEU		LEU	LEU	LEU	LEU	LEU	LEU	LEU
	34											ASX	ASN	THR			ASN		ALA	ALA	ALA	ALA	ALA	ALA	ALA
FR 2	35	TRP										TRP	TRP	TRP			TRP		TRP	TRP	TRP	TRP	TRP	TRP	TRP
	36											TYR	TYR	TYR			TYR		TYR	TYR	TYR	TYR	TYR	TYR	TYR
	37											GLX	GLN	GLN			GLN		GLN	GLN	GLN	GLN	GLN	GLN	GLX
	38											GLX	GLN	GLN			GLN		GLN	GLN	GLN	GLN	GLN	GLN	GLX
	39											LYS	LYS	LYS			LYS		LYS	LYS	LYS	LYS	LYS	LYS	LYS
	40											PRO	PRO	PRO			PRO		SER	PRO	PRO	PRO	PRO	PRO	PRO
	41											GLY	GLY	GLY			GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY
	42											LYS	LYS	LYS			LEU		LYS	LYS	LYS	LYS	LYS	GLU	GLX
	43						ALA					ALA	ALA	ALA			ALA		ALA	ALA	ALA	ALA	ALA	ALA	ALA
	44						PRO					PRO	PRO	PRO			PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO
	45						LYS					ASX	LYS	LYS			LYS		LYS	LYS	LYS	LYS	LYS	LYS	LYS
	46											LEU	VAL	GLU			LEU		LEU	LEU	LEU	LEU	LEU	VAL	LEU
	47											LEU	LEU	LEU			LEU		LEU	LEU	LEU	LEU	LEU	LEU	LEU
	48											ILE		ILE			ILE		ILE	ILE	ILE	ILE	MET	ILE	ILE
	49						GLY					TYR		TYR			TYR		TYR	TYR	TYR	TYR	TYR	TYR	TYR
CDR 2	50						ASP					ALA	ALA	ALA			GLY		SER	ALA	ALA	ASP	LYS	GLY	GLX
	51						ALA					ALA	ALA	ALA			ALA		ALA	ALA	ALA	ALA	ALA	ALA	
	52						SER					SER	SER	SER			SER		SER	SER	SER	SER	SER	SER	
	53						SER					ASX	SER	ASN			THR		SER	SER	SER	SER	SER	ASN	
	54						LEU					LEU	LEU	LEU			ARG		LEU	LEU	LEU	LEU	LEU	LEU	
	55						ASX					HIS	LYS	GLN			GLU		GLU	GLN	GLN	GLU	GLU	GLU	
	56						SER					SER	SER	SER			ALA		ASN	SER	SER	SER	SER	SER	
FR 3	57						GLY					GLY	GLY	GLY			GLY		GLY	GLY	GLY	GLY	GLY	GLY	
	58						VAL					VAL	VAL	VAL			VAL		VAL	VAL	VAL	VAL	VAL	VAL	
	59						VAL					PRO	PRO	PRO			PRO		PRO	PRO	PRO	PRO	PRO	PRO	
	60						SER					SER	SER	SER			SER		SER	SER	SER	SER	SER	SER	
	61						ARG					ARG	ARG	ARG			ARG		ARG	ARG	ARG	ARG	ARG	ARG	
	62						GLN					PHE	PHE	PHE			PHE		PHE	PHE	PHE	PHE	PHE	PHE	
	63						SER					SER	SER	SER			SER		SER	SER	SER	SER	ILE	SER	
	64						GLY					GLY	GLY	GLY			GLY		GLY	GLY	GLY	GLY	GLY	GLY	
	65						SER					SER	SER	SER			SER		SER	SER	SER	SER	SER	SER	
	66						GLY					GLY	GLY	GLY			GLY		GLY	GLY	GLY	GLY	GLY	GLY	
	67						SER					SER	SER	ALA			SER		SER	SER	SER	SER	SER	SER	
	68						GLY					GLY	GLY	GLY			GLY		GLY	GLY	GLY	GLY	GLY	GLY	
	69						THR					THR	THR	THR			THR		THR	THR	THR	THR	THR	THR	
	70						ASP					ASX	ASP	GLU			ASP		GLU	ASP	ASP	GLU	GLU	GLU	
	71						PRO					PHE	PHE	PHE			PHE		PHE	PHE	PHE	PHE	PHE	PHE	
	72						SER					THR	THR	THR			THR		THR	THR	THR	THR	THR	THR	
	73						LEU					PHE	LEU	LEU			PHE		LEU	LEU	LEU	LEU	LEU	LEU	
	74						ALA					THR	THR	THR			THR		THR	THR	THR	THR	THR	THR	
	75						VAL					ILE	ILE	ILE			ILE		ILE	ILE	ILE	ILE	ILE	ILE	
	76						SER					SER	SER	SER			SER		SER	SER	SER	SER	SER	SER	
	77						ARG					GLY	GLY	SER			SER		SER	SER	SER	SER	SER	SER	
	78											LEU	LEU	LEU			LEU		LEU	LEU	LEU	LEU	LEU	LEU	
	79											GLX	LEU	GLN			GLN		GLN	GLN	GLN	GLN	GLN	GLN	
	80											PRO	PRO	PRO			PRO		PRO	PRO	PRO	PRO	PRO	SER	
	81											GLX	GLU	GLU			GLU		GLU	GLU	GLU	ASP	ASP	ASP	
	82											ASP	ASP	ASP			ASP		ASP	ASP	ASP	ASP	ASP	ASP	
	83											PHE	PHE	PHE			ILE		PHE	PHE	PHE	PHE	PHE	PHE	
	84											ALA	ALA	ALA			ALA		ALA	ALA	ALA	ALA	ALA	ALA	
	85											THR	THR	THR			THR		THR	THR	THR	THR	THR	THR	
	86											TYR	TYR	TYR		TYR	TYR		TYR	TYR	TYR	TYR	TYR	TYR	
	87											TYR	TYR	TYR		TYR	TYR		PHE	TYR	TYR	TYR	TYR	TYR	
	88											CYS	CYS	CYS		CYS	CYS		CYS	CYS	CYS	CYS	CYS	CYS	
CDR 3	89											GLX	GLX	LEU		GLN	GLN		GLN	GLN	GLN	GLN	GLN	GLN	
	90											GLX	GLX	GLN		GLN	GLN		GLN	GLN	GLN	GLN	GLN	GLN	
	91						GLY					SER	SER	GLN		TYR	TYR		ALA	ALA	ALA	TYR	TYR	TYR	
	92						GLU					TYR	TYR	ASN		ASP	ASN		HIS	ASN	ASN	ASN	ASN	ASP	
	93						SER					SER	THR	SER		ASP	ASN		SER	SER	SER	SER	SER	SER	
	94						ASP					SER	THR	TYR		LEU	TRP		VAL	PHE	PHE	TYR	ASP	PHE	
	95						SER					PRO	PRO	PRO		PRO	PRO		PRO	PRO	PRO	SER	SER	PRO	
	95A						---					---	---	---		---	---		---	---	---	#	---	---	
	95B						---					---	---	---		---	---		---	---	---	---	---	---	
	95C						---					---	---	---		---	---		---	---	---	---	---	---	
	95D						---					---	---	---		---	---		---	---	---	---	---	---	
	95E						---					---	---	---		---	---		---	---	---	---	---	---	
	95F						---					---	---	---		---	---		---	---	---	---	---	---	
	96						ARG					THR	TYR	ARG		PRO	PRO		LEU	---	---	---	LYS	TYR	
	97						THR					THR	THR	SER		THR	THR		THR	---	---	---	MET	THR	
FR 4	98						PHE					PHE	PHE	PHE		PHE	PHE		PHE	---	---	---	PHE	PHE	
	99						GLY					GLY	GLY	GLY		GLY	GLY		GLY	---	---	---	GLY	GLY	
	100						GLN					GLX	PRO	GLN		PRO	GLN		GLY	---	---	---	GLN	GLN	
	101						GLY					GLY	GLY	GLY		GLY	GLY		GLY	---	---	---	GLY	GLY	
	102						THR					THR	THR	THR		THR	THR		THR	---	---	---	THR	THR	
	103						LYS					ARG	LYS	LYS		LYS	LYS		THR	---	---	---	LYS	LYS	
	104						VAL					LEU	VAL	VAL		VAL	VAL		VAL	---	---	---	VAL	LEU	
	105						GLU					GLX	GLU	GLU		ASP	GLU		ASP	---	---	---	GLU	GLU	
	106						VAL					ILE	MET	ILE		LEU	VAL		ILE	---	---	---	VAL	ILE	
	106A						---					---	---	---		---	---		---	---	---	---	---	---	
	107						LYS					LYS	THR	LYS		LYS	LYS		LYS	---	---	---	LYS	LYS	
	108											ARG	ARG	ARG			ARG		ARG	---	---	---	GLY	ARG	
	109											THR	THR	THR						---	---	---	THR	THR	

HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		48 HBJ 4	49 FRA	50 GR'	51 PAUL	52 MON	53' HEI	54 POT	55 S-GUI #	56 AMYLOID BAN #	57 BJ 19 #	58 BEL	59 JBL #	60 PAP	61 CAR	62 MEV	63 BI	64 AMYLOID ES305 #	65 CRA	66' DAV	67' FIN	68 KA	69 Vd 'CL	70 LUX	71 NE	72 Va' 'CL
FR 1	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ala
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	val	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	3	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN
	4	MET	MET	MET	MET	MET	MET	MET	MET	leu	leu	leu	MET	MET	MET	MET	MET	leu	MET	MET	MET	MET	leu	leu	leu	leu
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN
	7	SER	SER	SER	SER	SER	SER	SER	ala	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	ala	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	10	thr	thr	thr	thr	thr	thr	SER	SER	SER	SER	SER	SER	SER	thr	SER	pro	SER	SER	SER	SER	thr	phe	phe	SER	SER
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	thr	thr	val	ALA	ALA	ALA	ALA
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	tyr	SER	SER	SER	SER	SER	SER	val	val	SER	SER	SER	SER	SER
	15	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	leu	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	arg	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP
	18	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ser		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
	19	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	20	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	Ile	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	21	ILE	ILE	ILE	ILE	ILE		ILE.	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	#	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	22	THR	THR	THR	THR	THR		THR	THR	THR	THR	THR	ala	ala	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	23	CYS	CYS	CYS	CYS			CYS	CYS	CYS	CYS	CYS	CYS		CYS	CYS	CYS	CYS		CYS	CYS	CYS	CYS		CYS	CYS
CDR 1	24	ARG	ARG	ARG	ARG				ARG	ARG	GLN	GLX	ARG		ARG	ARG	GLN	GLN		ASP	ASP	GLU	ARG		GLX	ARG
	25	ALA	ALA	ALA	ALA				ALA	ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA		ALA	ALA	ALA	ALA		ALA	ALA
	26	SER	SER	SER	SER					SER	SER	SER	SER		SER	SER	SER	SER		SER	SER	SER	SER		SER	SER
	27	GLN	GLX	ARG	GLN					GLN	GLN	GLX	GLN		GLN	GLN	GLN	GLU		GLN	GLN	GLN	GLN		GLX	GLN
	27A	---	---	---	---					---	---	---	---		---	---	---	---		---	---	---	---		---	---
	27B	---	---	---	---					---	---	---	---		---	---	---	---		---	---	---	---		---	---
	27C	---	---	---	---					---	---	---	---		---	---	---	---		---	---	---	---		---	---
	27D	---	---	---	---					---	---	---	---		---	---	---	---		---	---	---	---		---	---
	27E	---	---	---	---					---	---	---	---		---	---	---	---		---	---	---	---		---	---
	27F	---	---	---	---					---	---	---	---		---	---	---	---		---	---	---	---		---	---
	28	SER	ASX	SER	SER					SER	ASP	ASX	SER		ASN	SER	ASP	ALA		ASX	ASX	THR	GLY		ASX	GLY
	29	VAL	ILE	ILE	ILE					VAL	ILE	ILE	ILE		ILE	SER	ILE	#		ILE	ILE	VAL	ILE		ILE	ILE
	30	ASN	ASX	SER	SER					TYR	ASN	SER	SER		SER	VAL	ARG			ASX	ASX	LEU	SER		SER	SER
	31	ASN	ASX	SER	SER					ASN	THR	LYS	THR		SER	ASP	ASN	ASN		SER	SER	SER	SER		ASX	SER
	32	TRP	TYR	TRP	SER					TYR	PHE		PHE		TRP	TYR	SER	TYR		TRP	TRP	TYR	TYR		TYR	ALA
	33	LEU	LEU	LEU						VAL	LEU		LEU		LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU		LEU	LEU
	34	ALA	ALA	ALA						ALA	ASN		#		ALA	ASN	ILE			ILE	ILE	ASN	ALA		ASX	ALA
FR 2	35	TRP	TRP	TRP						TRP	TRP		TRP		TRP	TRP	TRP	TRP		TRP	TRP	TRP	TRP		TRP	TRP
	36	TYR	TYR	TYR						PHE	TYR		TYR		TYR	TYR	TYR	TYR		TYR	TYR	TYR	TYR		TYR	TYR
	37	GLN	GLX	GLN						GLN	GLU		GLN		GLN	GLN	GLN			GLN	GLN	GLN	GLN		GLX	GLN
	38	GLU	GLX	GLY						GLN	GLN		GLN		GLN	GLN	GLN			GLN	GLN	GLN	GLN		GLX	GLN
	39	LYS	LYS	ARG						LYS	#		LYS		LYS	LYS	LYS			TYR	TYR	LYS	LYS		LYS	LYS
	40	PRO	PRO	PRO						PRO	PRO		PRO		PRO	PRO	PRO			PRO	PRO	PRO	PRO		PRO	PRO
	41	GLY								GLY	#	GLY	GLY		GLY	GLY	GLY					GLY	GLY		GLY	GLY
	42	GLX								LYS	LYS	LYS	LYS		LYS	LYS	LYS					LYS	LYS		GLX	LYS
	43	ALA								ALA	ALA	PRO	ALA		ALA	ALA	ALA					ALA	ALA		ALA	ALA
	44	PRO								PRO	PRO	PRO	PRO		PRO	PRO	PRO					PRO	PRO		PRO	PRO
	45	LYS								LYS	LYS	GLU	LYS		LYS	LYS	LYS					LYS	LYS		LYS	LYS
	46	LEU								SER	LEU	LEU	LEU		VAL	LEU	PHE					LEU	LEU		VAL	LEU
	47	LEU								LEU	LEU	LEU	LEU		LEU	LEU	LEU					LEU	LEU		MET	LEU
	48	ILE								ILE	ILE	ILE			ILE	ILE	ILE					ILE	ILE		ILE	ILE
	49	SER								TYR	TYR	TYR	TYR		TYR	PHE	TYR					TYR	TYR		TYR	TYR
CDR 2	50	LYS								ASP	ASP	ASP			LYS	ASP	ASP					ALA	ALA			ASP
	51	THR								ALA	ALA	ALA			SER	THR	ALA					ALA	ALA			ALA
	52	SER								SER	SER	SER			SER	SER	GLU					SER	SER			SER
	53	SER								THR	SER	THR			SER	ASN	ASN					SER	THR			SER
	54	LEU								LEU	LEU	LEU			LEU	LEU	LEU					LEU	LEU			LEU
	55	GLU								GLN	GLU	LYS			GLU	GLN	GLU					GLU	GLN			GLU
	56	ARG								SER	THR	THR			SER	SER	ILE					THR	SER			SER
FR 3	57	GLY								GLY	GLY	GLY			GLY	GLY	GLY					GLY	GLY			GLY
	58	VAL								VAL	VAL	VAL			VAL	VAL	VAL					VAL	VAL			VAL
	59	PRO								PRO	GLX	PRO			PRO	PRO						PRO	PRO			PRO
	60	SER								SER	SER	SER			SER	SER	SER					SER	SER			SER
	61	ARG								ASN	ARG	ARG			ARG	ARG	ARG					ARG	ARG			ARG
	62	PHE								PHE	PHE	PHE			PHE	PHE	PHE					PHE	PHE			PHE
	63	ALA								THR	LEU	SER			SER	SER	ARG					SER	SER			SER
	64	GLY								GLY	GLY	GLY			GLY	GLY	GLY					GLY	GLY			GLY
	65	SER								SER	SER	SER			SER	GLY	SER					GLN	SER			SER
	66	GLY								GLY	ASX	GLY			GLY	ARG	GLY					GLY	GLY			GLY
	67									SER	PHE	SER			SER	SER	SER					SER	SER			SER
	68									GLY	ASX	GLU			GLY	GLY	GLY					GLY	GLY			GLY
	69									THR	THR	THR			THR	THR	THR					THR	THR			THR
	70									ASP	LYS	HIS			ASP	ASP	ASP					ASX	GLU			ASP
	71									PHE		PHE			PHE	PHE	PHE					PHE	PHE			PHE
	72									ILE		THR			THR	THR	ALA					THR	THR			THR
	73									LEU		LEU			LEU	LEU	LEU					PHE	LEU			LEU
	74									THR		THR			THR	THR	SER					THR	THR			THR
	75									ILE		ILE			ILE	ILE	ILE					ILE	ILE			ILE
	76									SER					SER	SER	SER					SER	SER			SER
	77									SER		SER			SER	SER	SER					SER	SER			SER
	78									LEU		LEU			LEU	LEU	LEU					VAL	LEU			LEU
	79									GLN		GLN			GLX	GLN	GLN					GLX	GLN			GLN
	80									PRO		PRO			PRO	PRO	PRO					PRO	PRO			PRO
	81									GLU		ALA			ASX	ASP	GLU					GLX	GLU			GLU
	82									ASP		ASP			ASX	ASP	ASP					ASX	ASP			ASP
	83									PHE		PHE			PHE	PHE	PHE					PHE	PHE			PHE
	84									ALA		ALA			ALA	ALA	ALA					ALA	ALA			ALA
	85									THR		THR			THR	THR	THR					THR	THR			THR
	86									TYR		TYR			TYR	TYR	TYR					TYR	TYR			TYR
	87									TYR		TYR			TYR	TYR	TYR					TYR	TYR			TYR
	88									CYS		CYS			CYS	CYS	CYS					CYS	CYS			CYS
CDR 3	89									GLN		GLN			GLN	GLN	GLN					GLN	GLN			GLN
	90									GLN		GLN			GLN	GLN	GLN					GLX	GLN			GLN
	91									TYR	PHE	TYR			TYR	SER	TYR					TYR	LEU			PHE
	92									ASN	ASP	ASX			ASN	TYR	TYR					LEU	ASN			ASN
	93									SER	ASP	HIS			THR	THR	ASN					ASP	SER			SER
	94									TYR	LEU	PHE			PHE	ASN	LEU					LEU	TYR			TYR
	95									PRO	PRO	PRO			---	PRO	PRO					PRO	PRO			PRO
	95A									---	---	---			---	GLU	---					---	---			---
	95B									---	---	---			---	---	---					---	---			---
	95C									---	---	---			---	---	---					---	---			---
	95D									---	---	---			---	---	---					---	---			---
	95E									---	---	---			---	---	---					---	---			---
	95F									---	---	---			---	---	---					---	---			---
	96									TYR	TYR	LEU			PHE	VAL	TYR					ARG	---			---
	97									THR	THR	THR			THR	THR	THR					THR	---			---
FR 4	98									PHE	PHE	PHE			PHE	PHE	PHE					PHE	---			---
	99									GLY	GLY	GLY			GLY	GLY	GLY					GLY	---			---
	100									GLN	PRO	GLY			PRO	GLY	GLN					GLN	---			---
	101									GLY	GLY	GLY			GLY	GLY	GLY					GLY	---			---
	102									THR	THR	THR			THR	THR	THR					THR	---			---
	103									LYS	LYS	GLU			LYS	THR	LYS					LYS	---			---
	104									#	VAL	VAL			VAL	VAL	LEU					VAL	---			---
	105									#	GLU	GLU			ASP	ASP	GLU					ASP	---			---
	106									ILE	LEU	VAL			ILE	ILE	ILE					LEU	---			---
	106A									---	---	---			---	---	---					---	---			---
	107									LYS	LYS	LYS			LYS	LYS	LYS					LYS	---			---
	108									ARG					ARG	ARG	ARG					ARG	---			---
	109														THR		THR					THR	---			---

HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		73 NI	74 PW #	75 AMYLOID X	76 ALE	77 SHE	78 ADA	79 KUE	80 GO	81 BOL	82 RI #	83 Ve 'CL	84 OCO	85 V13 'CL	86 V18A 'CL	87 V19A 'CL	88 V19B 'CL	89 V18B 'CL	90 HF6-21/28	91 SAC	92* WAG	93 HBJ 1	94 AMYLOID 547	95 WEB	96 HOE
FR 1	0	---	---	---	---	---	---	---	---	---	pca	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	ASP	ASP	ASP	ASX	ASP	ASP	ASP	ASP	ASP	ASP	ala	ASX	val	ASP	ala	ala	ala	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	val	ILE	ILE	ILE	ILE	ILE	pro	gln	ILE	gln	val	ILE	ILE	ILE	ILE	ILE	ILE
	3	GLN	his	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	arg	GLX	trp	val	val	asp	val	val	GLN	GLN	leu	GLX	GLN	GLN
	4	MET	MET	MET	MET	leu	val	MET	MET	ile	MET	MET	MET	MET	MET	leu	MET	leu	MET	MET	MET	MET	MET	MET	MET
	5	THR	THR	THR	THR	THR	THR	THR	THR	met	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLX	GLX	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	thr	thr	thr	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	ala	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	glu	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER	SER	ala	ala	SER	leu	SER	SER	thr	SER	SER	SER
	10	SER	SER	SER		SER	SER	thr	phe	SER	thr	SER	SER	leu	SER	SER	SER	pro	SER	SER	SER	SER	SER	SER	thr
	11	LEU	LEU	LEU		LEU	LEU	gln	val	val	LEU	phe	LEU	LEU	thr	val	val	val	LEU	LEU	LEU	LEU	LEU	val	LEU
	12	SER	SER	SER	SER	SER	SER	pro	SER	SER	SER	SER	val	SER	SER	SER	glu	SER	pro	SER	SER	SER	SER	SER	SER
	13	ALA	ALA	ALA	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ser	ALA	ALA	ALA	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA
	14	thr	SER	SER	SER	SER	phe	SER	SER	SER	SER	SER	pro	SER	ala	ala	ala	pro	thr	SER	SER	SER	SER	SER	SER
	15	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	thr	VAL	thr	VAL	VAL	VAL	VAL	leu	VAL	VAL	VAL	VAL	VAL	VAL
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY			
	17	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASX	ASX	ASP	ASP	ASX	ASP	gly	gly	gly	gly	gly	ASP	ASP	ASP			
	18	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	gly	ARG	leu	ARG	thr	thr	thr	thr	pro	---					
	19	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	ile	VAL	ala	---					
	20	THR	THR	ile	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ser	---					
	21	leu	val	ILE	leu	phe	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	---					
	22	leu	arg		THR	THR	THR	THR	THR	THR	ser	THR	THR	ser	asn	asn	asn	asn	ser	---					
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	---					
CDR 1	24	GLU	ARG	GLX	ARG	GLX	GLX	ARG	ARG	ARG	ARG	ARG	GLX	ARG	GLN	GLN	GLN	GLN	ARG	---					
	25	ALA	ALA	ALA		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	MET	SER	ALA	ALA	ALA	SER	---					
	26	SER	SER			SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	---					
	27	GLN	GLX	GLX		GLX	GLX	GLN		GLX	GLX	GLN	GLX	GLN	GLN	GLU	GLU	GLN	GLN	---					
	27A	SER	---	---		---	---	---		---	---	---	---	---	ASN	---	---	SER	SER	---					
	27B	VAL	---	---		---	---	---		---	---	---	---	---	VAL	---	---	VAL	LEU	---					
	27C	LEU	---	---		---	---	---		---	---	---	---	---	---	---	---	---	VAL	---					
	27D	GLU	---	---		---	---	---		---	---	---	---	---	---	---	---	---	TRP	---					
	27E	---	---	---		---	---	---		---	---	---	---	---	---	---	---	---	SER	---					
	27F	---	---	---		---	---	---		---	---	---	---	---	---	---	---	---	---	---					
	28	SER	SER	ASX		ASX	ASX	SER		GLY	SER	GLY	ASX	GLY	TYR	SER	SER	TYR	ASP	---					
	29	GLY	ILE	ILE		ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	SER	ILE	ILE	ASP	GLY	---					
	30	ASN	GLY			SER		ASN			SER	SER	SER	SER	ASN	SER	SER	ASN	ARG	---					
	31	THR		PRO		ASX	ASX	ILE				SER	ASX	SER	ASN	SER	SER	ASN	SER	---					
	32	PHE	TYR	TYR		TYR	TYR	TRP				TYR	TYR	TYR	TYR	TYR	TRP	TRP	TYR	---					
	33	LEU	SER	LEU		LEU	LEU	LEU				LEU		LEU	LEU	LEU	LEU	LEU	LEU	---					
	34	ALA	ASX			ASX	ASX	ALA				ALA		ALA	SER	ASN	ALA	SER	GLU	---					
FR 2	35	TRP	TRP			TRP	TRP	TRP				TRP		TRP	TRP	TRP	TRP	TRP	TRP	---					
	36	TYR		TYR		TYR	TYR	TYR				TYR		TYR	PHE	TYR	TYR	TYR	TYR	---					
	37	GLN	GLN			HIS	GLX	GLN				GLN		GLN	GLN	GLN	GLN	GLN	GLN	---					
	38	GLN	GLN			GLX	TYR	GLN				GLN		GLN	GLN	GLN	GLN	GLN	GLN	---					
	39	LYS	LYS			LYS	LYS	LYS				LYS		LYS	LYS	LYS	LYS	LYS	LYS	---					
	40	PRO				PRO	PRO	PRO				PRO		PRO	PRO	LEU	PRO	PRO	PRO	---					
	41	LYS						GLU				GLY		GLY	GLY	GLY	GLY	GLY		---					
	42	LYS						LYS				LYS		LYS	GLN	GLN	GLN	GLN		---					
	43	ALA						ALA				ALA		ALA	PRO	PRO	PRO	PRO		---					
	44	PRO						PRO				PRO		PRO	PRO	PRO	PRO	PRO		---					
	45	LYS						LYS				LYS		GLU	LYS	LYS	LYS	LYS		---					
	46	LEU						LEU				LEU		LEU	LEU	LEU	LEU	LEU		---					
	47	LEU						LEU				LEU		LEU	LEU	LEU	LEU	LEU		---					
	48	ILE						ILE				ILE		ILE	ILE	ILE	ILE	ILE		---					
	49	TYR						TYR				TYR		TYR	TYR	TYR	TYR	TYR		---					
CDR 2	50	ASP						LYS				ALA		ALA	GLY	TYR	GLU	ASP		LYS					
	51	ALA						ALA				ALA		ALA	ALA	ALA	ALA	ALA		SER					
	52	SER						SER				SER		SER	SER	SER	SER	SER		---					
	53	ASN						THR				THR		THR	LYS	THR	LYS	LYS		---					
	54	LEU						LEU				LEU		LEU	LEU	LEU	LEU	LEU		---					
	55	GLU						GLU				GLN		GLN	ALA	ALA	ALA	ALA		---					
	56	THR						THR				SER		SER	SER	SER	SER	SER		---					
FR 3	57	GLY						GLY				GLY		GLY	GLY	GLY	GLY	GLY		---					
	58	VAL						VAL				VAL		VAL	VAL	VAL	VAL	VAL		---					
	59	PRO						PRO				PRO		PRO	PRO	PRO	PRO	PRO		---					
	60	SER						SER				SER		SER	SER	SER	SER	SER		---					
	61	ARG						ARG				ARG		ARG	ARG	ARG	ARG	ARG		---					
	62	PHE						PHE				PHE		PHE	PHE	PHE	PHE	PHE		---					
	63	SER						SER				SER		SER	SER	LYS	SER	SER		---					
	64	GLY						GLY				GLY		GLY	GLY	GLY	GLY	GLY		---					
	65	SER						SER				SER		SER	SER	SER	SER	SER		---					
	66	GLY						GLY				GLY		GLY	GLY	GLY	GLY	GLY		---					
	67	SER						SER				SER		SER	SER	SER	SER	SER		---					
	68	GLY						GLY				GLY		GLY	GLY	GLY	GLY	GLY		---					
	69	THR						THR				THR		THR	LYS	THR	THR	THR		---					
	70	ASP						GLN				ASP		ASP	GLN	GLU	GLN	GLN		---					
	71	PHE						PHE				PHE		PHE	PHE	TYR	PHE	PHE		---					
	72	THR						THR				THR		THR	THR	THR	THR	THR		---					
	73	PHE						LEU				LEU		LEU	LEU	LEU	LEU	LEU		---					
	74	THR						THR				THR		THR	THR	THR	THR	THR		---					
	75	ILE						ILE				ILE		ILE	ILE	ILE	ILE	ILE		---					
	76	SER						ASN				SER		SER	SER	SER	SER	SER		---					
	77	GLY						SER				CYS		CYS	GLY	GLY	GLY	GLY		---					
	78	LEU						LEU				LEU		LEU	VAL	VAL	VAL	VAL		---					
	79	GLX						GLN				GLN		GLN	GLN	GLN	GLN	GLN		---					
	80	PRO						PRO				SER		SER	CYS	CYS	CYS	CYS		---					
	81	GLX						ASP				GLU		GLU	ASP	ASP	ASP	ASP		---					
	82	ASX						ASP				ASP		ASP	ASP	ASP	ASP	ASP		---					
	83	PHE						PHE				PHE		PHE	ALA	ALA	ALA	ALA		---					
	84	ALA						ALA				ALA		ALA	ALA	ALA	ALA	ALA		---					
	85	VAL						THR				THR		THR	THR	THR	THR	THR		---					
	86	TYR						TYR				TYR		TYR	TYR	TYR	TYR	TYR		---					
	87	TYR						TYR				TYR		TYR	TYR	TYR	TYR	TYR		---					
	88	CYS						CYS				CYS		CYS	CYS	CYS	CYS	CYS		CYS					
CDR 3	89	GLN						GLN				GLN		GLN	ALA	GLN	GLN	GLN		GLX					
	90	GLX						GLN				GLN		GLN	GLY	HIS	GLY	GLY		GLX					
	91	TYR						TYR				TYR		TYR	TYR	GLY	GLY	SER		GLX					
	92	ASP						SER				TYR		TYR	TYR	TYR	TYR	TYR		ASX					
	93	THR						ARG				SER		SER	TYR	ILE	TYR	TYR		ALA					
	94	LEU						TYR				TYR		PHE	SER	TYR	ASN	SER		ALA					
	95	PRO						PRO				PRO		PRO	GLY	SER	SER	SER		THR					
	95A	---						---				---		---	SER	SER	GLY	GLY		---					
	95B	---						---				---		---	GLY	GLY	TRP	TRP		---					
	95C	---						---				---		---	THR	ASP	TYR	TYR		---					
	95D	---						---				---		---	ASP					---					
	95E	---						---				---		---						---					
	95F	---						---				---		---						---					
	96	SER						TYR				---		---						ILE					
	97	THR						THR				---		---						PRO					
FR 4	98	PHE						PHE				---		---						ILE					
	99	GLY						GLY				---		---						GLY					
	100	VAL						GLN				---		---						GLY					
	101	GLY						GLY				---		---						GLY					
	102	SER						THR				---		---						THR					
	103	LYS						LYS				---		---						LYS					
	104	VAL						LEU				---		---						VAL					
	105	GLU						ASP				---		---						ASX					
	106	SER						ILE				---		---						VAL					
	106A	---						---				---		---						---					
	107	LYS						LYS				---		---						LYS					
	108	ARG						ARG				---		---						ARG					
	109	THR						THR				---		---						THR					

HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

		97 LOD	98 HBJ 10	99 BEN	100 GR	101 MAA	102 MUK	103 AMYLOID 594	104* MAR	105 AMYLOID	106 BJ*	107 HBJ 6	108 PEN	109 AMYLOID MS #	110 CL*	111 GM131 CL #	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID
FR1	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---		1	1	1(PCA)
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASX	ASP	ASP	ASP	---		109	3 : 4	103(ASP) : 99(ASP)
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	#	---		107	4	101(ILE)
	3	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	glu		GLN	GLN	GLN	---		108	8	97(GLN) : 93(GLN)
	4		MET	MET	MET	leu	MET	MET	leu	MET	MET	MET	MET	MET	---		108	4	93(MET)
	5	THR	THR	THR	THR	THR	THR	THR	THR	pro	THR	THR		THR	---		108	3	106(THR)
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN			---		107	1 : 2	107(GLN) : 100(GLN)
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER					---		105	3	100(SER)
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO					---		105	3	103(PRO)
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER					---		105	4	99(SER)
	10	thr	SER	SER	SER	thr	SER	SER	SER	SER					---		104	5	81(SER)
	11		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU					---		103	5	91(LEU)
	12	SER	SER	SER	SER	SER	SER								---		102	4	98(SER)
	13	ALA	ALA	ALA	val	ALA									---		101	4	91(ALA)
	14	-	SER												---		97	7	86(SER)
	15	VAL													---		97	3	93(VAL)
	16														---		93	2	92(GLY)
	17														---		93	3 : 4	87(ASP) : 79(ASP)
	18										ARG				---		90	6	82(ARG)
	19										VAL				---		91	3	88(VAL)
	20										THR				---		91	4	87(THR)
	21										ILE				---		88	4	84(ILE)
	22										THR				---		88	7	75(THR)
	23										CYS				---		83	1	83(CYS)
CDR1	24										GLN				---		75	5	43(ARG)
	25										ALA				---		75	4	71(ALA)
	26										SER				---		72	4	67(SER)
	27										GLN				---		72	4	66(GLN) : 53(GLN)
	27A										---				---		4	2	3(SER)
	27B										---				---		4	2	3(VAL)
	27C										---				---		2	2	1(+)
	27D										---				---		2	2	1(+)
	27E										---				---		1	1	1(SER)
	27F										---				---				
	28										ASP				---		72	8	25(ASP) : 22(SER)
	29										ILE				---		71	5	61(ILE)
	30										ASN				---		68	10 : 11	35(SER)
	31										LYS				---		66	10	24(SER)
	32														---		66	7	33(TYR)
	33														---		64	4	60(LEU)
	34														---		60	7 : 8	24(ASN) : 22(ALA)
FR2	35														---		63	1	63(TRP)
	36														---		61	2	57(TYR)
	37														---		60	4	56(GLN) : 49(GLN)
	38														---		58	4	55(GLN) : 50(GLN)
	39														---		55	4	50(LYS)
	40														---		57	4	54(PRO)
	41														---		44	3	40(GLY)
	42														---		46	5	35(LYS)
	43														---		47	2	42(ALA)
	44														---		47	1	47(PRO)
	45														---		47	6	41(LYS)
	46														---		46	7	33(LEU)
	47														---		45	2	44(LEU)
	48														---		43	2	42(ILE)
	49														---		45	4	42(TYR)
CDR2	50														---		45	7 : 8	15(+)
	51														---		45	5	39(ALA)
	52														---		44	4	41(SER)
	53														---		43	5 : 6	18(SER)
	54														---		44	2	43(LEU)
	55														---		44	7	20(GLU)
	56														---		42	7	27(SER)
FR3	57														---		43	1	43(GLY)
	58														---		43	2	42(VAL)
	59														---		42	4	39(PRO)
	60														---		42	1	42(SER)
	61														---		43	3	41(ARG)
	62														---		43	3	41(PHE)
	63														---		43	7	36(SER)
	64														---		43	1	43(GLY)
	65														---		42	4	38(SER)
	66														---		43	3	41(GLY)
	67														---		41	3	38(SER)
	68														---		41	3	38(GLY)
	69														---		41	3	38(THR)
	70														---		41	5 : 6	25(ASP) : 23(ASP)
	71														---		40	4	36(PHE)
	72														---		40	4	37(THR)
	73														---		40	3	31(LEU)
	74														---		40	4	37(THR)
	75														---		40	3	38(ILE)
	76														---		39	2	37(SER)
	77														---		40	5	27(SER)
	78														---	LEU	40	2	35(LEU)
	79														---	GLN	40	2 : 3	39(GLN) : 35(GLN)
	80														---	PRO	40	3	33(PRO)
	81														---	GLU	40	3 : 5	29(GLU) : 26(GLU)
	82														---	ASP	40	1 : 2	40(ASP) : 37(ASP)
	83														---	PHE	40	4	28(PHE)
	84														---	ALA	40	2	39(ALA)
	85														---	THR	40	4	37(THR)
	86										TYR				---	TYR	42	2	41(TYR)
	87										TYR				---	TYR	41	2	40(TYR)
	88										CYS				---	CYS	42	1	42(CYS)
CDR3	89										GLN				---	GLN	43	3 : 4	40(GLN) : 37(GLN)
	90										GLN				---	GLN	43	3 : 4	39(GLN) : 34(GLN)
	91										TYR				---	HIS	45	10 : 11	24(TYR)
	92										GLU				ASP	ASP	46	8	15(ASN) : 13(+)
	93										ASN				ILE	HIS	46	9	20(SER)
	94										LEU				PHE	LEU	46	10	12(LEU)
	95										PRO				PRO	PRO	45	5	35(PRO)
	95A										---				---	---	5	3	2(+)
	95B										---				---	---	4	2	2(+)
	95C										---				---	---	4	3	2(TYR)
	95D										---				---	---	1	1	1(ASP)
	95E										---				---	---			
	95F										---				---	---			
	96										TYR				GLY	LEU	32	12	9(TYR)
	97														THR	THR	31	4	27(THR)
FR4	98														PHE	PHE	31	3	29(PHE)
	99														GLY	GLY	31	1	31(GLY)
	100														GLN	GLY	31	4 : 5	18(GLN) : 17(GLN)
	101														GLY	GLY	31	1	31(GLY)
	102														THR	THR	31	2	30(THR)
	103														LYS	THR	31	4	23(LYS)
	104														VAL	VAL	30	2	23(VAL)
	105														GLU	GLU	30	3 : 4	20(GLU) : 19(GLU)
	106														ILE	MET	31	7	15(ILE)
	106A														---	---			
	107														LYS	LYS	31	2	29(LYS)
	108														ARG	ARG	24	2	22(ARG)
	109														THR	THR	20	1	20(THR)

HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

VARIABILITY

FR1	0	
	1	3.2 : 4.4
	2	4.2
	3	8.9 : 9.3
	4	4.6
	5	3.1
	6	1. : 2.1
	7	3.2
	8	3.1
	9	4.2
	10	6.4
	11	5.7
	12	4.2
	13	4.4
	14	7.9
	15	3.1
	16	2.
	17	3.2 : 4.7
	18	6.6
	19	3.1
	20	4.2
	21	4.2
	22	8.2
	23	1.
CDR1	24	8.7
	25	4.2
	26	4.3
	27	4.4 : 5.4
	27A	
	27B	
	27C	
	27D	
	27E	
	27F	
	28	23. : 28.
	29	5.8
	30	19. : 21.
	31	28.
	32	14.
	33	4.3
	34	18. : 22.
FR2	35	1.
	36	2.1
	37	4.3 : 4.9
	38	4.2 : 4.8
	39	4.4
	40	4.2
	41	3.3
	42	6.8
	43	2.2
	44	1.
	45	6.9
	46	9.8
	47	2.
	48	2.
	49	4.3
CDR2	50	21. : 24.
	51	5.8
	52	4.3
	53	12. : 14.
	54	2.
	55	15.
	56	11.
FR3	57	1.
	58	2.
	59	4.3
	60	1.
	61	3.1
	62	3.1
	63	8.4
	64	1.
	65	4.4
	66	3.1
	67	3.2
	68	3.2
	69	3.2
	70	8.2 : 11.
	71	4.4
	72	4.3
	73	3.9
	74	4.3
	75	3.2
	76	2.1
	77	7.4
	78	2.3
	79	2.1 : 3.4
	80	3.6
	81	4.1 : 7.7
	82	1. : 2.2
	83	5.7
	84	2.1
	85	4.3
	86	2.
	87	2.1
	88	1.
CDR3	89	3.2 : 4.6
	90	3.3 : 5.1
	91	19. : 21.
	92	25. : 28.
	93	21.
	94	38.
	95	6.4
	95A	
	95B	
	95C	
	95D	
	95E	
	95F	
	96	43.
	97	4.6
FR4	98	3.2
	99	1.
	100	6.9 : 9.1
	101	1.
	102	2.1
	103	5.4
	104	2.6
	105	4.5 : 6.3
	106	14.
	106A	
	107	2.1
	108	2.2
	109	1.

ANTIBODY SPECIFICITIES: HUMAN KAPPA LIGHT CHAINS SUBGROUP I

8) **WEA:** ANTI-3,4-PYRUVYLATED GALACTOSE MONOCLONAL
25) **LOW:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
39) **LAY:** ANTI-HUMAN GAMMA G1 AND G3 GLOBULINS; PO IDIOTYPE
53) **HEI:** COLD AGGLUTININ WITH ANTI-GD (MEMBRANE-GLYCOLIPID-DEPENDENT) ACTIVITY
66) **DAV:** ANTI-HUMAN GAMMA G GLOBULIN
67) **FIN:** ANTI-HUMAN GAMMA G GLOBULIN
92) **WAG:** ANTI-DINITROPHENYL
104) **MAR:** ANTI-LIPOPROTEIN LIPASE

ALLOTYPE: HUMAN KAPPA LIGHT CHAINS SUBGROUP I

79) **KUE:** INV(2)

CLASS: HUMAN KAPPA LIGHT CHAINS SUBGROUP I

8) **WEA:** IGM-KAPPA
33) **F-GUI:** IGG3-KAPPA
55) **S-GUI:** IGG3-KAPPA
74) **PW:** IGG1-KAPPA
82) **RI:** IGG1-KAPPA

REFERENCE: HUMAN KAPPA LIGHT CHAINS SUBGROUP I

1) **ROY:** HILSCHMANN,N. & CRAIG,L.C. (1965) PROC.NAT.ACAD.SCI.USA,53,1403-1409; HILSCHMANN,N. (1967) Z.PHYSIOL.CHEM.,348,1077-1080; HILSCHMANN,N.,BARNIKOL,H.U.,HESS,M.,LANGER,B.,PONSTINGL,H.,STEINMETZ-KAYNE,M.,SUTER,L. & WATANABE,S. (1969) PROC. 5TH FEBS SYMP., 15,57-74. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED AND CORRECTED RESIDUES 65 AND 67 AS GIVEN IN THE TABLE)
2) **AU:** SCHIECHL,H. & HILSCHMANN,N. (1971) Z.PHYSIOL.CHEM.,352,111-115; (1972) Z.PHYSIOL.CHEM.,353,345-370. (CHECKED BY AUTHOR)
3) **REI:** PALM,W. & HILSCHMANN,N. (1973) Z.PHYSIOL.CHEM.,354,1651-1654; (1975) Z.PHYSIOL.CHEM.,356,167-191. (CHECKED BY AUTHOR)
4) **HAU:** WATANABE,S. & HILSCHMANN,N. (1970) Z.PHYSIOL.CHEM.,351,1291-1295. (CHECKED BY AUTHOR)
5) **HK101'CL:** BENTLEY,D.L. & RABBITTS,T.H. (1980) NATURE,288,730-733. (CHECKED BY AUTHOR 11/30/82)
6) **SCW:** EULITZ,M.,GOTZE,D. & HILSCHMANN,N. (1972) Z.PHYSIOL.CHEM.,353,487-491; EULITZ,M. & HILSCHMANN,N. (1974) Z.PHYSIOL.CHEM.,355,842-866. (CHECKED BY AUTHOR)
7) **AG:** TITANI,K.,SHINODA,T. & PUTNAM,F.W. (1969) J.BIOL.CHEM.,244,3550-3560. (CHECKED BY AUTHOR 06/15/83)
8) **WEA:** GONI,F. & FRANGIONE,B. (1983) PROC.NAT.ACAD.SCI.USA,80,4837-4841. (CHECKED BY AUTHOR 03/23/84)
9) **HK137'CL:** BENTLEY,D.L. & RABBITTS,T.H. (1983) CELL,32,181-189.
10) **HK134'CL:** BENTLEY,D.L. & RABBITTS,T.H. (1983) CELL,32,181-189.
11) **DAUDI'CL:** KLOBECK,H.G.,COMBRIATO,G. & ZACHAU,H.G. (1984) NUC.ACIDS RES.,12,18,6995-7006.
12) **WALKER'CL:** KLOBECK,H.G.,COMBRIATO,G. & ZACHAU,H.G. (1984) NUC.ACIDS RES.,12,18,6995-7006. (CHECKED BY AUTHOR 08/22/85 WHO CORRECTED RESIDUE 34)
13) **HF3-16/6:** ATKINSON,P.M.,LAMPMAN,G.W.,FURIE,B.C.,NAPARSTEK,Y.,SCHWARTZ,R.S.,STOLLAR,B.D. & FURIE,B. (1985) J.CLIN.INVEST.,75,1138-1143. (CHECKED BY AUTHOR 08/21/85)
14) **HF2-1/13B:** ATKINSON,P.M.,LAMPMAN,G.W.,FURIE,B.C.,NAPARSTEK,Y.,SCHWARTZ,R.S.,STOLLAR,B.D. & FURIE,B. (1985) J.CLIN.INVEST.,75,1138-1143. (CHECKED BY AUTHOR 08/21/85)
15) **HF2-18/2:** ATKINSON,P.M.,LAMPMAN,G.W.,FURIE,B.C.,NAPARSTEK,Y.,SCHWARTZ,R.S.,STOLLAR,B.D. & FURIE,B. (1985) J.CLIN.INVEST.,75,1138-1143. (CHECKED BY AUTHOR 08/21/85)
16) **HF2-1/17:** ATKINSON,P.M.,LAMPMAN,G.W.,FURIE,B.C.,NAPARSTEK,Y.,SCHWARTZ,R.S.,STOLLAR,B.D. & FURIE,B. (1985) J.CLIN.INVEST.,75,1138-1143. (CHECKED BY AUTHOR 08/21/85)
17) **BJ26:** ALESCIO-ZONTA,L. & BAGLIONI,C. (1970) EUR.J.BIOCHEM.,15,450-463. (CHECKED BY AUTHOR)
18) **RFZ:** SMITHIES,O.,GIBSON,D.,FANNING,E.M.,GOODFLIESH,R.M.,GILMAN,J.G. & BALLANTYNE,D.L. (1971) BIOCHEMISTRY,10,4912-4921. (CHECKED BY AUTHOR)
19) **PSM:** SEON,B.K. (1982) MOL.IMMUNOL.,19,83-86. (CHECKED BY AUTHOR 05/23/83)
20) **HOM:** CAVVIDOU,G.,KLEIN,M.,HORNE,C.,HOFMANN,T. & DORRINGTON,K.J. (1981) MOL.IMMUNOL.,18,793-805.
21) **ESM IGG:** KUAN,T.K.,TUNG,E.,WANG,I.Y. & WANG,A.C. (1981) IMMUNOL.,44,265-271. (CHECKED BY AUTHOR 05/26/83)
22) **ESM IGM:** KUAN,T.K.,TUNG,E.,WANG,I.Y. & WANG,A.C. (1981) IMMUNOL.,44,265-271. (CHECKED BY AUTHOR 05/26/83)
23) **WAT:** STEVENS,F.J.,WESTHOLM,F.A.,PANAGIOTOPOULOS,N.,SCHIFFER,M.,POPP,R.A. & SOLOMON,A. (1981) J.MOL.BIOL.,147,185-193. (CHECKED BY AUTHOR 05/26/1983)
24) **AMYLOID VIII-B:** GLENNER,G.G.,TERRY,W.,HERADA,M.,ISERSKY,C. & PAGE,D. (1971) SCIENCE,172,1150-1151. (CHECKED BY AUTHOR 09/22/78)
25) **LOW:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR WHO CORRECTED RESIDUE 16 AS GIVEN IN TABLE)
26) **DIE:** CAPRA,J.D. & KUNKEL,H.G. (1970) PROC.NAT.ACAD.SCI.USA,67,87-92. (CHECKED BY AUTHOR)
27) **CAR A:** CAPRA,J.D. & KUNKEL,H.G. (1970) PROC.NAT.ACAD.SCI.USA,67,87-92. (CHECKED BY AUTHOR)
28) **TEI:** CAPRA,J.D. & KUNKEL,H.G. (1970) PROC.NAT.ACAD.SCI.USA,67,87-92. (CHECKED BY AUTHOR)
29) **BJ48:** ALESCIO-ZONTA,L. & BAGLIONI,C. (1970) EUR.J.BIOCHEM.,15,450-463. (CHECKED BY AUTHOR)
30) **CON:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
31) **TRA:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
32) **AMYLOID LEP:** LIAN,J.B.,SKINNER,M.,BENSON,M.D. & COHEN,A.S. (1977) BIOCHIM.BIOPHYS.ACTA,491,167-176.
33) **F-GUI:** WANG,A.C.,FUDENBERG,H.H. & CREYSSEL,R. (1982) ACTA HAEMAT.,68,187-195. (CHECKED BY AUTHOR 05/26/83)
34) **OU(IOC):** KOHLER,H.,SHIMIZU,A.,PAUL,C. & PUTNAM,F.W. (1970) SCIENCE,169,56-59. (KAPLAN,A.P. & METZGER,H. (1969) BIOCHEMISTRY,8,3944-3951.) (CHECKED BY AUTHOR 06/15/83)
35) **DEE:** MILSTEIN,C. & DEVERSON,E.V. (1971) BIOCHEM.J.,123,945-958. (CHECKED BY AUTHOR)
36) **GAL(I):** LAURE,C.J.,WATANABE,S. & HILSCHMANN,N. (1973) Z.PHYSIOL.CHEM.,354,1503-1504. (CHECKED BY AUTHOR)
37) **JOH:** CAPRA,J.D. & KUNKEL,H.G. (1970) PROC.NAT.ACAD.SCI.USA,67,87-92. (CHECKED BY AUTHOR)
38) **KER:** MILSTEIN,C. (1966) BIOCHEM.J.,101,352-368. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED)
39) **LAY:** KAPLAN,A.P. & METZGER,H. (1969) BIOCHEMISTRY,8,3944-3951. (CHECKED BY AUTHOR); KLAPPER,D.G. & CAPRA,J.D. (1976) ANN.IMMUNOL.(INST.PASTEUR),127C,261-271. (CHECKED BY AUTHOR 08/01/79)
40) **BRA:** WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)
41) **WES:** KRATZIN,H.,YANG,C.Y.,KRUSCHE,J.U. & HILSCHMANN,N. (1980) Z.PHYSIOL.CHEM.,361,1591-1598.
42) **Vb'CL:** PECH,M.,JAENICHEN,H.-R.,POHLENZ,H.-D.,NEUMAIER,P.S.,KLOBECK,H.-G. & ZACHAU,H.G. (1984) J.MOL.BIOL.,176,189-204. (CHECKED BY AUTHOR 12/14/84)
43) **Vb"CL:** PECH,M.,JAENICHEN,H.-R.,POHLENZ,H.-D.,NEUMAIER,P.S.,KLOBECK,H.-G. & ZACHAU,H.G. (1984) J.MOL.BIOL.,176,189-204. (CHECKED BY AUTHOR 12/14/84)
44) **HK102'CL:** BENTLEY,D.L. & RABBITTS,T.H. (1980) NATURE,288,730-733. (CHECKED BY AUTHOR 11/30/82)
45) **EU:** GOTTLIEB,P.D.,CUNNINGHAM,B.A.,RUTISHAUSER,U. & EDELMAN,G.M. (1970) BIOCHEMISTRY,9,3155-3161. (CHECKED BY AUTHOR)
46) **DEN:** YANG,C.Y.,PAULY,E.,KRATZIN,H. & HILSCHMANN,N. (1981) Z.PHYSIOL.CHEM.,362,1131-1146.
47) **PAU:** DAYHOFF,M.O. (1972) ATLAS OF PROTEIN SEQUENCE & STRUCTURE,5,D-245. SUBMITTED BY SMITHIES,O.,GIBSON,D.M. AND FANNING,E.M. (CHECKED BY AUTHOR)
48) **HBJ4:** SMITH,G.P.,HOOD,L. & FITCH,W.M. (1971) ANN.REV.BIOCHEM.,40,969-1012.
49) **FRA:** MEINKE,G.C.,SIGRIST,P.H. & SPIEGELBERG,H.L. (1974) IMMUNOCHEM.,11,457-460. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED); MEINKE,G.C. & SPIEGELBERG,H.L. (1976) IMMUNOCHEM.,13,915-919. (CHECKED BY AUTHOR 10/17/77)
50) **GR':** FAIR,D.S.,SLEDGE,C.,KRUEGER,R.G.,MANN,K.G. & HOOD,L.E. (1975) BIOCHEMISTRY,14,5561-5568.
51) **PAUL:** SMITH,G.P.,HOOD,L. & FITCH,W.M. (1971) ANN.REV.BIOCHEM.,40,969-1012.
52) **MON:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
53) **HEI:** RIESEN,W.F.,MAJANIEMI,I.,HUSER,H.,BRAUN,D.G. & ROELCKE,D. (1978) SCAND.J.IMMUNOL.,8,145-148. (CHECKED BY AUTHOR 10/10/79)
54) **POT:** CAPRA,J.D. & KUNKEL,H.G. (1970) PROC.NAT.ACAD.SCI.USA,67,87-92. (CHECKED BY AUTHOR WHO CORRECTED RESIDUE 9 AS GIVEN IN TABLE)
55) **S-GUI:** WANG,A.C.,FUDENBERG,H.H. & CREYSSEL,R. (1982) ACTA HAEMAT.,68,187-195. (CHECKED BY AUTHOR 05/26/83)
56) **AMYLOID BAN:** DWULET,F.E.,O'CONNOR,T.P. & BENSON,M.D. (1986) MOL.IMMUNOL.,23,73-78.
57) **BJ19:** ALESCIO-ZONTA,L. & BAGLIONI,C. (1970) EUR.J.BIOCHEM.,15,450-463. (CHECKED BY AUTHOR)
58) **BEL:** MILSTEIN,C. (1969) PROC. 5TH FEBS SYMP.,15,43-56. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED AND CORRECTED RESIDUES 1,3,8,27,79 AND 82 AS GIVEN IN TABLE)
59) **JBL:** SEON,B.K. (1982) MOL.IMMUNOL.,19,83-86. (CHECKED BY AUTHOR 05/23/83)
60) **PAP:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
61) **CAR:** MILSTEIN,C.P. & DEVERSON,E.V. (1974) EUR.J.BIOCHEM.,49,377-391. (CHECKED BY AUTHOR)
62) **MEV:** EULITZ,M. & LINKE,R.P. (1982) Z.PHYSIOL.CHEM.,363,1347-1358. (CHECKED BY AUTHOR 10/10/83)

REFERENCE: HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

63) **BI:** BRAUN,H.,LEIBOLD,W.,BARNIKOL,H.U. & HILSCHMANN,N. (1971) Z.PHYSIOL.CHEM.,352,647-651; (1972) Z.PHYSIOL.CHEM.,353,1284-1306. (CHECKED BY AUTHOR WHO PROVIDED AN ADDITIONAL RESIDUE TO THOSE PUBLISHED AND CORRECTED RESIDUE 72 AS GIVEN IN TABLE)
64) **AMYLOID ES305:** WESTERMARK,P.,SLETTEN,K. & NATVIG,J.B. (1981) ACTA PATH.MICROBIOL.SCAND.,C89,199-203. (CHECKED BY AUTHOR 11/09/81)
65) **CRA:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
66) **DAV:** CAPRA,J.D. & KUNKEL,H.G. (1970) PROC.NAT.ACAD.SCI.USA,67,87-92. (CHECKED BY AUTHOR)
67) **FIN:** CAPRA,J.D. & KUNKEL,H.G. (1970) PROC.NAT.ACAD.SCI.USA,67,87-92. (CHECKED BY AUTHOR)
68) **KA:** SHINODA,T. (1975) J.BIOCHEM.,77,1277-1298. (CHECKED BY AUTHOR)
69) **Vd'CL:** PECH,M.,JAENICHEN,H.-R.,POHLENZ,H.-D.,NEUMAIER,P.S.,KLOBECK,H.-G. & ZACHAU,H.G. (1984) J.MOL.BIOL.,176,189-204. (CHECKED BY AUTHOR 12/14/84)
70) **LUX:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)
71) **NE:** MATTHEWS,J.B. & JEFFERIS,R. (1977) IMMUNOCHEM.,14,793-797. (CHECKED BY AUTHOR 08/10/79)
72) **Va''CL:** PECH,M.,JAENICHEN,H.-R.,POHLENZ,H.-D.,NEUMAIER,P.S.,KLOBECK,H.-G. & ZACHAU,H.G. (1984) J.MOL.BIOL.,176,189-204. (CHECKED BY AUTHOR 12/14/84)
73) **NI:** SHINODA,T. (1973) J.BIOCHEM.,73,433-446. (CHECKED BY AUTHOR)
74) **FAV:** PICK,A.I.,WANG,A.C.,FROHLICHMAN,R. & FUDENBERG,H.H. (1982) ACTA HAEMAT.,68,207-214. (CHECKED BY AUTHOR 05/26/83)
75) **AMYLOID X:** GLENNER,G.G.,TERRY,W.,HERADA,M.,ISERSKY,C. & PAGE,D. (1971) SCIENCE,172,1150-1151. (CHECKED BY AUTHOR 09/22/78)
76) **ALE:** MILSTEIN,C.,MILSTEIN,C.P.& FEINSTEIN,A. (1969) NATURE,221,151-154. (CHECKED BY AUTHOR)
77) **SHE:** MEINKE,G.C. & SPIEGELBERG,H.L. (1976) IMMUNOCHEM.,13,915-919. (CHECKED BY AUTHOR 10/17/77)
78) **ADA:** MEINKE,G.C. & SPIEGELBERG,H.L. (1976) IMMUNOCHEM.,13,915-919. (CHECKED BY AUTHOR 10/17/77)
79) **KUE:** EULITZ,M.,KLEY,H.P. & ZEITLER,H.J. (1979) Z.PHYSIOL.CHEM.,360,725-734. (CHECKED BY AUTHOR 07/17/79)
80) **GO:** WANG,A.C.,FUDENBERG,H.H. & CREYSSEL,R. (1974) EUR.J.IMMUNOL.,4,446-448. (CHECKED BY AUTHOR)
81) **BOL:** WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)
82) **RI:** PICK,A.I.,WANG,A.C.,FROHLICHMAN,R. & FUDENBERG,H.H. (1982) ACTA HAEMAT.,68,207-214. (CHECKED BY AUTHOR 05/26/83)
83) **Ve'CL:** PECH,M.,JAENICHEN,H.-R.,POHLENZ,H.-D.,NEUMAIER,P.S.,KLOBECK,H.-G. & ZACHAU,H.G. (1984) J.MOL.BIOL.,176,189-204. (CHECKED BY AUTHOR 12/14/84)
84) **OCO:** WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)
85) **V13'CL:** JAENICHEN,H.-R.,PECH,M.,LINDENMAIER,W.,WILDGRUBER,N. & ZACHAU,H.G. (1984) NUC.ACIDS RES.,12,5249-5263. (CHECKED BY AUTHOR 12/14/84)
86) **V18A'CL:** HEIDMANN,O. & ROUGEON,F. (1984) NATURE,311,74-76.
87) **V19A'CL:** HEIDMANN,O. & ROUGEON,F. (1984) NATURE,311,74-76.
88) **V19B'CL:** HEIDMANN,O. & ROUGEON,F. (1984) NATURE,311,74-76.
89) **V18B'CL:** HEIDMANN,O. & ROUGEON,F. (1984) NATURE,311,74-76.
90) **HF6-21/28:** ATKINSON,P.M.,LAMPMAN,G.W.,FURIE,B.C.,NAPARSTEK,Y.,SCHWARTZ,R.S.,STOLLAR,B.D. & FURIE,B. (1985) J.CLIN.INVEST.,75,1138-1143. (CHECKED BY AUTHOR 08/21/85)
91) **SAC:** SMITHIES,O.,GIBSON,O.M.,FANNING,E.M.,PERCY,M.E.,PARR,D.M. & CONNELL,G.E. (1971) SCIENCE,172,574-577. (CHECKED BY AUTHOR)
92) **WAG:** KAPLAN,A.P. & METZGER,H. (1969) BIOCHEMISTRY,8,3944-3951. (CHECKED BY AUTHOR)
93) **HBJ1:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.
94) **AMYLOID 547:** WESTERMARK,P.,SLETTEN,K. & NATVIG,J.B. (1981) ACTA PATH.MICROBIOL.SCAND.,C89,199-203. (CHECKED BY AUTHOR 11/09/81)
95) **WEB:** JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)
96) **HOE:** JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)
97) **LOD:** JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)
98) **HBJ10:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.
99) **BEN:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
100) **GR:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
101) **MAA:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
102) **MUK:** LITMAN,G.W.,GERBER-JENSON,B.,LITMAN,R.,MIDDAUGH,C.R. & SCHEFFEL,C. (1980) MOL.IMMUNOL.,17,337-344.
103) **AMYLOID 594:** WESTERMARK,P.,SLETTEN,K. & NATVIG,J.B. (1981) ACTA PATH.MICROBIOL.SCAND.,C89,199-203. (CHECKED BY AUTHOR 11/09/81)
104) **MAR:** KAPLAN,A.P. & METZGER,H. (1969) BIOCHEMISTRY,8,3944-3951. (CHECKED BY AUTHOR)
105) **AMYLOID:** COHEN,A.S.,SHIRAHAMA,T.,SKINNER,M.,BENSON,M.D. & CATHCART,E.S. (1973) PROTIDES BIOL.FLUIDS,20,73-80.
106) **BJ:** MILSTEIN,C. (1966) BIOCHEM.J.,101,352-368. (CHECKED BY AUTHOR)
107) **HBJ8:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.
108) **PEN:** MOULIN,A. & FOUGEREAU,M. (1973) NATURE NEW BIOLOGY,246,176-178.
109) **AMYLOID MS:** PICK,A.I.,SCHREIBMAN,S.,LAVIE,G. & FROHLICHMAN,R. (1973) PROTIDES BIOL.FLUIDS,20,63-72.
110) **CL*:** SOLOMON,A.,MCLAUGHLIN,C.L. & CAPRA,J.D. (1975) J.CLINICAL INVESTIGATION,55,579-586. (CHECKED BY AUTHOR)
111) **GM131'CL:** MORIN,J.W.,BLACK,A.,WU,M. & BEYCHOK,S. (1985) PROC.NAT.ACAD.SCI.USA,82,7025-7029.

NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP I

IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1: SET 1: ROY[1],AU[2],REI[3],HAU[4],HK101'CL[5],SCW[6],AG[7],WEA[8],HK137'CL[9],HK134'CL[10],DAUDI'CL[11],WALKER'CL[12],HF3-16/6[13],HF2-1/13B[14],HF2-18/2[15],HF2-1/17[16],BJ26[17],RFZ[18],PSM[19],HOM[20],ESM IGG[21],ESM IGM[22],WAT[23],AMYLOID VIII-B[24],LOW[25],DIE[26],CAR A[27],TEI[28],BJ48[29],CON[30],TRA[31],F-GUI[33],OU(IOC)[34],DEE[35]. (34 IDENTICAL)
SET 2: WES[41],Vb'CL[42],Vb''CL[43]. (3 IDENTICAL)
SET 3: HK102'CL[44],EU[45],DEN[46],PAU[47],HBJ4[48],FRA[49],GR'[50],PAUL[51],MON[52]. (9 IDENTICAL)
SET 4: AMYLOID BAN[56],BJ19[57],BEL[58]. (3 IDENTICAL)
SET 5: DAV[66],FIN[67]. (2 IDENTICAL)
SET 6: Vd'CL[69],LUX[70]. (2 IDENTICAL)

FR2: SET 1: ROY[1],AU[2],WALKER'CL[12],Vb'CL[42],Vb''CL[43],HK102'CL[44],KA[68],Vd'CL[69],Va''CL[72],Ve'CL[83]. (10 IDENTICAL)
SET 2: HK101'CL[5],HK134'CL[10]. (2 IDENTICAL)
SET 3: HK137'CL[9],AMYLOID BAN[56]. (2 IDENTICAL)
SET 4: V18A'CL[86]. (IDENTICAL TO 7 MOUSE V-KAPPA-III: PC1229(NZB)[1],PC2880(NZB)[2],PC7132(NZB)[3],MOPC70[5],PC2413(NZB)[11],50S10.1[27],V-21B18KB'CL[46]; AND 5 RABBIT V-KAPPA: K9-335[1],K9-338[2],K29-213[3],V20'CL[36],K18-167[64].)
SET 5: V19B'CL[88],V18B'CL[89]. (2 IDENTICAL HUMAN V-KAPPA-I; ALSO 4 HUMAN V-KAPPA-IV: VJI'CL[1],VKAPPA IV GERMLINE'CL[2],PB17IV'CL[3],LEN[4]; 1 MOUSE V-KAPPA-I: MCPC603[47]; 30 MOUSE V-KAPPA-III: MPC11'CL[6],TEPC111[7],PC3741(NZB)[8],TEPC124[9],MOPC321[12],PC7043(NZB)[13],PC7183(NZB)[14],PC6308(NZB)[15],PC6684(NZB)[17],PC7940(NZB)[18],PC7175(NZB)[19],PC2485(NZB)[20],PC4039(NZB)[21],PC7210(NZB)[23],H36-15[28],2242[29],V-21E1.5KB'CL[30],V-21C9.5KB'CL[31],PC7461(NZB)[33],PC2960(NZB)[34],97.C(A.BY)[35],10.A(A.TH)[39],H36-5[48],40.C(A.TH)[52],MOPC63[54],ABPC22[55],PC9245(NZB)[56],PC4050(NZB)[57],V-21B16KB'CL[58],11949[62]; 1 MOUSE V-KAPPA-VI: BFPC61A'CL[84]; AND 15 RABBIT V-KAPPA: K9-335-I[19],3368[20],BS-5[38],BS-1[39],K49-501[45],3547[47],K4820[57],K30-267[61],311[65],4422[66],17D9'CL[68],4192[71],4363[85],120[103],K-25[112].)

FR3: SET 1: HAU[4],HK101'CL[5],HK137'CL[9],HK134'CL[10],Vb'CL[42],Vb''CL[43],Va''CL[72]. (7 IDENTICAL)
SET 2: Ve'CL[83],V13'CL[85]. (2 IDENTICAL)
SET 3: V19B'CL[88],V18B'CL[89]. (2 IDENTICAL)

FR4: SET 1: AU[2],GAL(I)[36],CL*[110]. (3 IDENTICAL HUMAN V-KAPPA-I; ALSO 2 HUMAN V-KAPPA-II: GM 607 'CL[5],RPM1-6410'CL[16]; 7 HUMAN V-KAPPA-III: WOL[2],PAY[7],PIE[11],GLO[15],CUR[20],REE[57],VKAPPA3'CL[82]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)
SET 2: HAU[4]. (IDENTICAL TO 1 HUMAN V-KAPPA-III: POM[48].)
SET 3: AG[7],DEN[46],BI[63]. (3 IDENTICAL HUMAN V-KAPPA-I; ALSO 2 HUMAN V-KAPPA-II: NIM[3],FR[14]; 6 HUMAN V-KAPPA-III: NEU[5],GOT[6],GAR'[10],FLO[12],FR4[21],IARC/BL41'CL[28]; AND 1 HUMAN V-KAPPA-IV: LEN[4].)
SET 4: WEA[8],BJ48[29],LAY[39],EU[45]. (4 IDENTICAL)
SET 5: WALKER'CL[12],OU(IOC)[34]. (2 IDENTICAL HUMAN V-KAPPA-I; ALSO 1 HUMAN V-KAPPA-II: TEW[1].)
SET 6: WES[41],MEV[62]. (2 IDENTICAL)

IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:

CDR1: SET 1: AU[2],NE[71],SHE[77]. (3 IDENTICAL)
SET 2: WEA[8],GAL(I)[36]. (2 IDENTICAL)
SET 3: HK134'CL[10],Vb'CL[42],Vb''CL[43]. (3 IDENTICAL)
SET 4: HF3-16/6[13],HF2-1/13B[14],HF2-18/2[15],HF2-1/17[16]. (4 IDENTICAL)
SET 5: Vd'CL[69],Ve'CL[83]. (2 IDENTICAL)

CDR2: SET 1: HK101'CL[5],HK137'CL[9],HK134'CL[10],WALKER'CL[12],Vb'CL[42],Vb''CL[43]. (6 IDENTICAL)
SET 2: AG[7],NI[73]. (2 IDENTICAL)
SET 3: HK102'CL[44],Va''CL[72]. (2 IDENTICAL)
SET 4: Vd'CL[69],Ve'CL[83],V13'CL[85]. (3 IDENTICAL)
SET 5: V18A'CL[86]. (IDENTICAL TO 1 RABBIT V-KAPPA: 4153-I[24].)
SET 6: V19A'CL[87]. (IDENTICAL TO 1 RABBIT V-KAPPA: AH80-5[4].)

CDR3: SET 1: HK101'CL[5],HK134'CL[10]. (2 IDENTICAL)
SET 2: LAY[39]. (IDENTICAL TO 1 HUMAN V-KAPPA-III: POM[48].)
SET 3: Vb'CL[42],Vb''CL[43]. (2 IDENTICAL)

IDENTICAL SETS OF J-MINIGENES:

SET 1: AU[2]. (IDENTICAL TO 1 HUMAN V-KAPPA-II: RPM1-6410'CL[16]; 2 HUMAN V-KAPPA-III: PIE[11],VKAPPA3'CL[82]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)
SET 2: AG[7]. (IDENTICAL TO 1 HUMAN V-KAPPA-III: GOT[6].)
SET 3: WALKER'CL[12]. (IDENTICAL TO 1 HUMAN V-KAPPA-II: TEW[1].)
SET 4: DEN[46],BI[63]. (2 IDENTICAL HUMAN V-KAPPA-I; ALSO 1 HUMAN V-KAPPA-II: FR[14]; AND 3 HUMAN V-KAPPA-III: GAR'[10],FLO[12],IARC/BL41'CL[28].)

NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP I (cont'd)

GENERAL NOTES:

SEE SIGNAL PEPTIDE TABLE IF # OCCURS AT POSITION 0.

SPECIFIC NOTES:

5) **HK101'CL:** THE SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FOETAL LIVER DNA.

7) **AG:** THE AMINO ACID RESIDUES AT POSITIONS 39 AND 41 WERE REPORTED BY THE AUTHORS AS GLY AND LYS RESPECTIVELY; HOWEVER, THE PROOF WAS NOT ABSOLUTE. THUS, THEY ARE OMITTED.

9) **HK137'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FETAL DNA.

10) **HK134'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FETAL DNA.

17) **BJ26:** ACID RESIDUES AT POSITIONS 39 AND 41 OF BJ26 WERE REPORTED BY THE AUTHORS AS GLY AND LYS RESPECTIVELY. SINCE THIS PROTEIN WAS SEQUENCED BEFORE THE SEQUENCES OF MANY OTHER PROTEINS WERE KNOWN AT THESE TWO POSITIONS, WE HAVE OMITTED THEM.

33) **F-GUI:** THE SEQUENCES OF F-GUI AND S-GUI WERE FROM THE SAME PATIENT.

44) **HK102'CL:** THE SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FOETAL LIVER DNA.

55) **S-GUI:** THE SEQUENCES OF F-GUI AND S-GUI WERE FROM THE SAME PATIENT.

56) **AMYLOID BAN:** AMINO ACID RESIDUES FOUND AT POSITIONS 104 AND 105 ARE VAL,LEU AND GLN,GLU RESPECTIVELY.

57) **BJ18:** THE AMINO ACID RESIDUES AT POSITIONS 39 AND 41 WERE REPORTED BY THE AUTHORS AS GLY AND LYS RESPECTIVELY. SINCE THIS PROTEIN WAS SEQUENCED BEFORE THE SEQUENCES OF MANY OTHER PROTEINS WERE KNOWN AT THESE TWO POSITIONS, WE HAVE OMITTED THEM.

59) **JBL:** THE AMINO ACID RESIDUE FOUND AT POSITION 34 WAS ALA OR SER.

64) **AMYLOID ES305:** THE AMINO ACID RESIDUES AT POSITIONS 21 AND 29 WERE ILE OR LEU.

74) **PW:** THE SEQUENCE WAS FROM A PATIENT WITH TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER.

82) **RI:** THE SEQUENCE WAS FROM A PATIENT WITH TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER.

109) **AMYLOID MS:** THE AMINO ACID RESIDUE AT POSITION 2 MS WAS ILE OR LEU.

111) **GM131'CL:** FROM AN EPSTEIN-BARR VIRUS-TRANSFORMED HUMAN LYMPHOID CELL LINE

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
27C	(LEU,VAL)
27D	(TRP,GLU)
50	(ALA,ASP)
92	(TYR,ASP,ASN)
95A	(SER,GLY)
95B	(TRP,GLY)

HUMAN KAPPA LIGHT CHAINS SUBGROUP II

		INVARIANT RESIDUES	1 TEW	2 MIL	3 NIM	4 CUM	5 GM 607 'CL	6 BAT	7 BATES	8* ROB	9 SLO	10* WILS	11 GLI	12 AMYLOID TEW #	13 RAI	14* FR #	15 YOS	16 RPMI-6410 'CL #	17 MAN	18 KIR	19 HYL	20 MAG	21 TVE	22 EID	23 GAL (II)
FR 1	0		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP
	2	ILE(.97)	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	val		ILE	ILE	ILE	ILE	ILE	ILE
	3	VAL(.97)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL	VAL	VAL	VAL	VAL	VAL
	4		MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET	MET		MET	MET	MET	MET	MET	MET
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN	GLN	GLN	
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	SER	
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	PRO	PRO	PRO	
	9	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	10	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	12	PRO(.96)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	13	VAL(.96)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	gly	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	14	-THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR						
	15		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	leu	PRO						
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY						
	17		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	gln	GLU						
	18	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO						
	19	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA						
	20	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER						
	21	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE						
	22		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	gln	SER	SER	SER						
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS						
CDR 1	24	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG		ARG	ARG		ARG	ARG						ARG
	25		SER	SER	SER	SER	SER	SER	SER	ALA	SER	SER	SER	SER		SER		SER							
	26	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER		SER							
	27		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLX		GLN		GLN							
	27A		SER	ASN	SER	SER	SER	SER		ARG	SER	SER	SER			SER		SER							
	27B	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	—	LEU	LEU	LEU			LEU		LEU							
	27C		LEU	LEU	LEU	LEU	LEU	LEU	LEU	---	ARG	LEU	LEU			VAL		VAL							
	27D		HIS	GLX	TRP	ASP	HIS	HIS	HIS	—	HIS					TYR		TYR							
	27E		SER	—	SER	SER	SER	SER		—						ARG		SER							
	27F		---	—	---	GLY	---	---	---	—	ASX					—		---							
	28		ASP	SER	ASP	ASP	ASN	ASX	ASX	VAL						ASX		ASP							
	29		GLY	ASX	GLY	GLY	GLY	GLY	GLY	LEU						GLY		GLY							
	30		PHE	GLY	TYR	ASN	TYR	ASX	ASX							ASX		ASN							
	31		ASP	ASX	LYS	THR	ASN	ASX	ASX							THR		THR							
	32	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR							TYR		TYR							
	33	LEU	LEU	LEU	LEU	LEU	LEU	LEU								LEU		LEU							
	34		ASN	ASP	ASN	ASN	ASP	ASX								ASX		ASN							
FR 2	35	TRP	TRP	TRP	TRP	TRP	TRP	TRP								TRP		TRP							
	36		TYR	TYR	TYR	TYR	TYR	TYR								TYR		PHE							
	37		LEU	LEU	LEU	LEU	LEU	LEU								LEU		GLN							
	38		GLN	GLX	GLN	GLN	GLN	GLX								GLN		GLN							
	39		LYS	LYS	LYS	LYS	LYS	LYS								LYS		ARG							
	40		PRO	PRO	PRO	ALA	PRO	PRO								PRO		PRO							
	41	GLY	GLY	GLY	GLY	GLY	GLY	GLY								GLY		GLY							
	42		GLN	GLX	GLN	GLN	GLN	GLX								GLN		GLN							
	43	SER		SER	SER	SER	SER									SER		SER							
	44	PRO		PRO	PRO	PRO	PRO	PRO								PRO		PRO							
	45			GLX	GLN	GLN	GLN	GLX								GLU		ARG							
	46		LEU	LEU	LEU	LEU	LEU									LEU		ARG							
	47	LEU	LEU	LEU	LEU	LEU	LEU									LEU		LEU							
	48	ILE	ILE	ILE	ILE	ILE	ILE									ILE		ILE							
	49	TYR		TYR	TYR	TYR	TYR									TYR		TYR							
CDR 2	50			LEU	LEU	THR	LEU									LEU		LYS							
	51			GLY	GLY	LEU	GLY									SER		VAL							
	52	SER	SER	SER	SER	SER	SER									SER		SER							
	53		ASN	ASN	ASN	TYR	ASN									TYR		ASN							
	54	ARG	ARG	ARG	ARG	ARG	ARG									ARG		ARG							
	55		ALA	ALA	ALA	ALA	ALA									ASP		ASP							
	56	SER	SER	SER	SER	SER	SER									SER		SER							
FR 3	57	GLY	GLY	GLY	GLY	GLY	GLY									GLY		GLY							
	58	VAL	VAL	VAL	VAL	VAL	VAL									VAL		VAL							
	59	PRO	PRO	PRO	PRO	PRO	PRO									PRO		PRO							
	60		ASP	ASN	ASP	ASP	ASP									ASP		ASP							
	61	ARG	ARG	ARG	ARG	ARG	ARG									ARG		ARG							
	62	PHE	PHE	PHE	PHE	PHE	PHE								PHE	PHE		PHE							
	63	SER	SER	SER	SER	SER	SER								SER	SER		SER							
	64		GLY	GLY	GLY	GLY	GLY								GLY	ASP		GLY							
	65	SER	SER	SER	SER	SER	SER								SER	SER		SER							
	66	GLY	GLY	GLY	GLY	GLY	GLY								GLY	GLY		GLY							
	67	SER	SER	SER	SER	SER	SER								SER	SER		SER							
	68		GLY	GLY	ASP	GLY	GLY								GLY	GLY		GLY							
	69	THR	THR	THR	THR	THR	THR									THR		THR,							
	70		ASP	ASX	ASP	ASP	ASP									ASP		ASP							
	71	PHE	PHE	PHE	PHE	PHE	PHE								PHE	PHE		PHE							
	72	THR	THR	THR	THR	THR	THR								THR	THR		THR							
	73	LEU	LEU	LEU	LEU	LEU	LEU								LEU	LEU		LEU							
	74		LYS	LYS	ILE	LYS	LYS								ASX	LYS		LYS							
	75	ILE	ILE	ILE	ILE	ILE	ILE								ILE	ILE		ILE							
	76		SER	SER	SER	SER	SER								SER	THR		SER							
	77	ARG	ARG	ARG	ARG	ARG	ARG								ARG	ARG		ARG							
	78	VAL	VAL	VAL	VAL	VAL	VAL									VAL		VAL	VAL						
	79		GLU	GLX	GLU	GLN	GLU									GLN		GLU	GLX						
	80		ALA	ALA	PRO	ALA	ALA									ALA		ALA	ALA						
	81		GLU	GLX	GLU	GLU	GLU									GLU		GLU	GLX						
	82		ASP	ASX	ASP	ASP	ASP									ASP		ASP	ASX						
	83	VAL	VAL	VAL	VAL	VAL	VAL									VAL		VAL	VAL						
	84	GLY	GLY	GLY	GLY	GLY	GLY									GLY		GLY	GLY						
	85	VAL	VAL	VAL	VAL	VAL	VAL									VAL		VAL	VAL						
	86	TYR	TYR	TYR	TYR	TYR	TYR									TYR		TYR	TYR						
	87	TYR	TYR	TYR	TYR	TYR	TYR									TYR		TYR	TYR						
	88	CYS	CYS	CYS	CYS	CYS	CYS									CYS		CYS	CYS						
CDR 3	89	MET	MET	MET	MET	MET	MET									MET		MET							
	90		GLX	GLN	GLN	GLN	GLN									GLN		GLN							
	91		ALA	ALA	ALA	ARG	ALA									ALA		GLY							
	92		LEU	LEU	LEU	LEU	LEU									THR		THR							
	93		GLN	GLN	GLN	GLU	GLN									GLX		HIS							
	94		ALA	THR	SER	ILE	THR									SER		TRP							
	95		PRO	PRO	PRO	PRO	PRO									PRO		SER							
	95A		---	---	---	---	---									---		---							
	95B		---	---	---	---	---									---		---							
	95C		---	---	---	---	---									---		---							
	95D		---	---	---	---	---									—		---							
	95E		---	---	---	---	---									---		---							
	95F		---	---	---	---	---									---		---							
	96		ILE	LEU	PRO	TYR	GLN									TYR		TRP							
	97	THR	THR	THR	THR	THR	THR									THR		THR							
FR 4	98	PHE	PHE	PHE	PHE	PHE	PHE									PHE		PHE							
	99	GLY	GLY	GLY	GLY	GLY	GLY									GLY		GLY							
	100		GLN	GLY	GLN	GLN	GLN									GLN		GLN							
	101	GLY	GLY	GLY	GLY	GLY	GLY									GLY		GLY							
	102	THR	THR	THR	THR	THR	THR									THR		THR							
	103		ARG	ASN	LYS	LYS	LYS									LYS		LYS							
	104		LEU	VAL	LEU	LEU	VAL									LEU		VAL	VAL						
	105		GLU	GLU	GLU	GLU	GLU									GLX		GLU	GLU						
	106	ILE	ILE	ILE	ILE	ILE	ILE									ILE		ILE	ILE						
	106A		---	---	---	---	---									---		---	---						
	107		LYS	LYS	LYS	ARG	LYS									LYS		LYS	LYS						
	108	ARG	ARG	ARG	ARG	ARG	ARG									ARG		ARG							
	109	THR		THR	THR	THR										THR									

HUMAN KAPPA LIGHT CHAINS SUBGROUP II (cont'd)

		24* GIL	25 MEH	26 SC	27* TH	28 SYV	29 LUT	30 ROB 2	31 RAI 2	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
FR1	0	---	---	---	---	---	---	---	---				
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	31	1	31(ASP)	1.
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	30	2	29(ILE)	2.1
	3	VAL	VAL	VAL	VAL	VAL	VAL	VAL	met	30	2	29(VAL)	2.1
	4	MET	MET	MET	MET	MET	MET	leu	thr	30	3	28(MET)	3.2
	5	THR	THR	THR	THR	THR	THR			28	1	28(THR)	1.
	6	GLN	GLN	GLN	GLN	GLN	GLN			27	1	27(GLN)	1.
	7	SER	SER	SER	SER					25	1	25(SER)	1.
	8	PRO	PRO		PRO					24	1	24(PRO)	1.
	9	LEU	LEU							25	1	25(LEU)	1.
	10	SER								24	1	24(SER)	1.
	11	LEU								24	1	24(LEU)	1.
	12	ser								24	2	23(PRO)	2.1
	13									23	2	22(VAL)	2.1
	14									17	1	17(THR)	1.
	15									17	2	16(PRO)	2.1
	16									17	1	17(GLY)	1.
	17									17	2	16(GLU)	2.1
	18									17	1	17(PRO)	1.
	19									17	1	17(ALA)	1.
	20									17	1	17(SER)	1.
	21									17	1	17(ILE)	1.
	22									17	2	16(SER)	2.1
	23									17	1	17(CYS)	1.
CDR1	24									16	1	16(ARG)	1.
	25									14	2	13(SER)	2.2
	26									14	1	14(SER)	1.
	27									14	1 : 2	14(GLN) : 12(GLN)	1. : 2.3
	27A									12	3	10(SER)	
	27B									12	1	12(LEU)	
	27C									12	3	9(LEU)	
	27D									10	5	5(HIS)	
	27E									7	2	6(SER)	
	27F									2	2	1(+)	
	28									10	4	7(ASP) : 4(+)	5.7 : 10.
	29									10	3	8(GLY)	3.8
	30									9	4 : 5	5(ASN) : 3(ASP)	7.2 : 15.
	31									9	4	4(ASN) : 3(+)	9. : 12.
	32									9	1	9(TYR)	1.
	33									8	1	8(LEU)	1.
	34									8	2	6(ASN) : 4(+)	2.7 : 4.
FR2	35									8	1	8(TRP)	1.
	36									8	2	7(TYR)	2.3
	37									8	2	7(LEU)	2.3
	38									8	1 : 2	8(GLN) : 6(GLN)	1. : 2.7
	39									8	2	7(LYS)	2.3
	40									8	2	7(PRO)	2.3
	41									8	1	8(GLY)	1.
	42									8	1 : 2	8(GLN) : 6(GLN)	1. : 2.7
	43									6	1	6(SER)	1.
	44									7	1	7(PRO)	1.
	45									7	3	5(GLN) : 3(+)	4.2 : 7.
	46									7	2	6(LEU)	2.3
	47									7	1	7(LEU)	1.
	48									7	1	7(ILE)	1.
	49									6	1	6(TYR)	1.
CDR2	50									6	3	4(LEU)	4.5
	51									6	4	3(GLY)	8.
	52									7	1	7(SER)	1.
	53									7	2	5(ASN)	2.8
	54									7	1	7(ARG)	1.
	55									7	2	5(ALA)	2.8
	56									7	1	7(SER)	1.
FR3	57									7	1	7(GLY)	1.
	58									7	1	7(VAL)	1.
	59									7	1	7(PRO)	1.
	60									7	2	6(ASP)	2.3
	61									7	1	7(ARG)	1.
	62									8	1	8(PHE)	1.
	63									8	1	8(SER)	1.
	64									8	2	7(GLY)	2.3
	65									8	1	8(SER)	1.
	66									8	1	8(GLY)	1.
	67									8	1	8(SER)	1.
	68									8	2	7(GLY)	2.3
	69									7	1	7(THR)	1.
	70									7	1 : 2	7(ASP) : 6(ASP)	1. : 2.3
	71									8	1	8(PHE)	1.
	72									8	1	8(THR)	1.
	73									8	1	8(LEU)	1.
	74									8	3	6(LYS)	4.
	75									8	1	8(ILE)	1.
	76									8	2	7(SER)	2.3
	77									8	1	8(ARG)	1.
	78									8	1	8(VAL)	1.
	79									8	2	6(GLU) : 4(+)	2.7 : 4.
	80									8	2	7(ALA)	2.3
	81									8	1 : 2	8(GLU) : 6(GLU)	1. : 2.7
	82									8	1 : 2	8(ASP) : 6(ASP)	1. : 2.7
	83									8	1	8(VAL)	1.
	84									8	1	8(GLY)	1.
	85									8	1	8(VAL)	1.
	86									8	1	8(TYR)	1.
	87									8	1	8(TYR)	1.
	88									8	1	8(CYS)	1.
CDR3	89									7	1	7(MET)	1.
	90									7	1 : 2	7(GLN) : 6(GLN)	1. : 2.3
	91									7	3	5(ALA)	4.2
	92									7	2	5(LEU)	2.8
	93									7	3	5(GLN) : 4(GLN)	4.2 : 5.3
	94									7	5	2(+)	18.
	95									7	2	6(PRO)	2.3
	95A												
	95B												
	95C												
	95D												
	95E												
	95F												
	96									7	6	2(TYR)	21.
	97									7	1	7(THR)	1.
FR4	98									7	1	7(PHE)	1.
	99									7	1	7(GLY)	1.
	100									7	2	6(GLN)	2.3
	101									7	1	7(GLY)	1.
	102									7	1	7(THR)	1.
	103									7	3	5(LYS)	4.2
	104									8	2	4(+)	4.
	105									8	1 : 2	8(GLU) : 7(GLU)	1. : 2.3
	106									8	1	8(ILE)	1.
	106A												
	107									8	2	7(LYS)	2.3
	108									7	1	7(ARG)	1.
	109									4	1	4(THR)	1.

ANTIBODY SPECIFICITIES: HUMAN KAPPA LIGHT CHAINS SUBGROUP II

8) **ROB:** COLD AGGLUTININ WITH ANTI-PR1D ACTIVITY
10) **WILS:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
14) **FR:** ANTI-PHOSPHOCHOLINE(BINDING CONSTANT=6.4X10EXP4)
24) **GIL:** ANTI-IGG
27) **TH:** COLD AGGLUTININ WITH ANTI-PR2 ACTIVITY (RBC MEMBRANE ANTIGEN ON HUMAN, RAT AND GUINEA PIG ERYTHROCYTES INACTIVATED BY PROTEOLYTIC ENZYMES AND NEURAMINIDASE)

REFERENCE: HUMAN KAPPA LIGHT CHAINS SUBGROUP II

1) **TEW:** PUTNAM,F.W.,WHITLEY,E.J.,JR., PAUL,C.& DAVIDSON,J.N. (1973) BIOCHEMISTRY,12,3763-3780. (CHECKED BY AUTHOR 06/15/83)
2) **MIL:** DREYER,W.J.,GRAY,W.R. & HOOD,L. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,353-367.
3) **NIM:** EULITZ,M. & KLEY,H.-P. (1977) IMMUNOCHEM.,14,289-297. (CHECKED BY AUTHOR 10/18/77)
4) **CUM:** HILSCHMANN,N. & CRAIG,L.C. (1965) PROC.NAT.ACAD.SCI.USA,53,1403-1409; HILSCHMANN,N. (1967) Z.PHYSIOL.CHEM.,348,1718-1722; HILSCHMANN,N. (1969) NATURE.,56,195-205. (CHECKED BY AUTHOR)
5) **GM 607'CL:** KLOBECK,H.G.,SOLOMON,A. & ZACHAU,H.G. (1984) NATURE,309,73-76.
6) **BAT:** DAYHOFF,M.O. (1972) ATLAS OF PROTEIN SEQUENCE & STRUCTURE,5,D-248. SUBMITTED BY SMITHIES,O.,GIBSON,D.M. AND FANNING,E.M. (CHECKED BY AUTHOR)
7) **BATES:** SMITH,G.P.,HOOD,L. & FITCH,W.M. (1971) ANN.REV.BIOCHEM.,40,969-1012.
8) **ROB:** GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)
9) **SLO:** WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROEHLICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)
10) **WILS:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
11) **GLI:** FRANGIONE,B.,FRANKLIN,E.C. & PRELLI,F. (1976) SCAND.J.IMMUNOL.,5,623-627. (CHECKED BY AUTHOR 10/17/77)
12) **AMYLOID TEW:** TERRY,W.D.,PAGE,D.L.,KIMURA,S.,ISOBE,T.,OSSERMAN,E.F. & GLENNER,G.G. (1973) J.CLIN.INVEST.,52,1276-1281. (CHECKED BY AUTHOR 03/02/84)
13) **RAI:** MILSTEIN,C.P. & MILSTEIN,C. (1971) BIOCHEM.J.,121,211-215. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED)
14) **FR:** RIESEN,W.,RUDIKOFF,S.,ORIOL,R. & POTTER,M. (1975) BIOCHEMISTRY,14,1052-1057; RIESEN,W.F.,BRAUN,D.G. & JATON,J.C. (1976) PROC.NAT.ACAD.SCI.USA,73,2096-2100; RIESEN,W.F. & JATON,J.C. (1976) BIOCHEMISTRY,15,3829-3833. (CHECKED BY AUTHOR 12/05/77)
15) **YOS:** WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROEHLICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)
16) **RPM1-6410'CL:** HIETER,P.A.,MAX,E.E.,SEIDMAN,J.G.,MAIZEL,J.V.,JR. & LEDER,P. (1980) CELL,22,197-207; KLOBECK,H.G.MEINDL,A.,COMBRIATO,G., SOLOMON,A. & ZACHAU,H.G. (1985) NUC.ACIDS RES.,13,6499-6513.
17) **MAN:** MILSTEIN,C. (1969) PROC. 5TH FEBS SYMP.,15,43-56. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED)
18) **KIR:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)
19) **HYL:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)
20) **MAG:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)
21) **TVE:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)
22) **EID:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR)
23) **GAL(II):** MILSTEIN,C.,JARVIS,J.M. & MILSTEIN,C.P. (1969) J.MOL.BIOL.,46,599-602. (CHECKED BY AUTHOR)
24) **GIL:** ABRAHAM,G.N.,BROWN,P.,JOHNSTON,S.L.,NELLIS,L.,MARKS,S. & WELCH,E.H. (1978) IMMUNOLOGY,35,447-453. (CHECKED BY AUTHOR 07/23/79)
25) **MEH:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)
26) **SC:** SEON,B.K.,YAGI,Y. & PRESSMAN,D. (1973) J.IMMUNOL.,110,345-349. (CHECKED BY AUTHOR)
27) **TH:** GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)
28) **SYV:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)
29) **LUT:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,215-218. (CHECKED BY AUTHOR 12/05/77)
30) **ROB2:** MOULIN,A. & FOUGEREAU,M. (1973) NATURE NEW BIOLOGY,246,176-178.
31) **RAI2:** MOULIN,A. & FOUGEREAU,M. (1973) NATURE NEW BIOLOGY,246,176-178.

NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP II

IDENTICAL SETS OF FRAMEWORK SEGMENTS:
FR1: SET 1: TEW[1],MIL[2],NIM[3],CUM[4],GM 607 'CL[5],BAT[6],BATES[7],ROB[8],SLO[9],WILS[10],GLI[11],AMYLOID TEW[12],RAI[13]. (13 IDENTICAL)
FR2: SET 1: MIL[2],NIM[3],GM 607 'CL[5]. (3 IDENTICAL HUMAN V-KAPPA-II; ALSO 2 MOUSE V-KAPPA-II: VKAPPA 24B'CL[63],2S1.3[67].)
SET 2: MIL[2],FR[14]. (2 IDENTICAL)
FR3: SET 1: TEW[1],GM 607 'CL[5],RPM1-6410'CL[16]. (3 IDENTICAL)
FR4: SET 1: GM 607 'CL[5],RPM1-6410'CL[16]. (2 IDENTICAL HUMAN V-KAPPA-II; ALSO 3 HUMAN V-KAPPA-I: AU[2],GAL(I)[36],CL'[110]; 7 HUMAN V-KAPPA-III: WOL[2],PAY[7],PIE[11],GLO[15],CUR[20],REE[57],VKAPPA3'CL[82]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)
SET 2: NIM[3],FR[14]. (2 IDENTICAL HUMAN V-KAPPA-II; ALSO 3 HUMAN V-KAPPA-I: AG[7],DEN[46],BI[63]; 6 HUMAN V-KAPPA-III: NEU[5], GOT[6],GAR'[10],FLO[12],FR4[21],IARC/BL41'CL[28]; AND 1 HUMAN V-KAPPA-IV: LEN[4].)
SET 3: TEW[1]. (IDENTICAL TO 2 HUMAN V-KAPPA-I: WALKER'CL[12],OU(IOC)[34].)

IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:
CDR1:
CDR2: SET 1: MIL[2],NIM[3],GM 607 'CL[5]. (3 IDENTICAL)
CDR3:

IDENTICAL SETS OF J-MINIGENES:
SET 1: RPM1-6410'CL[16]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: AU[2]; 2 HUMAN V-KAPPA-III: PIE[11],VKAPPA3'CL[82]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)
SET 2: TEW[1]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: WALKER'CL[12].)
SET 3: FR[14]. (IDENTICAL TO 2 HUMAN V-KAPPA-I: DEN[46],BI[63]; AND 3 HUMAN V-KAPPA-III: GAR'[10],FLO[12],IARC/BL41'CL[28].)

SPECIFIC NOTES:
12) **AMYLOID TEW:** IT HAS THE SAME SEQUENCE AS THAT OF TEW SO FAR AS THE SEQUENCED POSITIONS ARE CONCERNED.
14) **FR:** AN IDIOTYPIC ANTIBODY TO FR NOT INHIBITABLE BY PHOSPHORYLCHOLINE REACTED BETTER WITH THE FR HEAVY CHAIN THAN WITH THE LIGHT CHAIN. THE CROSS-REACTION WITH MOPC167 WAS 10,000 TIMES WEAKER. (RIESEN,W.F. (1979) EUR.J.IMMUNOL.,9,421-425.)
16) **RPM1-6410'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN ADULT DNA.

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
27F	(GLY,ASN) : (GLY,ASP)
28	(ASP,ASN)
31	(THR,ASP)
34	(ASP,ASN)
45	(GLU,GLN)
79	(GLU,GLN)
94	(THR,SER)
104	(LEU,VAL)

HUMAN KAPPA LIGHT CHAINS SUBGROUP III

		INVARIANT RESIDUES	1 TI	2 WOL	3 SIE	4 NG9 'CL #	5 NEU	6 GOT	7 PAY	8 SON	9 WEI	10 GAR	11 PIE	12 FLO	13 LOP	14 SCA	15 GLO	16 SAL	17 WIL	18 MA	19 NIC	20 CUR	21 FR4	22 ORE	23 PER	24 CAM
FR1	0		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLX	GLU	GLU	GLU
	2		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE
	3	VAL(.96)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	4		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLX	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	10		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	11	LEU(.99)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	14	SER(.97)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	15	PRO(.98)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLX	GLX	GLU
	18		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
	19		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	20		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	21	LEU(.95)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	22	SER(.97)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	
CDR1	24		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
	25	ALA(.98)	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	26		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	GLY	SER	SER	SER	SER	SER	SER	SER	SER
	27		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLX	GLN
	27A		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	GLY	SER	SER	SER	SER	SER	SER	SER	SER		SER
	27B		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---
	27C		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---
	27D		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---
	27E		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---
	27F		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---
	28		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	ILE	VAL	SER	VAL	VAL	VAL	VAL	VAL	VAL		VAL
	29		SER	SER	SER	SER	SER	ARG	SER	SER	SER	SER	SER	SER	SER	SER	SER	VAL	SER	SER	SER	SER	ARG	SER		SER
	30		ASN	SER	ASN	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	ASN			SER	ASN	SER		SER
	31		SER	GLY	SER	SER	ARG	SER	SER	SER	SER	SER	SER	SER	ASN	ASN	SER	SER	SER			SER	ASN	SER		SER
	32		PHE	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	MET			TYR	TYR	TYR		TYR
	33		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	VAL	LEU			LEU	LEU	LEU		LEU
	34		ALA	GLY	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA			ALA	ALA	ALA		ALA
FR2	35	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP	TRP	TRP			TRP	TRP	TRP		TRP
	36	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR			TYR	TYR			TYR
	37		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN			GLN	GLN			GLN
	38		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN				GLN	GLN			GLN
	39		LYS	LYS	LYS	LYS	LYS	LYS	ARG	LYS	LYS	LYS	LYS	LYS	LYS		LYS	LYS				LYS	ARG			
	40		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO				PRO	PRO			
	41	GLY(.96)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY					GLY	GLY			
	42		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN					GLN	GLN			
	43		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA					ALA	ALA			
	44		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO					PRO	PRO			
	45		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG		ARG					ARG	LYS			
	46	LEU(.96)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU					LEU				
	47	LEU(.96)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU					LEU				
	48		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE			ILE					ILE				
	49		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR		TYR	TYR	TYR			TYR					TYR				
CDR2	50		VAL	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY			GLY					GLY				
	51		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA	ALA	ALA			ALA					ALA				
	52		SER	SER	SER	THR	SER	SER	SER	SER		SER	SER	SER			SER					SER				
	53		SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER			SER					SER				
	54	ARG(.95)	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG		ARG	ARG	ARG			ARG					ARG				
	55		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA	ALA	ALA			ALA					ALA	ALA			
	56		THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR			THR					THR	THR			
FR3	57	GLY(.96)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY			GLY					GLY	GLY			
	58		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE	ILE			ILE					ILE	ILE			
	59	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	PRO			PRO					PRO	PRO			
	60		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASN		ASP	ASP	ASP			ASP					ASP	ASP			
	61	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG		ARG	ARG	ARG			ARG					ARG	ARG			
	62	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE		PHE	PHE	PHE			PHE					PHE	PHE			
	63		SER	SER	SER	SER	THR	SER	SER	SER		SER	SER	SER			SER					SER	SER			
	64	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY			GLY					GLY	GLY			
	65	SER(.95)	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER			SER					SER	SER			
	66		GLY	GLY	GLY	ALA	GLY	GLY	GLY	GLY		GLY	GLY	GLY			GLY					GLY	GLY			
	67	SER(.95)	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER			SER					SER	SER			
	68	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY			GLY					GLY	GLY			
	69	THR(.95)	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR			THR					THR	THR			
	70		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	ASP			ASP					ASP	ASP			
	71	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE		PHE	PHE	PHE			PHE					PHE	PHE			
	72	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR			THR					THR	THR			
	73	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU			LEU					LEU	LEU			
	74	THR(.95)	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR	THR			THR					THR	THR			
	75	ILE(.95)	ILE	ILE	ILE	ILE	VAL	ILE	ILE	ILE		ILE	ILE	ILE			ILE					ILE	ILE			
	76		SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER			SER					SER	SER			
	77		GLY	GLY	GLY	ARG	ARG	ARG	ARG	ARG		ARG	ARG	ARG			ARG					ARG	ARG			
	78		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU	LEU			LEU					LEU	LEU			
	79		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU		GLU	GLU	GLU			GLU					GLU	GLU			
	80		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO	PRO	PRO			PRO					PRO	PRO			
	81	GLU(.95)	GLU	GLU	ASP	GLU	GLU	GLU	GLU	GLU		GLU	GLU	GLU			GLU					GLU	GLU			
	82	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP		ASP	ASP	ASP			ASP					ASP	ASP			
	83		PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE		PHE	PHE	PHE			PHE					PHE	PHE			
	84	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA	ALA	ALA			ALA					ALA	ALA			
	85	VAL(.95)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL	VAL	VAL			VAL					VAL	VAL			
	86	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR		TYR	TYR	TYR			TYR					TYR	TYR			
	87		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR		TYR	TYR	TYR			TYR					TYR	PHE			
	88	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		CYS	CYS	CYS			CYS					CYS	CYS			
CDR3	89	GLN(.95)	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN			GLN					GLN	GLN			
	90	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN	GLN	GLN			GLN					GLN	GLN			
	91		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR		TYR	TYR	TYR			TYR					TYR	TYR			
	92		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY	GLY			GLY					GLY	GLY			
	93		SER	SER	SER	ASN	ALA	SER	SER	SER		SER	SER	SER			SER					SER	GLY			
	94		SER	LEU	SER	SER	SER	SER	SER	SER		SER	SER	SER			SER					SER	SER			
	95		PRO	GLY	PRO	GLN	PRO	PRO	PRO	PRO		PRO	PRO	PRO			PRO					PRO	PRO			
	95A		---	---	---	---	---	---	---	PRO		---	---	---			---					---	---			
	95B		---	---	---	---	---	---	---	---		---	---	---			---					---	---			
	95C		---	---	---	---	---	---	---	---		---	---	---			---					---	---			
	95D		---	---	---	---	---	---	---	---		---	---	---			---					---	---			
	95E		---	---	---	---	---	---	---	---		---	---	---			---					---	---			
	95F		---	---	---	---	---	---	---	---		---	---	---			---					---	---			
	96		SER	ARG	GLN	---	CYS	ARG	LEU	TYR		TYR	TRP	TYR			LEU					ARG	GLN			
	97	THR(.95)	THR	THR	THR	---	THR	THR	THR	THR		THR	THR	THR			THR					THR	PRO			
FR4	98	PHE	PHE	PHE	PHE	---	PHE	PHE	PHE	PHE		PHE	PHE	PHE			PHE					PHE	PHE			
	99	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	GLY		GLY	GLY	GLY			GLY					GLY	GLY			
	100		GLN	GLN	GLN	---	GLN	GLN	GLN	GLY		GLN	GLN	GLN			GLN					GLN	GLN			
	101	GLY	GLY	GLY	GLY	---	GLY	GLY	GLY	GLY		GLY	GLY	GLY			GLY					GLY	GLY			
	102		THR	THR	SER	---	THR	THR	THR	THR		THR	THR	THR			THR					THR	THR			
	103		LYS	LYS	LYS	---	LYS	LYS	LYS	LYS		LYS	LYS	LYS			LYS					LYS	LYS			
	104		VAL	VAL	VAL	---	LEU	LEU	VAL	VAL		LEU	VAL	LEU			VAL					VAL	LEU			
	105		GLU	GLU	GLU	---	GLU	GLU	GLU	GLU		GLU	GLU	GLU			GLU					GLU	GLU			
	106		LEU	ILE	ILE	---	ILE	ILE	ILE	ILE		ILE	ILE	ILE			ILE					ILE	ILE			
	106A		---	---	---	---	---	---	---	---		---	---	---			---					---	---			
	107	LYS(.95)	LYS	LYS	LYS	---	LYS	LYS	LYS	LYS		LYS	LYS	LYS			LYS					LYS	LYS			
	108	ARG	ARG	ARG	ARG	---	ARG	ARG	ARG	ARG		ARG	ARG	ARG			ARG					ARG				
	109	THR	THR				THR	THR	THR	THR		THR	THR	THR			THR					THR				

HUMAN KAPPA LIGHT CHAINS SUBGROUP III (cont'd)

		25* STE	26* GJ	27* TAK	28 IARC/ BL41 'CL	29 RAD	30 DIL	31 CAS	32 MCE' #	33 KEA	34 SMI	35* AJ	36 BRO 'IGG	37 NIG	38 IKE	39 TIL	40 AMYLOID KSA	41 POL	42* CLA #	43* SHE' #	44 JH #	45 WIN	46 LEA	47 ARP	48* POM	49 VAND
FR 1	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	lys	GLU	GLU	GLU	GLU	GLU	GLU	GLU	asp	lys	GLU	GLU	GLU
	2	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	phe	ILE	ILE
	3	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	4	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	met	LEU	met	met	met	LEU	LEU	LEU	met	met
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	glu	GLN	GLN	GLN	GLN	glu	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ala	ala	asp	GLY	GLY	pro	ala	GLY	ala	#	#	ala	ala	ala	GLY	val	ala
	10	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13	LEU	LEU	LEU	LEU	LEU	LEU	LEU	phe	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU	val	val	LEU	LEU	LEU	LEU	val	val
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	15	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	GLU	GLU	GLX	GLU	asp	asp	asp	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLX	GLU	GLU	GLU	GLU	GLX	GLU	GLU	GLU
	18	ARG	ARG	ARG	ser	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG		ARG	ARG
	19	ALA	val	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	val	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	20	ala	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	THR	THR	THR	THR	THR	THR	THR	THR	ile	THR	THR
	21	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	22	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	23		CYS	CYS	CYS	CYS	CYS		CYS	CYS		CYS			CYS	CYS	CYS	CYS	CYS	CYS			CYS	CYS	CYS	CYS
CDR 1	24		ARG	ARG	ARG	ARG	ARG		ARG	ARG		ARG	ARG		ARG	ARG	ARG	ARG	ARG		THR		ARG	ARG	ARG	ARG
	25		ALA	ALA	ALA	ALA	ALA		ALA	ALA		ALA	ALA		ALA	ALA	ALA	ALA	ALA		ALA		ALA	ALA	ALA	ALA
	26		SER	SER	SER	SER	SER		SER	GLY		SER	SER		SER	SER	SER	SER	SER				SER	SER	SER	SER
	27			GLX	GLN	GLN	GLN		GLN	SER		GLN	GLN		GLN	GLN	GLX	GLX					GLX	GLN	GLN	GLN
	27A			SER	---	VAL	SER		---	---		SER	SER		SER	---	THR	---					---	---	SER	---
	27B			---	---	---	---		---	---		---	---		---	---	---	---					---	---	---	---
	27C			---	---	---	---		---	---		---	---		---	---	---	---					---	---	---	---
	27D			---	---	---	---		---	---		---	---		---	---	---	---					---	---	---	---
	27E			---	---	---	---		---	---		---	---		---	---	---	---					---	---	---	---
	27F			---	---	---	---		---	---		---	---		---	---	---	---					---	---	---	---
	28			VAL	SER	SER	LEU		SER	ASX		VAL	VAL		VAL	SER	LEU	SER					SER	HIS	ILE	ASX
	29			SER	VAL	SER	ASX			VAL		SER	ARG		SER	VAL		VAL					VAL	VAL	SER	ILE
	30				SER	ASN	SER			ALA			SER		ASN	SER		ARG					SER	ILE	ASN	GLY
	31			SER	SER	SER	LYS			LYS			SER		SER	SER							LEU	ALA	SER	SER
	32			TYR	ASN	TYR	SER			SER			TYR		TYR	TYR		TYR					TYR	GLY	TYR	ASN
	33			LEU	LEU	LEU	LEU			LEU			LEU		LEU	LEU		LEU					LEU	TYR	LEU	LEU
	34			ALA	ALA	ALA	SER			ALA			ASP		ALA	ALA		ALA					ALA	ALA	ALA	ALA
FR 2	35				TRP	TRP	TRP			TRP			TRP		TRP	TRP								TRP	TRP	TRP
	36				TYR	TYR	TYR			TYR			TYR		TYR	TYR	TYR							TYR	TYR	TYR
	37				GLN	GLN	GLX			GLX			GLN		GLN	GLN	GLN							GLN	GLN	GLX
	38				GLN	GLN	GLX			GLX			GLN		GLN		GLN							GLN	GLN	GLX
	39				LYS	LYS	LYS			LYS			LYS		ARG		SER							LYS	LYS	LYS
	40				ARG	PRO	PRO			PRO			PRO		PRO		PRO							PRO	PRO	PRO
	41				GLY	GLY	GLY										GLY								SER	GLY
	42				GLN	GLN	GLX										GLN								GLY	ARG
	43				SER	ALA	THR										ALA								SER	ALA
	44				PRO	PRO	PRO										PRO								PRO	PRO
	45				ARG	ARG	ARG										ARG								ARG	ARG
	46				LEU		LEU										LEU								LEU	LEU
	47				LEU		LEU										LEU								LEU	
	48				ILE		ILE										ILE								ILE	
	49				ARG		TYR										TYR								TYR	
CDR 2	50				ASP												GLY								GLY	
	51				ALA												THR								ALA	
	52				SER												SER								SER	
	53				SER												THR								THR	
	54				ARG												ARG								ARG	
	55				ALA	ALA											ALA								ALA	
	56				ASN	THR											THR								THR	
FR 3	57				GLY	GLY											GLY								GLY	
	58				ILE	THR											ILE								ILE	
	59				PRO	PRO											PRO								PRO	
	60				ASP	VAL											ALA								ALA	
	61				ARG	ARG											ARG								ARG	
	62				PHE	PHE											PHE								PHE	
	63				SER	SER											SER								SER	
	64				GLY	GLY											GLY								GLY	
	65				SER	SER																			SER	
	66				GLY	GLU																			GLY	
	67				SER	SER																			SER	
	68				GLY	GLY																			GLY	
	69				THR	THR																			THR	
	70				ASP	ASP																			GLU	
	71				PHE	PHE																			PHE	
	72				THR	THR																			THR	
	73				LEU	LEU																			LEU	
	74				ILE	THR																			THR	
	75				ILE	ILE																			ILE	
	76				SER	SER																			SER	
	77				ARG	ARG																			SER	
	78				LEU	LEU																			LEU	
	79				GLU	GLU																			GLN	
	80				PRO	PRO																			SER	
	81				GLU	GLU																			GLU	
	82				ASP	ASP																			ASP	
	83				PHE	PHE																			PHE	
	84				ALA	ALA																			ALA	
	85				VAL	VAL																			VAL	
	86				TYR	TYR																			TYR	
	87				TYR	TYR																			TYR	
	88				CYS	CYS																			CYS	
CDR 3	89				GLN	GLN																			GLN	
	90				GLN	GLN																			GLN	
	91				TYR	TYR																			TYR	
	92				SER	GLU																			ASN	
	93				THR	THR																			ASN	
	94				SER	SER																			TRP	
	95				PRO	PRO																			PRO	
	95A				---	---																			---	
	95B				---	---																			---	
	95C				---	---																			---	
	95D				---	---																			---	
	95E				---	---																			---	
	95F				---	---																			---	
	96				TYR	THR																			PRO	
	97				THR	THR																			THR	
FR 4	98				PHE	PHE																			PHE	
	99				GLY	GLY																			GLY	
	100				GLN	GLN																			GLN	
	101				GLY	GLY																			GLY	
	102				THR	THR																			THR	
	103				LYS	ARG																			ARG	
	104				LEU	LEU																			VAL	
	105				GLU	ASP																			GLU	
	106				ILE	ILE																			ILE	
	106A				---	---																			---	
	107				LYS	LYS																			LYS	
	108				ARG																				ARG	
	109																									

HUMAN KAPPA LIGHT CHAINS SUBGROUP III (cont'd)

		50 AMYLOID SO124	51 DOV	52 SHM	53 GRA	54* GOE II	55 LOW'	56 VER	57 REE	58 WE #	59 HOW	60 HS4	61 HBJ 5	62* TEH	63* CRA (III)	64* PLA	65* PIN	66 MCE	67 HAC	68 K-EV15 CL	69 BER	70* BOR	71* DRI	72* WAL	73* GOL	74* GAG
FR1	0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1	GLU	GLU	GLU	GLU	asp	GLU	GLU	GLU	asp	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU
	2	ILE	ILE	ILE	met	ILE	ILE	thr	leu	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	thr	ILE	ILE	ILE	ILE	ILE	ILE
	3	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	gln	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	thr	VAL	VAL	VAL	VAL	VAL	VAL
	4	met	met	met	met	LEU	met	LEU	LEU	met	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLX	GLN	GLN	GLN	GLN
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	
	9	ala	ala	ala	ala	ala	ala	GLY	ala	ser	GLY	GLY	asx	GLY	GLY	GLY	GLY	GLY	GLY	ala	GLY	ala				
	10	THR	THR	THR	THR	ser	gly	THR	THR	ser	THR	THR	THR	THR	THR	THR	THR	THR	THR	phe	THR	THR				
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	met						
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER						
	13	val	val	val	met	val	val	LEU	val	ala	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		ala						
	14	SER	SER	SER	SER	thr	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER			thr						
	15	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	val	PRO	PRO	PRO	PRO	PRO	PRO	PRO			PRO						
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY							GLY						
	17	GLU	GLU	GLU	GLU	GLU	gly	GLU	GLU	asp	GLU	GLU	GLX							asp						
	18	ARG	ser	gly	ARG	ARG	thr	val	glu											lys						
	19	val	ALA	ALA	ALA	ALA	ALA	val	val	val										val						
	20	THR	THR	THR	THR	ser	THR	THR	THR	#										asn						
	21	LEU	LEU	LEU	LEU	LEU	LEU	ile	LEU	ile										ile						
	22	SER	SER	SER	SER	SER	SER	tyr	SER	thr										SER						
	23	CYS	CYS	CYS		CYS	CYS	CYS	CYS											CYS						
CDR1	24	ARG	ARG	ARG			SER	LYS	ARG											LYS						
	25	ALA	ALA	ALA			ALA	SER	ALA	ALA										ALA						
	26		SER	SER			GLY		SER	SER										SER						
	27		GLU	GLU			GLN		GLU	GLN										GLN						
	27A		---	---			---	---	---	---										---						
	27B		---	---			---	---	---	---										---						
	27C		---	---			---	---	---	---										---						
	27D		---	---			---	---	---	---										---						
	27E		---	---			---	---	---	---										---						
	27F		---	---			---	---	---	---										---						
	28		SER	THR			SER	LEU	SER	THR										ASP						
	29		VAL	VAL			VAL	SER	LEU	#										ILE						
	30		SER	ALA			ASP		SER	GLY										ASP						
	31		THR	LYS			GLX		ASP											ASP						
	32		ASX	SER			THR		ASN											ASP						
	33		LEU	LEU			VAL	LEU	VAL	#										MET						
	34		ALA	ALA			ALA	ALA	ALA											ASN						
FR2	35		TRP	TRP			TRP	TRP	TRP											TRP						
	36		TYR	TYR			TYR	TYR	TYR											TYR						
	37		GLX	GLX			GLX	GLN	GLN											GLN						
	38		GLX	GLX			GLX	GLN	HIS											GLN						
	39		LYS				LYS		LYS											LYS						
	40		PRO				PRO		PRO											PRO						
	41								GLY											GLY						
	42								GLN											GLU						
	43								ALA											ALA						
	44							LEU	PRO											ALA						
	45							ARG	ARG											ILE						
	46							LEU	LEU											PHE						
	47							LEU	LEU											ILE						
	48							ILE	LEU											ILE						
	49							TYR	TYR											GLN						
CDR2	50							GLY	ALA											GLU						
	51							VAL	THR											ALA						
	52							SER	SER											THR						
	53							THR	THR											THR						
	54							ARG	ARG											LEU						
	55							ALA	PRO											VAL						
	56							ALA	THR											PRO						
FR3	57							GLY	ASP											GLY						
	58							ILE	ILE											ILE						
	59							PRO	PRO											PRO						
	60							ASP	ALA											PRO						
	61							ARG	ARG											ARG						
	62							PHE	PHE											PHE						
	63							THR	SER											SER						
	64							GLY	GLY											GLY						
	65							ARG	SER											SER						
	66							ALA	MET											GLY						
	67							SER	SER											TYR						
	68							GLY	GLY											GLY						
	69							THR	THR											THR						
	70							ASP	GLU											ASP						
	71							PHE	PHE											PHE						
	72							THR	THR											THR						
	73							LEU	LEU											LEU						
	74							THR	THR											THR						
	75							ILE	ILE											ILE						
	76							SER	PHE											ASN						
	77							ASP	ARG											ASN						
	78							LEU	VAL											ILE						
	79							GLU	GLU											GLU						
	80							PRO	SER											SER						
	81							GLU	GLU											GLU						
	82							ASP	ASP											ASP						
	83							PHE	SER											ALA						
	84							ALA	ALA											ALA						
	85							VAL	VAL											TYR						
	86							TYR	TYR											TYR						
	87							TYR	TYR											PHE						
	88							CYS	CYS											CYS						
CDR3	89							GLN	GLN											LEU						
	90							GLN	GLN											GLN						
	91							TYR	HIS											HIS						
	92							ASP	GLU											ASP						
	93							THR	---											ASN						
	94							SER	---											PHE						
	95							PRO	---											PRO						
	95A							---	---											---						
	95B							---	---											---						
	95C							---	---											---						
	95D							---	---											---						
	95E							---	---											---						
	95F							---	---											---						
	96							LEU	---											---						
	97							THR	THR											---						
FR4	98							PHE	PHE											---						
	99							GLY	GLY											---						
	100							GLY	GLN											---						
	101							GLY	GLY											---						
	102							THR	THR											---						
	103							LYS	LYS											---						
	104							LEU	VAL											---						
	105							ASP	GLU											---						
	106							VAL	ILE											---						
	106A							---	---											---						
	107							ARG	LYS											---						
	108								ARG											---						
	109																			---						

HUMAN KAPPA LIGHT CHAINS SUBGROUP III (cont'd)

		75 DOB	76 HS6	77 HBJ 12	78 BUR (K)	79 LEG	80 B6	81 AMYLOID WR #	82 VKAPPA3 CL #	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
FR1	0	---	---	---	---	---							
	1	GLU	GLU	GLU	GLU	GLU				79	3 : 4	74(GLU) : 73(GLU)	3.2 : 4.3
	2	ILE	ILE	ILE	ILE	ILE				79	5	74(ILE)	5.3
	3	ile	VAL	VAL	VAL	VAL				79	4	76(VAL)	4.2
	4	met	LEU	val	LEU	LEU				79	3	65(LEU)	3.6
	5	THR	THR	THR						77	1	77(THR)	1.
	6	GLN	GLN	GLN						77	2	75(GLN) : 69(GLN)	2.1 : 2.2
	7	SER								75	1	75(SER)	1.
	8	PRO								74	1	74(PRO)	1.
	9	ala								69	6 : 7	46(GLY)	9. : 11.
	10									70	4	66(THR)	4.2
	11									68	2	67(LEU)	2.
	12									67	1	67(SER)	1.
	13									67	5	52(LEU)	6.4
	14									68	2	64(SER)	2.1
	15									68	2	65(PRO)	2.
	16									62	1	62(GLY)	1.
	17									62	3 : 4	56(GLU) : 50(GLU)	3.3 : 5.
	18						ARG			58	7	51(ARG)	8.
	19						ALA			60	2	52(ALA)	2.3
	20						ala			59	5	53(THR)	5.6
	21						LEU			60	2	57(LEU)	2.1
	22						SER			60	3	58(SER)	3.1
	23						CYS			50	1	50(CYS)	1.
CDR1	24						ARG			51	4	47(ARG)	4.3
	25						ALA			52	2	51(ALA)	2.
	26						SER			49	2	46(SER)	2.1
	27						GLN			47	3	43(GLN) : 37(GLN)	3.3 : 3.8
	27A						SER			32	4	29(SER)	
	27B						---						
	27C						---						
	27D						---						
	27E						---						
	27F						---						
	28						LEU			47	7 : 8	25(VAL)	13. : 15.
	29						SER			44	6	27(SER)	9.8
	30						GLY			40	7	24(SER)	12.
	31						ASN			39	10	24(SER)	16.
	32						TYR			40	8	28(TYR)	11.
	33						LEU			41	4	36(LEU)	4.6
	34						ALA			41	5	37(ALA)	5.5
FR2	35						TRP	TRP		38	1	38(TRP)	1.
	36						TYR	TYR		39	1	39(TYR)	1.
	37						GLN	GLN		39	1 : 2	39(GLN) : 33(GLN)	1. : 2.4
	38						GLN	GLN		37	2 : 3	36(GLN) : 30(GLN)	2.1 : 3.7
	39						LYS	LYS		33	3	29(LYS)	3.4
	40						PRO	PHE		34	3	32(PRO)	3.2
	41						GLY	GLY		27	2	26(GLY)	2.1
	42						GLN	GLN		27	4	24(GLN) : 23(GLN)	4.5 : 4.7
	43						ALA	ALA		26	3	23(ALA)	3.4
	44						PRO	PRO		27	3	25(PRO)	3.2
	45						ARG			26	3	24(ARG)	3.3
	46						LEU	LEU		24	2	23(LEU)	2.1
	47						LEU	LEU		23	2	22(LEU)	2.1
	48						MET	ILE		22	3	20(ILE)	3.3
	49						TYR	PHE		22	4	19(TYR)	4.6
CDR2	50						GLY	ASP		21	5	16(GLY)	6.6
	51						VAL			20	3	16(ALA)	3.8
	52						SER			20	2	18(SER)	2.2
	53						SER	SER		21	2	16(SER)	2.6
	54						ARG	#		20	2	19(ARG)	2.1
	55						ALA	ALA		23	3	21(ALA)	3.3
	56						THR			22	4	19(THR)	4.6
FR3	57						GLY	GLY		23	2	22(GLY)	2.1
	58						ILE	VAL		23	3	21(ILE)	3.3
	59						PRO		PRO	23	1	23(PRO)	1.
	60						ASP		ASP	23	5	17(ASP)	6.8
	61						ARG		ARG	23	1	23(ARG)	1.
	62						PHE		PHE	23	1	23(PHE)	1.
	63						SER		SER	23	2	21(SER)	2.2
	64						GLY		GLY	23	1	23(GLY)	1.
	65						SER		SER	22	2	21(SER)	2.1
	66						GLY		ALA	22	4	17(GLY)	5.2
	67						SER		SER	22	2	21(SER)	2.1
	68						GLY		GLY	22	1	22(GLY)	1.
	69						ALA		THR	22	2	21(THR)	2.1
	70								ASP	21	2	19(ASP)	2.2
	71								PHE	21	1	21(PHE)	1.
	72								THR	21	1	21(THR)	1.
	73								LEU	21	1	21(LEU)	1.
	74								THR	21	2	20(THR)	2.1
	75								ILE	21	2	20(ILE)	2.1
	76								SER	21	3	19(SER)	3.3
	77						ARG		ARG	22	5	16(ARG)	6.9
	78						LEU		LEU	22	3	20(LEU)	3.3
	79						GLX		GLU	22	2	21(GLU) : 20(GLU)	2.1 : 2.2
	80						PRO		PRO	22	2	19(PRO)	2.3
	81						GLU		GLU	22	2	21(GLU)	2.1
	82						ASP		ASP	22	1	22(ASP)	1.
	83						PHE		PHE	22	3	20(PHE)	3.3
	84						ALA		ALA	22	1	22(ALA)	1.
	85						VAL		VAL	22	2	21(VAL)	2.1
	86						TYR		TYR	22	1	22(TYR)	1.
	87						TYR		TYR	22	2	20(TYR)	2.2
	88						CYS		CYS	22	1	22(CYS)	1.
CDR3	89						GLN		GLN	22	2	21(GLN)	2.1
	90						GLN		GLN	22	1	22(GLN)	1.
	91						TYR		TYR	22	2	20(TYR)	2.2
	92						GLY		GLY	22	5	16(GLY)	6.9
	93						SER		ASN	21	5	12(SER)	8.8
	94						SER		SER	21	4	18(SER)	4.7
	95						PRO		GLN	21	3	18(PRO)	3.5
	95A						---		---	1	1	1(PRO)	
	95B						---		---				
	95C						---		---				
	95D						---		---				
	95E						---		---				
	95F						---		---				
	96						PHE		TRP	19	10	4(TYR)	48.
	97						THR		THR	20	2	19(THR)	2.1
FR4	98						PHE		PHE	20	1	20(PHE)	1.
	99						GLY		GLY	20	1	20(GLY)	1.
	100						GLN		GLN	20	2	18(GLN)	2.2
	101						GLY		GLY	20	1	20(GLY)	1.
	102						SER		THR	20	2	18(THR)	2.2
	103						LYS		LYS	20	2	18(LYS)	2.2
	104						LEU		VAL	20	2	11(VAL)	3.6
	105						GLU		GLU	20	2	18(GLU)	2.2
	106						ILE		ILE	20	3	18(ILE)	3.3
	106A						---		---				
	107						LYS		LYS	20	2	19(LYS)	2.1
	108								ARG	16	1	16(ARG)	1.
	109									10	1	10(THR)	1.

ANTIBODY SPECIFICITIES: HUMAN KAPPA LIGHT CHAINS SUBGROUP III

2) **WOL:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE
3) **SIE:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE
5) **NEU:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE (KUNKEL,H.G.,WINCHESTER,R.J.,JOSLIN,F.G. & CAPRA,J.D. (1974) J.EXP.MED.,139,128)
6) **GOT:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
7) **PAY:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
8) **SON:** CRYOGLOBULIN WITH ANTI-LOW-DENSITY LIPOPROTEIN ACTIVITY; B IDIOTYPE
9) **WEI':** CRYOGLOBULIN WITH ANTI-LOW-DENSITY LIPOPROTEIN ACTIVITY; B IDIOTYPE
10) **GAR':** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
11) **PIE:** AUTOANTIBODY WHICH BINDS SPECIFICALLY TO INTERMEDIATE FILAMENTS
12) **FLO:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
13) **LOP:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
14) **SCA:** CRYOGLOBULIN WITH ANTI-LOW-DENSITY LIPOPROTEIN ACTIVITY; B IDIOTYPE
15) **GLO:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE; CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
18) **MA:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY (GROUP 1)
19) **NIC:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP SMALL I ACTIVITY
20) **CUR:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
22) **DRE:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
23) **PER:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
25) **STE:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
26) **GJ:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY (ATYPICAL)
27) **TAK:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
35) **AJ:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
42) **CLA:** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
43) **SHE':** CRYOGLOBULIN WITH ANTI-IGG ACTIVITY; B IDIOTYPE
48) **POM:** ANTI-HUMAN GAMMA G1 GLOBULIN; PO IDIOTYPE
54) **GOEII:** ANTI-MEASLES VIRUS (WOODFOLK STRAIN); ANTI-SUBACUTE SCLEROSING PANENCEPHALITIS VIRUS (LEC STRAIN)
62) **TEH:** ANTI-HUMAN GAMMA G GLOBULIN
63) **CRA(III):** ANTI-HUMAN GAMMA G GLOBULIN
64) **PLA:** ANTI-HUMAN GAMMA G GLOBULIN
65) **PIN:** ANTI-HUMAN GAMMA G GLOBULIN
70) **BOR:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
71) **DRI:** ANTI-HUMAN GAMMA G GLOBULIN
72) **WAL:** ANTI-HUMAN GAMMA G GLOBULIN
73) **GOL:** ANTI-HUMAN GAMMA G GLOBULIN
74) **GAG:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY

CLASS: HUMAN KAPPA LIGHT CHAINS SUBGROUP III

5) **NEU:** IGM-KAPPA
6) **GOT:** IGM-KAPPA
7) **PAY:** IGM-KAPPA
8) **SON:** IGM-KAPPA
9) **WEI':** IGM-KAPPA
10) **GAR':** IGM-KAPPA
11) **PIE:** IGM-KAPPA
12) **FLO:** IGM-KAPPA
13) **LOP:** IGM-KAPPA
14) **SCA:** IGM-KAPPA
15) **GLO:** IGM-KAPPA
20) **CUR:** IGM-KAPPA
42) **CLA:** IGM-KAPPA
43) **SHE':** IGM-KAPPA

REFERENCE: HUMAN KAPPA LIGHT CHAINS SUBGROUP III

1) **TI:** SUTER,L.,BARNIKOL,H.U.,WATANABE,S. & HILSCHMANN,N. (1969) Z.PHYSIOL.CHEM.,350,275-278; (1972) Z.PHYSIOL.CHEM.,353,189-208. (CHECKED BY AUTHOR)
2) **WOL:** ANDREWS,D.W. & CAPRA,J.D. (1981) PROC.NAT.ACAD.SCI.USA,78,3799-3803. (CHECKED BY AUTHOR 08/25/81); ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5816-5822.
3) **SIE:** CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR); ANDREWS,D.W. & CAPRA,J.D. (1981) PROC.NAT.ACAD.SCI.USA,78,3799-3803. (CHECKED BY AUTHOR 08/25/81 WHO SUGGESTED THAT THE SEQUENCE DETERMINED IN 1975 WAS INCORRECT AND SHOULD BE DELETED); ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5816-5822.
4) **NG9'CL:** BENTLEY,D.L. (1984) NATURE,307,77-80.
5) **NEU:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
6) **GOT:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); PONS-ESTEL,B.,GONI,F.,SOLOMON,A. & FRANGIONE,B. (1984) J.EXP.MED.,160,893-904; GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
7) **PAY:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
8) **SON:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); PONS-ESTEL,B.,GONI,F.,SOLOMON,A. & FRANGIONE,B. (1984) J.EXP.MED.,160,893-904.
9) **WEI':** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84)
10) **GAR':** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); PONS-ESTEL,B.,GONI,F.,SOLOMON,A. & FRANGIONE,B. (1984) J.EXP.MED.,160,893-904; GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
11) **PIE:** PONS-ESTEL,B.,GONI,F.,SOLOMON,A. & FRANGIONE,B. (1984) J.EXP.MED.,160,893-904. (CHECKED BY AUTHOR 05/16/86)
12) **FLO:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
13) **LOP:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84)
14) **SCA:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84)
15) **GLO:** CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR); LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
16) **SAL:** CAPRA,J.D.,KEHOE,J.M.,WINCHESTER,R.J. & KUNKEL,H.G. (1971) ANN.N.Y.ACAD.SCI.,190,371-381. (CHECKED BY AUTHOR)
17) **WIL:** CAPRA,J.D.,KEHOE,J.M.,WINCHESTER,R.J. & KUNKEL,H.G. (1971) ANN.N.Y.ACAD.SCI.,190,371-381. (CHECKED BY AUTHOR)
18) **MA:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
19) **NIC:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
20) **CUR:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84); GONI,F.,CHEN,P.P.,PONS-ESTEL,B.,CARSON,D.A. & FRANGIONE,B. (1985) J.IMMUNOL.,135,4073-4079.
21) **FR4:** MILSTEIN,C. (1969) FEBS LETTERS,2,301-304. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED)
22) **DRE:** GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)
23) **PER:** GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)
24) **CAM:** HOPPER,J.E.,NOYES,C.,HSU,R.,HEINRIKSON,R. & GALLAGHER,W. (1979) J.IMMUNOL.,122,2007-2010. (CHECKED BY AUTHOR 01/26/83)
25) **STE:** EDMAN,P. & COOPER,A.G. (1968) FEBS LETTERS,2,33-35. (CHECKED BY AUTHOR)
26) **GJ:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)
27) **TAK:** GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)
28) **IARC/BL41'CL:** KLOBECK,H.G.MEINDL,A.,COMBRIATO,G.,SOLOMON,A. & ZACHAU,H.G. (1985) NUC.ACIDS RES.,13,6499-6513.
29) **RAD:** MILSTEIN,C. (1969) FEBS LETTERS,2,301-304. (CHECKED BY AUTHOR)
30) **DIL:** DAYHOFF,M.O. (1972) ATLAS OF PROTEIN SEQUENCE & STRUCTURE,5,D-250. SUBMITTED BY SMITHIES,O.,GIBSON,D.M. AND FANNING,E.M. (CHECKED BY AUTHOR 07/24/79)

REFERENCE: HUMAN KAPPA LIGHT CHAINS SUBGROUP III (cont'd)

31) **CAS:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)

32) **MCE':** MIDDAUGH,C.R.,KEHOE,J.M.,PRYSTOWSKY,M.B.,GERBER-JENSON,B.,JENSON,J.C. & LITMAN,G.W. (1978) IMMUNOCHEM.,15,171-187. (CHECKED BY AUTHOR 10/22/80)

33) **KEA:** WANG,A.C. & FUDENBERG,H.H. (1975) IMMUNOL.COMMUN.,4,483-497. (CHECKED BY AUTHOR 09/23/77); WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROEHLICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)

34) **SMI:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)

35) **AJ:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)

36) **BRO'IGG:** HOPPER,J.E.,NOYES,C.,HEINRIKSON,R. & KESSEL,J.W. (1976) J.IMMUNOL.,116,743-746. (CHECKED BY AUTHOR 01/26/83)

37) **NIG:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)

38) **IKE:** CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR)

39) **TIL:** PINK,J.R.L.,WANG,A.C. & FUDENBERG,H.H. (1971) ANN.REV.MED.,22,145-170. (CHECKED BY AUTHOR)

40) **AMYLOID KSA:** SLETTEN,K.,WESTERMARK,P.,PITKANEN,P.,THYRESSON,N. & OLSTAD,O.K. (1983) SCAND.J.IMMUNOL.,18,557-560. (CHECKED BY AUTHOR 04/26/84)

41) **POL:** WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)

42) **CLA:** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84)

43) **SHE':** LEDFORD,D.K.,GONI,F.,PIZZOLATO,M.,FRANKLIN,E.C.,SOLOMON,A. & FRANGIONE,B. (1983) J.IMMUNOL.,131,1322-1325. (CHECKED BY AUTHOR 03/23/84)

44) **JH:** JEMMERSON,R.,KAPLAN,B.,DENTON,M.D.,ANDERAS,P.,ANDERSON,B. & MARGOLIASH,E. (1979) BIOCHEMISTRY,18,4676-4683.

45) **WIN:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)

46) **LEA:** WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)

47) **ARP:** WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROEHLICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)

48) **POM:** KLAPPER,D.G. & CAPRA,J.D. (1976) ANN.IMMUNOL.(INST.PASTEUR),127C,261-271. (CHECKED BY AUTHOR 08/01/79)

49) **VAND:** SEON,B.K.,GAILANI,S.,HENDERSON,E.S. & PRESSMAN,D. (1977) IMMUNOCHEM.,14,567-572. (CHECKED BY AUTHOR 08/28/78)

50) **AMYLOID SO124:** SLETTEN,K.,WESTERMARK,P.,PITKANEN,P.,THYRESSON,N. & OLSTAD,O.K. (1983) SCAND.J.IMMUNOL.,18,557-560. (CHECKED BY AUTHOR 04/26/84)

51) **DOV:** WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROEHLICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)

52) **SHM:** WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROEHLICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)

53) **GRA:** NIALL,H.D. & EDMAN,P. (1967) NATURE,216,262-263. (CHECKED BY AUTHOR 07/25/79)

54) **GOEII:** STROSBERG,A.D.,KARCHER,D. & LOWENTHAL,A. (1975) J.IMMUNOL.,115,157-160. (CHECKED BY AUTHOR)

55) **LOW':** WANG,A.C.,TUNG,E.,WANG,I.,FUDENBERG,H.H.,PICK,A.I. & FROEHLICHMAN,R. (1980) CANCER IMMUNOL.IMMUNOTHER.,9,81-86. (CHECKED BY AUTHOR 03/18/81)

56) **VER:** CHERSI,A. & NATALI,P.G. (1978) IMMUNOCHEMISTRY,15,585-589. (CHECKED BY AUTHOR 09/13/79)

57) **REE:** PRELLI,F.,TUMMOLO,D.,SOLOMON,A. & FRANGIONE,B. (1986) J.IMMUNOL., IN PRESS.

58) **WE:** DWORSKY,E.,SLETTEN,K.,HARBOE,M. & WETTELAND,P. (1980) SCAND.J.IMMUNOL.,12,281-287. (CHECKED BY AUTHOR 02/28/1984)

59) **HOW:** KAPLAN,A.P. & METZGER,H. (1969) BIOCHEMISTRY,8,3944-3951. (CHECKED BY AUTHOR)

60) **HS4:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.

61) **HBJ5:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.

62) **TEH:** JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)

63) **CRA(III):** JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)

64) **PLA:** JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)

65) **PIN:** JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)

66) **MCE:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)

67) **HAC:** HOOD,L. & TALMAGE,D.W. (1970) SCIENCE,168,325-334.

68) **K- EV15'CL:** STAVNEZER,J.,KEKISH,O.,BATTER,D.,GRENIER,J.,BALAZS,I.,HENDERSON,E. & ZEGERS,B.J.M. (1985) NUC.ACIDS RES.,13,3495-3514.

69) **BER:** WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)

70) **BOR:** GERGELY,J.,WANG,A.C. & FUDENBERG,H.H. (1973) VOX SANG.,24,432-440. (CHECKED BY AUTHOR)

71) **DRI:** CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR)

72) **WAL:** CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR)

73) **GOL:** CAPRA,J.D. (1975) ADV.IMMUNOLOGY,20,1-40. (CHECKED BY AUTHOR)

74) **GAG:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)

75) **DOB:** HOOD,L. & TALMAGE,D.W. (1970) SCIENCE,168,325-334.

76) **HS8:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.

77) **HBJ12:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.

78) **BUR(K):** MOULIN,A. & FOUGEREAU,M. (1973) NATURE NEW BIOLOGY,246,176-178. (CHECKED BY AUTHOR)

79) **LEG:** MOULIN,A. & FOUGEREAU,M. (1973) NATURE NEW BIOLOGY,246,176-178. (CHECKED BY AUTHOR)

80) **B6:** MILSTEIN,C. (1969) FEBS LETTERS,2,301-304. (CHECKED BY AUTHOR)

81) **AMYLOID WR:** WESTERMARK,P.,SLETTEN,K.,PITKANEN,P.,NATVIG,J.B. & LINDHOLM,C.E. (1982) MOL.IMMUNOL.,19,447-450. (CHECKED BY AUTHOR 08/01/83)

82) **VKAPPA3'CL:** BENTLEY,D.L. & RABBITTS,T.H. (1981) CELL,24,613-623. (CHECKED BY AUTHOR 12/07/81)

NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP III

IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1: SET 1: TI[1],WOL[2],SIE[3],NG9'CL[4],NEU[5],GOT[6],PAY[7],SON[8],WEI'[9],GAR'[10],PIE[11],FLO[12],LOP[13],SCA[14],GLO[15],SAL[16],WIL[17],MA[18],NIC[19],CUR[20],FR4[21],DRE[22],PER[23],CAM[24]. (24 IDENTICAL)

SET 2: GJ[26],TAK[27]. (2 IDENTICAL)

SET 3: RAD[29],DIL[30],CAS[31]. (3 IDENTICAL)

SET 4: KEA[33],SMI[34]. (2 IDENTICAL HUMAN V-KAPPA-III; ALSO 1 MOUSE V-KAPPA-V: Vg'CL[122].)

SET 5: DRE[22],PER[23],BRO'IGG[36]. (3 IDENTICAL)

SET 6: CLA[42],SHE'[43]. (2 IDENTICAL)

FR2: SET 1: TI[1],WOL[2],SIE[3],NG9'CL[4],NEU[5],GOT[6],SON[8],GAR'[10],PIE[11],FLO[12],GLO[15],CUR[20]. (12 IDENTICAL HUMAN V-KAPPA-III; ALSO 1 MOUSE V-KAPPA-IV: Vh'CL[12]; AND 1 MOUSE V-KAPPA-V: Vg'CL[122].)

FR3: SET 1: TI[1],WOL[2]. (2 IDENTICAL)

SET 2: GOT[6],PAY[7],GAR'[10],PIE[11],FLO[12],GLO[15],CUR[20]. (7 IDENTICAL)

FR4: SET 1: WOL[2],PAY[7],PIE[11],GLO[15],CUR[20],REE[57],VKAPPA3'CL[82]. (7 IDENTICAL HUMAN V-KAPPA-III; ALSO 3 HUMAN V-KAPPA-I: AU[2],GAL(I)[36],CL*[110]; 2 HUMAN V-KAPPA-II: GM 607 'CL[5],RPM1-6410'CL[16]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)

SET 2: POM[48]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: HAU[4].)

SET 3: NEU[5],GOT[6],GAR'[10],FLO[12],FR4[21],IARC/BL41'CL[28]. (6 IDENTICAL HUMAN V-KAPPA-III; ALSO 3 HUMAN V-KAPPA-I: AG[7],DEN[46],BI[63]; 2 HUMAN V-KAPPA-II: NIM[3],FR[14]; AND 1 HUMAN V-KAPPA-IV: LEN[4].)

SET 4: SON[8]. (IDENTICAL TO 1 HUMAN V-KAPPA-IV: VJI'CL[1].)

IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:

CDR1: SET 1: SIE[3],IKE[38]. (2 IDENTICAL)

SET 2: NG9'CL[4],PAY[7],SON[8],WEI'[9],GAR'[10],PIE[11],FLO[12],GLO[15],CUR[20],DRE[22],CAM[24]. (11 IDENTICAL)

SET 3: TIL[39]. (IDENTICAL TO 1 MOUSE V-KAPPA-V: Vg'CL[122].)

CDR2: SET 1: WOL[2],SIE[3],NEU[5],GOT[6],PAY[7],SON[8],GAR'[10],PIE[11],FLO[12],GLO[15],CUR[20]. (11 IDENTICAL)

SET 2: POM[48]. (IDENTICAL TO 1 MOUSE V-KAPPA-IV: Vh'CL[12].)

CDR3: SET 1: POM[48]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: LAY[39].)

SET 2: GOT[6],CUR[20]. (2 IDENTICAL)

SET 3: PAY[7],GLO[15]. (2 IDENTICAL)

SET 4: GAR'[10],FLO[12]. (2 IDENTICAL)

IDENTICAL SETS OF J-MINIGENES:

SET 1: PIE[11],VKAPPA3'CL[82]. (2 IDENTICAL HUMAN V-KAPPA-III; ALSO 1 HUMAN V-KAPPA-I: AU[2]; 1 HUMAN V-KAPPA-II: RPM1-6410'CL[16]; AND 1 HUMAN V-KAPPA-IV: PB17IV'CL[3].)

SET 2: GOT[6]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: AG[7].)

SET 3: GAR'[10],FLO[12],IARC/BL41'CL[28]. (3 IDENTICAL HUMAN V-KAPPA-III; ALSO 2 HUMAN V-KAPPA-I: DEN[46],BI[63]; AND 1 HUMAN V-KAPPA-II: FR[14].)

SET 4: WOL[2],CUR[20]. (2 IDENTICAL)

SET 5: PAY[7],GLO[15]. (2 IDENTICAL)

SPECIFIC NOTES:

4) **NG9'CL:** THE AMINO ACID SEQUENCE IS TRANSLATED FROM THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN CDNA.

32) **MCE':** IT IS A CRYOIMMUNOGLOBULIN. THE AUTHORS ORIGINALLY DESIGNATED IT AS MCE, BUT IN ORDER TO DIFFERENTIATE IT FROM ANOTHER MCE SEQUENCED BY CAPRA ET AL., IT IS DENOTED AS MCE'.

42) **CLA:** THE AMINO ACID RESIDUES FOUND AT POSITION 9 WERE GLY AND ALA.

43) **SHE':** THE AMINO ACID RESIDUES FOUND AT POSITION 9 WERE GLY AND ALA.

NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP III (cont'd)

44) **JH:** THE NAME WAS GIVEN TO US BY THE AUTHORS. IT IS NOT INCLUDED IN THE PAPER.

58) **WE:** AT POSITIONS 20,29 AND 33 OF AMINO ACID SEQUENCE WERE FOUND BOTH LEU AND ILE. IN THE SAME SEQUENCE TWO RESIDUES WERE FOUND IN POSITIONS 1,3,4,9,10,15,17,19,20,21,22 AND 29. THE SECOND RESIDUES WERE GLU,VAL,LEU,GLY,THR,PRO,GLU,ALA,THR,LEU,SER AND VAL, RESPECTIVELY. A DETERMINATION WAS NOT MADE IN THE ARTICLE AS TO WHETHER THE SEQUENCE BELONGED TO SUBGROUP I OR TO SUBGROUP III.

81) **AMYLOID WR:** AMINO ACID RESIDUES FOUND AT POSITION 54 ARE LEU AND ALA.

82) **VKAPPA3'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF CDNA FROM A MOUSE-HUMAN HYBRID CELL LINE.

HUMAN KAPPA LIGHT CHAINS SUBGROUP IV

		INVARIANT RESIDUES	1 VJI 'CL	2 VKAPPA IV GERMLINE 'CL	3* PB17IV 'CL	4 LEN	5* R.K.	6** L. TH.	7** TUR	8 AH	9 DA	10 DA-H	11 DA-N	12 JAH	13 SCH	14 JUV	15 AMYLOID GAB	# OF SEQUENCES	# OF AMINO ACIDS
FR1	0		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	1	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	15	1
	2		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	leu	ILE	15	2
	3	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	15	1
	4		MET	MET	MET	MET	MET	leu	MET	MET	MET	MET	leu	MET	MET	MET	MET	15	2
	5		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	gln	THR	THR	THR	THR	15	2
	6	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	15	1
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		14	1
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	15	1
	9		ASP	ASP	ASP	asn	ASX	ASX	ser	glx	ASP	ASP	ASP	ASP	ala	asn	ASX	15	5
	10		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	thr			13	2
	11	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		14	1
	12	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		14	1
	13	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		14	1
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER					11	1
	15		LEU	LEU	LEU	LEU	LEU	LEU	LEU	pro	pro	pro	LEU					11	2
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY					11	1
	17		GLU	GLU	GLU	GLU	GLX	GLX	GLU	asp	asp	asp	asp					11	2 : 3
	18		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	gln	gln	leu					11	3
	19	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA				ALA	12	1
	20	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR				THR	12	1
	21		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	val	val	leu				ILE	12	3
	22		ASN	ASN	ASN	ASN	ASX	ASX	ser	ASX	ser	ser	ser				asp	12	3
	23	CYS	CYS	CYS	CYS	CYS			CYS	CYS	CYS	CYS	CYS				CYS	10	1
CDR1	24		LYS	LYS	LYS	LYS			ARG	ARG	GLN	GLN	GLN				LYS	10	3
	25		SER	SER	SER	SER			SER	ARG	ALA	ALA	ALA					9	3
	26		SER	SER	SER	SER			SER	ALA			SER					7	2
	27		GLN	GLN	GLN	GLN			GLN	GLX			GLN					7	1 : 2
	27A		SER	SER	SER	SER			SER	ARG			---					6	2
	27B		VAL	VAL	ILE	VAL			VAL	VAL			VAL					7	2
	27C	LEU	LEU	LEU	LEU	LEU			LEU				LEU					6	1
	27D	TYR	TYR	TYR	TYR	TYR							TYR					5	1
	27E		SER	SER	SER	SER				TYR								5	2
	27F	SER	SER	SER	SER	SER												4	1
	28		ASN	ASN	ASP	ASN							ASP					5	2
	29		ASN	ASN	ASN	SER												4	2
	30	LYS	LYS	LYS	LYS	LYS							LYS					5	1
	31	ASN	ASN	ASN	ASN	ASN												4	1
	32	TYR	TYR	TYR	TYR	TYR												4	1
	33	LEU	LEU	LEU	LEU	LEU												4	1
	34	ALA	ALA	ALA	ALA	ALA												4	1
FR2	35	TRP	TRP	TRP	TRP	TRP												4	1
	36	TYR	TYR	TYR	TYR	TYR												4	1
	37	GLN	GLN	GLN	GLN	GLN												4	1
	38	GLN	GLN	GLN	GLN	GLN												4	1
	39	LYS	LYS	LYS	LYS	LYS												4	1
	40	PRO	PRO	PRO	PRO	PRO					PRO							5	1
	41	GLY	GLY	GLY	GLY	GLY					GLY							5	1
	42	GLN	GLN	GLN	GLN	GLN					GLN							5	1
	43		PRO	PRO	PRO	PRO					ALA							5	2
	44	PRO	PRO	PRO	PRO	PRO					PRO							5	1
	45	LYS	LYS	LYS	LYS	LYS					LYS							5	1
	46	LEU	LEU	LEU	LEU	LEU					LEU							5	1
	47	LEU	LEU	LEU	LEU	LEU					LEU							5	1
	48	ILE	ILE	ILE	ILE	ILE					ILE							5	1
	49	TYR	TYR	TYR	TYR	TYR					TYR							5	1
CDR2	50	TRP	TRP	TRP	TRP	TRP					TRP							5	1
	51		ALA	ALA	ALA	ALA					GLY							5	2
	52		SER	SER	SER	SER					ARG							5	2
	53	THR	THR	THR	THR	THR												4	1
	54	ARG	ARG	ARG	ARG	ARG												4	1
	55	GLU	GLU	GLU	GLU	GLU												4	1
	56	SER	SER	SER	SER	SER												4	1
FR3	57	GLY	GLY	GLY	GLY	GLY												4	1
	58	VAL	VAL	VAL	VAL	VAL												4	1
	59	PRO	PRO	PRO	PRO	PRO												4	1
	60	ASP	ASP	ASP	ASP	ASP												4	1
	61	ARG	ARG	ARG	ARG	ARG												4	1
	62	PHE	PHE	PHE	PHE	PHE												4	1
	63	SER	SER	SER	SER	SER					SER							5	1
	64	GLY	GLY	GLY	GLY	GLY					GLY							5	1
	65	SER	SER	SER	SER	SER					SER							5	1
	66	GLY	GLY	GLY	GLY	GLY					GLY							5	1
	67	SER	SER	SER	SER	SER					SER							5	1
	68		GLY	GLY	GLY	GLY					LYS							5	2
	69	THR	THR	THR	THR	THR												4	1
	70	ASP	ASP	ASP	ASP	ASP												4	1
	71	PHE	PHE	PHE	PHE	PHE												4	1
	72	THR	THR	THR	THR	THR												4	1
	73	LEU	LEU	LEU	LEU	LEU												4	1
	74	THR	THR	THR	THR	THR												4	1
	75	ILE	ILE	ILE	ILE	ILE												4	1
	76	SER	SER	SER	SER	SER												4	1
	77	SER	SER	SER	SER	SER												4	1
	78	LEU	LEU	LEU	LEU	LEU												4	1
	79	GLN	GLN	GLN	GLN	GLN												4	1
	80	ALA	ALA	ALA	ALA	ALA												4	1
	81	GLU	GLU	GLU	GLU	GLU												4	1
	82	ASP	ASP	ASP	ASP	ASP												4	1
	83	VAL	VAL	VAL	VAL	VAL												4	1
	84	ALA	ALA	ALA	ALA	ALA												4	1
	85	VAL	VAL	VAL	VAL	VAL												4	1
	86	TYR	TYR	TYR	TYR	TYR												4	1
	87	TYR	TYR	TYR	TYR	TYR												4	1
	88	CYS	CYS	CYS	CYS	CYS												4	1
CDR3	89	GLN	GLN	GLN	GLN	GLN												4	1
	90	GLN	GLN	GLN	GLN	GLN												4	1
	91	TYR	TYR	TYR	TYR	TYR												4	1
	92		ASP	TYR	TYR	TYR												4	2
	93		THR	SER	ASN	SER												4	3
	94		ILE	THR	LEU	THR												4	3
	95	PRO	PRO	PRO	PRO	PRO												4	1
	95A		---	---	---	---													
	95B		---	---	---	---													
	95C		---	---	---	---													
	95D		---	---	---	---													
	95E		---	---	---	---													
	95F		---	---	---	---													
	96		---	---	TRP	TYR												2	2
	97		THR	---	THR	SER												3	2
FR4	98	PHE	PHE	---	PHE	PHE												3	1
	99	GLY	GLY	---	GLY	GLY												3	1
	100		GLY	---	GLN	GLN												3	2
	101	GLY	GLY	---	GLY	GLY												3	1
	102	THR	THR	---	THR	THR												3	1
	103	LYS	LYS	---	LYS	LYS												3	1
	104		VAL	---	VAL	LEU					LEU							4	2
	105	GLU	GLU	---	GLU	GLU					GLU							4	1
	106	ILE	ILE	---	ILE	ILE					ILE							4	1
	106A		---	---	---	---					---								
	107		LYS	---	LYS	LYS					ARG							4	2
	108	ARG	ARG	---	ARG	ARG												3	1
	109			---		THR												1	1

HUMAN KAPPA LIGHT CHAINS SUBGROUP IV (cont'd)

		OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
FR1	0		
	1	15(ASP)	1.
	2	14(ILE)	2.1
	3	15(VAL)	1.
	4	13(MET)	2.3
	5	14(THR)	2.1
	6	15(GLN)	1.
	7	14(SER)	1.
	8	15(PRO)	1.
	9	10(ASP) : 7(ASP)	7.5 : 11.
	10	11(SER)	2.4
	11	14(LEU)	1.
	12	14(ALA)	1.
	13	14(VAL)	1.
	14	11(SER)	1.
	15	8(LEU)	2.8
	16	11(GLY)	1.
	17	7(GLU) : 5(GLU)	3.1 : 6.6
	18	8(ARG)	4.1
	19	12(ALA)	1.
	20	12(THR)	1.
	21	9(ILE)	4.
	22	7(ASN) : 4(+)	5.1 : 9.
	23	10(CYS)	1.
CDR1	24	5(LYS)	6.
	25	5(SER)	5.4
	26	6(SER)	2.3
	27	7(GLN) : 6(GLN)	1. : 2.3
	27A	5(SER)	
	27B	6(VAL)	
	27C	6(LEU)	
	27D	5(TYR)	
	27E	4(SER)	
	27F	4(SER)	
	28	3(ASN)	3.3
	29	3(ASN)	2.7
	30	5(LYS)	1.
	31	4(ASN)	1.
	32	4(TYR)	1.
	33	4(LEU)	1.
	34	4(ALA)	1.
FR2	35	4(TRP)	1.
	36	4(TYR)	1.
	37	4(GLN)	1.
	38	4(GLN)	1.
	39	4(LYS)	1.
	40	5(PRO)	1.
	41	5(GLY)	1.
	42	5(GLN)	1.
	43	4(PRO)	2.5
	44	5(PRO)	1.
	45	5(LYS)	1.
	46	5(LEU)	1.
	47	5(LEU)	1.
	48	5(ILE)	1.
	49	5(TYR)	1.
CDR2	50	5(TRP)	1.
	51	4(ALA)	2.5
	52	4(SER)	2.5
	53	4(THR)	1.
	54	4(ARG)	1.
	55	4(GLU)	1.
	56	4(SER)	1.
FR3	57	4(GLY)	1.
	58	4(VAL)	1.
	59	4(PRO)	1.
	60	4(ASP)	1.
	61	4(ARG)	1.
	62	4(PHE)	1.
	63	5(SER)	1.
	64	5(GLY)	1.
	65	5(SER)	1.
	66	5(GLY)	1.
	67	5(SER)	1.
	68	4(GLY)	2.5
	69	4(THR)	1.
	70	4(ASP)	1.
	71	4(PHE)	1.
	72	4(THR)	1.
	73	4(LEU)	1.
	74	4(THR)	1.
	75	4(ILE)	1.
	76	4(SER)	1.
	77	4(SER)	1.
	78	4(LEU)	1.
	79	4(GLN)	1.
	80	4(ALA)	1.
	81	4(GLU)	1.
	82	4(ASP)	1.
	83	4(VAL)	1.
	84	4(ALA)	1.
	85	4(VAL)	1.
	86	4(TYR)	1.
	87	4(TYR)	1.
	88	4(CYS)	1.
CDR3	89	4(GLN)	1.
	90	4(GLN)	1.
	91	4(TYR)	1.
	92	3(TYR)	2.7
	93	2(SER)	6.
	94	2(THR)	6.
	95	4(PRO)	1.
	95A		
	95B		
	95C		
	95D		
	95E		
	95F		
	96	1(+)	4.
	97	2(THR)	3.
FR4	98	3(PHE)	1.
	99	3(GLY)	1.
	100	2(GLN)	3.
	101	3(GLY)	1.
	102	3(THR)	1.
	103	3(LYS)	1.
	104	2(+)	4.
	105	4(GLU)	1.
	106	4(ILE)	1.
	106A		
	107	3(LYS)	2.7
	108	3(ARG)	1.
	109	1(THR)	

ANTIBODY SPECIFICITIES: HUMAN KAPPA LIGHT CHAINS SUBGROUP IV

3) **PB17IV'CL:** ANTI-STREPTOCOCCUS GROUP A CARBOHYDRATE WITH SPECIFICITY FOR N-ACETYL GLUCOSAMINE

5) **R.K.:** COLD AGGLUTININ WITH ANTI-PR1H ACTIVITY (RBC MEMBRANE ANTIGEN ON HUMAN ERYTHROCYTES INACTIVATED BY PROTEOLYTIC ENZYMES AND NEURAMINIDASE)

6) **L.TH.:** COLD AGGLUTININ WITH ANTI-PR2 ACTIVITY (RBC MEMBRANE ANTIGEN ON HUMAN, RAT AND GUINEA PIG ERYTHROCYTES INACTIVATED BY PROTEOLYTIC ENZYMES AND NEURAMINIDASE)

7) **TUR:** COLD AGGLUTININ WITH ANTI-PR ACTIVITY

REFERENCE: HUMAN KAPPA LIGHT CHAINS SUBGROUP IV

1) **VJI'CL:** KLOBECK,H.G.,BORNKAMMM,G.W.,COMBRIATO,G.,MOCIKAT,R.,POHLENZ,H.D. & ZACHAU,H.G. (1985) NUC.ACIDS RES.,13,6515-6529. (CHECKED BY AUTHOR 02/25/86)

2) **VKAPPA IV GERMLINE'CL:** KLOBECK,H.G.,BORNKAMMM,G.W.,COMBRIATO,G.,MOCIKAT,R.,POHLENZ,H.D. & ZACHAU,H.G. (1985) NUC.ACIDS RES.,13, 6515-6529.

3) **PB17IV'CL:** MARSH,P.,MILLS,F. & GOULD,H. (1985) NUC.ACIDS RES.,13,6531-6544. (CHECKED BY AUTHOR 03/19/86 WHO CORRECTED A MISPRINT IN THE ORIGINAL PAPER FOR RESIDUE 50)

4) **LEN:** SCHNEIDER,M. & HILSCHMANN,N. (1974) Z.PHYSIOL.CHEM.,355,1164-1168. (CHECKED BY AUTHOR)

5) **R.K.:** WANG,A.C.,FUDENBERG,H.H.,WELLS,J.V. & ROELCKE,D. (1973) NATURE NEW BIOLOGY,243,126-128. (CHECKED BY AUTHOR)

6) **L.TH.:** WANG,A.C.,FUDENBERG,H.H.,WELLS,J.V. & ROELCKE,D. (1973) NATURE NEW BIOLOGY,243,126-128. (CHECKED BY AUTHOR)

7) **TUR:** CAPRA,J.D.,KEHOE,J.M.,WILLIAMS,R.C.,JR.,FEIZI,T. & KUNKEL,H.G. (1972) PROC.NAT.ACAD.SCI.USA,69,40-43. (CHECKED BY AUTHOR)

8) **AH:** PICK,A.I.,WANG,A.C.,FROHLICHMAN,R. & FUDENBERG,H.H. (1982) ACTA HAEMAT.,68,207-214. (CHECKED BY AUTHOR 05/26/83)

9) **DA:** WANG,A.C.,ZHANG,H.S.,BONEWALD,L.,TUNG,E.,BOUVET,J.P. & LIACOPOULOS,P. (1985) MIAMI WINTER SYMP.,17,335-336. (CHECKED BY AUTHOR 02/25/86 WHO CORRECTED RESIDUES AS SHOWN)

10) **DA-H:** BOUVET,J.P.,LIACOPOULOS,P.,PILLOT,J.,BANDA,R.,TUNG,E. & WANG,A.C. (1980) J.IMMUNOL.,125,213-220. (CHECKED BY AUTHOR 08/04/80); BOUVET,J.P.,LIACOPOULOS,P.,PILLOT,J.,BANDA,R.,TUNG,E. & WANG,A.C. (1982) J.IMMUNOL.,129,1519-1524.

11) **DA-N:** BOUVET,J.P.,LIACOPOULOS,P.,PILLOT,J.,BANDA,R.,TUNG,E. & WANG,A.C. (1980) J.IMMUNOL.,125,213-220. (CHECKED BY AUTHOR 08/04/80); BOUVET,J.P.,LIACOPOULOS,P.,PILLOT,J.,BANDA,R.,TUNG,E. & WANG,A.C. (1982) J.IMMUNOL.,129,1519-1524.

12) **JAH:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,219-222. (CHECKED BY AUTHOR 12/05/77)

13) **SCH:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,219-222. (CHECKED BY AUTHOR 12/05/77)

14) **JUV:** SLETTEN,K.,HANNESTAD,K. & HARBOE,M. (1974) SCAND.J.IMMUNOL.,3,219-222. (CHECKED BY AUTHOR 12/05/77)

15) **AMYLOID GAB:** PRAS,M.,FRANGIONE,B. & FRANKLIN,E.C. (1980) IN AMYLOID AND AMYLOIDOSIS,G.G.GLENNER,P.P.E COSTA & F.DE FREITAS EDS., EXCERPTA MEDICA AMSTERDAM OXFORD-PRINCETON,249-252. (CHECKED BY AUTHOR 11/18/81)

NOTES: HUMAN KAPPA LIGHT CHAINS SUBGROUP IV

IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1: SET 1: VJI'CL[1],VKAPPA IV GERMLINE'CL[2],PB17IV'CL[3],R.K.[5]. (4 IDENTICAL)
SET 2: LEN[4],R.K.[5]. (2 IDENTICAL)
SET 3: DA[9],DA-H[10]. (2 IDENTICAL)

FR2: SET 1: VJI'CL[1],VKAPPA IV GERMLINE'CL[2],PB17IV'CL[3],LEN[4]. (4 IDENTICAL HUMAN V-KAPPA-IV; ALSO 2 HUMAN V-KAPPA-I: V19B'CL[88], V18B'CL[89]; 1 MOUSE V-KAPPA-I: MCPC603[47]; 30 MOUSE V-KAPPA-III: MPC11'CL[6],TEPC111[7],PC3741(NZB)[8],TEPC124[9], MOPC321[12],PC7043(NZB)[13],PC7183(NZB)[14],PC6308(NZB)[15],PC6684(NZB)[17],PC7940(NZB)[18],PC7175(NZB)[19], PC2485(NZB)[20],PC4039(NZB)[21],PC7210(NZB)[23],H36-15[26],2242[29],V-21E1.5KB'CL[30],V-21C9.5KB'CL[31], PC7461(NZB)[33],PC2960(NZB)[34],97.C(A.BY)[35],10.A(A.TH)[39],H36-5[48],40.C(A.TH)[52],MOPC63[54],ABPC22[55], PC9245(NZB)[56],PC4050(NZB)[57],V-21B16KB'CL[58],11949[62]; 1 MOUSE V-KAPPA-VI: BFPC61A'CL[64]; AND 15 RABBIT V-KAPPA: K9-335-I[19],3368[20],BS-5[38],BS-1[39],K49-501[45],3547[47],K4820[57],K30-267[61],311[65],4422[66],17D9'CL[68], 4192[71],4363[85],120[103],K-25[112].)

FR3: SET 1: VJI'CL[1],VKAPPA IV GERMLINE'CL[2],PB17IV'CL[3],LEN[4]. (4 IDENTICAL)

FR4: SET 1: PB17IV'CL[3]. (IDENTICAL TO 3 HUMAN V-KAPPA-I: AU[2],GAL(I)[38],CL*[110]; 2 HUMAN V-KAPPA-II: GM 607 'CL[5], RPM1-6410'CL[16]; AND 7 HUMAN V-KAPPA-III: WOL[2],PAY[7],PIE[11],GLO[15],CUR[20],REE[57],VKAPPA3'CL[82].)
SET 2: LEN[4]. (IDENTICAL TO 3 HUMAN V-KAPPA-I: AG[7],DEN[46],BI[63]; 2 HUMAN V-KAPPA-II: NIM[3],FR[14]; AND 6 HUMAN V-KAPPA-III: NEU[5],GOT[6],GAR'[10],FLO[12],FR4[21],IARC/BL41'CL[28].)
SET 3: VJI'CL[1]. (IDENTICAL TO 1 HUMAN V-KAPPA-III: SON[8].)

IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:

CDR1: SET 1: VJI'CL[1],VKAPPA IV GERMLINE'CL[2]. (2 IDENTICAL)

CDR2: SET 1: VJI'CL[1],VKAPPA IV GERMLINE'CL[2],PB17IV'CL[3],LEN[4]. (4 IDENTICAL HUMAN V-KAPPA-IV; ALSO 1 MOUSE V-KAPPA-VI: KPNI6 'CL[70].)

CDR3:

IDENTICAL SETS OF J-MINIGENES:

SET 1: PB17IV'CL[3]. (IDENTICAL TO 1 HUMAN V-KAPPA-I: AU[2]; 1 HUMAN V-KAPPA-II: RPM1-6410'CL[16]; AND 2 HUMAN V-KAPPA-III: PIE[11],VKAPPA3'CL[82].)

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
22	(SER,ASP,ASN)
96	(TRP,TYR)
104	(LEU,VAL)

HUMAN LAMBDA LIGHT CHAINS SUBGROUP I

		INVARIANT RESIDUES	1* NEWM	2 HA	3 LR	4 NIG -64	5 NEW	6 BL2 CL	7 WAH	8 NIG -77	9 VOR	10 RHE	11 LOC	12 OKA	13 AMYLOID EPS	14 HBJ 7	15 COX	16* KOH	17 HS 92	18 HS 78	19 NIG -51	20 HS 94	21 HBJ 11	22 BJ 98	23 MZ
FR1	0		---	---	---	---	---	---	---	---	---	---	---	---		---	---	---	---	---	---	---	---		
	1	PCA(.95)	PCA	PCA	PCA	PCA	PCA	gln	PCA	PCA	PCA	PCA	PCA	PCA		PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA		
	2	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER		
	3	VAL(.95)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	ala	VAL	VAL	VAL		
	4	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		
	5	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	
	6	GLN(.95)	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN		
	7	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		
	10		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	11		VAL	VAL	VAL	VAL	VAL	VAL	ala	ala	ala	ala	ala	ala	leu	ala	ala	ala	VAL	VAL	ala	VAL	ala		
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	
	13		GLY	GLY	GLY	ala	ala	ala	GLY	GLY	GLY	GLY	GLY	GLY	ala	GLY	GLY	GLY	GLY	GLY	GLY	ala	GLY	ala	
	14		ALA	thr	ALA	ALA	ALA	ALA	thr	thr	thr	thr	thr	thr	ALA	thr	thr	thr	ALA	ALA	val	ALA	thr	ALA	
	15	PRO(.95)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ser	PRO	PRO	PRO	PRO	PRO	PRO		
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		
	17		GLN	GLN	glu	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN	GLN	GLN		
	18		ARG	ARG	ARG	glu	lys	lys	ARG	ARG	ARG	ARG	ARG	ARG	ARG	gly	ARG	ser	ARG	thr	ser	ARG	ARG		
	19	VAL(.95)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL	VAL			VAL	ala
	20		THR	THR	glu	THR	THR	THR	THR	THR	THR	THR	THR	THR	ser	THR	THR	THR		THR	ile			THR	ile
	21	ILE(.95)	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE			ILE			ser	ILE
	22	SER(.95)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER			SER			ile	SER
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS				CYS			CYS	CYS
CDR1	24		THR	SER	SER	SER	SER	SER	PHE	SER	SER	THR	SER	SER	SER	SER	SER				SER				SER
	25	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY				GLY				GLY
	26		SER	GLY		SER	GLY	SER	SER	SER	GLY	SER	SER	SER	SER	SER	SER				SER				SER
	27		SER	SER		SER	SER	SER	SER	THR	ASN	ALA	SER	GLY	SER		SER				SER				SER
	27A		---	---		---	---	---	---	---	---	---	---	---	---		---				---				—
	27B		---	---		---	---	---	---	---	—	---	---	---	---		---				---				---
	27C		---	---		---	---	---	---	---	---	---	---	---	---		---				---				---
	27D		SER	SER		SER	THR	SER	SER	SER	PHE	THR	SER	SER	---		SER				SER				SER
	27E		ASN	ASN		ASN	ASN	ASN	ASN	ASN	ASP	ASP	ASN	ASN	SER		ASN				ASN				ASN
	27F		ILE	GLY		---	---	---	---	---	---	---	ILE	---	ASN		---				---				---
	28		GLY	THR		ILE	ILE	ILE	ILE	ILE	ILE	ILE	GLY	ILE	ILE		LEU				ILE				MET
	29		ALA	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLU	GLY	GLY		GLY				GLY				
	30		GLY	ASN		ASP	ASN	ASN	ARG	SER	ARG	SER	THR	SER	LYS		SER				ARG				
	31		ASN	ASN		ASN	ASN	ASP	TYR	ASN	ASN	ASN	ASN	HIS	ASN		ASN				ASN				
	32		HIS	TYR		PHE	TYR	TYR	TYR	THR	SER	SER	SER	THR	TYR		GLN				THR				
	33	VAL	VAL	VAL		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL				VAL				
	34		LYS	TYR		SER	SER	SER	TYR	THR	ASN	ILE	SER	ASN	ASP		ASN				ASN				
FR2	35	TRP	TRP	TRP		TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP				TRP				
	36		TYR	TYR		TYR	HIS	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR		TYR				TYR				
	37		GLN	GLN		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	HIS	GLN		ARG				GLN				
	38		GLN	GLN		GLN	HIS	GLN	GLN	HIS	VAL	GLN	HIS	GLN	GLN		HIS				GLN				
	39		LEU	LEU		LEU	LEU	VAL	LEU	LEU	HIS	VAL	LEU	PHE	LEU		LEU				VAL				
	40	PRO	PRO	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO				PRO				
	41	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY				GLY				
	42		THR	THR		THR	THR	THR	THR	THR	THR	LYS	THR	THR	THR		THR				ALA				
	43		ALA	ALA		ALA	ALA	ALA	THR	ALA	ALA	ALA	ALA	ALA	ALA		ALA				ALA				
	44	PRO	PRO	PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO				PRO				
	45		LYS	LYS		LYS	LYS	LYS	LYS	LYS	ARG	LYS	LYS	LYS	LYS		LYS				LYS				
	46	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU				LEU				
	47		LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		VAL				LEU				
	48		ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE				VAL				
	49		PHE	TYR		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	PHE		TYR				TYR				
CDR2	50		HIS	ARG		ASP	GLU	ASP	LYS	SER	SER	TYR	GLU	ARG	ASN		SER				SER				
	51		ASN	ASP		ASN	ASP	ASN	ASP	ASN	SER	ASN	ASP	ASN	ASN		ASP				ASN				
	52		ASN	ASP		ASN	ASN	ASN	ASN	ASP	ASP	ASP	ASN	ASP	ASN		SER				ASN				
	53		ALA	LYS		LYS	LYS	LYS	GLN	GLN	GLN	LEU	SER	GLN	LYS		GLN				GLN				
	54		ARG	ARG		ARG	ARG	ARG	ARG	ARG	ARG	LEU	ARG	ARG	ARG		ARG				TRP				
	55		---	PRO		PRO	PRO	PRO	PRO	PRO	SER	PRO	ALA	PRO			PRO				PRO				
	56	SER	---	SER		SER	SER	SER	SER	SER	SER	SER	SER	SER			SER				SER				
FR3	57	GLY	---	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY			GLY				GLY				
	58		---	VAL		ILE	ILE	ILE	VAL	VAL	VAL	VAL	VAL	VAL			VAL				VAL				
	59		---	PRO		PRO	PRO	PRO	PRO	PRO	PRO	SER	SER	PRO			PRO				PRO				
	60		---	ASP		ASP	ASP	ASP	ASP	HIS	ASP	ASP	ASP	ASP			ASP				ASP				
	61	ARG	---	ARG		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG		ARG				ARG				
	62		PHE	PHE		PHE	ILE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE		ILE				PHE				
	63	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER				SER				
	64		VAL	GLY		GLY	ALA	GLY	GLY	GLY	GLY	ALA	ALA	GLY	GLY		ALA				GLY				
	65	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER				SER				
	66	LYS	LYS	LYS		LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS		LYS				LYS				
	67	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER				SER				
	68	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY				GLY				
	69		SER	THR		THR	THR	THR	THR	ALA	THR	THR	THR	THR	THR		THR				THR				
	70	SER	SER	SER		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER				SER				
	71	ALA	ALA	ALA		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA		ALA				ALA				
	72		THR	SER		THR	THR	THR	SER	SER	SER	SER	SER	SER	THR		SER				SER				
	73	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU				LEU				
	74		ALA	ALA		GLY	ALA	GLY	ALA	ALA	ALA	ALA	ALA	ALA	GLY		ALA				ALA				
	75	ILE	ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE				ILE				
	76		THR	SER		THR	THR	THR	SER	SER	SER	SER	SER	SER	THR		SER				SER				
	77	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY				GLY				
	78	LEU	LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU				LEU				
	79		GLN	ARG		GLN	ARG	GLN	ARG	GLN	GLN	GLU	GLN	GLN	GLN		GLN				HIS				
	80		ALA	SER		THR	THR	THR	SER	SER	SER	SER	PRO	SER	THR		SER				SER				
	81		GLU	GLU		GLY	GLY	GLY	GLU	GLU	GLU	GLU	GLU	GLU	GLY		GLU				GLU				
	82		ASP	ASP		ASP	ASP	ASP	ASP	ASP	ASN	ASP	ASP	ASP	ASP		ASP				ASP				
	83	GLU	GLU	GLU		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU		GLU				GLU				
	84		ALA	ALA		ALA	ALA	ALA	ALA	THR	ALA	ALA	THR	ALA	ALA		SER				ALA				
	85		ASP	HIS		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ILE		ASP				ASP				
	86	TYR	TYR	TYR		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR		TYR				TYR				
	87		TYR	HIS		TYR	TYR	TYR	TYR	TYR	PHE	TYR	TYR	TYR	TYR		TYR				PHE				
	88	CYS	CYS	CYS		CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		CYS				CYS				
CDR3	89		GLN	ALA		GLY	ALA	GLY	ALA	ALA	ALA	ALA	ALA	ALA	GLY		ALA				ALA				
	90		SER	ALA		THR	THR	THR	ALA	THR	THR	ALA	ALA	ALA	THR		SER				THR				
	91		TYR	TRP		TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TYR		TRP				TRP				
	92		ASP	ASP		ASP	ASP	ASN	ASP	ASP	ASP	ASN	ASP	ASP	ASP		ASP				ASP				
	93		ARG	TYR		SER	SER	ASN	ASP	ASP	ASP	ASP	ASP	ASP	ASN		ASP				ASP				
	94		SER	ARG		SER	SER	SER	SER	SER	SER	SER	SER	SER	ARG		SER				SER				
	95		LEU	LEU		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	ARG		LEU				LEU				
	95A		---	SER		SER	ASN	SER	---	ASN	ASP	ASP	ASP	ASP	---		ASP				ASP				
	95B		—	ALA		VAL	ALA	GLY	---	GLY	GLY	GLU	VAL	GLY	---		GLY				GLY				
	95C		---	---		---	---	---	---	---	---	---	---	---	---		---				---				
	95D		---	---		---	---	---	---	---	---	---	---	---	---		---				---				
	95E		---	---		---	---	---	---	—	---	---	---	---	---		---				---				
	95F		---	---		---	---	---	---	---	---	---	---	---	---		---				---				
	96		ARG	VAL		GLY	VAL	TRP	TRP	PRO	PRO	PRO	ALA	PRO	SER		PRO				PRO				
	97		VAL	VAL		MET	VAL	VAL	VAL	VAL	VAL	GLY	VAL	VAL	VAL		VAL				VAL				
FR4	98	PHE	PHE	PHE		PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE		PHE				PHE				
	99	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY				GLY				
	100		GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	THR	GLY	GLY		GLY				GLY				
	101	GLY	GLY	GLY		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		GLY				GLY				
	102	THR	THR	THR		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR				THR				
	103		LYS	GLN		ARG	LYS	LYS	THR	LYS	LYS	LYS	LYS	LYS	ASN		LYS				LYS				
	104		LEU	LEU		VAL	VAL	LEU	LEU	VAL	VAL	LEU	VAL	LEU	VAL		VAL				LEU				
	105	THR	THR	THR		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		THR				THR				
	106	VAL	VAL	VAL		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL				VAL				
	106A		LEU	LEU		LEU	LEU	LEU	LEU	GLN	LEU	LEU	LEU	LEU	VAL		LEU				LEU				
	107		ARG	ARG		GLY	GLY	GLY	SER	GLY	GLY	GLY	GLY	GLY	GLY		GLY				GLY				
	108	GLN	GLN	GLN			GLN		GLN	GLN	GLN	GLN	GLN	GLN	GLN		GLN				GLN				
	109	PRO	PRO	PRO			PRO		PRO	PRO	PRO	PRO	PRO	PRO	PRO		PRO				PRO				

HUMAN LAMBDA LIGHT CHAINS SUBGROUP I (cont'd)

		24 FUL #	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
FR1	0					
	1		20	2	19(PCA)	2.1
	2		20	1	20(SER)	1.
	3		21	2	20(VAL)	2.1
	4		21	1	21(LEU)	1.
	5		22	1	22(THR)	1.
	6		21	1 : 2	21(GLN) : 20(GLN)	1. : 2.1
	7		21	1	21(PRO)	1.
	8		21	1	21(PRO)	1.
	9		21	1	21(SER)	1.
	10					
	11		21	3	11(ALA)	5.7
	12		22	1	22(SER)	1.
	13		22	2	16(GLY)	2.8
	14		22	3	11(THR)	6.
	15		21	2	20(PRO)	2.1
	16		21	1	21(GLY)	1.
	17		21	2	20(GLN) : 19(GLN)	2.1 : 2.2
	18		21	6	14(ARG)	9.
	19		20	2	19(VAL)	2.1
	20		20	4	16(THR)	5.
	21		19	2	18(ILE)	2.1
	22		19	2	18(SER)	2.1
	23	CYS	19	1	19(CYS)	1.
CDR1	24	SER	18	3	15(SER)	3.8
	25	GLY	18	1	18(GLY)	1.
	26	ASN	17	3	13(SER)	3.9
	27	SER	16	5	12(SER)	6.7
	27A	---				
	27B	---				
	27C	---				
	27D	SER	15	3	12(SER)	
	27E		15	3	12(ASN)	
	27F		4	3	2(ILE)	
	28		15	5	10(ILE)	7.5
	29		14	3	12(GLY)	3.5
	30		14	7	4(SER)	25.
	31		14	4	11(ASN)	5.1
	32		14	6	5(TYR)	17.
	33		14	1	14(VAL)	1.
	34		14	7	4(+)	25.
FR2	35		14	1	14(TRP)	1.
	36		14	2	13(TYR)	2.2
	37		14	3	12(GLN)	3.5
	38		14	3	9(GLN)	4.7
	39		14	4	9(LEU)	6.2
	40		14	1	14(PRO)	1.
	41		14	1	14(GLY)	1.
	42		14	3	12(THR)	3.5
	43		14	2	13(ALA)	2.2
	44		14	1	14(PRO)	1.
	45		14	2	13(LYS)	2.2
	46		14	1	14(LEU)	1.
	47		14	2	13(LEU)	2.2
	48		14	2	13(ILE)	2.2
	49		14	2	12(TYR)	2.3
CDR2	50		14	8	4(SER)	28.
	51		14	3	8(ASN)	5.3
	52		14	3	8(ASN)	5.3
	53		14	5	6(GLN)	12.
	54		14	3	12(ARG)	3.5
	55		12	3	10(PRO)	3.6
	56		12	1	12(SER)	1.
FR3	57		12	1	12(GLY)	1.
	58		12	2	9(VAL)	2.7
	59		12	2	10(PRO)	2.4
	60		12	2	11(ASP)	2.2
	61		13	1	13(ARG)	1.
	62		14	2	12(PHE)	2.3
	63		14	1	14(SER)	1.
	64		14	3	9(GLY)	4.7
	65		14	1	14(SER)	1.
	66		14	1	14(LYS)	1.
	67		14	1	14(SER)	1.
	68		14	1	14(GLY)	1.
	69		14	3	12(THR)	3.5
	70		14	1	14(SER)	1.
	71		14	1	14(ALA)	1.
	72		14	2	9(SER)	3.1
	73		14	1	14(LEU)	1.
	74		14	2	11(ALA)	2.5
	75		14	1	14(ILE)	1.
	76		14	2	9(SER)	3.1
	77		14	1	14(GLY)	1.
	78		14	1	14(LEU)	1.
	79		14	4	9(GLN)	6.2
	80		14	4	8(SER)	7.
	81		14	2	10(GLU)	2.8
	82		14	2	13(ASP)	2.2
	83		14	1	14(GLU)	1.
	84		14	3	11(ALA)	3.8
	85		14	3	12(ASP)	3.5
	86		14	1	14(TYR)	1.
	87		14	3	11(TYR)	3.8
	88		14	1	14(CYS)	1.
CDR3	89		14	3	10(ALA)	4.2
	90		14	3	7(THR)	6.
	91		14	2	12(TRP)	2.3
	92		14	2	12(ASP)	2.3
	93		14	5	8(ASP)	8.8
	94		14	2	12(SER)	2.3
	95		14	2	13(LEU)	2.2
	95A		11	3	6(ASP)	
	95B		11	4	6(GLY)	
	95C					
	95D					
	95E					
	95F					
	96		14	7	6(PRO)	18.
	97		14	3	12(VAL)	3.5
FR4	98		14	1	14(PHE)	1.
	99		14	1	14(GLY)	1.
	100		14	2	13(GLY)	2.2
	101		14	1	14(GLY)	1.
	102		14	1	14(THR)	1.
	103		14	5	10(LYS)	7.
	104		14	2	7(+)	4.
	105		14	1	14(THR)	1.
	106		14	1	14(VAL)	1.
	106A		14	3	12(LEU)	
	107		14	3	11(GLY)	3.8
	108		12	1	12(GLN)	1.
	109		12	1	12(PRO)	1.

ANTIBODY SPECIFICITIES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP I

1) **NEWM:** ANTI-3-(3'-HYDROXY-3',7',11',15',TETRAMETHYL HEXADECYL) 2-METHYL 1,4 NAPHTHOQUINONE(VIT.K1OH)
16) **KOH:** ANTI-HUMAN GAMMA G GLOBULIN

REFERENCE: HUMAN LAMBDA LIGHT CHAINS SUBGROUP I

1) **NEWM:** CHEN,B.L. & POLJAK,R.J. (1974) BIOCHEMISTRY,13,1295-1302. (CHECKED BY AUTHOR 01/24/78)
2) **HA:** SHINODA,T.,TITANI,K. & PUTNAM,F.W. (1970) J.BIOL.CHEM.,245,4475-4487. (CHECKED BY AUTHOR 06/15/83)
3) **LR:** CAULIN-GLASER,T.,PRELLI,F. & FRANKLIN,E.C. (1982) J.LAB.CLIN.MED.,99,845-851. (CHECKED BY AUTHOR 12/10/82)
4) **NIG-64:** TONOIKE,H.,KAMETANI,F.,HOSHI,A.,SHINODA,T. & ISOBE,T. (1985) BIOCHEM.BIOPHYS.RES.COMMUN.,126,1228-1234.
5) **NEW:** LANGER,B.,STEINMETZ-KAYNE,M. & HILSCHMANN,N. (1968) Z.PHYSIOL.CHEM.,349,945-951.
6) **BL2 'CL:** TSUJIMOTO,Y. & CROCE,C.M. (1984) NUC.ACIDS RES.,12,8407-8414.
7) **WAH:** TAKAHASHI,Y.,TAKAHASHI,N.,TETAERT,D. & PUTNAM,F.W. (1983) PROC.NAT.ACAD.SCI.USA,80,3686-3690. (CHECKED BY AUTHOR 06/15/83)
8) **NIG-77:** TONOIKE,H.,KAMETANI,F.,HOSHI,A.,SHINODA,T. & ISOBE,T. (1985) BIOCHEM.BIOPHYS.RES.COMMUN.,126,1228-1234.
9) **VOR:** ENGELHARD,M.,HESS,M. & HILSCHMANN,N. (1974) Z.PHYSIOL.CHEM.,355,85-88; ENGELHARD,M. & HILSCHMANN,N. (1975) Z.PHYSIOL.CHEM.,356, 1413-1444.
10) **RHE:** FUREY,W. JR.,WANG,B.C.,YOO,C.S. & SAX,M. (1983) J.MOL.BIOL.,167,661-692. (CHECKED BY AUTHOR 05/15/84)
11) **LOC:** ZHU,D.,KIM,H.S. & DEUTSCH,H.F. (1983) MOL.IMMUNOL.,20,1107-1116.
12) **OKA:** ZHU,D.,KIM,H.S. & DEUTSCH,H.F. (1983) MOL.IMMUNOL.,20,1107-1116.
13) **AMYLOID EPS:** TOFT,K.G.,SLETTEN,K. & HUSBY,G. (1985) BIOL.CHEM.HOPPE-SEYLER,366,617-625.
14) **HBJ7:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.
15) **COX:** ZHU,D.,KIM,H.S. & DEUTSCH,H.F. (1983) MOL.IMMUNOL.,20,1107-1116.
16) **KOH:** KAPLAN,A.P. & METZGER,H. (1969) BIOCHEMISTRY,8,3944-3951.
17) **HS92:** HOOD,L. & EIN,D. (1968) NATURE,220,764-767; (1968) SCIENCE,1662,679-681.
18) **HS78:** HOOD,L. & EIN,D. (1968) NATURE,220,764-767; (1968) SCIENCE,1662,679-681.
19) **NIG-51:** TAKAHASHI,N.,TAKAYASU,T.,SHINODA,T.,ITO,S.,OKUYAMA,T. & SHIMIZU,A. (1980) BIOMED.RES.,1,321-333. (CHECKED BY AUTHOR 01/28/81)
20) **HS94:** HOOD,L. & EIN,D. (1968) NATURE,220,764-767; (1968) SCIENCE,1662,679-681.
21) **HBJ11:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145.
22) **BJ98:** BAGLIONI,C. (1967) BIOCHEM.BIOPHYS.RES.COMMUN.,26,82-89.
23) **MZ:** MILSTEIN,C.,FRANGIONE,B. & PINK,J.R.L. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,31-36. (CHECKED BY AUTHOR 10/17/77)
24) **FUL:** SOX,H.C.,JR. & HOOD,L. (1970) PROC.NAT.ACAD.SCI.USA,66,975-982.

NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP I

IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1: SET 1: WAH[7],NIG-77[8],VOR[9],RHE[10],LOC[11],OKA[12]. (6 IDENTICAL)
FR2: SET 1: NEWM[1],AMYLOID EPS[13]. (2 IDENTICAL)
SET 2: HA[2],NIG-64[4]. (2 IDENTICAL)
SET 3: NIG-77[8],LOC[11]. (2 IDENTICAL)
FR3: SET 1: NIG-64[4],BL2 'CL[6]. (2 IDENTICAL)
FR4: SET 1: NEWM[1]. (IDENTICAL TO 1 HUMAN V-LAMBDA-II: WH[3]; AND 1 HUMAN V-LAMBDA-V: BO[1].)
SET 2: NEW[5],VOR[9],COX[15]. (3 IDENTICAL HUMAN V-LAMBDA-I; ALSO 1 HUMAN V-LAMBDA-VI: AMYLOID-AR[1]; AND 6 MOUSE V-LAMBDA: MOPC315[25],TEPC952[26],MA8-13[27],5-7[29],MOPC315-26'CL'[30],MOPC315-37'CL[32].)
SET 3: BL2 'CL[6],RHE[10],OKA[12],NIG-51[19]. (4 IDENTICAL HUMAN V-LAMBDA-I; ALSO 5 HUMAN V-LAMBDA-II: MES[2],ES492[8],TRO[14], VIL[17],WIN[21]; 4 HUMAN V-LAMBDA-III: HIL[1],CAP[4],BAU[12],DEL[14]; 1 HUMAN V-LAMBDA-IV: SH[1]; 3 HUMAN V-LAMBDA-VI: SUT[2],THO[4],LBV'CL[5]; AND 24 MOUSE V-LAMBDA: MOPC104E[1],J558[2],XS104[3],HOPC1[4],J698[5],H2061[6],W3159[7], Y5431[8],Y5485[9],Y5830[10],Y5669[11],MOPC511(L)[12],S178[13],Y5444[14],Y5606[15],S176[16],H2020[17],RPC20[18],IG 303LAMBDA'CL[19],S43'CL[21],S2H5'CL[38],S2E9'CL[39],S1F12'CL[40],IG 25LAMBDA'CL[41].)
SET 4: LOC[11]. (IDENTICAL TO 1 HUMAN V-LAMBDA-V: MCG[3].)

IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:

CDR1:
CDR2: SET 1: NIG-64[4],BL2 'CL[6]. (2 IDENTICAL)
CDR3: SET 1: VOR[9],NIG-51[19]. (2 IDENTICAL)

IDENTICAL SETS OF J-MINIGENES:

SET 1: NEW[5]. (IDENTICAL TO 1 HUMAN V-LAMBDA-VI: AMYLOID-AR[1].)
SET 2: BL2 'CL[6]. (IDENTICAL TO 2 HUMAN V-LAMBDA-VI: SUT[2],THO[4]; AND 24 MOUSE V-LAMBDA: MOPC104E[1],J558[2],XS104[3],HOPC1[4], J698[5],H2061[6],W3159[7],Y5431[8],Y5485[9],Y5830[10],Y5669[11],MOPC511(L)[12],S178[13],Y5444[14],Y5606[15],S176[16], H2020[17],RPC20[18],IG 303LAMBDA'CL[19],S43'CL[21],S2H5'CL[38],S2E9'CL[39],S1F12'CL[40],IG 25LAMBDA'CL[41].)
SET 3: VOR[9],COX[15]. (2 IDENTICAL)
SET 4: OKA[12],NIG-51[19]. (2 IDENTICAL)

SPECIFIC NOTES:

24) **FUL:** SOX AND HOOD HAVE REPORTED FOUR HUMAN V KAPPA AND ONE V LAMBDA CHAINS WITH ASN-SER/THR TO CONTAIN CARBOHYHDRATE.

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
34	(SER,ASN)
104	(LEU,VAL)

HUMAN LAMBDA LIGHT CHAINS SUBGROUP II

		INVARIANT RESIDUES	1 NIG -84	2 MES	3 WH	4 NEI	5 KAR	6 RIM	7 SLA	8 ES492	9 WEIR	10 TOG	11 SM #	12 HS 68	13 HS 77	14 TRO	15 BOH	16 NIG -58	17 VIL	18 HBJ 15	19 HBJ 8	20 HS 70	21 WIN	22 BUR	23 PRE	24 HS 86
0	FR1		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
1			PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	his	PCA	PCA	PCA	PCA	PCA	PCA	PCA
2		SER(.96)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
3			ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	pro
4		LEU(.96)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
5			THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ala	ser	THR	THR	THR	ala
6		GLN(.96)	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN
7			PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ser	PRO
8			ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	arg	arg	arg	ALA	ALA	ALA	ALA	pro	arg	pro	ALA
9		SER(.96)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	arg	SER	SER	SER
10			---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
11			VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	ala	VAL
12		SER(.96)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
13			GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ala	glu	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
14		SER(.96)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
15		PRO(.96)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	leu	PRO	PRO	PRO	PRO	PRO	PRO	PRO
16		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
17			GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	glu
18			SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	thr	SER	SER	SER	SER	SER	SER
19			ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	val	val	leu	ILE	ILE	ILE	ILE	val	val	val	ILE
20		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR
21			ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	val	ILE	ILE			ILE	ILE	ILE	ILE				ILE	ILE	ILE	
22			SER	SER	SER	SER	SER	SER	SER		SER	SER	SER			SER	SER	SER	SER				SER	SER	SER	
23		CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		CYS	CYS	CYS			CYS	CYS	CYS	CYS				CYS	CYS	CYS	
24	CDR1		THR	THR	THR	THR				ALA	ALA	THR	THR			THR	ALA	SER	THR				THR	ILE		
25			GLY	GLY	GLY	GLY				GLY	GLY	GLY	GLY			GLY	GLY	GLY	GLY				GLY	GLY		
26			THR	THR	THR	THR				THR	HIS	THR	ASX			THR	THR	ALA	THR				SER	THR		
27			THR	SER	SER	THR				HIS	THR	THR	SER			SER	SER	PRO	SER				TYR	SER		
27A			---	---	---	---				---	---	---	---			---	---	---	---				---	---		
27B			---	---	---	---				---	---	---	---			---	---	---	---				---	---		
27C			---	---	---	---				---	---	---	---			---	---	---	---				---	---		
27D			SER	SER	SER	SER				SER	SER	ASN	SER			SER	SER	CYS	SER				SER	SER		
27E			ASP	ASP	ASP	ASP				ASP	ASP	ASP	VAL			ASP	ASP	ASP	ASP				ASN	ASN		
27F			VAL	VAL	VAL	VAL				VAL	VAL	ILE	VAL			VAL	VAL	VAL	VAL				VAL	VAL		
28			GLY	GLY	GLY	GLY				ASN	ALA	GLY	GLY			GLY	GLY	ASP	GLY				THR	GLY		
29			GLY	GLY	SER	SER				PHE	ASP	SER	---			ALA	GLY	GLY	GLY				GLY	ASP		
30			TYR	TYR	TYR	TYR				THR	SER	TYR	---			TYR	ASN	CYS	TYR				TYR	TYR		
31			ASP	ASN	ASN	ASN				ASX	ASN	SER	---			ASN	HIS	GLU	ASN				ASN	LYS		
32			PHE	TYR	PHE	PHE				ALA	SER	TYR	---			SER	PHE	SER	TYR				HIS	TYR		
33			VAL	VAL	VAL	VAL					ILE	VAL	---			VAL	VAL	VAL	VAL				VAL	VAL		
34			SER	SER	SER	SER					SER	SER	---			SER	SER	SER	SER				SER	SER		
35	FR2	TRP	TRP	TRP	TRP	TRP				TRP	TRP	TRP	---			TRP	TRP	TRP	TRP				TRP	TRP		
36			TYR	PHE	TYR	TYR				TYR	PHE	TYR	---			TYR	TYR	TYR	PHE				TYR	TYR		
37			GLN	GLN	GLN	GLN				GLN	GLN	GLN	---			GLN	GLN	GLN	GLN				GLN	GLX		
38			GLN	GLN	GLN	GLN				LEU	GLN	GLN	---			GLN	GLN	GLN	GLN				GLN	GLX		
39			HIS	HIS	HIS	ASN				HIS	HIS	TYR	---			HIS	HIS	HIS	HIS				ASP	HIS		
40		PRO	PRO	PRO	PRO	PRO				PRO	PRO	PRO	---			PRO	PRO	PRO	PRO				PRO	PRO		
41			GLY	GLY	GLY	GLY				GLY	ASP	GLY	---			GLY	GLY	GLY	GLY				GLY	GLY		
42			LYS	LYS	LYS	LYS				ILE	LYS	LYS	---			LYS	LYS	LYS	THR				LYS	LYS		
43			ALA	ALA	ALA	ALA				ALA	ALA	ALA	---			ALA	ALA	ALA	ALA				VAL	ALA		
44		PRO	PRO	PRO	PRO	PRO				PRO	PRO	PRO	---			PRO	PRO	PRO	PRO				PRO	PRO		
45			LYS	LYS	LYS	LYS				LYS	LYS	LYS	---			LYS	LYS	LYS	LYS				LYS	LYS		
46			LEU	LEU	LEU	LEU				LEU	LEU	VAL	---			LEU	LEU	LEU	LEU				LEU	LEU		
47			LEU	MET	ILE	MET				MET	LEU	LEU	---			MET	ILE	ILE	ILE				MET	ILE		
48		ILE	ILE	ILE	ILE	ILE				ILE	ILE	ILE	---			ILE	ILE	ILE	ILE				ILE	ILE		
49			TYR	PHE	TYR	TYR				PHE	TYR	PHE	---			PHE	TYR	TYR	SER				TYR	TYR		
50	CDR2		ASP	ASP	ASP	GLU				ASP	ALA	ASP	---			ASP	GLY	GLY	GLU				ASP	GLU		
51			VAL	VAL	VAL	GLY				VAL	VAL	VAL	---			VAL	VAL	PHE	VAL				VAL	VAL		
52			ASN	SER	THR	ASN				SER	THR	ASN	---			THR	ASN	SER	ARG				ASP	SER		
53			SER	GLU	TYR	LYS				ASN	PHE	SER	---			LYS	LYS	ASN	ASN				LYS	SER		
54			ARG	ARG	ARG	ARG				ARG	ARG	ARG	---			ARG	ARG	ARG	ARG				ARG	ARG		
55			PRO	PRO	PRO	PRO				PRO	PRO	PRO	---			PRO	PRO	PRO	PRO				PRO	PRO		
56		SER	SER	SER	SER	SER				SER	SER	SER	---			SER	SER	SER	SER				SER	SER		
57	FR3		GLY	GLY	GLY	GLY				GLY	GLY	GLY	---			GLY	GLY	GLY	GLY				GLY	GLY		
58			ILE	VAL	ILE	VAL				VAL	ILE	VAL	---			VAL	VAL	VAL	VAL				VAL	VAL		
59			SER	SER	SER	SER				SER	PRO	SER	---			PRO	PRO	PRO	SER				PRO	PRO		
60			ASN	ASP	SER	ASN				ASN	LEU	HIS	---			ASP	TYR	LEU	ASP				ASP	ASP		
61		ARG	ARG	ARG	ARG	ARG				ARG	ARG	ARG	---			ARG	ARG	ARG	ARG				ARG	ARG		
62			PHE	PHE	PHE	PHE				PHE	PHE	PHE	---			LEU	PHE	PHE	PHE		PHE		PHE	PHE		
63		SER	SER	SER	SER	SER				SER	SER	SER	---			SER	SER	SER	SER		SER		SER	SER		
64		GLY	GLY	GLY	GLY	GLY				GLY	GLY	GLY	---			GLY	GLY	GLY	GLY		GLY		GLY	GLY		
65		SER	SER	SER	SER	SER				SER	SER	SER	---			SER	SER	SER	SER		SER		SER	SER		
66			LYS	LYS	ARG	LYS				LYS	LYS	LYS	---			LYS	LYS	LYS	LYS		LYS		LYS	LYS		
67			SER	SER	SER	SER				SER	SER	SER	---			SER	SER	SER	SER				SER	SER		
68			GLY	GLY	GLY	GLY				GLY	GLY	GLY	---			GLY	GLY	GLY	ALA				ALA	GLY		
69			ASN	ASN	ASN	LYS				ASN	ASN	ASN	---			ASP	ASN	ASP	ASN				ASN	ASX		
70			THR	THR	THR	THR				THR	THR	THR	---			THR	THR	ALA	THR				THR	THR		
71		ALA	ALA	ALA	ALA	ALA				ALA	ALA	ALA	---			ALA	ALA	ALA	ALA				ALA	ALA		
72			SER	SER	SER	SER				SER	SER	SER	---			SER	SER	SER	SER				SER	SER		
73		LEU	LEU	LEU	LEU	LEU				LEU	LEU	LEU	---			LEU	LEU	LEU	LEU				LEU	LEU		
74		THR	THR	THR	THR	THR				THR	THR	THR	---			THR	THR	THR	THR				THR	THR		
75			ILE	ILE	ILE	ILE				ILE	ILE	ILE	---			ILE	ILE	ILE	ILE				ILE	ILE		
76		SER	SER	SER	SER	SER				SER	SER	SER	---			SER	SER	SER	SER				SER	SER		
77		GLY	GLY	GLY	GLY	GLY				GLY	GLY	GLY	---			GLY	GLY	GLY	GLY				GLY	GLY		
78			LEU	LEU	LEU	LEU				LEU	LEU	LEU	---			LEU	LEU	LEU	LEU				LEU	LEU		
79			GLN	GLN	GLN	GLN				GLN	LEU	GLN	---			ARG	GLN	GLN	GLN				GLN	GLN		
80			ALA	ALA	ALA	VAL				ALA	PRO	ALA	---			ALA	ALA	VAL	ALA				ALA	ALA		
81			GLU	GLU	GLU	GLU				GLU	ASP	GLU	---			ASP	GLU	GLU	GLU				ASN	GLU		
82			ASP	ASP	ASP	ASP				ASP	ASP	ASP	---			ASP	ASP	ASP	ASP				ASN	ASP		
83		GLU	GLU	GLU	GLU	GLU				GLU	GLU	GLU	---			GLU	GLU	GLU	GLU				GLU	GLU		
84		ALA	ALA	ALA	ALA	ALA				ALA	ALA	ALA	---			ALA	ALA	ALA	ALA				ALA	ALA		
85			ASP	ASP	ASP	ASP				ASP	ASP	HIS	---			ASP	HIS	ASP	ASP				ASP	ASX		
86		TYR	TYR	TYR	TYR	TYR				TYR	TYR	TYR	---			TYR	TYR	TYR	TYR				TYR	TYR		
87			TYR	TYR	TYR	TYR				TYR	PHE	PHE	---			TYR	TYR	TYR	TYR				TYR	TYR		
88		CYS	CYS	CYS	CYS	CYS				CYS	CYS	CYS	---			CYS	CYS	CYS	CYS				CYS	CYS		
89	CDR3		SER	SER	SER	CYS				SER	MET	SER	---			CYS	CYS	SER	SER				SER	CYS		
90			SER	SER	SER	SER				SER	SER	SER	---			SER	SER	SER	SER				SER	SER		
91			PHE	TYR	TYR	TYR				PHE	TYR	TYR	---			TYR	TYR	TYR	TYR				TYR	TYR		
92			THR	ALA	THR	ALA				THR	LEU	ARG	---			ALA	ALA	ALA	THR				GLY	ILE		
93			THR	GLY	SER	GLY				ASP	SER	THR	---			GLY	GLY	ASP	SER				GLY	GLY		
94			THR	SER	ASX	ASX				THR	ASP	SER	---			ARG	ARG	SER	SER				THR	SER		
95			ASN	ASN	SER	SER				THR	ALA	GLY	---			TYR	PHE	SER	ASN				TYR	---		
95A			SER	THR	THR	THR				GLN	SER	THR	---			SER	THR	---	SER				SER	---		
95B			ARG	---	---	---				LEU	---	---	---			---	---	---	---				---	---		
95C			---	---	---	---				---	---	---	---			---	---	---	---				---	---		
95D			---	---	---	---				---	---	---	---			---	---	---	---				---	---		
95E			---	---	---	---				---	---	---	---			---	---	---	---				---	---		
95F			---	---	---	---				---	---	---	---			---	---	---	---				---	---		
96			ALA	VAL	ARG	ARG				VAL	PHE	ILE	---			VAL	TRP	VAL	VAL				LEU	TYR		
97			VAL	ILE	LEU	VAL	VAL	VAL		VAL	VAL	ILE	---			ILE	VAL	ILE	VAL				ILE	VAL		
98	FR4	PHE	PHE	PHE	PHE	PHE	PHE	PHE		PHE	PHE	PHE	---			PHE	PHE	PHE	PHE				PHE	PHE		
99		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	---			GLY	GLY	GLY	GLY				GLY	GLY	GLY	
100			GLY	GLY	GLY	GLY	THR	THR	THR	GLY	SER	GLY	---			GLY	GLY	ALA	GLY				GLY	THR	THR	
101		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	---			GLY	GLY	GLY	GLY				GLY	GLY	GLY	
102		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	---			THR	THR	THR	THR				THR	THR	THR	
103			LYS	LYS	LYS	ARG	GLN	ARG	LYS	LYS	LYS	TYR	---			LYS	ASN	LYS	LYS				LYS	LYS	LYS	
104			LEU	LEU	LEU	VAL	VAL			LEU	VAL	VAL	---			LEU	LEU	LEU	LEU				LEU	VAL		
105			SER	THR	THR	THR	THR			THR	THR	THR	---			THR	THR	THR	THR				THR	ILE		
106		VAL	VAL	VAL	VAL	VAL				VAL	VAL	VAL	---			VAL	VAL	VAL	VAL				VAL	VAL		
106A		LEU	LEU	LEU	LEU	LEU				LEU	LEU	LEU	---			LEU	LEU	LEU	LEU				LEU	LEU		
107			GLY	GLY	ARG	SER				GLY	ARG	ARG	---			GLY	GLY	ARG	GLY				GLY	GLY		
108			GLN	GLN		GLN				GLN	GLN		GLN				GLN		GLN				GLN	GLX		
109		PRO	PRO	PRO		PRO				PRO	PRO		PRO				PRO		PRO				PRO	PRO		

HUMAN LAMBDA LIGHT CHAINS SUBGROUP II (cont'd)

		25 WAL	26 4A CL	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
FR1	0	---	---				
	1	PCA	gln	26	3	24(PCA)	3.3
	2	SER	thr	26	2	25(SER)	2.1
	3	val	val	26	3	23(ALA)	3.4
	4	LEU	val	26	2	25(LEU)	2.1
	5	THR	THR	26	3	23(THR)	3.4
	6	GLN	GLN	26	1 : 2	26(GLN) : 25(GLN)	1. : 2.1
	7	PRO	glu	26	3	24(PRO)	3.3
	8	pro	pro	26	3	18(ALA)	4.3
	9	SER	SER	26	2	25(SER)	2.1
	10	---	---				
	11	ala	leu	26	3	23(VAL)	3.4
	12	SER	thr	26	2	25(SER)	2.1
	13	GLY	val	26	4	23(GLY)	4.5
	14	thr	SER	26	2	25(SER)	2.1
	15-	PRO	PRO	26	2	25(PRO)	2.1
	16	GLY	GLY	26	1	26(GLY)	1.
	17	GLN	gly	26	4	23(GLN)	4.5
	18	arg	thr	26	3	23(SER)	3.4
	19		val	25	3	18(ILE)	4.2
	20		THR	25	1	25(THR)	1.
	21		leu	19	3	17(ILE)	3.4
	22		thr	18	2	17(SER)	2.1
	23		CYS	18	1	18(CYS)	1.
CDR1	24		ALA	15	4	9(THR)	6.7
	25		SER	15	2	14(GLY)	2.1
	26		SER	15	5	10(THR)	7.5
	27		THR	15	5	7(SER)	11.
	27A		---				
	27B		---				
	27C		---				
	27D		GLY	15	4	12(SER)	
	27E		ALA	15	4	11(ASP)	
	27F		VAL	15	2	14(VAL)	
	28		THR	15	5	10(GLY)	7.5
	29		SER	14	5	6(GLY)	12.
	30		GLY	14	6	9(TYR)	9.3
	31		TYR	14	7	8(ASN) : 7(ASN)	12. : 14.
	32		TYR	14	5	5(TYR)	14.
	33		PRO	13	3	11(VAL)	3.5
	34		ASN	13	2	12(SER)	2.2
FR2	35		TRP	14	1	14(TRP)	1.
	36		PHE	14	2	10(TYR)	2.8
	37		GLN	14	1 : 2	14(GLN) : 13(GLN)	1. : 2.2
	38		GLN	14	2 : 3	13(GLN) : 12(GLN)	2.2 : 3.5
	39		LYS	14	5	10(HIS)	7.
	40		PRO	14	1	14(PRO)	1.
	41		GLY	14	2	13(GLY)	2.2
	42		GLN	14	4	11(LYS)	5.1
	43		ALA	14	2	13(ALA)	2.2
	44		PRO	14	1	14(PRO)	1.
	45		ARG	14	2	13(LYS)	2.2
	46		ALA	14	3	12(LEU)	3.5
	47		LEU	14	3	5(−)	8.4
	48		ILE	14	1	14(ILE)	1.
	49		TYR	14	3	9(TYR)	4.7
CDR2	50		SER	14	5	7(ASP)	10.
	51		THR	14	4	11(VAL)	5.1
	52		SER	14	5	5(SER)	14.
	53		ASN	14	6	4(+)	21.
	54		LYS	14	2	13(ARG)	2.2
	55		HIS	14	2	13(PRO)	2.2
	56		SER	14	1	14(SER)	1.
FR3	57		TRP	14	2	13(GLY)	2.2
	58		THR	14	3	10(VAL)	4.2
	59		PRO	14	2	7(+)	4.
	60		ALA	14	7	5(ASP)	20.
	61		ARG	14	1	14(ARG)	1.
	62		PHE	15	2	14(PHE)	2.1
	63		SER	15	1	15(SER)	1.
	64		GLY	15	1	15(GLY)	1.
	65		SER	15	1	15(SER)	1.
	66		LEU	15	3	13(LYS)	3.5
	67		LEU	14	2	13(SER)	2.2
	68		GLY	14	2	12(GLY)	2.3
	69		GLY	14	4	10(ASN) : 9(ASN)	5.6 : 6.2
	70		LYS	14	3	12(THR)	3.5
	71		ALA	14	1	14(ALA)	1.
	72		ALA	14	2	13(SER)	2.2
	73		LEU	14	1	14(LEU)	1.
	74		THR	14	1	14(THR)	1.
	75		LEU	14	2	13(ILE)	2.2
	76		SER	14	1	14(SER)	1.
	77		GLY	14	1	14(GLY)	1.
	78		VAL	14	2	13(LEU)	2.2
	79		GLN	14	3	12(GLN)	3.5
	80		PRO	14	3	10(ALA)	4.2
	81		GLU	14	3	11(GLU)	3.8
	82		ASP	14	2	13(ASP)	2.2
	83		GLU	14	1	14(GLU)	1.
	84		ALA	14	1	14(ALA)	1.
	85		GLU	14	3 : 4	11(ASP) : 10(ASP)	3.8 : 5.6
	86		TYR	14	1	14(TYR)	1.
	87		TYR	14	2	12(TYR)	2.3
	88		CYS	14	1	14(CYS)	1.
CDR3	89		LEU	14	4	8(SER)	7.
	90		LEU	14	2	13(SER)	2.2
	91		TYR	14	2	12(TYR)	2.3
	92		TYR	14	7	5(ALA)	20.
	93		GLY	14	4	7(GLY)	8.
	94		GLY	14	5 : 6	5(SER)	14. : 17.
	95		ALA	13	7	3(+)	30.
	95A		---	11	3	5(+)	
	95B		---	2	2	1(+)	
	95C		---				
	95D		---				
	95E		---				
	95F		---				
	96		---	13	8	5(VAL)	21.
	97	VAL	---	16	3	10(VAL)	4.8
FR4	98	PHE	---	16	1	16(PHE)	1.
	99	GLY	---	18	1	18(GLY)	1.
	100	SER	---	18	4	10(GLY)	7.2
	101	GLY	---	18	1	18(GLY)	1.
	102	THR	---	18	1	18(THR)	1.
	103	LYS	---	18	5	13(LYS)	6.9
	104	VAL	---	15	2	9(LEU)	3.3
	105	THR	---	15	3	13(THR)	3.5
	106		---	13	1	13(VAL)	1.
	106A		---	13	1	13(LEU)	
	107		---	13	3	8(GLY)	4.9
	108		---	10	1 : 2	10(GLN) : 9(GLN)	1. : 2.2
	109		---	10	1	10(PRO)	1.

REFERENCE: HUMAN LAMBDA LIGHT CHAINS SUBGROUP II

1) **NIG-84:** TONOIKE,H.,KAMETANI,F.,HOSHI,A.,SHINODA,T. & ISOBE,T. (1985) FEBS LETTERS,185,139-141.
2) **MES:** ZHU,D.,KIM,H.S. & DEUTSCH,H.F. (1983) MOL.IMMUNOL.,20,1107-1116.
3) **WH:** KIEFER,C.R.,PATTON,H.M.,JR.,MCQUIRE,B.S.,JR. & GARVER,F.A. (1980) J.IMMUNOL.,124,301-306. (CHECKED BY AUTHOR 02/11/80)
4) **NEI:** GARVER,F.A. & HILSCHMANN,N. (1971) FEBS LETTERS,16,128-132; (1972) EUR.J.BIOCHEM.,26,10-32. (CHECKED BY AUTHOR)
5) **KAR:** FETT,J.W. & DEUTSCH,H.F. (1976) IMMUNOCHEM.,13,149-155. (CHECKED BY AUTHOR)
6) **RIM:** FETT,J.W. & DEUTSCH,H.F. (1976) IMMUNOCHEM.,13,149-155. (CHECKED BY AUTHOR)
7) **SLA:** FETT,J.W. & DEUTSCH,H.F. (1976) IMMUNOCHEM.,13,149-155. (CHECKED BY AUTHOR)
8) **ES492:** TVETERAAS,T.,SLETTEN,K. & WESTERMARK,P. (1985) BIOCHEM.J.,232,183-190.
9) **WEIR:** FETT,J.W. & DEUTSCH,H.F. (1976) IMMUNOCHEM.,13,149-155. (CHECKED BY AUTHOR); JABUSCH,J.R. & DEUTSCH,H.F. (1982) MOL.IMMUNOL., 19,901-906.
10) **TOG:** NABESHIMA,Y. & IKENAKA,T. (1979) MOL.IMMUNOL.,16,439-444. (CHECKED BY AUTHOR 10/10/79)
11) **SM:** GARVER,F.A.,CHANG,L.,MENDICINO,J.,ISOBE,T. & OSSERMAN,E.F. (1975) PROC.NAT.ACAD.SCI.USA,72,4559-4563. (CHECKED BY AUTHOR 05/31/83)
12) **HS68:** HOOD,L. & EIN,D. (1968) NATURE,220,764-767.
13) **HS77:** HOOD,L. & EIN,D. (1968) NATURE,220,764-767.
14) **TRO:** SCHOLZ,R. & HILSCHMANN,N. (1975) Z.PHYSIOL.CHEM.,356,1333-1335.
15) **BOH:** KOHLER,H.,RUDOFSKY,S. & KLUSKENS,L. (1975) J.IMMUNOLOGY,114,415-421. (CHECKED BY AUTHOR)
16) **NIG-58:** TAKAYASU,T.,TAKAHASHI,N.,SHINODA,T.,OKUYAMA,T. & TOMIOKA,H. (1980) J.BIOCHEM.,89,421-436. (CHECKED BY AUTHOR 05/24/84)
17) **VIL:** PONSTINGL,H. & HILSCHMANN,N. (1969) Z.PHYSIOL.CHEM.,350,1148-1152; (1971) Z.PHYSIOL.CHEM.,352,859-877. (CHECKED BY AUTHOR)
18) **HBJ15:** STANTON,T.,SLEDGE,C.,CAPRA,J.D.,WOODS,R.,CLEM,W. & HOOD,L. (1974) J.IMMUNOL.,112,633-640.
19) **HBJ8:** HOOD,L.,GRAY,W.R.,SANDERS,B.G. & DREYER,W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,32,133-145; STANTON,T.,SLEDGE, C.,CAPRA,J.D.,WOODS,R.,CLEM,W. & HOOD,L. (1974) J.IMMUNOL.,112,633-640.
20) **HS70:** HOOD,L. & EIN,D. (1968) NATURE,220,764-767.
21) **WIN:** CHEN,B.L.,CHIU,Y.Y.H.,HUMPHREY,R.L. & POLJAK,R.J. (1978) BIOCHIM.BIOPHYS.ACTA,537,9-21. (CHECKED BY AUTHOR 07/16/79)
22) **BUR:** LIU,V.Y.S.,LOW,T.L.K.,INFANTE,A. & PUTNAM,F.W. (1976) SCIENCE,193,1017-1020; INFANTE,A. & PUTNAM,F.W. (1979) J.BIOL.CHEM.,254,9006-9016. (CHECKED BY AUTHOR 07/28/79)
23) **PRE:** FETT,J.W. & DEUTSCH,H.F. (1976) IMMUNOCHEM.,13,149-155. (CHECKED BY AUTHOR)
24) **HS86:** HOOD,L. & EIN,D. (1968) NATURE,220,764-767.
25) **WAL:** FETT,J.W. & DEUTSCH,H.F. (1976) IMMUNOCHEM.,13,149-155. (CHECKED BY AUTHOR)
26) **4A'CL:** ANDERSON,M.L.M.,SZAJNERT,M.F.,KAPLAN,J.C.,MCCOLL,L. & YOUNG,B.D. (1984) NUC.ACIDS RES.,12,6647-6661. (CHECKED BY AUTHOR 05/16/85)

NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP II

IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1: SET 1: NIG-84[1],MES[2],WH[3],NEI[4],KAR[5],RIM[6],SLA[7]. (7 IDENTICAL)
SET 2: TRO[14],BOH[15]. (2 IDENTICAL)
FR2: SET 1: WH[3],BOH[15],NIG-58[16],BUR[22]. (4 IDENTICAL)
FR3:
FR4: SET 1: WH[3]. (IDENTICAL TO 1 HUMAN V-LAMBDA-I: NEWM[1]; AND 1 HUMAN V-LAMBDA-V: BO[1].)
SET 2: MES[2],ES492[8],TRO[14],VIL[17],WIN[21]. (5 IDENTICAL HUMAN V-LAMBDA-II; ALSO 4 HUMAN V-LAMBDA-I: BL2 'CL[6],RHE[10], OKA[12],NIG-51[19]; 4 HUMAN V-LAMBDA-III: HIL[1],CAP[4],BAU[12],DEL[14]; 1 HUMAN V-LAMBDA-IV: SH[1]; 3 HUMAN V-LAMBDA-VI: SUT[2],THO[4],LBV'CL[5]; AND 24 MOUSE V-LAMBDA: MOPC104E[1],J558[2],XS104[3],HOPC1[4],J698[5],H2061[6], W3159[7],Y5431[8],Y5485[9],Y5830[10],Y5669[11],MOPC511(L)[12],S178[13],Y5444[14],Y5606[15],S176[16],H2020[17], RPC20[18],IG 303LAMBDA'CL[19],S43'CL[21],S2H5'CL[38],S2E9'CL[39],S1F12'CL[40],IG 25LAMBDA'CL[41].)
SET 3: NIG-84[1]. (IDENTICAL TO 1 HUMAN V-LAMBDA-III: GAR[7].)

IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:

CDR1: SET 1: MES[2],VIL[17]. (2 IDENTICAL HUMAN V-LAMBDA-II; ALSO 1 HUMAN V-LAMBDA-V: MCG[3].)
CDR2: SET 1: NIG-84[1],TOG[10]. (2 IDENTICAL)
CDR3:

IDENTICAL SETS OF J-MINIGENES:

SET 1: MES[2],TRO[14]. (2 IDENTICAL HUMAN V-LAMBDA-II; ALSO 1 HUMAN V-LAMBDA-III: BAU[12].)
SET 2: ES492[8],VIL[17]. (2 IDENTICAL HUMAN V-LAMBDA-II; ALSO 1 HUMAN V-LAMBDA-III: DEL[14].)

SPECIFIC NOTES:

11) SM: IT HAS O-LINKED CARBOHYDRATE ATTACHED TO SER AT POSITION 22 AND N-LINKED CARBOHYDRATE ATTACHED TO ASX AT POSITION 25.

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
47	(ILE,MET)
53	(LYS,ASN)
59	(PRO,SER)
95	(SER,ASN)
95A	(THR,SER)
95B	(LEU,ARG)

HUMAN LAMBDA LIGHT CHAINS SUBGROUP III

		INVARIANT RESIDUES	1 HIL	2 YO	3 PS	4 CAP	5 LOY A	6 LOY G	7 GAR	8 CH	9 X (PET)	10 KERN	11 TA	12 BAU	13 AMYLOID 758	14 DEL	15 LYN	16 NIG -68	17 AMYLOID 808	18 MOT #	19 WIG	20 WHI	21 OU	22 LON
FR1	0		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	#	---	---	---	---
	1		SER	SER	SER	SER	SER	SER	SER	SER	---	---	SER	---	---	---	---	---	---	phe	SER	—	---	---
	2	TYR(.96)	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	phe	TYR	TYR	TYR
	3		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	asp	ala	ala	gly	asp	val	GLU	asp	asp	GLU	gly	val	GLX	ser
	4	LEU(.96)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU	LEU
	5		THR	THR	THR	THR	THR	THR	lys	THR	THR	THR	THR	THR	THR	ser	THR	THR	THR	THR	ser	THR	THR	THR
	6		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLX	GLN
	7		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ala		PRO	PRO	ala	PRO	PRO
	8	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	10		---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	11		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	leu	met	VAL	VAL	leu	VAL	VAL	VAL	leu	VAL	VAL
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	13	VAL(.96)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	leu	VAL	VAL	VAL	VAL
	14		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	ala	phe	SER	SER	ala	SER	ala	SER	SER
	15	PRO(.95)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ala	PRO	PRO	PRO	PRO
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	
	17		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLX	GLN	GLN	GLN		GLN	GLN	GLX	GLX	
	18		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	pro	THR	THR	THR	THR	THR		
	19	ALA(.95)	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	gly	ALA	ALA	ALA	ALA	ALA		
	20		ARG	ARG	ARG	ARG	ser	ser	ARG	ARG	ser	val		ser	ser	ARG	thr	tyr		met	ser	ARG		
	21	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		ILE	ILE		ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE	ILE		
	22	THR	THR	THR	THR	THR	THR	THR	THR		THR	THR		THR	THR	THR	THR	THR	THR	THR	THR	THR		
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		CYS	CYS		CYS		CYS	CYS	CYS		CYS	CYS	CYS		
CDR1	24		SER	SER	SER	SER	SER	SER	SER		SER	SER		SER	SER	GLY	SER	SER		GLU		GLX		
	25		ALA	GLY	GLY	GLY	GLY	GLY	GLY		GLY	GLY		GLY	GLY	GLY	GLY	GLY		GLY				
	26		ASN	ASP	ASP	ASP	ASP	ASX	ASP		ASP	ASP		ASP	GLX	ASP	ASP	ASP		ASN		ASX		
	27		ALA	ALA	ALA	ALA	---	---	VAL		LYS	ASN		LYS	ASX	GLY	ALA	ASN		ASP		ASX		
	27A		---	---	---	---	---	---	---		---	---		---	---	---	---	---		---		---		
	27B		---	---	---	---	---	---	---		---	---		---	---	---	---	---		---		---		
	27C		---	---	---	---	---	---	---		---	---		---	---	---	---	---		---		---		
	27D		---	---	---	---	---	---	---		---	---		---	---	---	---	---		---		---		
	27E		---	---	---	---	---	---	---		---	---		---	---	---	---	---		---		---		
	27F		---	---	---	---	---	---	---		---	---		---	---	---	---	---		---		---		
	28		LEU	LEU	LEU	LEU	LEU	LEU	LEU		LEU	LEU		LEU	LEU	ILE	LEU	LEU		ILE		ILE		
	29		PRO	PRO	THR	PRO	---	---	PRO		GLY	GLU		GLY		GLY	SER	GLY		GLY		GLX		
	30		ASN	ASP	ASN	ALA	GLX	GLX	LYS		ASP	LYS		GLU		GLY	ASP	ASN		GLU		GLX		
	31		GLN	LEU	LYS	GLU	GLX	GLX	LYS		LYS	THR		GLN		LYS	LYS	GLU		ARG				
	32		TYR	TYR	TYR	TYR			TYR		ASP	PHE		TYR		SER	TYR	PHE		SER			TYR	
	33		ALA	VAL	ALA	ALA			ALA		VAL	VAL		VAL		VAL	VAL	VAL		VAL			VAL	
	34		TYR		TYR	TYR			TYR		CYS	SER		CYS		HIS		SER		HIS			CYS	
FR2	35	TRP	TRP	TRP	TRP	TRP			TRP		TRP	TRP		TRP		TRP	TRP	TRP		TRP			TRP	
	36		TYR	TYR		TYR			TYR		TYR	PHE		TYR		TYR	TYR	TYR		TYR				
	37		GLN	GLN		GLN			GLN		GLN	GLN		GLN		GLN	GLX	GLN		GLN				
	38		GLN	GLN		GLN			GLU		GLN	GLN		GLN		GLN	HIS	GLN		GLN				
	39		LYS	LYS		LYS			ARG		ARG	ARG		LYS		LYS	LYS	ARG		LYS				
	40		PRO			PRO			SER		PRO	PRO		PRO		PRO	PRO	PRO		PRO				
	41	GLY	GLY			GLY.			GLY		GLY	GLY		GLY		GLY	GLY	GLY		GLY				
	42		ARG			GLN			GLN		GLN	GLN		GLN		GLN		GLX		GLN				
	43		ALA			ALA			ALA		SER	SER		SER		ALA		SER		ALA				
	44	PRO	PRO			PRO			PRO		PRO	PRO		PRO		PRO	PRO	PRO		PRO				
	45		VAL			VAL			VAL		VAL	LEU		VAL		VAL	LEU	ALA		VAL				
	46		MET			MET			LEU		LEU	LEU		LEU		LEU		LEU		PRO				
	47	VAL	VAL			VAL			VAL		VAL	VAL		VAL		VAL	VAL	VAL		VAL				
	48		ILE			ILE			VAL		ILE	ILE		ILE		VAL	ILE	ILE		ILE				
	49		TYR			TYR			TYR		TYR	TYR		TYR		HIS	TYR	TYR		TYR				
CDR2	50		LYS			GLU			GLU		GLN	HIS		HIS		GLU	GLX	ASX		ASP				
	51		ASP			THR			ASP	ASP	ASP	THR		ASP		ASP		THR		ASP				
	52		THR			ASN			SER	THR	ASN	SER		SER		ASN	THR	SER		ALA				
	53		GLN			LYS			GLY	GLY	GLN	GLU		LYS		ASP	LYS	LYS		ASP				
	54	ARG	ARG			ARG			ARG	ARG	ARG	ARG		ARG		ARG	ARG	ARG		ARG				
	55		PRO			PRO			PRO	PRO	SER	PRO		PRO		PRO	PRO	PRO		PRO				
	56		SER			SER			SER	SER	SER	SER		SER		ALA		SER		SER				
FR3	57		GLY			GLY			GLU	THR	GLY	GLU		GLY		GLY	GLY	GLY		GLY				
	58		ILE			ILE			ILE	ILE	ILE	ILE		ILE		ILE		ILE		VAL				
	59	PRO	PRO			PRO			PRO	PRO	PRO	PRO		PRO		PRO		PRO		PRO				
	60		GLN			GLU			GLU	GLU	GLU	GLU		GLU		GLU	GLX	GLU		ALA				
	61	ARG	ARG			ARG			ARG	ARG	ARG	ARG		ARG		ARG	ARG	ARG		ARG				
	62	PHE	PHE			PHE			PHE	PHE	PHE	PHE		PHE		PHE	PHE	PHE		PHE				
	63	SER	SER			SER			SER	SER	SER	SER		SER		SER		SER		SER				
	64		SER			GLY			GLY	GLY	GLY	GLY		GLY		GLY		GLY		GLY				
	65		SER			SER			SER	SER	SER	SER		SER		SER		SER		TYR				
	66		THR			THR			SER	THR	ASN	SER		ASN		ASN		LYS		ASN				
	67	SER	SER			SER			SER	SER	SER	SER		SER		SER		SER		SER				
	68	GLY	GLY			GLY			GLY	GLY	GLY	GLY		GLY		GLY		GLY		GLY				
	69		THR			THR			THR	THR	ASN	ALA		THR		ASN		ASN		ASN				
	70		THR			THR			LYS	THR	THR	THR		THR		THR		THR		SER				
	71		VAL			VAL			ALA	ALA	ALA	ALA		ALA		ALA		ALA		ALA				
	72		THR			THR			THR	THR	THR	THR		THR		ALA		THR		ILE				
	73	LEU	LEU			LEU			LEU	LEU	LEU	LEU		LEU		LEU		LEU		LEU				
	74	THR	THR			THR			THR	THR	THR	THR		THR		THR		THR		THR				
	75	ILE	ILE			ILE			ILE	ILE	ILE	ILE		ILE		ILE		ILE		ILE				
	76		SER			SER			SER	SER	SER	SER		SER		SER		SER		ASN				
	77		GLY			GLY			GLY	GLY	GLY	GLY		GLY		ARG		GLY		ARG				
	78		VAL			VAL			ALA	VAL	THR	ALA		THR		VAL		THR		VAL				
	79		GLN			GLN			GLN	GLN	GLN	GLN		GLN		GLU		GLU		GLU				
	80		ALA			ALA			VAL	ALA	ALA	SER		ALA		ALA		SER		ALA				
	81		GLU			GLU			GLU	ASN	MET	VAL		MET		GLY		MET		GLY				
	82		ASP			ASP			ASP	ASX	ASP	ASP		ASP		ASP		ASX		ASP				
	83		GLU			GLU			GLU	GLX	GLU	GLU		GLU		GLU		GLU		GLU				
	84	ALA	ALA			ALA			ALA	ALA	ALA	ALA		ALA		ALA		ALA		ALA				
	85		ASP			ASP			ASP	ASX	ASP	ASP		ASP		ASP		ASX		ASP				
	86	TYR	TYR			TYR			TYR	TYR	TYR	TYR		TYR		TYR		TYR		TYR				
	87		TYR			TYR			TYR	TYR	TYR	PHE		TYR		TYR		TYR		PHE				
	88	CYS	CYS			CYS			CYS	CYS	CYS	CYS		CYS		CYS		CYS		CYS				
CDR3	89		GLN			SER			SER	GLX	GLN	GLN		GLN		GLU		GLX		GLN				
	90		ALA			SER			THR	SER	ALA	THR		ALA		VAL		ALA		SER				
	91		TRP			ALA			ASP	ALA	TRP	TRP		TRP		TRP		TRP		TRP				
	92		ASP			ASP			ILE	ASN	ASP	ASP		ASP		ASP		ASX		ASP				
	93		ASN			SER			ASN	SER	SER	THR		SER		ASP		GLX		ASN				
	94		SER			SER			GLY	ARG	MET	ILE		TYR		ARG		ILE		GLY				
	95		ALA.			GLN			TYR		SER	THR		THR		THR		ARG		SER				
	95A		---			GLY			---		---	---		---		ALA		ASP		TYR				
	95B		---			---			---		---	---		---		HIS		---		GLU				
	95C		---			---			---		---	---		---		---		---		---				
	95D		---			---			---		---	---		---		---		---		---				
	95E		---			---			---		---	---		---		---		---		---				
	95F		---			---			---		---	---		---		---		---		---				
	96		SER			MET			PRO		VAL	ALA		VAL		VAL		VAL		VAL				
	97		ILE			VAL			LEU		VAL	ILE		ILE		VAL		VAL		VAL	VAL			
FR4	98	PHE	PHE			PHE			PHE		PHE	PHE		PHE		PHE		PHE		PHE	PHE			
	99	GLY	GLY			GLY			GLY	GLY	GLY	GLY		GLY		GLY		GLY		GLY	GLY			
	100		GLY			GLY.			GLY	GLY	GLY	GLY		GLY		GLY		GLY		THR	ALA			
	101	GLY	GLY			GLY			GLY	GLY	GLY	GLY		GLY		GLY		GLY		GLY	GLY			
	102	THR	THR			THR			THR	THR	THR	THR		THR		THR		THR		THR	THR			
	103		LYS			LYS			LYS	LYS	ARG	LYS		LYS		LYS		LYS		MET	THR			
	104		LEU			LEU			LEU	LEU	LEU	LEU		LEU		LEU		LEU		VAL				
	105		THR			THR			SER	THR	THR	THR		THR		THR		THR		THR				
	106	VAL	VAL			VAL			VAL	VAL	VAL	VAL		VAL		VAL		VAL		VAL				
	106A	LEU	LEU			LEU			LEU	LEU	LEU	LEU		LEU		LEU		LEU		LEU				
	107		GLY			GLY			GLY		SER	SER		GLY		GLY				GLY				
	108	GLN	GLN			GLN			GLN		GLN	GLN		GLN						GLN				
	109	PRO	PRO			PRO			PRO		PRO	PRO		PRO						PRO				

HUMAN LAMBDA LIGHT CHAINS SUBGROUP III (cont'd)

		23 SG	24 GIM	25 111	26 119	27 VIN	28 MIL	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
FR 1	0	---	---	---	---	---					
	1	tyr	---	---	---	---		12	3	10(SER)	3.6
	2	TYR	TYR	TYR	TYR	TYR		27	2	26(TYR)	2.1
	3	val	val	GLU	GLX			26	6 : 7	13(GLU) : 11(GLU)	12. : 17.
	4	LEU	LEU	LEU	LEU			28	2	25(LEU)	2.1
	5	THR	THR	THR	THR			26	3	23(THR)	3.4
	6	GLN	GLX	GLN	GLX			28	1 : 2	26(GLN) : 22(GLN)	1. : 2.4
	7	PRO	PRO	PRO	PRO			25	2	23(PRO)	2.2
	8	PRO	PRO	PRO	PRO			26	1	26(PRO)	1.
	9	SER	SER					24	1	24(SER)	1.
	10	---	---								
	11	VAL	VAL					24	3	20(VAL)	3.6
	12	SER	SER					24	1	24(SER)	1.
	13	VAL	VAL					24	2	23(VAL)	2.1
	14							22	3	18(SER)	3.7
	15							22	2	21(PRO)	2.1
	16							21	1	21(GLY)	1.
	17							20	1 : 2	20(GLN) : 17(GLN)	1. : 2.4
	18					met		21	3	19(THR)	3.3
	19							20	2	19(ALA)	2.1
	20							18	6	8(ARG)	14.
	21						ILE	19	1	19(ILE)	1.
	22						THR	19	1	19(THR)	1.
	23						CYS	17	1	17(CYS)	1.
CDR 1	24						GLY	17	3 : 4	13(SER)	3.9 : 5.2
	25						GLY	16	2	15(GLY)	2.1
	26						ASP	17	3	14(ASP) : 12(ASP)	3.8 : 4.3
	27						GLU	15	7	5(ALA)	21.
	27A										
	27B										
	27C										
	27D										
	27E										
	27F										
	28							16	2	13(LEU)	2.5
	29							13	5 : 6	5(GLY)	13. : 16.
	30							15	6 : 7	5(GLU) : 3(+)	18. : 35.
	31							14	6	5(LYS)	17.
	32							13	4	8(TYR)	6.5
	33							13	2	9(VAL)	2.9
	34							11	4	4(TYR)	11.
FR 2	35							13	1	13(TRP)	1.
	36							11	2	10(TYR)	2.2
	37							11	1 : 2	11(GLN) : 10(GLN)	1. : 2.2
	38							11	3	9(GLN)	3.7
	39							11	2	7(LYS)	3.1
	40							10	2	9(PRO)	2.2
	41							10	1	10(GLY)	1.
	42							9	2 : 3	8(GLN) : 7(GLN)	2.3 : 3.9
	43							9	2	5(ALA)	3.6
	44							10	1	10(PRO)	1.
	45							10	3	7(VAL)	4.3
	46							9	3	6(LEU)	4.5
	47							10	1	10(VAL)	1.
	48							10	2	8(ILE)	2.5
	49							10	2	9(TYR)	2.2
CDR 2	50							10	5 : 6	4(GLU) : 3(GLU)	13. : 20.
	51							10	2	7(ASP)	2.9
	52							11	4	4(SER)	11.
	53							11	5	4(LYS)	14.
	54							11	1	11(ARG)	1.
	55							11	2	10(PRO)	2.2
	56							10	2	9(SER)	2.2
FR 3	57							11	3	8(GLY)	4.1
	58							10	2	9(ILE)	2.2
	59							10	1	10(PRO)	1.
	60							11	3	9(GLU) : 8(GLU)	3.7 : 4.1
	61							11	1	11(ARG)	1.
	62							11	1	11(PHE)	1.
	63							10	1	10(SER)	1.
	64							10	2	9(GLY)	2.2
	65							10	2	9(SER)	2.2
	66							10	4	4(ASN)	10.
	67							10	1	10(SER)	1.
	68							10	1	10(GLY)	1.
	69							10	3	5(THR)	6.
	70							10	3	8(THR)	3.8
	71							10	2	8(ALA)	2.5
	72							10	3	8(THR)	3.8
	73							10	1	10(LEU)	1.
	74							10	1	10(THR)	1.
	75							10	1	10(ILE)	1.
	76							10	2	9(SER)	2.2
	77							10	2	8(GLY)	2.5
	78							10	3	5(VAL)	6.
	79							10	2	7(GLN)	2.9
	80							10	3	7(ALA)	4.3
	81							10	5	3(+)	17.
	82							10	1 : 2	10(ASP) : 8(ASP)	1. : 2.5
	83							10	1 : 2	10(GLU) : 9(GLU)	1. : 2.2
	84							10	1	10(ALA)	1.
	85							10	1 : 2	10(ASP) : 8(ASP)	1. : 2.5
	86							10	1	10(TYR)	1.
	87							10	2	8(TYR)	2.5
	88							10	1	10(CYS)	1.
CDR 3	89							10	3	7(GLN) : 5(GLN)	4.3 : 6.
	90							10	4	4(ALA)	10.
	91							10	3	7(TRP)	4.3
	92							10	3	8(ASP) : 7(ASP)	3.8 : 4.3
	93							10	5	4(SER)	13.
	94							10	8	2(+)	30.
	95							9	6	3(THR)	18.
	95A							4	4	1(+)	
	95B							2	2	1(+)	
	95C										
	95D										
	95E										
	95F										
	96							9	5	5(VAL)	9.
	97							10	3	6(VAL)	5.
FR 4	98							10	1	10(PHE)	1.
	99							11	1	11(GLY)	1.
	100							11	3	9(GLY)	3.7
	101							11	1	11(GLY)	1.
	102							11	1	11(THR)	1.
	103							11	4	8(LYS)	5.5
	104							10	2	9(LEU)	2.2
	105							10	2	9(THR)	2.2
	106							10	1	10(VAL)	1.
	106A							10	1	10(LEU)	
	107							8	2	6(GLY)	2.7
	108							7	1	7(GLN)	1.
	109							7	1	7(PRO)	1.

ANTIBODY SPECIFICITIES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP III

7) **GAR:** ANTI-RIBOFLAVIN

- **REFERENCE: HUMAN LAMBDA LIGHT CHAINS SUBGROUP III**

1) **HIL:** LOPEZ DE CASTRO,J.A., CHIU,Y.Y.H. & POLJAK,R.J. (1978) BIOCHEMISTRY,17,1718-1723. (CHECKED BY AUTHOR 07/16/79)
2) **YO:** TISCHENDORF,F.W.,TISCHENDORF,M.M. & WITTMANN-LIEBOLD,B. (1976) Z.NATURFORSCH,31C,758-760.
3) **PS:** KOCHWA,S.,TERRY,W.D.,CAPRA,J.D. & YANG,N.Y. (1971) ANN.N.Y.ACAD.SCI.,190,49-70. (CHECKED BY AUTHOR)
4) **CAP:** ZHU,D.,KIM,H.S. & DEUTSCH,H.F. (1983) MOL.IMMUNOL.,20,1107-1116.
5) **LOY A:** WOLFENSTEIN-TODEL,C.,FRANKLIN,E.C. & RUDDERS,R.A. (1974) J.IMMUNOL.,112,871-876. (CHECKED BY AUTHOR)
6) **LOY G:** WOLFENSTEIN-TODEL,C.,FRANKLIN,E.C. & RUDDERS,R.A. (1974) J.IMMUNOL.,112,871-876. (CHECKED BY AUTHOR)
7) **GAR:** KIEFER,C.R.,MCGUIRE,B.S.,JR.,OSSERMAN,E.F. & GARVER,F.A. (1983) J.IMMUNOL.,131,1871-1875. (CHECKED BY AUTHOR 02/20/84)
8) **CH:** OKADA,Y.,NOZU,Y.,TITANI,K.,WATANABE,S.,HARA,H. & KITAGAWA,M. (1972) IMMUNOCHEM.,9,207-210.
9) **X(PET):** MILSTEIN,C.,CLEGG,J.B. & JARVIS,J.M. (1968) BIOCHEM.J.,110,631-652. (CHECKED BY AUTHOR)
10) **KERN:** PONSTINGL,H.,HESS,M. & HILSCHMANN,N. (1968) Z.PHYSIOL.CHEM.,349,867-871; (1971) Z.PHYSIOL.CHEM.,352,247-266. (CHECKED BY AUTHOR)
11) **TA:** TONNELLE,C. (1973) BIOCHIM.BIOPHYS.RES.COMM.,55,1112-1116. (CHECKED BY AUTHOR 11/16/80)
12) **BAU:** BACZKO,K.,BRAUN,D.G.,HESS,M. & HILSCHMANN,N. (1970) Z.PHYSIOL.CHEM.,351,763-767; BACZKO,K.,BRAUN,D.G. & HILSCHMANN,N. (1974) Z.PHYSIOL.CHEM.,355,131-154. (CHECKED BY AUTHOR)
13) **AMYLOID 758:** NATVIG,J.B.,WESTERMARK,P.,SLETTEN,K.,HUSBY,G. & MICHAELSON,T. (1981) SCAND.J.IMMUNOL.,14,89-94. (CHECKED BY AUTHOR 06/01/83)
14) **DEL:** EULITZ,M. (1974) EUR.J.BIOCHEM.,50,49-69. (CHECKED BY AUTHOR 10/18/77)
15) **LYN:** MEINKE,G.C.,SIGRIST,P.H. & SPIEGELBERG,H.L. (1974) IMMUNOCHEM.,11,457-460. (CHECKED BY AUTHOR WHO PROVIDED ADDITIONAL RESIDUES TO THOSE PUBLISHED); MEINKE,G.C. & SPIEGELBERG,H.L. (1976) IMMUNOCHEM.,13,915-919. (CHECKED BY AUTHOR 10/17/77)
16) **NIG-68:** KAMETANI,F.,YOSHIMURA,K.,TONOIKE,H.,HOSHI,A.,SHINODA,T. & ISOBE,T. (1985) BIOCHEM.BIOPHYS.RES.COMMUN.,126,848-852.
17) **AMYLOID 808:** WESTERMARK,P.,NATVIG,J.B.,ANDERS,R.F.,SLETTEN,K. & HUSBY,G. (1976) SCAND.J.IMMUNOL.,5,31-36. (CHECKED BY AUTHOR 06/01/83)
18) **MOT:** KOJIMA,M.,ODANI,S. & IKENAKA,T. (1980) MOL.IMMUNOL.,17,1407-1414.
19) **WIG:** FETT,J.W. & DEUTSCH,H.F. (1976) IMMUNOCHEM.,13,149-155. (CHECKED BY AUTHOR)
20) **WHI:** WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)
21) **DU:** BUCHWALD,B.M. (1971) CAN.J.BIOCHEM.,49,900-902. (CHECKED BY AUTHOR)
22) **LON:** JOHNSTON,S.L.,ABRAHAM,G.N. & WELCH,E.H. (1975) BIOCHEM.BIOPHYS.RES.COMMUN.,66,842-847. (CHECKED BY AUTHOR 10/17/77)
23) **SG:** TISCHENDORF,F.W.,TISCHENDORF,M.M. & WITTMANN-LIEBOLD,B. (1976) Z.NATURFORSCH,31C,758-760.
24) **GIM:** HESS,M.,HILSCHMANN,N.,RIVET,L.,RIVET,C. & ROPARTZ,C. (1971) NATURE NEW BIOLOGY,234,58-61. (CHECKED BY AUTHOR)
25) **111:** LANGER,B.,STEINMETZ-KAYNE,M. & HILSCHMANN,N. (1968) Z.PHYSIOL.CHEM.,349,945-951. (CHECKED BY AUTHOR)
26) **119:** HESS,M.,HILSCHMANN,N.,RIVET,L.,RIVET,C. & ROPARTZ,C. (1971) NATURE NEW BIOLOGY,234,58-61. (CHECKED BY AUTHOR)
27) **VIN:** PINK,J.R.L. & MILSTEIN,C. (1969) PROC. 5TH FEBS SYMP.,15,177-182. (CHECKED BY AUTHOR)
28) **MIL:** LANGER,B.,STEINMETZ-KAYNE,M. & HILSCHMANN,N. (1968) Z.PHYSIOL.CHEM.,349,945-951. (CHECKED BY AUTHOR)

NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP III

IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1: SET 1: HIL[1],YO[2],PS[3],CAP[4]. (4 IDENTICAL)
SET 2: LOY A[5],LOY G[6]. (2 IDENTICAL)

FR2:

FR3:

FR4: SET 1: HIL[1],CAP[4],BAU[12],DEL[14]. (4 IDENTICAL HUMAN V-LAMBDA-III; ALSO 4 HUMAN V-LAMBDA-I: BL2 'CL[6],RHE[10],OKA[12], NIG-51[19]; 5 HUMAN V-LAMBDA-II: MES[2],ES492[8],TRO[14],VIL[17],WIN[21]; 1 HUMAN V-LAMBDA-IV: SH[1]; 3 HUMAN V-LAMBDA-VI: SUT[2],THO[4],LBV'CL[5]; AND 24 MOUSE V-LAMBDA: MOPC104E[1],J558[2],XS104[3],HOPC1[4],J698[5],H2061[6], W3159[7],Y5431[8],Y5485[9],Y5830[10],Y5669[11],MOPC511(L)[12],S178[13],Y5444[14],Y5606[15],S176[16],H2020[17], RPC20[18],IG 303LAMBDA'CL[19],S43'CL[21],S2H5'CL[38],S2E9'CL[39],S1F12'CL[40],IG 25LAMBDA'CL[41].)
SET 2: GAR[7]. (IDENTICAL TO 1 HUMAN V-LAMBDA-II: NIG-84[1].)
SET 3: KERN[10]. (IDENTICAL TO 1 HUMAN V-LAMBDA-VI: NIG-48[10].)

IDENTICAL SETS OF J-MINIGENES:

SET 1: BAU[12]. (IDENTICAL TO 2 HUMAN V-LAMBDA-II: MES[2],TRO[14].)
SET 2: DEL[14]. (IDENTICAL TO 2 HUMAN V-LAMBDA-II: ES492[8],VIL[17].)

SPECIFIC NOTES:

18) **MOT:** THERE ARE TWO RESIDUES IN FRONT OF POSITION 1; THEY ARE VAL AND THR.

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
30	(ASP,ASN,GLN)
81	(MET,GLU)
94	(ILE,ARG,SER,GLY)
95A	(TYR,ALA,GLY,ASP)
95B	(HIS,GLU)

HUMAN LAMBDA LIGHT CHAINS SUBGROUP IV

		INVARIANT RESIDUES	1 SH	2 NEV	3 USH	4 PFA	5 FRA'	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
	0		---	---	---	---	---				
	1		---	---	---	---	---				
	2	SER	SER	SER	SER	SER	---	4	1	4(SER)	1.
	3		GLU	GLU	GLU	GLU	ala	5	2	4(GLU)	2.5
	4	LEU	LEU	LEU	LEU	LEU	LEU	5	1	5(LEU)	1.
	5		THR	THR	THR	THR	val	5	2	4(THR)	2.5
	6	GLN	GLN	GLN	GLN	GLN	GLN	5	1	5(GLN)	1.
	7		ASP	ASX	pro	pro	pro	5	2 : 3	3(PRO)	3.3 : 5.
	8		PRO	PRO	PRO	PRO	ala	5	2	4(PRO)	2.5
	9		ALA	ALA	ser	ser	ser	5	2	3(SER)	3.3
	10		---	---	---	---	---				
FR1	11	VAL	VAL	VAL	VAL	VAL	VAL	5	1	5(VAL)	1.
	12		SER	SER	SER	SER	glx	5	2	4(SER)	2.5
	13	-	VAL	VAL	VAL	VAL	gly	5	2	4(VAL)	2.5
	14		ALA	ALA	ser	ser	ALA	5	2	3(ALA)	3.3
	15		LEU	LEU	pro	pro	pro	5	2	3(PRO)	3.3
	16	GLY	GLY	GLY	GLY	GLY	GLY	5	1	5(GLY)	1.
	17		GLN	GLX	GLN	GLN	GLX	5	1 : 2	5(GLN) : 3(GLN)	1. : 3.3
	18	-	THR	THR	THR	THR	ser	5	2	4(THR)	2.5
	19		VAL	VAL	ala	ala	ile	5	3	2(+)	7.5
	20		ARG	ARG	ser	val	ala	5	4	2(ARG)	10.
	21	ILE	ILE	ILE	ILE	ILE	ILE	5	1	5(ILE)	1.
	22		THR	THR	THR	THR	gly	5	2	4(THR)	2.5
	23	CYS	CYS		CYS	CYS	CYS	4	1	4(CYS)	1.
	24		GLN		SER	SER	ILE	4	3	2(SER)	6.
	25	GLY	GLY		GLY	GLY	GLY	4	1	4(GLY)	1.
	26		ASP		ASP	ASP	ILE	4	2	3(ASP)	2.7
	27		SER		LYS	LYS	SER	4	2	2(+)	4.
	27A		---		---	---	---				
	27B		---		---	---	---				
	27C		---		---	---	---				
CDR1	27D		---		---	---	---				
	27E		---		---	---					
	27F		---		---	---	ASX	1	1	1(ASN) : 1(ASP)	
	28		LEU		LEU	LEU	ILE	4	2	3(LEU)	2.7
	29		ARG		GLY	GLY	GLY	4	2	3(GLY)	2.7
	30		GLY		ASP	GLN	ALA	4	4	1(+)	16.
	31		TYR		ASN	ALA	TYR	4	3	2(TYR)	6.
	32		ASP		TYR		ASX	3	2 : 3	2(ASP) : 1(+)	3. : 9.
	33		ALA		ALA		TYR	3	2	2(ALA)	3.
	34		ALA		SER		ILE	3	3	1(+)	9.
	35	TRP	TRP		TRP	TRP		3	1	3(TRP)	1.
	36	TYR	TYR		TYR	TYR		3	1	3(TYR)	1.
	37	GLN	GLN		GLN			2	1	2(GLN)	1.
	38	GLN	GLN		GLN			2	1	2(GLN)	1.
	39	LYS	LYS		LYS			2	1	2(LYS)	1.
	40		PRO					1	1	1(PRO)	
FR2	41		GLY					1	1	1(GLY)	
	42		GLN					1	1	1(GLN)	
	43		ALA					1	1	1(ALA)	
	44		PRO					1	1	1(PRO)	
	45		LEU					1	1	1(LEU)	
	46		LEU					1	1	1(LEU)	
	47		VAL					1	1	1(VAL)	
	48		ILE					1	1	1(ILE)	
	49		TYR					1	1	1(TYR)	
	50		GLY					1	1	1(GLY)	
	51		ARG					1	1	1(ARG)	
CDR2	52		ASN					1	1	1(ASN)	
	53		ASN					1	1	1(ASN)	
	54		ARG					1	1	1(ARG)	
	55		PRO					1	1	1(PRO)	
	56		SER					1	1	1(SER)	
	57		GLY					1	1	1(GLY)	
	58		ILE					1	1	1(ILE)	
	59		PRO					1	1	1(PRO)	
	60		ASP					1	1	1(ASP)	
	61		ARG					1	1	1(ARG)	
	62		PHE					1	1	1(PHE)	
	63		SER					1	1	1(SER)	
	64		GLY					1	1	1(GLY)	
	65		SER					1	1	1(SER)	
	66		SER					1	1	1(SER)	
	67		SER					1	1	1(SER)	
	68		GLY					1	1	1(GLY)	
	69		HIS					1	1	1(HIS)	
	70		THR					1	1	1(THR)	
	71		ALA					1	1	1(ALA)	
FR3	72		SER					1	1	1(SER)	
	73		LEU					1	1	1(LEU)	
	74		THR					1	1	1(THR)	
	75		ILE					1	1	1(ILE)	
	76		THR					1	1	1(THR)	
	77		GLY					1	1	1(GLY)	
	78		ALA					1	1	1(ALA)	
	79		GLN					1	1	1(GLN)	
	80		ALA					1	1	1(ALA)	
	81		GLU					1	1	1(GLU)	
	82		ASP					1	1	1(ASP)	
	83		GLU					1	1	1(GLU)	
	84		ALA					1	1	1(ALA)	
	85		ASP					1	1	1(ASP)	
	86		TYR					1	1	1(TYR)	
	87		TYR					1	1	1(TYR)	
	88		CYS					1	1	1(CYS)	
	89		ASN					1	1	1(ASN)	
	90		SER					1	1	1(SER)	
	91		ARG					1	1	1(ARG)	
	92		ASP					1	1	1(ASP)	
	93		SER					1	1	1(SER)	
	94		SER					1	1	1(SER)	
	95		GLY					1	1	1(GLY)	
CDR3	95A		LYS					1	1	1(LYS)	
	95B		HIS					1	1	1(HIS)	
	95C		---								
	95D		---								
	95E		---								
	95F		---								
	96		VAL					1	1	1(VAL)	
	97		LEU					1	1	1(LEU)	
	98		PHE					1	1	1(PHE)	
	99		GLY					1	1	1(GLY)	
	100		GLY					1	1	1(GLY)	
	101		GLY					1	1	1(GLY)	
	102		THR					1	1	1(THR)	
FR4	103		LYS					1	1	1(LYS)	
	104		LEU					1	1	1(LEU)	
	105		THR					1	1	1(THR)	
	106		VAL					1	1	1(VAL)	
	106A		LEU					1	1	1(LEU)	
	107		GLY					1	1	1(GLY)	
	108		GLN					1	1	1(GLN)	
	109		PRO					1	1	1(PRO)	

REFERENCE: HUMAN LAMBDA LIGHT CHAINS SUBGROUP IV

1) **SH:** TITANI,K.,WIKLER,M.,SHINODA,T. & PUTNAM,F.W. (1970) J.BIOL.CHEM.,245,2171-2176. (CHECKED BY AUTHOR 06/15/83)
2) **NEV:** WANG,A.C.,WELLS,J.V.,FUDENBERG,H.H. & GERGELY,J. (1974) IMMUNOCHEM.,11,341-345. (CHECKED BY AUTHOR)
3) **USH:** TISCHENDORF,F.W.,TISCHENDORF,M.M. & WITTMANN-LIEBOLD,B. (1976) Z.NATURFORSCH,31C,758-760.
4) **PFA:** TISCHENDORF,F.W.,TISCHENDORF,M.M. & WITTMANN-LIEBOLD,B. (1976) Z.NATURFORSCH,31C,758-760.
5) **FRA':** WANG,A.C. & FUDENBERG,H.H. (1974) J.IMMUNOGENETICS,1,303-313. (CHECKED BY AUTHOR)

NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP IV

IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1: SET 1: SH[1],NEV[2]. (2 IDENTICAL)
FR2:
FR3:
FR4: SET 1: SH[1]. (IDENTICAL TO 4 HUMAN V-LAMBDA-I: BL2'CL[6],RHE[10],OKA[12],NIG-51[19]; 5 HUMAN V-LAMBDA-II: MES[2],ES492[8],TRO[14],VIL[17],WIN[21]; 4 HUMAN V-LAMBDA-III: HIL[1],CAP[4],BAU[12],DEL[14]; 3 HUMAN V-LAMBDA-VI: SUT[2],THO[4],LBV'CL[5]; AND 24 MOUSE V-LAMBDA: MOPC104E[1],J558[2],XS104[3],HOPC1[4],J698[5],H2061[6],W3159[7],Y5431[8],Y5485[9],Y5830[10],Y5669[11],MOPC511(L)[12],S178[13],Y5444[14],Y5606[15],S176[16],H2020[17],RPC20[18],IG 303LAMBDA'CL[19],S43'CL[21],S2H5'CL[38],S2E9'CL[39],S1F12'CL[40],IG 25LAMBDA'CL[41].)

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
19	(VAL,ALA)
27	(LYS,SER)
30	(ALA,GLY,ASP,GLN)
32	(TYR,ASP,ASN)
34	(ILE,ALA,SER)

HUMAN LAMBDA LIGHT CHAINS SUBGROUP V

	•	INVARIANT RESIDUES	1 BO	2 HBJ 2	3* MCG	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
	0		---	---	---				
	1	PCA	PCA	PCA	PCA	3	1	3(PCA)	1.
	2	SER	SER	SER	SER	3	1	3(SER)	1.
	3	ALA	ALA	ALA	ALA	3	1	3(ALA)	1.
	4	LEU	LEU	LEU	LEU	3	1	3(LEU)	1.
	5	THR	THR	THR	THR	3	1	3(THR)	1.
	6	GLN	GLN	GLN	GLN	3	1	3(GLN)	1.
	7	PRO	PRO	PRO	PRO	3	1	3(PRO)	1.
	8	PRO	PRO	PRO	PRO	3	1	3(PRO)	1.
	9	SER	SER	SER	SER	3	1	3(SER)	1.
	10		---	---	---				
FR1	11	ALA	ALA	ALA	ALA	3	1	3(ALA)	1.
	12	SER	SER	SER	SER	3	1	3(SER)	1.
	13	GLY	GLY	GLY	GLY	3	1	3(GLY)	1.
	14	SER	SER	SER	SER	3	1	3(SER)	1.
	15		PRO	PRO	leu	3	2	2(PRO)	3.
	16	GLY	GLY	GLY	GLY	3	1	3(GLY)	1.
	17	GLN	GLN	GLN	GLN	3	1	3(GLN)	1.
	18	SER	SER	SER	SER	3	1	3(SER)	1.
	19	VAL	VAL	VAL	VAL	3	1	3(VAL)	1.
	20	THR	THR	THR	THR	3	1	3(THR)	1.
	21	ILE	ILE	ILE	ILE	3	1	3(ILE)	1.
	22	SER	SER	SER	SER	3	1	3(SER)	1.
	23	CYS	CYS	CYS	CYS	3	1	3(CYS)	1.
	24	THR	THR	THR	THR	3	1	3(THR)	1.
	25	GLY	GLY	GLY	GLY	3	1	3(GLY)	1.
	26	THR	THR	THR	THR	3	1	3(THR)	1.
	27	SER	SER		SER	2	1	2(SER)	1.
	27A		---		---				
	27B		---		---				
	27C		---		---				
	27D	SER	SER		SER	2	1	2(SER)	
CDR1	27E	ASP	ASP		ASP	2	1	2(ASP)	
	27F	VAL	VAL		VAL	2	1	2(VAL)	
	28	GLY	GLY		GLY	2	1	2(GLY)	1.
	29		ASP		GLY	2	2	1(+)	4.
	30		ASN		TYR	2	2	1(+)	4.
	31		LYS		ASN	2	2	1(+)	4.
	32	TYR	TYR		TYR	2	1	2(TYR)	1.
	33	VAL	VAL		VAL	2	1	2(VAL)	1.
	34	SER	SER		SER	2	1	2(SER)	1.
	35	TRP	TRP		TRP	2	1	2(TRP)	1.
	36	TYR	TYR		TYR	2	1	2(TYR)	1.
	37	GLN	GLN		GLN	2	1	2(GLN)	1.
	38	GLN	GLN		GLN	2	1	2(GLN)	1.
	39	HIS	HIS		HIS	2	1	2(HIS)	1.
	40		PRO		ALA	2	2	1(+)	4.
	41	GLY	GLY		GLY	2	1	2(GLY)	1.
FR2	42		ARG		LYS	2	2	1(+)	4.
	43	ALA	ALA		ALA	2	1	2(ALA)	1.
	44	PRO	PRO		PRO	2	1	2(PRO)	1.
	45	LYS	LYS		LYS	2	1	2(LYS)	1.
	46		LEU		VAL	2	2	1(+)	4.
	47		VAL		ILE	2	2	1(+)	4.
	48	ILE	ILE		ILE	2	1	2(ILE)	1.
	49		PHE		TYR	2	2	1(+)	4.
	50	GLU	GLU		GLU	2	1	2(GLU)	1.
	51	VAL	VAL		VAL	2	1	2(VAL)	1.
	52		SER		ASN	2	2	1(+)	4.
CDR2	53		GLY		LYS	2	2.	1(+)	4.
	54	ARG	ARG		ARG	2	1	2(ARG)	1.
	55	PRO	PRO		PRO	2	1	2(PRO)	1.
	56	SER	SER		SER	2	1	2(SER)	1.
	57	GLY	GLY		GLY	2	1	2(GLY)	1.
	58	VAL	VAL		VAL	2	1	2(VAL)	1.
	59	PRO	PRO		PRO	2	1	2(PRO)	1.
	60	ASP	ASP		ASP	2	1	2(ASP)	1.
	61	ARG	ARG		ARG	2	1	2(ARG)	1.
	62	PHE	PHE		PHE	2	1	2(PHE)	1.
	63	SER	SER		SER	2	1	2(SER)	1.
	64	GLY	GLY		GLY	2	1	2(GLY)	1.
	65	SER	SER		SER	2	1	2(SER)	1.
	66	LYS	LYS		LYS	2	1	2(LYS)	1.
	67	SER	SER		SER	2	1	2(SER)	1.
	68		ASP		GLY	2	2	1(+)	4.
	69	ASN	ASN		ASN	2	1	2(ASN)	1.
	70	THR	THR		THR	2	1	2(THR)	1.
	71	ALA	ALA		ALA	2	1	2(ALA)	1.
FR3	72	SER	SER		SER	2	1	2(SER)	1.
	73	LEU	LEU		LEU	2	1	2(LEU)	1.
	74	THR	THR		THR	2	1	2(THR)	1.
	75	VAL	VAL		VAL	2	1	2(VAL)	1.
	76	SER	SER		SER	2	1	2(SER)	1.
	77	GLY	GLY		GLY	2	1	2(GLY)	1.
	78	LEU	LEU		LEU	2	1	2(LEU)	1.
	79		ARG		GLN	2	2	1(+)	4.
	80	ALA	ALA		ALA	2	1	2(ALA)	1.
	81	GLU	GLU		GLU	2	1	2(GLU)	1.
	82	ASP	ASP		ASP	2	1	2(ASP)	1.
	83	GLU	GLU		GLU	2	1	2(GLU)	1.
	84	ALA	ALA		ALA	2	1	2(ALA)	1.
	85	ASP	ASP		ASP	2	1	2(ASP)	1.
	86	TYR	TYR		TYR	2	1	2(TYR)	1.
	87	TYR	TYR		TYR	2	1	2(TYR)	1.
	88	CYS	CYS		CYS	2	1	2(CYS)	1.
	89	SER	SER		SER	2	1	2(SER)	1.
	90	SER	SER		SER	2	1	2(SER)	1.
	91	TYR	TYR		TYR	2	1	2(TYR)	1.
	92		VAL		GLU	2	2	1(+)	4.
	93		ASP		GLY	2	2	1(+)	4.
	94		ASN		SER	2	2	1(+)	4.
CDR3	95		ASN		ASP	2	2	1(+)	4.
	95A	ASN	ASN		ASN	2	1	2(ASN)	
	95B		---		---				
	95C		---		---				
	95D		---		---				
	95E		---		---				
	95F		---		---				
	96	PHE	PHE		PHE	2	1	2(PHE)	1.
	97	VAL	VAL		VAL	2	1	2(VAL)	1.
	98	PHE	PHE		PHE	2	1	2(PHE)	1.
	99	GLY	GLY		GLY	2	1	2(GLY)	1.
	100		GLY		THR	2	2	1(+)	4.
	101	GLY	GLY		GLY	2	1	2(GLY)	1.
	102	THR	THR		THR	2	1	2(THR)	1.
	103	LYS	LYS		LYS	2	1	2(LYS)	1.
FR4	104		LEU		VAL	2	2	1(+)	4.
	105	THR	THR		THR	2	1	2(THR)	1.
	106	VAL	VAL		VAL	2	1	2(VAL)	1.
	106A	LEU	LEU		LEU	2	1	2(LEU)	
	107		ARG		GLY	2	2	1(+)	4.
	108	GLN	GLN		GLN	2	1	2(GLN)	1.
	109	PRO	PRO		PRO	2	1	2(PRO)	1.

ANTIBODY SPECIFICITIES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP V

3) **MCG:** ANTI-EPSILON-DNP-LYS. EPSILON-DNP-AMINOCAPROATE. DNP-LEU. TRIACETIN. SODIUM MERTHIOLATE. METHADONE. 1,10-PHENANTHROLINE. CAFFEINE. THEOPHYLLINE. DI-DNP-LYS. DNP-TRP. DNP-PHE. DI-DNP-TYR. COLCHICINE. P-NITROANILINE. P-NITROPHENYLPHOSPHORYL CHOLINE. 5-ACETYLURACIL. MENADIONE. MEPERIDINE. TRIBUTYRIN. OMEGA-BROMOHEPTANOATE. O-CHLOROMERCURIPHENOL. P-CHLOROMERCURIPHENOL. PHENYLMERCURIC COMPOUNDS. METHYL-MERCURIC CHLORIDE.

REFERENCE: HUMAN LAMBDA LIGHT CHAINS SUBGROUP V

1) **BO:** WIKLER.M. & PUTNAM.F.W. (1970) J.BIOL.CHEM..245.4488-4507. (CHECKED BY AUTHOR 06/15/83)
2) **HBJ2:** HOOD.L..GRAY.W.R..SANDERS.B.G. & DREYER.W.J. (1967) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL..32.133-145.
3) **MCG:** FETT.J.W. & DEUTSCH.H.F. (1974) BIOCHEMISTRY.13.4102-4114. (CHECKED BY AUTHOR)

NOTES: HUMAN LAMBDA LIGHT CHAINS SUBGROUP V

IDENTICAL SETS OF FRAMEWORK SEGMENTS:
FR1: SET 1: BO[1].HBJ2[2]. (2 IDENTICAL)
FR2:
FR3: -
FR4: SET 1: BO[1]. (IDENTICAL TO 1 HUMAN V-LAMBDA-I: NEWM[1]; AND 1 HUMAN V-LAMBDA-II: WH[3].)
SET 2: MCG[3]. (IDENTICAL TO 1 HUMAN V-LAMBDA-I: LOC[11].)

IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:
CDR1: SET 1: MCG[3]. (IDENTICAL TO 2 HUMAN V-LAMBDA-II: MES[2].VIL[17].)
CDR2:
CDR3:

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
29	(GLY.ASP)
30	(TYR.ASN)
31	(LYS.ASN)
40	(PRO.ALA)
42	(LYS.ARG)
46	(LEU.VAL)
47	(ILE.VAL)
49	(TYR.PHE)
52	(SER.ASN)
53	(LYS.GLY)
68	(GLY.ASP)
79	(ARG.GLN)
92	(VAL.GLU)
93	(GLY.ASP)
94	(SER.ASN)
95	(ASP.ASN)
100	(THR.GLY)
104	(LEU.VAL)
107	(ARG.GLY)

HUMAN LAMBDA LIGHT CHAINS SUBGROUP VI

	•	INVARIANT RESIDUES	1 AMYLOID -AR #	2 SUT #	3 AMYLOID -RS	4 THO #	5 LBV CL #	6 GIO	7 YAM	8 WAN	9 WIN	10 NIG -48 #	11 JAM	12 MOR	13 KIN	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
	0		---	---	---	---	---	---	---	---	---	---	---	---	---				
	1		ASP	ASP	ASP	asn	asn	asn	ASP	asn	asn	asn	ASP	asn	asn	13	2	8(ASN)	3.3
	2		PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	leu	PHE	leu	PHE	13	2	11(PHE)	2.4
	3		MET	MET	MET	MET	MET	MET	MET	ile	MET	MET	MET	MET	MET	13	2	12(MET)	2.2
	4	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	13	1	13(LEU)	1.
	5		THR	THR	THR	THR	THR	THR	THR	THR	THR	ile	THR	THR	lou	13	3	11(THR)	3.5
	6		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	glu	GLN	GLN	13	2	12(GLN)	2.2
	7	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	13	1	13(PRO)	1.
	8		HIS	HIS	HIS	HIS	HIS	HIS	HIS			pro	HIS	HIS	HIS	11	2	10(HIS)	2.2
	9	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	13	1	13(SER)	1.
FR1	10		---	---	---	---	---	---	---	---	---	---	---	---	---				
	11		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	leu	VAL	13	2	12(VAL)	2.2
	12	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	13	1	13(SER)	1.
	13		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	asp		12	2	11(GLU)	2.2
	14	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER	SER		11	1	11(SER)	1.
	15	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		12	1	12(PRO)	1.
	16	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		12	1	12(GLY)	1.
	17		LYS	LYS	gln	LYS	LYS	LYS	gly	LYS	LYS	LYS	LYS	LYS		12	3	10(LYS)	3.6
	18		THR	THR	THR	THR	THR	THR	lys	THR	THR	THR		THR		11	2	10(THR)	2.2
	19		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		ile		11	2	10(VAL)	2.2
	20		THR	ile	THR	THR	THR	THR	ser	THR	THR	THR		ile		11	3	8(THR)	4.1
	21		PHE	ile	ile	ile	ile	ile	ilo	ile		met		ile		10	3	8(ILE)	3.8
	22	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER		SER		10	1	10(SER)	1.
	23	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		CYS		CYS		10	1	10(CYS)	1.
	24		THR	THR	THR	THR	THR	THR	SER			THR		THR		9	2	8(THR)	2.3
	25		GLY	ARG	GLY	ARG	GLY					ARG		ALA		7	3	3(+)	7.
	26		SER	SER	SER	SER	ASN					THR		ASN		7	3	4(SER)	5.3
	27		GLY	ASP	GLY	SER	SER					SER		GLY		7	3	3(−)	7.
	27A		GLY	GLY	ASP	GLY	GLY		ALA			---		GLY		7	3	5(GLY)	
	27B		SER	THR	SER	SER	SER					---		ASN		6	3	4(SER)	
CDR1	27C		---	---	---	---	---					---		---					
	27D		---	---	---	---	---					---		---					
	27E		---	---	---	---	---					ASP		---		1	1	1(ASP)	
	27F		---	---	---	---	---					SER		---		1	1	1(SER)	
	28	ILE	ILE	ILE	ILE	ILE	ILE					ILE		ILE		7	1	7(ILE)	1.
	29		ALA	ALA	ALA	ALA	ALA					ALA		GLY		7	2	6(ALA)	2.3
	30		ASP	GLY		SER	SER					SER		SER		6	3	4(SER)	4.5
	31		SER	TYR	SER	TYR	ASN					ASN		HIS		7	4	2(+)	14.
	32		PHE	TYR	TYR	TYR	TYR					TYR		PRO		7	3	5(TYR)	4.2
	33	VAL	VAL	VAL	VAL	VAL	VAL					VAL		VAL		7	1	7(VAL)	1.
	34	GLN	GLN	GLN		GLN	GLN					GLN				5	1	5(GLN)	1.
	35	TRP	TRP	TRP		TRP	TRP					TRP		TRP		6	1	6(TRP)	1.
	36	TYR	TYR	TYR		TYR	TYR					TYR		TYR		6	1	6(TYR)	1.
	37		GLN	GLN		GLN	GLN					ARG		LYS		6	3	4(GLN)	4.5
	38		GLN	GLN		LEU	GLN					GLN				5	2	4(GLN)	2.5
	39	ARG	ARG	ARG		ARG	ARG					ARG				5	1	5(ARG)	1.
	40		PRO	PRO		PRO	ARG					PRO		PRO		6	2	5(PRO)	2.4
FR2	41		GLY	GLY		GLY	VAL					GLY		ASP		6	3	4(GLY)	4.5
	42		SER	ARG		SER	SER					GLY		SER		6	3	4(SER)	4.5
	43	ALA	ALA	ALA		ALA	ALA					ALA				5	1	5(ALA)	1.
	44	PRO	PRO	PRO		PRO	PRO					PRO				5	1	5(PRO)	1.
	45	THR	THR	THR		THR	THR					THR				5	1	5(THR)	1.
	46		THR	THR		THR	ILE					THR				5	2	4(THR)	2.5
	47		VAL	VAL		VAL	VAL					LEU				5	2	4(VAL)	2.5
	48	ILE	ILE	ILE		ILE	ILE					ILE				5	1	5(ILE)	1.
	49		TYR	PHE		TYR	TYR					TYR				5	2	4(TYR)	2.5
	50		ASP	GLU		GLU	GLU					ASP				5	2	3(GLU)	3.3
	51		ASP	ASP		ASP	ASP					THR				5	2	4(ASP)	2.5
CDR2	52		ASN	THR		ASN	ASN					ASN				5	2	4(ASN)	2.5
	53	GLN	GLN	GLN		GLN	GLN					GLN				5	1	5(GLN)	1.
	54	ARG	ARG	ARG		ARG	ARG					ARG				5	1	5(ARG)	1.
	55	PRO	PRO	PRO		PRO	PRO					PRO				5	1	5(PRO)	1.
	56		SER	SER		SER	LEU					TYR				5	3	3(SER)	5.
	57	GLY	GLY	GLY		GLY	GLY					GLY				5	1	5(GLY)	1.
	58	VAL	VAL	VAL		VAL	VAL					VAL				5	1	5(VAL)	1.
	59	PRO	PRO	PRO		PRO	PRO					PRO				5	1	5(PRO)	1.
	60		ASP	ASP		ASP	ASP					ASN				5	2	4(ASP)	2.5
	61	ARG	ARG	ARG		ARG	ARG					ARG				5	1	5(ARG)	1.
	62	PHE	PHE	PHE		PHE	PHE					PHE				5	1	5(PHE)	1.
	63	SER	SER	SER		SER	SER					SER				5	1	5(SER)	1.
	64	GLY	GLY	GLY		GLY	GLY					GLY				5	1	5(GLY)	1.
	65	SER	SER	SER		SER	SER					SER				5	1	5(SER)	1.
	66		#	#		#	#					#							
	67	SER	SER	SER		SER	SER					SER				5	1	5(SER)	1.
	68		ALA	SER		SER	SER					SER				5	2	4(SER)	2.5
	69	ASN	ASN	ASN		ASN	ASN					ASN				5	1	5(ASN)	1.
	70	SER	SER	SER		SER	SER					SER				5	1	5(SER)	1.
	71	ALA	ALA	ALA		ALA	ALA					ALA				5	1	5(ALA)	1.
FR3	72	SER	SER	SER		SER	SER					SER				5	1	5(SER)	1.
	73	LEU	LEU	LEU		LEU	LEU					LEU				5	1	5(LEU)	1.
	74	THR	THR	THR		THR	THR					THR				5	1	5(THR)	1.
	75		ILE	ILE		VAL	ILE					ILE				5	2	4(ILE)	2.5
	76	SER	SER	SER		SER	SER					SER				5	1	5(SER)	1.
	77		GLY	GLY		GLU	GLY					GLY				5	2	4(GLY)	2.5
	78	LEU	LEU	LEU		LEU	LEU					LEU				5	1	5(LEU)	1.
	79		LYS	GLN		LYS	LYS					THR				5	3	3(LYS)	5.
	80		THR	THR		THR	THR					ASN				5	2	4(THR)	2.5
	81		GLU	GLU		GLU	GLU					ASP				5	2	4(GLU)	2.5
	82	ASP	ASP	ASP		ASP	ASP					ASP				5	1	5(ASP)	1.
	83		GLU	GLU		GLU	GLU					THR				5	2	4(GLU)	2.5
	84	ALA	ALA	ALA		ALA	ALA					ALA				5	1	5(ALA)	1.
	85		ASP	ASP		ASP	ASP					MET				5	2	4(ASP)	2.5
	86	TYR	TYR	TYR		TYR	TYR					TYR				5	1	5(TYR)	1.
	87		TYR	TYR		TYR	TYR					PHE				5	2	4(TYR)	2.5
	88	CYS	CYS	CYS		CYS	CYS					CYS				5	1	5(CYS)	1.
	89	GLN	GLN	GLN		GLN	GLN					GLN				5	1	5(GLN)	1.
	90	SER	SER	SER		SER	SER					SER				5	1	5(SER)	1.
	91		TYR	TYR		TYR	PHE					TYR				5	2	4(TYR)	2.5
	92		ASN	ASP		ASP	ASP					ASP				5	2	4(ASP)	2.5
	93		SER	ARG		SER	ASN					SER				5	3	3(SER)	5.
CDR3	94		ASN	ASP		ASN	THR					SER				5	4	2(ASN)	10.
	95		HIS	HIS		ASN	ASN					ASN				5	2	3(ASN)	3.3
	95A		HIS	---		---	GLN					LEU				3	3	1(+)	
	95B		---	---		---	---					---							
	95C		---	---		---	---					---							
	95D		---	---		---	---					---							
	95E		---	---		---	---					---							
	95F		---	---		---	---					---							
	96		VAL	TRP		TRP	GLY					TRP				5	3	3(TRP)	5.
	97	VAL	VAL	VAL		VAL	VAL					VAL				5	1	5(VAL)	1.
	98	PHE	PHE	PHE		PHE	PHE					PHE				5	1	5(PHE)	1.
	99	GLY	GLY	GLY		GLY	GLY					GLY				5	1	5(GLY)	1.
	100	GLY	GLY	GLY		GLY	GLY					GLY				5	1	5(GLY)	1.
	101	GLY	GLY	GLY		GLY	GLY					GLY				5	1	5(GLY)	1.
FR4	102	THR	THR	THR		THR	THR					THR				5	1	5(THR)	1.
	103	LYS	LYS	LYS		LYS	LYS					LYS				5	1	5(LYS)	1.
	104		VAL	LEU		LEU	LEU					LEU		LEU		6	2	5(LEU)	2.4
	105	THR	THR	THR		THR	THR					THR		THR		6	1	6(THR)	1.
	106	VAL	VAL	VAL		VAL	VAL					VAL		VAL		6	1	6(VAL)	1.
	106A	LEU	LEU	LEU		LEU	LEU					LEU		LEU		6	1	6(LEU)	
	107		GLY	GLY		GLY	GLY					SER		SER		6	2	4(GLY)	3.
	108	GLN	GLN	GLN		GLN	GLN					GLN		GLN		6	1	6(GLN)	1.
	109	PRO	PRO	PRO		PRO	PRO					PRO		PRO		6	1	6(PRO)	1.

HUMAN HEAVY CHAINS SUBGROUP I

		INVARIANT RESIDUES	1 EU	2* SIE	3 HG3 'CL #	4* WOL	5 CA	6 ND 'CL #	7 MOT #	8 BRO 'IGG	9 THO	10* STE	11 BEN (I)	12 ZUC #	13 DI	14 BOT #	15 OMM 'CL #
FR1	0		---	---	#	---	---	---	---	---	---	---	---	---	---	---	---
	1		PCA	PCA	gln	PCA	PCA	gln	PCA	glu	glu	PCA	glu	PCA	PCA	asp	gln
	2		VAL	VAL	VAL	VAL	VAL	thr	VAL	VAL	VAL	VAL	leu	VAL	VAL	ser	VAL
	3		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	his	GLN	GLN	GLN	pro	his
	4	LEU(.96)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	LEU	LEU	LEU
	5		VAL	VAL	VAL	met	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		glu	gln
	6		GLN	GLN	GLN	GLN	glu	GLN	GLN	GLN	GLN	glu	GLN	glu		GLN	glu
	7	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		SER	SER
	8		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ser	GLY	GLY	GLY	GLY	GLY
	9		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	gly	his	pro
	10		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	asp		GLU	gly
	11		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	leu		VAL	leu
	12		LYS	LYS	LYS	LYS	arg	arg	LYS	LYS	LYS	LYS	ser	val	LYS	gly	gly
	13		LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS		LYS	LYS	ile	LYS
	14		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO		leu	PRO
	15		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		lys	pro
	16		SER	SER	ala	SER	ala	ala	SER	glu	glu	ala		gly		glu	---
	17		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER		---		thr	---
	18		VAL	VAL	VAL	VAL	VAL	VAL	ala	leu	leu	met		---		glu	---
	19		LYS	arg	LYS	arg	LYS	arg	arg	LYS	arg	LYS		---	LYS	ala	---
	20		VAL	VAL	VAL	VAL	ile	VAL	leu	ile	ile	VAL		---		glu	---
	21		SER	thr	SER	SER	SER	SER	SER	SER	SER	SER		---		asp	---
	22		CYS	CYS	CYS	CYS	CYS	CYS	CYS			CYS		---		arg	---
	23		LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	LYS	arg		---		ile	---
	24		ALA	thr	ALA	thr	thr	ALA	val	gly	gly	ALA		---		ile	---
	25		SER	SER	SER	SER	SER	SER	SER	SER	phe			---		lys	---
	26		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY			---		glu	---
	27		GLY	GLY	tyr	GLY	tyr	tyr	asp	tyr	tyr			---		glu	---
	28		THR	THR	THR	THR	THR	THR	asp					---		glu	---
	29		PHE	PHE	PHE	PHE	PHE	PHE	PHE					---		ala	---
	30		SER	SER	asn	val	SER	ile	asn					---		arg	---
CDR1	31		ARG	GLY	SER	ASP	HIS	ASP	THR					---		LEU	---
	32		SER	TYR	TYR	TYR	TYR	SER	TYR					---		SER	---
	33		ALA	THR	TYR	LYS	ALA	TYR	ASP					---		GLY	---
	34		ILE	ILE	MET	GLY	MET	ILE	ILE					---		ARG	---
	35		ILE	SER	HIS	LEU		HIS	HIS					---	SER	ASP	---
	35A		—	—	—	—		—	—					---	---	---	---
	35B		—	—	—	—		—	—					---	---	---	---
FR2	36		TRP	TRP	TRP	TRP		TRP	TRP					---	TRP	MET	---
	37		VAL	VAL	VAL	VAL		ILE	VAL					---	ILE	GLN	---
	38		ARG	ARG	ARG	ARG		ARG	ARG					---	ARG	VAL	---
	39		GLN	GLN	GLN	GLN		GLN	GLN					---	GLN	THR	---
	40		ALA	ALA	ALA	ALA		ALA	ALA					---	PRO	SER	---
	41		PRO	PRO	PRO	PRO		PRO	PRO					---	PRO	GLN	---
	42		GLY	GLY	GLY	GLY		GLY	GLY					---	GLY	PRO	---
	43		GLN	ARG	GLN	LYS		HIS	ARG					---	LYS	---	---
	44	GLY	GLY	GLY	GLY	GLY		GLY	GLY					---	GLY	---	---
	45	LEU	LEU	LEU	LEU	LEU		LEU	LEU					---	LEU	---	---
	46	GLU	GLU	GLU	GLU	GLU		GLU	GLU					---	GLU	---	---
	47	TRP	TRP	TRP	TRP	TRP		TRP	TRP					---	TRP	---	---
	48		MET	VAL	MET	VAL		VAL	MET					---	VAL	---	---
	49		GLY	GLY	GLY	GLY		GLY	ALA					---	GLY	---	---
CDR2	50		GLY	SER	ILE	GLN		TRP	VAL					---	GLU	---	---
	51		ILE	PRO	ILE	ILE		ILE	VAL					---	ILE	---	---
	52		VAL	ALA	ASN	PRO		ASN	HIS					---	ASP	---	---
	52A		PRO	LYS	PRO	LEU		PRO	PRO					---	---	---	---
	52B		—	—	—	—		—	—					---	---	---	---
	52C		—	—	—	—		—	—					---	—	---	---
	53		MET	TRP	SER	ARG		ASN	SER					---	TYR	---	---
	54		PHE	THR	GLY	PHE		SER	ASP					---	SER	---	---
	55		GLY	ASP	GLY	ASN		GLY	ASP					---	GLY	---	---
	56		PRO	PRO	SER	GLY		GLY	ARG					---	THR	---	---
	57		PRO	PHE	THR	GLU		THR	THR					---	THR	---	---
	58		ASN	GLN	SER	VAL		ASN	THR					---	ASP	---	---
	59		TYR	GLY	TYR	LYS		TYR	TYR					---	TYR	---	---
	60		ALA	VAL	ALA	ASN		ALA	GLY					---		---	---
	61		GLN	TYR	GLN	PRO		PRO	PRO					---		---	---
	62		LYS	ILE	LYS	GLY		ARG	ARG					---		---	---
	63		PHE	LYS	PHE	SER		PHE	SER					---		---	---
	64		GLN	TRP	GLN	VAL		GLN	GLN					---	LYS	---	---
	65		GLY	GLU	GLY	VAL		GLY	ALA					---	SER	---	---
FR3	66	ARG	ARG	ARG	ARG	ARG		ARG	ARG					---	ARG	---	---
	67		VAL	VAL	VAL	VAL		VAL	PHE					---		---	---
	68		THR	THR	THR	SER		THR	THR					---		---	---
	69		ILE	VAL	MET	VAL		MET	VAL					---	MET	---	---
	70		THR	SER	THR	SER		THR	THR					---	SER	---	---
	71		ALA	LEU	ARG	LEU		ARG	ARG					---	LEU	---	---
	72		ASP	LYS	ASP	LYS		ASP	ASP					---	ASP	---	---
	73		GLU	PRO	THR	PRO		ALA	SER					---	THR	---	---
	74	SER	SER	SER	SER	SER		SER	SER					---	SER	---	---
	75		THR	PHE	THR	PHE		PHE	THR					---	VAL	---	---
	76		ASN	ASN	SER	ASN		SER	THR					---	ASN	---	---
	77		THR	GLN	THR	GLN		THR	THR					---	LEU	---	---
	78		ALA	ALA	VAL	ALA		ALA	VAL					---	PHE	---	---
	79		TYR	TYR	TYR	HIS		TYR	TYR					---	SER	---	---
	80		MET	MET	MET	MET		MET	MET					---	LEU	---	---
	81		GLU	GLU	GLU	GLU		ASP	GLU					---	SER	---	---
	82		LEU	LEU	LEU	LEU		LEU	LEU					---	LEU	---	---
	82A		SER	VAL	SER	SER		ARG	THR					---	THR	---	---
	82B		SER	ASN	SER	SER		SER	ALA					---	SER	---	---
	82C		LEU	LEU	LEU	LEU		LEU	LEU					---	VAL	---	---
	83		ARG	PHE	ARG	PHE		ARG	ILE					---	THR	---	---
	84		SER	ASN	SER	SER		SER	SER					---	ALA	---	---
	85		GLU	GLU	GLU	GLU		ASP	ALA					---	ALA	---	---
	86		ASP	ASP	ASP	ASP		ASP	ASP					---	ASP	---	---
	87		THR	GLY	THR	THR		SER	THR					---	THR	---	---
	88	ALA	ALA	ALA	ALA	ALA		ALA	ALA					---	ALA	---	---
	89		PHE	VAL	VAL	VAL		VAL	ILE					---	VAL	---	---
	90		TYR	TYR	TYR	TYR		PHE	TYR					---	TYR	---	---
	91		PHE	TYR	TYR	TYR		TYR	TYR					---	TYR	---	---
	92	CYS	CYS	CYS	CYS	CYS		CYS	CYS					---	CYS	---	---
	93		ALA	ALA	ALA	ALA		ALA	ALA					---	ALA	---	---
	94		GLY	ARG	ARG	ARG		LYS	ARG					---	ARG	---	---
CDR3	95		GLY	GLU	#	GLU		SER	GLY					---		---	---
	96		TYR	TRP	—	TYR		ASP	ALA					---		---	---
	97		GLY	LYS	---	GLY		PRO	HIS					---		---	---
	98		ILE	GLY	---	PHE		PHE	TYR					---		---	---
	99		TYR	GLN	---	ASP		TRP	SER					---		---	---
	100		SER	VAL	---	THR		SER	ASP					---		---	---
	100A		—	ASN	---	SER		ASP	THR					---		---	---
	100B		—	VAL	---	ASP		TYR	ASP					---		---	---
	100C		---	ASN	---	TYR		TYR	ASP					---		---	---
	100D		---	PRO	---	TYR		ASN	SER					---		---	---
	100E		---	---	---	—		PHE	GLY					---		---	---
	100F		---	---	---	—		ASP	THR					---		---	---
	100G		---	---	---	---		TYR	SER					---		---	---
	100H		---	---	---	---		SER	LEU					---		---	---
	100I		---	---	---	---		TYR	---					---		---	---
	100J		---	---	---	---		THR	---					---		---	---
	100K		—	PHE	—	TYR		LEU	---					---		---	---
	101		PRO	ASP	---	TYR		ASP	GLY					---		---	---
	102		GLU	TYR	---	TYR		VAL	PRO					---	ARG	---	---
FR4	103		GLU	TRP	---	TRP		TRP	TRP					---	TRP	---	---
	104		TYR	GLY	---	GLY		GLY	GLY					---	GLY	---	---
	105		ASN	GLN	—	GLN		GLN	GLN					---	SER	---	---
	106	GLY	GLY	GLY	---	GLY		GLY	GLY					---	GLY	---	---
	107		GLY	VAL	---	THR		THR	THR					---	GLY	---	---
	108		LEU	LEU	---	LEU		THR	LEU					---	LEU	---	---
	109		VAL	VAL	---	VAL		VAL	LEU					---	VAL	---	---
	110		THR	THR	---	THR		THR	ILE					---	THR	---	---
	111	VAL	VAL	VAL	---	VAL		VAL	VAL					—	VAL	---	---
	112		SER	SER	---	SER		SER	SER					SER	SER	---	---
	113	SER	SER	SER	---	SER		SER	SER					SER	SER	---	---

	16* MAR	17 FI	18 VU	19 WAR	20 VIL	21 DUN	22 ADA	23 NOR	24 SAW
0	---	---	---	---	---	---	---	---	---
1	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA	PCA
2	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
3	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	glu
4	LEU	LEU	LEU	LEU	LEU	LEU			LEU

HUMAN HEAVY CHAINS SUBGROUP I (cont'd)

		25* KOH	26 RIC	27 WIS #	28 VAU #	29 LEB #	30 SAC #	31 DEE	32 LEA	33 HAR	34 HUS	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID	VARIABILITY
FR1	0	---	---	---	---	---	---		---	---					
	1	gln	PCA	PCA	PCA	PCA	gly		---	---		30	5	21(PCA)	7.1
	2	VAL	VAL	met	VAL	VAL	ala		---	---		30	6	25(VAL)	7.2
	3	GLN	leu	GLN	---	---	---		pca	pca		29	6	22(GLN)	7.9
	4	LEU		---	---	---	---		LEU	LEU		25	2	24(LEU)	2.1
	5			---	---	---	---					14	4	11(VAL)	5.1
	6			---	---	---	---					14	2	10(GLN)	2.8
	7			---	---	---	---					14	1	14(SER)	1.
	8			---	---	---	---					15	2	14(GLY)	2.1
	9			---	---	---	---					15	4	12(ALA)	5.
	10			---	---	---	---					14	3	12(GLU)	3.5
	11			---	---	---	---					14	2	12(VAL)	2.3
	12			---	---	---	---					15	5	9(LYS)	8.3
	13			---	---	---	---					14	2	13(LYS)	2.2
	14			---	---	---	---					14	2	13(PRO)	2.2
	15			---	---	---	---					14	3	12(GLY)	3.5
	16			---	---	---	---					12	4	4(+)	12.
	17			---	---	---	---	VAL				11	2	10(SER)	2.2
	18			---	---	---	---	arg				12	5	7(VAL)	8.6
	19			---	---	---	---	ile				13	3	6(+)	6.5
	20			---	---	---	---					12	4	6(VAL)	8.
	21			---	---	---	---					11	3	9(SER)	3.7
	22			---	---	---	---					9	2	8(CYS)	2.3
	23			---	---	---	---					11	3	9(LYS)	3.7
	24			---	---	---	---					11	5	4(ALA)	14.
	25			---	---	---	---					10	3	8(SER)	3.8
	26			---	---	---	---					10	2	9(GLY)	2.2
	27			---	---	---	---					10	4	5(TYR)	8.
	28			---	---	---	---					8	3	6(THR)	4.
	29			---	---	---	---					8	2	7(PHE)	2.3
	30			---	---	---	---					8	5	3(SER)	13.
CDR1	31			---	---	---	---					8	7	2(ASP)	28.
	32			---	---	---	---					8	2	5(TYR)	3.2
	33			---	---	---	---					8	6	2(+)	24.
	34			---	---	---	---					8	4	4(ILE)	8.
	35			---	---	---	---					8	5	3(HIS)	13.
	35A			---	---	---	---								
	35B			---	---	---	---								
FR2	36			---	---	---	---					8	2	7(TRP)	2.3
	37			---	---	---	---					8	3	5(VAL)	4.8
	38			---	---	---	---					8	2	7(ARG)	2.3
	39			---	---	---	---					8	2	7(GLN)	2.3
	40			---	---	---	---					8	3	6(ALA)	4.
	41			---	---	---	---					8	2	7(PRO)	2.3
	42			---	---	---	---					8	2	7(GLY)	2.3
	43			---	---	---	---					7	4	2(+)	14.
	44			---	---	---	---					7	1	7(GLY)	1.
	45			---	---	---	---					7	1	7(LEU)	1.
	46			---	---	---	---					7	1	7(GLU)	1.
	47			---	---	---	---					7	1	7(TRP)	1.
	48			---	---	---	---					7	2	4(VAL)	3.5
	49			---	---	---	---					7	2	6(GLY)	2.3
CDR2	50			---	---	---	---					7	7	1(+)	49.
	51			---	---	---	---					7	3	5(ILE)	4.2
	52			---	---	---	---					7	6	2(ASN)	21.
	52A			---	---	---	---					6	3	4(PRO)	
	52B			---	---	---	---								
	52C			---	---	---	---								
	53			---	---	---	---					7	6	2(SER)	21.
	54			---	---	---	---					7	5	2(+)	18.
	55			---	---	---	---					7	3	4(GLY)	5.3
	56			---	---	---	---					7	5	2(+)	18.
	57			---	---	---	---					7	4	4(THR)	7.
	58			---	---	---	---					7	6	2(ASN)	21.
	59			---	---	---	---					7	3	5(TYR)	4.2
	60			---	---	---	---					6	4	3(ALA)	8.
	61			---	---	---	---					6	3	3(PRO)	6.
	62			---	---	---	---					6	4	2(+)	12.
	63			---	---	---	---					6	3	3(PHE)	6.
	64			---	---	---	---					7	4	4(GLN)	7.
	65			---	---	---	---					7	5	3(GLY)	12.
FR3	66			---	---	---	---					7	1	7(ARG)	1.
	67			---	---	---	---					6	2	5(VAL)	2.4
	68			---	---	---	---					6	2	5(THR)	2.4
	69			---	---	---	---					7	3	3(+)	7.
	70			---	---	---	---					7	2	4(THR)	3.5
	71			---	---	---	---					7	3	3(+)	7.
	72			---	---	---	---					7	2	5(ASP)	2.8
	73			---	---	---	---					7	5	2(+)	18.
	74			---	---	---	---					7	1	7(SER)	1.
	75			---	---	---	---					7	3	3(+)	7.
	76			---	---	---	---					7	3	4(ASN)	5.3
	77			---	---	---	---					7	3	4(THR)	5.3
	78			---	---	---	---					7	3	4(ALA)	5.3
	79			---	---	---	---					7	3	5(TYR)	4.2
	80			---	---	---	---					7	2	6(MET)	2.3
	81			---	---	---	---					7	3	5(GLU)	4.2
	82			---	---	---	---				MET	8	2	7(LEU)	2.3
	82A			---	---	---	---				ASN	8	5	3(SER)	
	82B			---	---	---	---				SER	8	3	6(SER)	
	82C			---	---	---	---				LEU	8	2	7(LEU)	
	83			---	---	---	---				ARG	8	4	4(ARG)	8.
	84			---	---	---	---				VAL	8	4	5(SER)	6.4
	85			---	---	---	---				GLX	8	3 : 4	5(GLU) : 4(GLU)	4.8 : 8.
	86			---	---	---	---				ASX	8	1 : 2	8(ASP) : 7(ASP)	1. : 2.3
	87			---	---	---	---				THR	8	3	6(THR)	4.
	88			---	---	---	---				ALA	8	1	8(ALA)	1.
	89			---	---	---	---				VAL	8	3	6(VAL)	4.
	90			---	---	---	---	TYR			TYR	9	2	8(TYR)	2.3
	91			---	---	---	---	TYR			TYR	9	2	8(TYR)	2.3
	92			---	---	---	---	CYS			CYS	9	1	9(CYS)	1.
	93			---	---	---	---	THR			ALA	9	2	8(ALA)	2.3
	94			---	---	---	---	GLY			ARG	9	3	6(ARG)	4.5
CDR3	95			---	---	---	---	ARG			ASX	7	5	2(+)	18.
	96			---	---	---	---	GLY			ARG	7	6	2(TYR)	21.
	97			---	---	---	---	MET			ASX	7	6	2(GLY)	21.
	98			---	---	---	---				ASX	6	5	2(PHE)	15.
	99			---	---	---	---				TYR	6	5	2(TYR)	15.
	100			---	---	---	---				GLY	6	5	2(SER)	15.
	100A			---	---	---	---				ASX	5	4	2(ASN) : 2(ASP)	
	100B			---	---	---	---				PHE	5	4	2(ASP)	
	100C			---	---	---	---				---	4	3	2(TYR)	
	100D			---	---	---	---				---	4	4	1(+)	
	100E			---	---	---	---				---	2	2	1(+)	
	100F			---	---	---	---				---	2	2	1(+)	
	100G			---	---	---	---				---	2	2	1(+)	
	100H			---	---	---	---				---	2	2	1(+)	
	100I			---	---	---	---				---	1	1	1(TYR)	
	100J			---	---	---	---				---	1	1	1(THR)	
	100K			---	---	---	---				---	3	3	1(+)	
	101			---	---	---	PRO				ASX	7	4 : 5	3(ASP) : 2(+)	9.3 : 18.
	102			---	---	---	GLX				TYR	8	5 : 6	3(TYR)	13. : 16.
FR4	103			---	---	---	GLX				TRP	8	2 : 3	6(TRP)	2.7 : 4.
	104			---	---	---	THR				GLY	8	3	6(GLY)	4.
	105			---	---	---	ASX				GLX	8	3 : 5	5(GLN) : 4(GLN)	4.8 : 10.
	106			GLY	---	---	---				GLY	8	1	8(GLY)	1.
	107			VAL	---	---	LEU				THR	9	4	4(THR)	9.
	108			#	---	---	VAL				LEU	8	3	6(LEU)	4.
	109			#	---	---	ILE				VAL	8	3	6(VAL)	4.
	110			THR	---	---	THR				THR	9	2	8(THR)	2.3
	111			VAL	---	---	VAL				VAL	9	1	9(VAL)	1.
	112			SER	---	---	SER				THR	10	2	9(SER)	2.2
	113			SER	---	---	SER				SER	10	1	10(SER)	1.

ANTIBODY SPECIFICITIES: HUMAN HEAVY CHAINS SUBGROUP I

2) **SIE:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE
4) **WOL:** ANTI-HUMAN GAMMA G GLOBULIN; WA IDIOTYPE
10) **STE:** COLD AGGLUTININ WITH ANTI-BLOOD GROUP I ACTIVITY
16) **MAR:** ANTI-LIPOPROTEIN LIPASE
25) **KOH:** ANTI-HUMAN GAMMA G GLOBULIN

CLASS: HUMAN HEAVY CHAINS SUBGROUP I

1) **EU:** IGG1-KAPPA
2) **SIE:** IGM-KAPPA
4) **WOL:** IGM-KAPPA
5) **CA:** IGG1-
6) **ND'CL:** IGE-
7) **MOT:** IGG-
8) **BRO'IGG:** IGG-KAPPA
10) **STE:** IGG1-
11) **BEN(I):** IGG3-
12) **ZUC:** IGG3-
13) **DI:** IGM-
14) **BOT:** IGM-
15) **OMM'CL:** IGG3-
16) **MAR:** IGM-
19) **WAR:** IGG1-
20) **VIL:** IGG3-LAMBDA
21) **DUN:** IGG4-
22) **ADA:** IGA-
23) **NOR:** IGA-
24) **SAW:** IGG2-
25) **KOH:** IGM-LAMBDA
26) **RIC:** IGG3-
27) **WIS:** IGG3-
28) **VAU:** IGG1-
29) **LEB:** IGG1-
30) **SAC:** IGG1-KAPPA
34) **HUS:** IGG3-

REFERENCE: HUMAN HEAVY CHAINS SUBGROUP I

1) **EU:** CUNNINGHAM,B.A.,RUTISHAUSER,U.,GALL,W.E.,GOTTLIEB,P.D.,WAXDAL,M.J. & EDELMAN,G.M. (1970) BIOCHEMISTRY,9,3161-3170. (CHECKED BY AUTHOR)
2) **SIE:** ANDREWS,D.W. & CAPRA,J.D. (1981) PROC.NAT.ACAD.SCI.USA,78,3799-3803; ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5816-5822. (CHECKED BY AUTHOR 11/15/82); ANDREWS,D.W., & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5822-5830.
3) **HG3'CL:** RECHAVI,G.,RAM,D.,GLAZER,L.,ZAKUT,R. & GIVOL,D. (1983) PROC.NAT.ACAD.SCI.USA,80,855-859. (CHECKED BY AUTHOR 01/04/83)
4) **WOL:** ANDREWS,D.W. & CAPRA,J.D. (1981) PROC.NAT.ACAD.SCI.USA,78,3799-3803; ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5816-5822. (CHECKED BY AUTHOR 11/15/82); ANDREWS,D.W. & CAPRA,J.D. (1981) BIOCHEMISTRY,20,5822-5830.
5) **CA:** PITCHER,S.E. & KONIGSBERG,W. (1970) J.BIOL.CHEM.,245,1267-1274. (CHECKED BY AUTHOR)
6) **ND'CL:** BENNICH,H. & VON BAHR-LINDSTROM,H. (1974) PROGRESS IN IMMUNOLOGY,1,49-58; BENNICH,H.H.,JOHANSSON,S.G.O. & VON BAHR-LINDSTROM,H. (1978) IN IMMEDIATE HYPERSENSITIVITY: MODERN CONCEPTS AND DEVELOPMENTS, BACH,M.K., ED.,PP.1-36, MARCEL DEKKER,NEW YORK; KENTEN,J.H.,MOLGAARD,H.V.,HOUGHTON,M.,DERBYSHIRE,R.B.,VINEY,J.,BELL,L.O. & GOULD,H.J. (1982) PROC.NAT.ACAD.SCI.USA,79,6661-6665.
7) **MOT:** KOJIMA,M.,ODANI,S. & ONO,T. (1982) MOL.IMMUNOL.,19,1095-1103; KOJIMA,M.,KOIDE,T.,ODANI,S. & ONO,T. (1986) MOL.IMMUNOL.,23,169-174. (CHECKED BY AUTHOR 08/08/86)
8) **BRO'IGG:** HOPPER,J.E.,NOYES,C.,HEINRIKSON,R. & KESSEL,J.W. (1976) J.IMMUNOL.,116,743-746; HOPPER,J.E. & BRAHN,E. (1977) J.IMMUNOL.,119, 847-849. (CHECKED BY AUTHOR 08/25/78 WHO POINTED OUT THAT BRO' IS SAME AS BRIGG AND SUGGESTED THAT IT SHOULD BE RENAMED AS BROIGG)
9) **THO:** HOPPER,J.E. & BRAHN,E. (1977) J.IMMUNOL.,119,847-849. (CHECKED BY AUTHOR 08/25/78)
10) **STE:** FISHER,C.E.,PALM,W.H. & PRESS,E.M. (1969) FEBS LETTERS,5,20-22. (CHECKED BY AUTHOR)
11) **BEN(I):** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
12) **ZUC:** FRANGIONE,B. & MILSTEIN,C. (1969) NATURE,224,597-599. (CHECKED BY AUTHOR)
13) **DI:** KOHLER,H.,SHIMIZU,A.,PAUL,C.,MOORE,V. & PUTNAM,F.W. (1970) NATURE,227,1318-1320; FLORENT,G.,LEHMAN,D. & PUTNAM,F.W. (1974) BIOCHEMISTRY,13,2482-2498. (CHECKED BY AUTHOR 06/15/83)
14) **BOT:** BARNIKOL-WATANABE,S.,MIHAESCO,E.,MIHAESCO,C.,BARNIKOL,H.U. & HILSCHMANN,N. (1984) Z.PHYSIOL.CHEM.,365,105-118.
15) **OMM'CL:** ALEXANDER,A.,STEINMETZ,M.,BARRITAULT,D.,FRANGIONE,B.,FRANKLIN,E.C.,HOOD,L. & BUXBAUM,J.N. (1982) PROC.NAT.ACAD.SCI.USA,79, 3260-3264. (CHECKED BY AUTHOR 06/17/83)
16) **MAR:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
17) **FI:** MONTGOMERY,P.C.,BELLO,A.C. & ROCKEY,J.H. (1970) BIOCHIM.BIOPHYS.ACTA,200,258-266. (CHECKED BY AUTHOR)
18) **VU:** MONTGOMERY,P.C.,BELLO,A.C. & ROCKEY,J.H. (1970) BIOCHIM.BIOPHYS.ACTA,200,258-266. (CHECKED BY AUTHOR)
19) **WAR:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
20) **VIL:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
21) **DUN:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
22) **ADA:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
23) **NOR:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
24) **SAW:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
25) **KOH:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
26) **RIC:** KAPLAN,A.P.,HOOD,L.,TERRY,W.D. & METZGER,H. (1971) IMMUNOCHEMISTRY,8,801-811. (CHECKED BY AUTHOR)
27) **WIS:** FRANKLIN,E.C.,PRELLI,F. & FRANGIONE,B. (1979) PROC.NAT.ACAD.SCI.USA,76,452-456. (CHECKED BY AUTHOR 07/18/79)
28) **VAU:** FRANKLIN,E.C.,KYLE,R.,SELIGMANN,M. & FRANGIONE,B. (1979) MOL.IMMUNOL.,16,919-921. (CHECKED BY AUTHOR 12/10/82)
29) **LEB:** FRANKLIN,E.C.,KYLE,R.,SELIGMANN,M. & FRANGIONE,B. (1979) MOL.IMMUNOL.,16,919-921. (CHECKED BY AUTHOR 12/10/82)
30) **SAC:** PARR,D.M. (1981) MOL.IMMUNOL.,18,257-259. (CHECKED BY AUTHOR 03/02/82)
31) **DEE:** FRANGIONE,B. & MILSTEIN,C. (1967) NATURE,216,939-941. (CHECKED BY AUTHOR)
32) **LEA:** FRANGIONE,B. & FRANKLIN,E.C. (1977) PROG.IMMUNOL.,3,278-288. (CHECKED BY AUTHOR 07/18/79)
33) **HAR:** FRANGIONE,B. & FRANKLIN,E.C. (1977) PROG.IMMUNOL.,3,278-288. (CHECKED BY AUTHOR 07/18/79)
34) **HUS:** WANG,A.C. & FUDENBERG,H.H. (1975) ARCH.BIOCHEM.BIOPHYS.,168,657-664. (CHECKED BY AUTHOR 09/23/77)

NOTES: HUMAN HEAVY CHAINS SUBGROUP I

IDENTICAL SETS OF FRAMEWORK SEGMENTS:
FR1: SET 1: VAU[28],LEB[29]. (2 IDENTICAL)
FR2: SET 1: EU[1],HG3'CL[3]. (2 IDENTICAL)
SET 2: WOL[4]. (IDENTICAL TO 2 HUMAN V-H-III: TIL[4],TEI[10].)
FR3: SET 1: ND'CL[6]. (IDENTICAL TO 1 HUMAN V-H-III: U266'CL[106].)
FR4: SET 1: WOL[4]. (IDENTICAL TO 2 HUMAN V-H-II: MCE'[4],NZU[15]; 4 HUMAN V-H-III: TIL[4],DOB[31],WEA[33],NIE[34]; AND 1 MOUSE V-H-IIIA: MOPC47A[48].)
SET 2: ND'CL[6]. (IDENTICAL TO 1 HUMAN V-H-II: HIG1'CL[10]; 1 HUMAN V-H-III: U266'CL[106]; AND 1 MOUSE V-H-IIA: HDEX12[15].)

IDENTICAL SETS OF COMPLEMENTARITY DETERMINING REGIONS:
CDR1:
CDR2:
CDR3: SET 1: HG3'CL[3]. (IDENTICAL TO 1 HUMAN V-H-III: LAMBDA-VH26'CL[2]; 1 MOUSE V-H-IB: PJ14'CL[22]; AND 5 MOUSE V-H-IIB: 186-2'CL[3], 186-1'CL[5],102'CL[15],23'CL[18],3'CL[26].)
SET 2: ND'CL[6]. (IDENTICAL TO 1 HUMAN V-H-III: U266'CL[106].)

IDENTICAL SETS OF J-MINIGENES:
SET 1: ND'CL[6]. (IDENTICAL TO 1 HUMAN V-H-II: HIG1'CL[10]; AND 1 HUMAN V-H-III: U266'CL[106].)

NOTES: **HUMAN HEAVY CHAINS SUBGROUP I (cont'd)**

SPECIFIC NOTES:

3) **HG3'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN FETAL LIVER GENOMIC DNA.

6) **ND'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF MOUSE CDNA. IT CORRESPONDS TO THE AMINO ACID SEQUENCE DETERMINED EARLIER EXCEPT THAT THE AMINO ACID SEQUENCE DETERMINATION GAVE PCA AT POSITION 1, VAL AT 2, VAL AT 34, GLY AT 35, ILE AT 48 AND HIS AT 49.

7) **MOT:** PAPAIN CLEAVES BETWEEN ARG 56 AND THR 57, AND BETWEEN ARG 62 AND SER 63.

12) **ZUC:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.

14) **BOT:** IT WAS FROM A CASE OF IGM HEAVY CHAIN DISEASE.

15) **OMM'CL:** THE AMINO ACID SEQUENCE IS OBTAINED BY TRANSLATING THE NUCLEOTIDE SEQUENCE OF A CLONE OF HUMAN CELL LINE CDNA. IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.

27) **WIS:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE. ITS RESIDUES AT POSITIONS 108 AND 109 ARE ASN AND CYS RESPECTIVELY, WHICH DO NOT CORRESPOND TO THE USUAL RESIDUES FOUND AT THESE POSITIONS IN HUMAN HEAVY CHAIN SUBGROUP I.

28) **VAU:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.

29) **LEB:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.

30) **SAC:** IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
16	(ALA,SER)
19	(LYS,ARG)
33	(TYR,ALA)
43	(LYS,ARG,GLN)
50	(TRP,ILE,VAL,SER,GLY,GLU,GLN)
54	(PHE,SER)
56	(PRO,GLY)
62	(LYS,ARG)
69	(VAL,MET)
71	(LEU,ARG)
73	(PRO,THR)
75	(PHE,THR)
95	(GLY,GLU)
100D	(TYR,PRO,SER,ASN)
100E	(PHE,GLY)
100F	(THR,ASP)
100G	(TYR,SER)
100H	(LEU,SER)
100K	(TYR,PHE,LEU)
101	(PRO,ASP)

HUMAN HEAVY CHAINS SUBGROUP II

		INVARIANT RESIDUES	1 COR	2 DAW	3 OU	4 MCE #	5 CE-1 'CL #	6 HE	7 SUP-T1 VH-JA 'CL #	8* NEWM	9 WAH	10 HIG1 'CL	11 CAR	12 SA	13 IO	14 SPA #	15 NZU #	16 ERI	# OF SEQUENCES	# OF AMINO ACIDS	OCCURRENCES OF MOST COMMON AMINO ACID
0	FR1		---	---	---	---	---	---	---	---	---	---			---	---					
1			PCA	PCA	PCA	PCA	gln	PCA	gln	PCA	arg	gln			PCA	PCA			12	3	8(PCA)
2			VAL	VAL	VAL	ile	VAL	VAL	VAL	VAL	leu	VAL			VAL	glu			12	4	9(VAL)
3			THR	THR	THR	THR	asn	THR	gln	gln	gln	gln			THR	glu			12	4	6(THR)
4			LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			LEU	val			12	2	11(LEU)
5			ARG	ARG	thr	lys	ARG	lys	gln	glx	gln	gln				ARG			11	4 : 5	4(+) : 4(ARG)
6			GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLX	GLU	gln				GLU			11	2	10(GLU) : 9(GLU)
7			SER	SER	SER	SER	SER	asn	SER	SER	SER	trp				SER			11	3	9(SER)
8		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY				---			10	1	10(GLY)
9			PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	ala				---			10	2	9(PRO)
10			ALA	ALA	ALA	thr	ALA	thr	gly	gly	gly	gly				---			10	3	4(+)
11		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU				---			10	1	10(LEU)
12		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL				---			10	1	10(VAL)
13			LYS	arg	LYS	LYS	LYS	LYS	LYS	arg	LYS	LYS				---			10	2	8(LYS)
14			PRO	PRO	PRO	PRO	ala	PRO	PRO	PRO	PRO	PRO				---			10	2	9(PRO)
15			THR	THR	lys	THR	THR	THR	ser	ser	ser	ser				---			10	3	5(THR)
16			GLN	GLN	GLN	glu	his	glu	glu	GLN	glu	glu				---			10	3	5(GLU)
17			THR	THR	pro	THR	THR	THR	THR	THR	THR	THR				---			10	2	9(THR)
18		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU				---			10	1	10(LEU)
19			THR	THR	THR	THR	THR	THR	ser	ser	ser	ser				---			10	2	6(THR)
20		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU			---			11	1	11(LEU)
21		THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR		---			12	1	12(THR)
22		CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS		---			12	1	12(CYS)
23			THR	THR	THR	THR	THR	THR	THR	THR	ile	ala	THR	THR		---			12	3	10(THR)
24			PHE	PHE	PHE	PHE	PHE	leu	val	val	val	val	val	val		---			12	3	6(VAL)
25			SER	SER	SER	SER	SER	SER	SER	SER	SER	phe	SER	SER		---			12	2	11(SER)
26		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY		---			12	1	12(GLY)
27			PHE	PHE	PHE	PHE	leu	leu	tyr	ser	gly	gly		gly		---			11	5	4(PHE)
28			SER	SER	SER	SER	SER	SER	SER	thr	pro	SER		SER		---			11	3	9(SER)
29			LEU	LEU	LEU	LEU	val	LEU	ile	phe	ile	phe				---			10	4	5(LEU)
30			SER	SER	SER	SER	asn	thr	SER	SER	arg	SER				---			10	4	7(SER)
31	CDR1		SER	GLY	THR	THR	THR	THR	SER	ASN	ARG	GLY				---			10	5	4(THR)
32			THR	GLU	SER	SER	ARG	ASP	GLY	ASP	THR	TYR				---			10	7	2(÷)
33			GLY	THR	ARG	GLY	GLY	GLY	TYR	TYR	GLY	TYR				---			10	4	5(GLY)
34			MET	MET	MET	VAL	MET	VAL	TYR	TYR	TYR	TRP				---			10	4	4(MET)
35			CYS	CYS	ARG	GLY	SER	ALA	TRP	THR	TYR	SER				---			10	8	2(÷)
35A			VAL	VAL	VAL	VAL	VAL	VAL	GLY	---	TRP	---				---			8	3	6(VAL)
35B			GLY	ALA	SER	GLY	SER	GLY	---	---	GLY	---				---			7	3	4(GLY)
36	FR2	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP				---			10	1	10(TRP)
37			ILE	ILE	ILE	ILE	ILE	ILE	ILE	VAL	ILE	ILE				---			10	2	9(ILE)
38		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG				---			10	1	10(ARG)
39			GLN	GLN	ARG	GLN	GLN	GLN	GLN	GLN	GLN	GLN				---			10	2	9(GLN)
40			PRO	PRO	PRO	ARG	PRO	GLY	PRO	PRO	PRO	PRO				---			10	3	8(PRO)
41		PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO				---			10	1	10(PRO)
42		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY				---			10	1	10(GLY)
43			LYS	GLU	LYS	LYS	LYS	ARG	LYS	ARG	LYS	ARG				---			10	3	6(LYS)
44			GLY	ALA	ALA	ALA	ALA	ALA	GLY	GLY	GLY	GLY				---			10	2	5(÷)
45		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU				---			10	1	10(LEU)
46		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU				---			10	1	10(GLU)
47		TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP				---			10	1	10(TRP)
48			LEU	LEU	LEU	LEU	LEU	LEU	ILE	ILE	ILE	ILE				---			10	2	6(LEU)
49			ALA	ALA	ALA	ALA	ALA	ALA	GLY	GLY	GLY	GLY				---			10	2	6(ALA)
50	CDR2		ARG	TRP	ARG	PHE	ARG	TRP	SER	TYR	GLY	GLU				---			10	7	3(ARG)
51			ILE	ASP	ILE	ILE	ILE	LEU	ILE	VAL	VAL	ILE				---			10	4	6(ILE)
52			ASP	ILE	ASX	ASN	ASP	LEU	TYR	PHE	TYR	ASN				---			10	6	3(ASN) : 3(ASP)
52A			---	---	---	---	---	TYR	HIS	---	---	---				---			2	2	1(÷)
52B			---	---	---	---	---	---	---	---	---	---				---					
52C			---	---	---	---	---	---	---	---	---	---				---					
53			TRP	LEU	ASX	TRP	TRP	TRP	---	TYR	TYR	HIS				---			9	5	4(TRP)
54			ASP	ASN	ASX	ASP	ASP	ASP	SER	HIS	THR	SER				---			10	5	5(ASP) : 4(ASP)
55			ASP	ASP	ASP	ASP	ASP	ASP	GLY	GLY	GLY	GLY				---			10	2	6(ASP)
56			ASP	ASP	LYS	ASP	ASP	ASP	SER	THR	SER	SER				---			10	4	5(ASP)
57			LYS	LYS	PHE	ASN	LYS	LYS	THR	SER	ILE	THR				---			10	6	4(LYS)
58			TYR	TYR	TYR	ARG	TYR	ARG	TYR	ASP	TYR	ASN				---			10	4	6(TYR)
59			TYR	TYR	TRP	TYR	TYR	PHE	TYR	ASP	TYR	TYR				---			10	4	7(TYR)
60			ASX	GLY	SER	SER	GLY	SER	ASN	THR	ASN	LYS				---			10	5 : 6	3(+) : 3(SER)
61			THR	ALA	THR	PRO	THR	PRO	PRO	THR	PRO	THR				---			10	3	5(THR)
62			SER	SER	SER	SER	SER	SER	SER	PRO	SER	SER				---			10	2	9(SER)
63		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU				---			10	1	10(LEU)
64			GLU	GLU	ARG	ARG	GLU	LYS	LYS	ARG	ARG	LYS				---			10	3	4(ARG)
65			THR	THR	THR	SER	THR	SER	SER	SER	GLY	SER				---			10	3	5(SER)
66	FR3	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG				---			10	1	10(ARG)
67			LEU	LEU	LEU	LEU	LEU	LEU	VAL	VAL	VAL	VAL				---			10	2	6(LEU)
68			THR	ALA	SER	THR	THR	THR	THR	THR	THR	THR				---			10	3	8(THR)
69			ILE	VAL	ILE	GLY	ILE	VAL	ILE	MET	ILE	ILE				---			10	4	6(ILE)
70			SER	SER	SER	THR	SER	THR	SER	LEU	SER	SER				---			10	3	7(SER)
71			LYS	LYS	LYS	LYS	LYS	ARG	VAL	VAL	VAL	LEU				---			10	4	5(LYS)
72			ASP	ASP	ASN	ASP	ASP	ASP	ASP	ASP	ASP	ASP				---			10	2	9(ASP)
73			THR	THR	ASP	THR	THR	THR	THR	THR	THR	THR				---			10	2	9(THR)
74		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER				---			10	1	10(SER)
75			ARG	LYS	LYS	ARG	LYS	LYS	LYS	LYS	ARG	LYS				---			10	2	7(LYS)
76		ASN	ASN	ASN	ASN	ASN	ASN	ASN	ASN	ASN	ASN	ASN				---			10	1	10(ASN)
77			GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	LEU				---			10	2	9(GLN)
78			VAL	VAL	VAL	VAL	VAL	VAL	PHE	PHE	PHE	PHE				---			10	2	6(VAL)
79			VAL	VAL	VAL	VAL	VAL	VAL	SER	SER	SER	SER				---			10	2	6(VAL)
80		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU				---			10	1	10(LEU)
81			THR	SER	ILE	THR	LYS	THR	LYS	ARG	ASN	LYS				---			10	6	3(÷)
82			MET	MET	MET	ILE	VAL	MET	LEU	LEU	LEU	LEU				---			10	4	4(÷)
82A			---	ASN	ILE	THR	THR	THR	SER	SER	ARG	SER				---			9	5	3(÷)
82B			---	THR	ASN	ASN	ASN	ASN	SER	SER	SER	SER				---			9	3	4(÷)
82C			---	VAL	VAL	MET	MET	MET	VAL	VAL	MET	VAL				---	MET		10	2	5(÷)
83			ASP	GLY	ASN	ASP	ASP	ASP	THR	THR	SER	THR				---	ASP		11	5	5(ASP)
84			PRO	PRO	PRO	PRO	PRO	PRO	ALA	ALA	ALA	ALA				---	PRO		11	2	7(PRO)
85			VAL	GLY	VAL	VAL	ALA	VAL	ALA	ALA	ALA	ALA				---	VAL		11	3	5(÷)
86		ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP	ASP				---	ASP		11	1	11(ASP)
87			THR	THR	THR	SER	THR	THR	THR	THR	THR	THR				---	SER		11	2	9(THR)
88			ALA	ALA	ALA	GLY	ALA	ALA	ALA	ALA	ALA	ALA				---	GLY		11	2	9(ALA)
89			THR	THR	THR	THR	THR	THR	VAL	VAL	MET	VAL				---	THR		11	3	7(THR)
90		TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR	TYR				---	TYR		11	1	11(TYR)
91			TYR	TYR	TYR	PHE	TYR	TYR	TYR	TYR	TYR	TYR				---	PHE		11	2	9(TYR)
92		CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS				---	CYS		11	1	11(CYS)
93			ALA	ALA	ALA	ALA	ALA	VAL	ALA	ALA	ALA	ALA				---	ALA		11	2	10(ALA)
94			ARG	ARG	ARG	HIS	ARG	HIS	ARG	ARG	ARG	ARG				---	HIS		11	2	8(ARG)
95	CDR3		ILE	SER	VAL	ARG	MET	ARG	VAL	ASN	GLY	GLY				---	ARG		11	7	3(ARG)
96			THR	CYS	VAL	PRO	GLN	HIS	ARG	LEU	ASN	LEU				---	PRO		11	9	2(÷)
97			VAL	GLY	ASN	PRO	VAL	PRO	ARG	ILE	PRO	LEU				---	PRO		11	7	4(PRO)
98			ILE	SER	SER	TRP	THR	ARG	ARG	ALA	PRO	ARG				---	TRP		11	7	3(ARG)
99			PRO	GLN	VAL	ARG	MET	THR	---	GLY	PRO	GLY				---	ARG		10	7	2(÷)
100			ALA	TYR	MET	PHE	VAL	LEU	---	CYS	TYR	GLY				---	PHE		10	8	2(+)
100A			PRO	PHE	ALA	THR	ARG	ALA	---	ILE	TYR	TRP				---	THR		10	8	2(+)
100B			ALA	---	GLY	GLY	GLU	---	---	---	ASP	ASN				---	SER		7	6	2(GLY)
100C			GLY	---	TYR	ASN	VAL	---	---	---	ILE	ASP				---	ASP		7	6	2(ASP)
100D			TYR	---	TYR	LEU	MET	---	---	---	GLY	VAL				---	LEU		7	5	2(÷)
100E			---	---	TYR	GLY	ILE	---	---	---	THR	ASP				---	GLY		6	5	2(GLY)
100F			---	---	TYR	GLY	THR	---	---	---	GLY	TYR				---	SER		6	4	2(÷)
100G			---	---	TYR	---	SER	---	TYR	---	SER	TYR				---	---		5	2	3(TYR)
100H			---	---	---	---	ASN	---	SER	---	ASP	TYR				---	---		4	4	1(+)
100I			---	---	---	---	---	---	SER	---	ASP	GLY				---	---		3	3	1(÷)
100J			---	---	---	---	ALA	---	ALA	---	GLY	MET				---	---		4	3	2(ALA)
100K			MET	---	MET	PHE	PHE	PHE	SER	---	ILE	---				---	PHE		8	4	4(PHE)
101			ASP	ASP	ASP	ASP	ASP	ASP	LYS	ASP	ASP	ASP				---	SER	ASP	12	3	10(ASP)
102			VAL	TYR	VAL		ILE	VAL	ILE	VAL	VAL	VAL				---	PRO	VAL	11	4	7(VAL)
103	FR4		TRP	TRP	TRP	TRP	TRP	TRP	ILE	TRP	TRP	TRP				---	TRP	TRP	12	2	11(TRP)
104			GLY	GLY	GLY	GLY	GLY	GLY	PHE	GLY	GLY	GLY				---	GLY	GLY	12	2	11(GLY)
105			ARG	GLN	LYS	GLN	GLN	GLN	GLY	GLN	GLN	GLN				---	GLN	ARG	12	4	8(GLN)
106			GLY	GLY	GLY	GLY	GLY	GLY	SER	GLY	GLY	GLY				---	GLY	GLY	12	2	11(GLY)
107			THR	ILE	THR	THR	THR	THR	GLY	SER	THR	THR				---	THR	THR	12	4	9(THR)
108			PRO	LEU	THR	LEU	MET	LYS	THR	LEU	THR	THR				---	LEU	THR	12	5	5(THR)
109			VAL	VAL	VAL	VAL	VAL	VAL	ARG	VAL	VAL	VAL				---	VAL	VAL	12	2	11(VAL)
110			THR	THR	THR	THR	THR	ALA	LEU	THR	HIS	THR				---	THR	THR	12	4	9(THR)
111			VAL	VAL	VAL	VAL	VAL	VAL	SER	VAL	VAL	VAL				---	VAL	VAL	12	2	11(VAL)
112			SER	SER	SER	SER	SER	SER	ILE	SER	SER	SER				---	SER	SER	12	2	11(SER)
113						SER	SER		ARG	SER	SER	SER				---	SER	ALA	8	3	6(SER)

HUMAN HEAVY CHAINS SUBGROUP II (cont'd)

		VARIABILITY
FR1	0	
	1	4.5
	2	5.3
	3	8.
	4	2.2
	5	11. : 14.
	6	2.2 : 2.4
	7	3.7
	8	1.
	9	2.2
	10	7.5
	11	1.
	12	1.
	13	2.5
	14	2.2
	15	6.
	16	6.
	17	2.2
	18	1.
	19	3.3
	20	1.
	21	1.
	22	1.
	23	3.6
	24	6.
	25	2.2
	26	1.
	27	14.
	28	3.7
	29	8.
	30	5.7
CDR1	31	13.
	32	35.
	33	8.
	34	10.
	35	40.
	35A	
	35B	
FR2	36	1.
	37	2.2
	38	1.
	39	2.2
	40	3.8
	41	1.
	42	1.
	43	5.
	44	4.
	45	1.
	46	1.
	47	1.
	48	3.3
	49	3.3
CDR2	50	23.
	51	6.7
	52	20.
	52A	
	52B	
	52C	
	53	11.
	54	10. : 13.
	55	3.3
	56	8.
	57	15.
	58	6.7
	59	5.7
	60	17. : 20.
	61	6.
	62	2.2
	63	1.
	64	7.5
	65	6.
FR3	66	1.
	67	3.3
	68	3.8
	69	6.7
	70	4.3
	71	8.
	72	2.2
	73	2.2
	74	1.
	75	2.9
	76	1.
	77	2.2
	78	3.3
	79	3.3
	80	1.
	81	20.
	82	10.
	82A	
	82B	
	82C	
	83	11.
	84	3.1
	85	6.6
	86	1.
	87	2.4
	88	2.4
	89	4.7
	90	1.
	91	2.4
	92	1.
	93	2.2
	94	2.8
CDR3	95	26.
	96	50.
	97	19.
	98	26.
	99	35.
	100	40.
	100A	
	100B	
	100C	
	100D	
	100E	
	100F	
	100G	
	100H	
	100I	
	100J	
	100K	
	101	3.6
	102	6.3
FR4	103	2.2
	104	2.2
	105	6.
	106	2.2
	107	5.3
	108	12.
	109	2.2
	110	5.3
	111	2.2
	112	2.2
	113	4.

ANTIBODY SPECIFICITIES: HUMAN HEAVY CHA[illegible]UBGROUP II

8) NEWM: ANTI-3-(3'-HYDROXY-3',7',11',15',TETRAMETHYL HEXADECYL) 2-METHYL 1,4 NAPHTHOQUINONE(VIT.K1OH)

CLASS: HUMAN HEAVY CHAINS SUBGROUP II

1) COR: IGG1-
2) DAW: IGG1-LAMBDA
3) OU: IGM-KAPPA
4) MCE': IGM-KAPPA
6) HE: IGG1-
8) NEWM: IGG1-LAMBDA
9) WAH: IGD-LAMBDA
12) SA: IGG2-LAMBDA
15) NZU: IGM-
16) ERI: IGD-

REFERENCE: HUMAN HEAVY CHAINS SUBGROUP II

1) COR: PRESS,E.M. & HOGG,N.M. (1970) BIOCHEM.J.,117,641-660. (CHECKED BY AUTHOR)
2) DAW: PRESS,E.M. & HOGG,N.M. (1970) BIOCHEM.J.,117,641-660. (CHECKED BY AUTHOR)
3) OU: PUTNAM,F.W.,SHIMIZU,A.,PAUL.,C.,SHINODA,T. & KOHLER,H. (1971) ANN.N.Y.ACAD.SCI.,190,83-103. (CHECKED BY AUTHOR 06/15/83)
4) MCE': GERBER-JENSON,B.,KAZIN,A.,KEHOE,J.M.,SCHEFFEL,C.,ERICKSON,B.W. & LITMAN,G.W. (1981) J.IMMUNOL.,126,1212-1216. (CHECKED BY AUTHOR 12/15/80)
5) CE-1 'CL: TAKAHASHI,N.,NOMA,T. & HONJO,T. (1984) PROC.NAT.ACAD.SCI.USA,81,5194-5198.
6) HE: CUNNINGHAM,B.A.,GOTTLIEB,P.D.,PFLUMM,M.N. & EDELMAN,G.M. (1971) PROGRESS IN IMMUNOLOGY (B.AMOS,ED.),ACADEMIC PRESS,N.Y.,PP.3-24. (CHECKED BY AUTHOR)
7) SUP-T1 VH-JA'CL: DENNY,C.T.,YOSHIKAI,Y.,MAK,T.W.,SMITH,S.D.,HOLLIS,G.F. & KIRSCH,I.R. (1986) NATURE,320,549-551.
8) NEWM: POLJAK,R.J.,AMZEL,L.M.,CHEN,B.L.,PHIZACKERLEY, R.P. & SAUL,F. (1974) PROC.NAT.ACAD.SCI.USA,71,3440-3444. (CHECKED BY AUTHOR WHO CORRECTED RESIDUES 6,9,15,16,24,26,27,29 THROUGH 35B,59,60 AS GIVEN IN TABLE OF THE FIRST EDITION OF THIS BOOK, AND HAS MORE RECENTLY REVISED RESIDUES 5,24,28,29,30,31,33,34,35,35A,35B,59,60 AND 101); POLJAK,R.J.,AMZEL,L.M.,CHEN,B.L.,CHIU,Y.Y., PHIZACKERLEY,R.P.,SAUL,F. & YSERN,X. (1976) COLD SPRING HARBOR SYMP. QUANTITATIVE BIOL.,41,639-645; POLJAK,R.J.,NAKASHIMA,Y.,CHEN, B.L. & KONIGSBERG,W. (1977) BIOCHEMISTRY,16,3412-3420. THE SEQUENCE LISTED IN THE LAST REFERENCE IS GIVEN IN THE TABLE. (CHECKED BY AUTHOR, W.K., 09/30/78)
9) WAH: PUTNAM,F.W.,TAKAHASHI,N.,TETAERT,D.,DEBUIRE,B. & LIN,L.C. (1981) PROC.NAT.ACAD.SCI.USA,78,6168-6172. (CHECKED BY AUTHOR 11/30/81); TAKAHASHI,N.,TETAERT,D.,DEBUIRE,B.,LIN,L. & PUTNAM,F.W. (1982) PROC.NAT.ACAD.SCI.USA,79,2850-2854.
10) HIG1'CL: KUDO,A.,ISHIHARA,T.,NISHIMURA,Y. & WATANABE,T. (1985) GENE,33,181-189. (CHECKED BY AUTHOR 10/01/85)
11) CAR: FRANGIONE,B. (1968) PH.D. THESIS, UNIVERSITY OF CAMBRIDGE. (CHECKED BY AUTHOR)
12) SA: MILSTEIN,C. & FRANGIONE,B. (1971) BIOCHEM.J.,121,217-225. (CHECKED BY AUTHOR)
13) IO: MONTGOMERY,P.C.,BELLO,A.C. & ROCKEY,J.H. (1970) BIOCHIM.BIOPHYS.ACTA,200,258-266. (CHECKED BY AUTHOR)
14) SPA: FRANGIONE,B. & FRANKLIN,E.C. (1979) J.IMMUNOL.,122,1177-1179. (CHECKED BY AUTHOR 07/18/79)
15) NZU: ERICKSON,B.W.,GERBER-JENSON,B.,WANG,A.C. & LITMAN,G.W. (1981) MOL.IMMUNOL.,19,357-365. (CHECKED BY AUTHOR 11/30/81)
16) ERI: MILSTEIN,C.P. & DEVERSON,E.V. (1980) IMMUNOLOGY,40,657-664. (CHECKED BY AUTHOR 11/30/82)

NOTES: HUMAN HEAVY CHAINS SUBGROUP II

IDENTICAL SETS OF FRAMEWORK SEGMENTS:

FR1:
FR2: SET 1: SUP-T1 VH-JA'CL[7],WAH[9]. (2 IDENTICAL)
FR3:
FR4: SET 1: MCE'[4],NZU[15]. (2 IDENTICAL HUMAN V-H-II; ALSO 1 HUMAN V-H-I: WOL[4]; 4 HUMAN V-H-III: TIL[4],DOB[31],WEA[33],NIE[34]; AND 1 MOUSE V-H-IIIA: MOPC47A[48].)
SET 2: HIG1'CL[10]. (IDENTICAL TO 1 HUMAN V-H-I: ND'CL[6]; 1 HUMAN V-H-III: U266'CL[106]; AND 1 MOUSE V-H-IIA: HDEX12[15].)

IDENTICAL SETS OF J-MINIGENES:

SET 1: HIG1'CL[10]. (IDENTICAL TO 1 HUMAN V-H-I: ND'CL[6]; AND 1 HUMAN V-H-III: U266'CL[106].)

SPECIFIC NOTES:

4) MCE': IT IS A CRYOIMMUNOGLOBULIN AND IS DESIGNATED BY THE AUTHORS AS MCE. IN ORDER TO DIFFERENTIATE IT FROM ANOTHER MCE SEQUENCED BY CAPRA ET AL., IT IS DENOTED AS MCE'.
5) CE-1 'CL: CELL LINE CESS
7) SUP-T1 VH-JA'CL: IT IS FROM A PATIENT SUFFERING FROM CHILDHOOD T-CELL LYMPHOMA WITH Inv(14)(q11.2;q32.2). THE INVERSION ON CHROMOSOME 14 BRINGS THE VH GENE AND JA MINIGENE TOGETHER, GIVING RISE TO A HYBRID MOLECULE CONTAINING PART OF THE IMMUNOGLOBULIN GENE AND PART OF THE T-LYMPHOCYTE RECEPTOR FOR ANTIGEN GENE.
14) SPA: IT WAS FROM A CASE OF HEAVY CHAIN DISEASE.
15) NZU: IT IS A CRYOIMMUNOGLOBULIN.

+ THE FOLLOWING WERE EQUALLY AND MOST FREQUENTLY OCCURRING:

AT POSITION	RESIDUES
5	(ARG,GLN)
10	(ALA,GLY)
32	(THR,SER,ASP)
35	(CYS,SER)
44	(ALA,GLY)
52A	(TYR,HIS)
60	(SER,ASN)
81	(LYS,THR)
82	(LEU,MET)
82A	(THR,SER)
82B	(SER,ASN)
82C	(VAL,MET)
85	(VAL,ALA)
96	(PRO,LEU)
99	(PRO,ARG,GLY)
100	(TYR,PHE)
100A	(ALA,THR)
100D	(TYR,LEU)
100F	(TYR,GLY)
100H	(TYR,SER,ASP,ASN)
100I	(SER,GLY,ASP)

HUMAN HEAVY CHAINS SUBGROUP III

		INVARIANT RESIDUES	1* TUR	2 LAMBDA -VH26 'CL #	3* POM	4 TIL	5 WAS	6 HF2-1/13B	7 HF2-1/17	8 HF2-18/2	9 H11 'CL	10 TEI	11 BRO 'IGM	12 GR'	13 WAT	14* LAY	15 GRA' #	16* FR #	17 MU	18 VIN	19 HF3-16/6	20 BEN (III)	21 ZAP	22 JON	23 KEA
FR1	0		---	#	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1		GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	GLU	ala	GLU	GLU	GLU	GLU	GLU	GLU	GLU	asp	GLU
	2		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL
	3		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN
	4	LEU(.97)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	5		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	val	val	val	val	val	LEU	val	val	val	val	LEU	val	val	val	val
	6		GLU	GLU	GLU	GLU	GLU	GLU	GLU	gln	GLU	GLU	GLU	GLU	GLU	GLU	GLU	asp	GLU	GLU	gln	GLU	GLU	GLU	GLX
	7	SER(.97)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	8	GLY(.99)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	9	GLY(.98)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	10		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	ala	GLY	GLY
	11		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	12		VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	ile	VAL	ala	VAL	VAL	VAL
	13		GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	lys	GLN	GLN	GLN	GLN	lys	lys
	14	PRO(.95)	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO	PRO
	15	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	16		GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	17	SER(.97)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	18	LEU(.97)	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	gly	LEU	LEU
	19		ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG
	20		LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU	LEU
	21		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	gln	ala	SER	SER	SER
	22	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS	CYS
	23		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	24		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA
	25	SER(.98)	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER
	26	GLY(.97)	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY	GLY
	27	PHE(.98)	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE
	28		THR	THR	THR	THR	ser	lys	lys	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	THR	ser	THR	THR	THR	ile
	29		PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	PHE	ile	PHE	PHE	val	PHE	PHE	PHE	PHE	PHE
	30		SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	SER	asx	SER	SER	SER	thr	SER	SER	SER	SER	SER	pro
CDR1	31		ARG	SER	SER	THR	THR	SER	SER	SER	SER	THR	TYR	ALA	THR	ALA	LYS	ASX	ARG	THR	PRO	THR	THR	THR	
	32		VAL	TYR	SER	TYR	ASP	TYR	TYR	TYR	TYR	SER	TYR	ASX	TYR	SER	THR	PHE	GLY	ASN	SER	THR	THR	ALA	TYR
	33		LEU	ALA	ALA	VAL	ALA	ALA	ALA	ALA	TRP	ALA	ASN	TYR	THR	ALA	VAL	TYR	GLY	TYR	ALA	PHE	SER	TRP	
	34		SER	MET	MET	MET	MET	MET	MET	MET	MET	VAL	MET	MET	MET	MET	TYR	MET	LEU	MET	MET	MET	ARG	MET	
	35		SER	SER	SER	SER	TYR	SER	SER	SER	HIS	TYR	ASN		VAL	SER	GLU	ASP	GLU		SER	ARG	PHE	LYS	
	35A		---	---	---	---	---	---	---	---	---	---	---	---		---	---	---			---	---	---	---	
	35B		---	---	---	---	---	---	---	---	---	---	---	---		---	---	---			---	---	---	---	
FR2	36	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP	TRP		TRP	TRP	TRP	TRP		TRP	TRP	TRP	TRP	
	37	VAL(.95)	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL	VAL		VAL	VAL	VAL	VAL		VAL	VAL	VAL	VAL	
	38	ARG(.97)	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	ARG	PRO		ARG	ARG	ARG	ARG		ARG	ARG	ARG	ARG	
	39	GLN(.97)	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLN	GLY		GLN	GLN	GLN	GLN		GLN		GLN	GLN	
	40		ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	ALA	VAL	ALA		ALA	ALA	ALA	ALA		ALA		ALA	ALA	
	41		PRO	PRO	PRO	PRO	PRO				PRO	PRO	THR	PRO		PRO	PRO	PRO	PRO				PRO	PRO	
	42	GLY	GLY	GLY	GLY	GLY	GLY				GLY	GLY	GLY	GLY		GLY	GLY	GLY	GLY				GLY	GLY	
	43	LYS(.97)	LYS	LYS	LYS	LYS	LYS				LYS	LYS	LYS	ARG		LYS	LYS	LYS	LYS				LYS	LYS	
	44		GLY	GLY	GLY	GLY	GLY				GLY	GLY	GLY	GLY		GLY	GLY	GLY	ALA				GLY	GLY	
	45	LEU(.97)	LEU	LEU	LEU	LEU	LEU				LEU	LEU	LEU	LEU		LEU	LEU	LEU	LEU				LEU	LEU	
	46		GLU	GLU	GLU	GLX	GLU				VAL	GLU	GLU			GLU	GLU	GLU	GLX				GLU	GLU	
	47	TRP	TRP	TRP	TRP	TRP	TRP				TRP	TRP	TRP			TRP	TRP	TRP	TRP				TRP	TRP	
	48		VAL	VAL	VAL	VAL	VAL				VAL	VAL	VAL			VAL	VAL	VAL	VAL				VAL	VAL	
	49		SER	SER	ALA	GLY	ALA				SER	GLY	SER			ALA	THR	ALA	LEU				GLU	VAL	
CDR2	50		GLY	ALA	TRP	ALA	TRP				ARG	TRP	ALA			TRP	TYR	ALA	VAL				PHE	TRP	
	51		ARG	ILE	LYS	ILE	LYS				ILE	ARG	ILE			LYS	VAL	ARG	PHE				ARG	ARG	
	52		LEU	SER	TYR	GLX	TYR				ASN	TYR	GLY			TYR	GLY		SER				VAL	VAL	
	52A		ASN	GLY	GLU	GLY	GLN				SER	GLU	---			GLU	GLN		VAL				GLN	GLU	
	52B	LYS	---	---	---	---	---				---	---	---			---	---		---				---	---	
	52C		---	---	---	---	---				---	---	---			---	---		---				---	---	
	53		ALA	SER	ASN	LEU	GLU				ASP	GLY	THR			ASN	VAL		THR				GLY	GLN	
	54		SER	GLY	GLY	SER	ALA				GLY	SER	ALA			GLY	SER						SER	VAL	
	55		SER	GLY	ASN	VAL	SER				SER	SER	GLY			ASN	LYS						ALA	VAL	
	56		ASN	SER	ASP	SER	ASN				SER	LEU	ASP			ASP	PHE						ILE	GLU	
	57		LEU	THR	LYS	GLX	SER				THR	THR	GLN			LYS	SER		LYS				SER	LYS	
	58		HIS	TYR	HIS	SER	HIS				THR	HIS	TYR			HIS			PHE				HIS	ALA	
	59		PHE	TYR	TYR	TYR	PHE				TYR	TYR	TYR			TYR	TYR		TYR				TYR	PHE	
	60		ALA	GLY	ALA	ALA	ALA				ALA	ALA	ALA			ALA	ALA		THR				ALA	ALA	
	61		VAL	ASP	ASP	ASX	ASP				ASP	VAL	ASP			ASP	VAL		GLU				ASP	ASN	
	62		SER	SER	SER	SER	THR				SER	SER	SER			SER	SER		SER				SER	SER	
	63		ALA	VAL	VAL	VAL	VAL				VAL	VAL	VAL			VAL	VAL		LEU				VAL	VAL	
	64		GLN	LYS	ASN	LYS	ASN				LYS	GLN	LYS			ASN	GLN		ASN				GLN	ASN	
	65	GLY(.97)	GLY	GLY	GLY	GLY	GLY				GLY	GLY	GLY			GLY	GLY						ALA	GLY	
FR3	66	ARG	ARG	ARG	ARG	ARG	ARG				ARG	ARG	ARG			ARG	ARG						ARG	ARG	
	67	PHE(.97)	PHE	PHE	PHE	PHE	PHE				PHE	PHE	PHE			PHE	PHE						PHE	PHE	
	68		THR	THR	THR	THR	THR				THR	THR	THR			THR	THR						THR	THR	
	69	ILE(.97)	ILE	ILE	ILE	ILE	ILE				ILE	ILE	ILE			ILE	ILE						ILE	ILE	
	70	SER(.97)	SER	SER	SER	SER	SER				SER	SER	SER			SER	SER						SER	SER	
	71	ARG	ARG	ARG	ARG	ARG	ARG				ARG	ARG	ARG			ARG	ARG						ARG	ARG	
	72		ASN	ASP	ASN	ASP	ASN				ASP	ASN	ASN			ASN	ASN						ASN	ASN	
	73		ASP	ASN	ASP	ASP	ASP				ASN	ASP	ASP			ASP	ASP						ASP	ASP	
	74		SER	SER	SER	SER	SER				ALA	SER	SER			SER	SER						SER	SER	
	75		LYS	LYS	LYS	LYS	LYS				LYS	LYS	LYS			LYS	LYS						LYS	LYS	
	76		ASN	ASN	ASN	ASN	ASN				ASN	ASN	ASN			ASN	ASN						ASN	ASN	
	77		THR	THR	THR	THR	THR				THR	THR	THR			THR	THR						THR	THR	
	78		LEU	LEU	LEU	---	LEU				LEU	LEU	LEU			LEU	LEU						LEU	LEU	
	79		TYR	TYR	TYR	---	TYR				TYR	TYR	TYR			TYR	TYR						TYR	TYR	
	80		LEU	LEU	LEU	---	LEU				LEU	LEU	LEU			LEU	LEU						LEU	LEU	
	81		GLN	GLN	LEU	---	GLN				GLN	GLN	ASN			GLN	GLU						GLN	GLN	
	82	MET(.95)	MET	MET	MET	MET	MET				MET	MET	MET			MET	MET	MET					MET	MET	MET
	82A		LEU	ASN	ASN	ASN	ASN				ASN	LEU	ASN			ASN	LYS	ASN					ASN	ILE	ASN
	82B		SER	SER	SER	SER	ARG				SER	SER	SER			GLY	THR	SER					THR	SER	ASN
	82C		LEU	LEU	LEU	LEU	LEU				LEU	LEU	LEU			LEU	GLY	LEU					GLY	VAL	LEU
	83		GLN	ARG	GLN	ARG	GLU				ARG	GLU	ARG			GLN	GLU	ARG					GLU	THR	ARG
	84		ALA	ALA	ALA	ALA	ALA				ALA	PRO	ALA			ALA	PRO	ALA		ALA			ALA	PRO	VAL
	85		GLU	GLU	GLU	GLU	GLU				GLU	GLU	GLU			GLU	GLU	GLX		GLU			GLU	GLU	GLX
	86		ASP	ASP	ASP	ASP	ASP				ASP	ASP	ASP			VAL	ASP	ASX		ASP			ASP	ASP	ASX
	87		THR	THR	THR	THR	THR				THR	THR	THR			SER	THR	THR		THR			THR	THR	THR
	88		ALA	ALA	ALA	ALA	ALA				ALA	ALA	ALA			ALA	ALA	ALA		ALA			ALA	ALA	ALA
	89		LEU	VAL	LEU	VAL	VAL				VAL	VAL	VAL			ILE	VAL	VAL		VAL			VAL	VAL	VAL
	90	TYR(.98)	TYR	TYR	TYR	TYR	TYR				TYR	TYR	TYR			TYR	TYR	TYR		TYR			TYR	TYR	TYR
	91	TYR(.95)	TYR	TYR	TYR	TYR	TYR				TYR	TYR	TYR			TYR	TYR	TYR		TYR			TYR	TYR	TYR
	92	CYS	CYS	CYS	CYS	CYS	CYS				CYS	CYS	CYS			CYS	CYS	CYS		CYS			CYS	CYS	CYS
	93		ALA	ALA	ALA	ALA	ALA				ALA	ALA	ALA			ALA	ALA	ALA		ALA			ALA	ALA	ALA
	94		ARG	LYS	ARG	LYS	ARG				ARG	ARG	ARG			ARG	ARG	ARG		ARG			ARG	ARG	ARG
CDR3	95		LEU	#	ASP	GLY	PHE				---	VAL	SER			ASP	HIS						THR	VAL	ASX
	96		SER	---	ALA	LYS	ARG				---	THR	PRO			ALA	ILE						ARG	VAL	ARG
	97		VAL	---	GLY	VAL	GLN				---	PRO	VAL			GLY	TYR						PRO	VAL	LEU
	98		THR	---	PRO	SER	PRO				---	ALA	SER			PRO	VAL						GLY	SER	GLY
	99		ALA	---	TYR	ALA	PHE				---	ALA	LEU			TYR	THR						GLY	THR	PRO
	100		VAL	---	VAL	TYR	VAL				---	ALA	VAL			VAL	LEU						TYR	SER	THR
	100A		ALA	---	SER	TYR	GLN				---	SER	ASP			SER	TYR						PHE	---	ALA
	100B		PHE	---	PRO	PHE	---				---	LEU	GLY			PRO	TYR						SER	---	CYS
	100C		---	---	THR	---	---				---	THR	TRP			THR	TYR						---	---	SER
	100D		---	---	PHE	---	---				---	---	LEU			PHE	---						---	---	VAL
	100E		---	---	---	---	---				---	---	TYR			---	---						---	---	
	100F		---	---	---	---	---				---	---	TYR			---	---						---	---	
	100G		---	---	---	---	---				---	---	TYR			---	---						---	---	
	100H		---	---	---	---	---				---	---	TYR			---	---						---	---	
	100I		---	---	---	---	---				---	---	GLY			---	---						---	---	
	100J		---	---	---	---	PHE				---	PHE	---			---	PHE						---	MET	
	100K		---	---	PHE	---	PHE				---	SER	---			PHE	MET						---		
	101		ASP	---	ALA	ASX	ASP				---	ALA	SER			ALA	ALA						ASP	ASP	
	102		VAL	---	HIS	TYR	VAL				---	VAL				HIS	VAL						VAL	VAL	
FR4	103		TRP	---	TYR	TRP	PHE				---	TRP	TRP			TRP	TRP						TRP	TRP	
	104	GLY(.97)	GLY	---	GLY	GLY	GLY				---	GLY	GLY			GLY	GLY						GLY	GLY	
	105		GLN	---	GLN	GLX	GLN				---	GLN	GLN			GLN	GLN						GLN	GLN	
	106	GLY(.97)	GLY	---	GLY	GLY	GLY				---	GLY	GLY			GLY	GLY						GLY	GLY	
	107		THR	---	THR	THR	THR				---	THR	THR			THR	THR						THR	THR	
	108		LYS	---	LEU	LEU	LEU				---	LEU	LEU			LEU	LEU						LEU	PRO	
	109	VAL(.97)	VAL	---	VAL	VAL	VAL				---	VAL				VAL							VAL	VAL	
	110		SER	---	THR	THR	THR				---	THR				THR							SER	THR	
	111			---		VAL					---														
	112	SER		---		SER					---														
	113			---		SER					---														

MAIL ROOM
OCT 20 1993
77
PAT. & TRADEMARK OFF.

GP1806

PATENT DOCKET NO. 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#23
11-12-93

In re Application of)
Paul J. Carter et al.)
Serial No. 07/715,272)
Filed: 14 June 1991)
For: Immunoglobulin Variants)

Group Art Unit: 1806
Examiner: L. Feisee

RECEIVED
NOV 05 1993
GROUP 1800

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-1896

NOTICE OF APPEAL

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Applicant hereby appeals to the Board of Appeals and Interferences from the decision dated May 19, 1993, of the Primary Examiner finally rejecting claims 1-11 and 17-21.

The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $270 to cover the fees for this appeal and to charge the deposit account for any further fees in regard to this patent application. A duplicate copy of this Notice is enclosed for this purpose.

Respectfully submitted,

GENENTECH, INC.

Janet E. Hasak

Janet E. Hasak
Reg. No. 28,616

Dated: October 15, 1993

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Louise Strasbaugh
Louise Strasbaugh

Date: October 15, 1993

RP14167 11/04/93 07715272 07-0630 140 119 270.00CH

w020.u

GP1806

PATENT DOCKET 709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

18D1/10/25/93

#22 [illegible]

In re Application of

Paul J. Carter et al.

Serial No. 07/715,272

Filed: 14 June 1991

For: Immunoglobulin Variants

Group Art Unit: 1806

Examiner: L. Feisee

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-1896

PETITION AND FEE FOR EXTENSION OF TIME (37 CFR 1.136(a))

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Office action dated May 19, 1993 for an additional month, from September 19, 1993 to October 19, 1993. The extended time for response does not exceed the statutory period.

Please charge Deposit Account Number 07-0630 in the amount of $250 to cover the cost of the second month extension fee less the first month extension fee paid in relation to the request for a one month extension of time filed on September 20, 1993. Any deficiency or overpayment should be charged or credited to this deposit account. <u>A duplicate of this sheet is enclosed.</u>

Respectfully submitted,

GENENTECH, INC.

Janet E. Hasak

Janet E. Hasak
Reg. No. 28,616

Date: October 15, 1993

RP14166 11/04/93 07715272 07-0630 140 116 250.00CH

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Louise Strasbaugh

Louise Strasbaugh

Date: October 15, 1993

w021.u

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/715,272	06/14/91	CARTER	[illegible]

EXAMINER	
[illegible]	
ART UNIT	PAPER NUMBER
1806	21

1802/1021

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

DATE MAILED: 10/21/93

Below is a communication from the EXAMINER in charge of this application

COMMISSIONER OF PATENTS AND TRADEMARKS

ADVISORY ACTION

☒ THE PERIOD FOR RESPONSE:

a) ☒ is extended to run 4 months or continues to run ______ from the date of the final rejection

b) ☐ expires three months from the date of the final rejection or as of the mailing date of this Advisory Action, whichever is later. In no event however, will the statutory period for the response expire later than six months from the date of the final rejection.

Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a), the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee. Any extension fee pursuant to 37 CFR 1.17 will be calculated from the date of the originally set shortened statutory period for response or as set forth in b) above.

☐ Appellant's Brief is due in accordance with 37 CFR 1.192(a).

☒ Applicant's response to the final rejection, filed 9/23/93 has been considered with the following effect, but it is not deemed to place the application in condition for allowance:

1. ☒ The proposed amendments to the claim and /or specification will not be entered and the final rejection stands because:

   a. ☐ There is no convincing showing under 37 CFR 1.116(b) why the proposed amendment is necessary and was not earlier presented.

   b. ☒ They raise new issues that would require further consideration and/or search. (See Note).

   c. ☒ They raise the issue of new matter. (See Note).

   d. ☒ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.

   e. ☐ They present additional claims without cancelling a corresponding number of finally rejected claims.

NOTE: The language "of a human immunoglobulin subgroup" has not been defined with respect to the variable domain of a consensus antibody.

2. ☐ Newly proposed or amended claims ______ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.

3. ☒ Upon the filing an appeal, the proposed amendment ☐ will be entered ☒ will not be entered and the status of the claims will be as follows:

Claims allowed: ~~None~~ 12 and 13
Claims objected to: None
Claims rejected: 1-11, 17-21

However;

☒ Applicant's response has overcome the following rejection(s): The rejection under 35 USC 112 first paragraph regarding "at least a portion", and "reasonably".

4. ☒ The affidavit, exhibit or request for reconsideration has been considered but does not overcome the rejection because the introduction of the language "human subgroup" is defined in patent but not in the application. Also, it is not clear that the "consensus antibody" of the application would be identical to the antibody of Queen et al. The declaration [illegible] not add [illegible] different than that alread

5. ☐ The affidavit or exhibit will not be considered because applicant has not shown good and sufficent reasons why it was not earlier presented.

☐ The proposed drawing correction ☐ has ☐ has not been approved by the examiner.

☐ Other

DAVID L. LACEY
SUPERVISORY PATENT EXAMINER
GROUP 180
10/21/93

303 (REV 5-86)

aF

PATENT DOCKET 709

24

1-5-94

DEC 17 1993 PAT. & TRADEMARK OFF.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of) Group Art Unit: 1806

Paul J. Carter et al.) Examiner: L. FEISEE

1809

Serial No. 07/715272

Filed: June 14, 1991

For: Immunoglobulin Variants

RECEIVED
DEC 29 1993
GROUP 1800

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 225-1896

AMENDMENT PURSUANT TO 37 CFR § 1.116(a)

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

O/C to enter 1/11/94

Sir:

Pursuant to 37 CFR § 1.116(a), please cancel claims 1-11, 17, and 19-21 of the above application. Claim 18 was canceled in the Amendment filed September 20, 1993. Applicants trust that the above-mentioned application with allowed claims 12 and 13 will be in condition for allowance following the entry of this amendment and look forward to receiving the Notice to this effect.

Respectfully submitted,
GENENTECH, INC.
Janet E. Hasak

Janet E. Hasak
Reg. No. 28,616

Dated: December 13, 1993

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service in first class envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on the date shown below.

Dated: 13 DEC 1993

Louise Strasbaugh
Louise Strasbaugh

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.

EXAMINER

ART UNIT	PAPER NUMBER
	25

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Wendy Lee

(2) Lila Feisee

(3)

(4)

Date of interview 1/11/94

Type: ☒ Telephonic ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description:

Agreement ☐ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed:

Identification of prior art discussed:

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:

Finality would be withdrawn.
Action forth coming.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1 – 7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Examiner's Signature

PTOL-413 (REV. 1-84)

ORIGINAL FOR INSERTION IN RIGHT HAND FLAP OF FILE WRAPPER

UNITED STA S DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/715,272	06/14/91	CARTER	P 709

EXAMINER	
FEISEE, L	
ART UNIT	**PAPER NUMBER**
1806	26

18M2/0203

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

DATE MAILED: 02/03/94

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 12/17/93 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice of Draftsman's Patent Drawing Review, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ ______

Part II SUMMARY OF ACTION

1. ☒ Claims 12 and 13 are pending in the application.

   Of the above, claims ______ are withdrawn from consideration.
2. ☒ Claims 1-11, 14-21 have been cancelled.
3. ☐ Claims ______ are allowed.
4. ☒ Claims 12 and 13 are rejected.
5. ☐ Claims ______ are objected to.
6. ☐ Claims ______ are subject to restriction or election requirement.
7. ☒ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on ______. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on ______, has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed ______, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. ______ ; filed on ______.
13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

PTOL-326 (Rev. 2/93)

The finality of the previous Office action is withdrawn in view of the following new grounds of rejection.

Claims 12 and 13 are pending in this application, and these claims are humanized light and heavy chain variable regions of a previously referenced antibody 4D5.

Claims 12 and 13 are rejected under 35 U.S.C. § 103 as being unpatentable over Hudziak et. al. or Fendly et. al. in view of Queen et. al.

Hudziak et. al. and Fendly et. al. both teach the production and characterization of the 4D5 antibody (see Hudziak et. al. 1166-1167 and Fendly et. al. pages 1553-1554). Hudziak et. al. suggests the possible therapeutic role of the 4D5 antibody in human neoplasias which overexpress p185-HER2 (pages 1171, last paragraph) while Fendly et. al. disclose the possible use of anti-p185 HER2 antibodies for <u>in vivo</u> radioimaging for detection of relevant primary tumors. They do not describe the production of these antibodies in the humanized form.

Queen et. al. teach the production of antibodies against IL-2 receptor in the humanized form, using computer modeling in order to determine the modification of certain framework regions in conjunction with CDR grafting. The antibodies produced are than to be used for <u>in vivo</u> administration to human patients, either for diagnosis or therapy. It is known in the art that murine and even chimeric antibodies have characteristics which may severely limit their use in human therapy. As foreign proteins, murine and chimeric antibodies may elicit immune reactions that reduce or destroy their therapeutic efficacy and/or evoke allergic or hypersensitivity reactions in patients. The probable need for readministration of such therapeutic modalities in neoplastic disorders increases these risks. The result would be tissue injury by virtue of antigen-antibody deposition.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to make humanized antibodies having the sequences of the 4D5 antibody. The methods of Queen et. al. were clear and self explanatory, and resulted in a high affinity antibody. One of ordinary skill in the art would have been motivated to humanize the 4D5 antibody in light of its potential therapeutic and diagnostic applicability.

Although the claims are drawn to specific amino acid sequences, it is maintained that the differences in amino acid sequence which would have been obtained using the method of Queen et. al. would not have been patentably distinct from the claimed amino acid sequences. Absent sufficient factual evidence to the contrary the claims are obvious over the cited prior art.

35 U.S.C. § 101 reads as follows:

> **Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title.**

Claims 12 and 13 are rejected under 35 U.S.C. § 101 because the claimed invention lacks patentable utility. These claims are drawn to a light chain variable region polypeptide and a heavy chain variable region polypeptide which in and of themselves have no patentable utility. The specification does not disclose any practical utility for the individual polypeptides and does not present evidence that these polypeptides are capable of binding in any particular manner when not in association with each other.

Claims 12 and 13 are directed to an invention not patentably distinct from claims 1, 3-9, and 40 of commonly assigned 07/977,453.

Specifically, the claims of the instant invention are drawn to the humanized version of the 4D5 antibody which is disclosed in copending application.

Commonly assigned 07/977,453, discussed above, would form the basis for a rejection of the noted claims under 35 U.S.C. § 103 if the commonly assigned case qualifies as prior art under 35 U.S.C. § 102(f) or (g) and the conflicting inventions were not commonly owned at the time the invention in this application was made. In order for the examiner to resolve this issue, the assignee is required under 37 C.F.R. 1.78(c) and 35 U.S.C. § 132 to either show that the conflicting inventions were commonly owned at the time the invention in this application was made or to name the prior inventor of the conflicting subject matter. Failure to comply with this requirement will result in a holding of abandonment of the application. A showing that the inventions were commonly owned at the time the invention in this application was made will preclude a rejection under 35 U.S.C. § 103 based upon the commonly assigned case as a reference under 35 U.S.C. § 102(f) or (g).

Claims 12 and 13 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1,3-9, and 40 of copending application Serial No. 07/977,453 in view of Queen et. al.. The instant claims are drawn to the heavy chain and light chain variable regions of the 4D5 antibody. Copending application 07/977,453 claims an antibody with the same characteristics as 4D5, and also states within the claims that 4D5 antibody was useful for diagnosis and therapy of tumors expressing the p185 HER2 antigen on their surface. The induction of HAMA responses upon repeated administration of rodent antibodies has led to the desirability of producing antibodies which are even more "near human" than chimeric antibodies. Queen et. al. describes the production of antibodies which contain essentially the CDR of rodents and are grafted into human framework regions. These antibodies are also mutated in certain framework residues in order to produce functional and high affinity molecules. The procedure in Queen et. al. clearly teaches the particular framework residues that need to be changed in order to yield high affinity antibodies, and they teach how to determine the appropriate residues using computer modeling programs. This protocol is adaptable to any number of antibodies. Therefore, not only was the production of non-immunogenic 4D5 antibodies desirable, but the procedure for producing the antibodies was also well known and practiced. It would have been <u>prima facie</u> obvious to one of ordinary skill in the art at the time the invention was made to use the claims of the copending application in combination with the reference of Queen et. al. in order to obtain high affinity functional humanized antibodies.

The obviousness-type double patenting rejection is a judicially established doctrine based upon public policy and is primarily intended to prevent prolongation of the patent term by prohibiting claims in a second patent not patentably distinct from claims in a first patent. In re Vogel, 164 U.S.P.Q. 619 (CCPA 1970). A timely filed terminal disclaimer in compliance with 37 C.F.R. 1.321(b) would overcome an actual or provisional rejection on this ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 C.F.R. 1.78(d).

Claims 12 and 13 are provisionally rejected under 35 U.S.C. § 103 as being obvious over copending application Serial No. 07/977,453 in view of Queen et. al.

The instant claims are drawn to the heavy chain and light chain variable regions of the 4D5 antibody. Copending application 07/977,453 discloses an antibody with the same characteristics as 4D5, and also discloses that 4D5 antibody is useful for diagnosis and therapy of tumors expressing the p185 HER2 antigen on their surface. The induction of HAMA responses upon repeated administration of rodent antibodies has led to the desirability of producing antibodies which are even more "near human" than chimeric antibodies. Queen et. al. describes the production of antibodies which contain essentially the CDR of rodents and are grafted into human framework regions. These antibodies are also mutated in certain framework residues in order to produce functional and high affinity molecules. The procedure in Queen et. al. clearly teaches the particular framework residues that need to be changed in order to yield high affinity antibodies, and they teach how to determine the appropriate residues using computer modeling programs. This protocol is adaptable to any number of antibodies. Therefore, not only was the production of non-immunogenic 4D5 antibodies desirable, but the procedure for producing the antibodies was also well known and practiced. It would have been <u>prima facie</u> obvious to one of ordinary skill in the art at the time the invention was made to use the claims of the copending application in combination with the reference of Queen et. al. in order to obtain high affinity functional humanized antibodies.

Copending application Serial No. 07/977,453 has a common assignee with the instant application. Based upon the earlier effective U.S. filing date of the copending application, it would constitute prior art under 35 U.S.C. § 102(e) if patented. This provisional rejection under 35 U.S.C. § 103 is based upon a presumption of future patenting of the conflicting application.

This provisional rejection might be overcome either by a showing under 37 C.F.R. 1.132 that any unclaimed invention disclosed in the copending application was derived from the inventor of this application and is thus not the invention "by another", or by a showing of a date of invention prior to the effective U.S. filing date of the copending application.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lila Feisee whose telephone number is (703) 308-2731.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Feisee/lf
January 11, 1994

PAULA K. HUTZELL
PRIMARY EXAMINER
GROUP 1800

TO SEPARATE, .D TOP AND BOTTOM EDGES, SNAP–APART AN ISCARD CARBON

FORM PTO-892 (REV. 2-92)
U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

SERIAL NO. 07/715,272
GROUP ART UNIT 1806
ATTACHMENT TO PAPER NUMBER 26

NOTICE OF REFERENCES CITED

APPLICANT(S) Carter et al.

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

R	Queen et al. PNAS 86:10029-10033 (1989)
S	Fendley et al. Cancer Research 5:1550-1558
T	Hudziak et al. Molecular and Cellular Biology 1989 p. 1165-1172
U	

EXAMINER

DATE 1/11/94

ith this office action.
on 707.05 (a).)

* A copy of this reference

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/715,272	06/14/91	CARTER	

EXAMINER P 709

FEISEE, L

18M2/0906

ART UNIT	PAPER NUMBER
1806	27

GENENTECH, INC.
ATTN: CAROLYN R. ADLER
460 POINT SAN BRUNO BLVD.
SOUTH SAN FRANCISCO, CA 94080

DATE MAILED: 09/06/94

NOTIFICATION OF DEFECTIVE NOTICE OF APPEAL OR DEFECTIVE BRIEF

1. ☐ The Notice of Appeal filed ____________ is:
   - A. ☐ Not acceptable for reason(s) that:
     - (1) ☐ The Appeal fee required by 35 U.S.C. 41 (a)(6) and 37 CFR 1.17(e) was not submitted with the Notice of Appeal.
     - (2) ☐ The submitted fee of $ __________ is insufficient. The appeal fee required by 37 CFR 1.17(e) is $ ____________.
     - (3) ☐ The Notice of Appeal was not timely filed.
     - (4) ☐ The Appeal fee received on ____________ was not timely filed.
     - (5) ☐ The Appeal is not in compliance with 37 CFR 1.191 in that the claims have not been finally or twice rejected.
     - (6) ☐ A Notice of Allowability was mailed by the Office on ____________.
   - B. ☐ Defective and should be corrected as indicated. Applicant is given a TIME LIMIT of ONE MONTH from the date of this letter OR the TIME REMAINING IN THE RESPONSE PERIOD OF THE LAST OFFICE ACTION, whichever is longer, to complete the appeal. NO EXTENSION OF THIS ONE MONTH PERIOD MAY BE GRANTED UNDER 37 CFR 1.136(a) or (b) BUT THE PERIOD FOR RESPONSE SET IN THE LAST ACTION MAY POSSIBLY BE EXTENDED. If the appeal is not timely completed, the application will be abandoned.
     - (1) ☐ The Notice of Appeal is not signed.
     - (2) ☐ Identification of the appealed claim or claims is required under 37 CFR 1.191 (b).

2. ☐ The Brief filed ________________ is NOT acceptable for the reason(s) indicated below.

   The Appeal in this application will be dismissed unless the applicant makes the Brief acceptable. Extensions of time may be obtained under 37 CFR 1.136(a).
   - A. ☐ The Brief and/or Brief fee is untimely. See 37 CFR 1.192.
   - B. ☐ The requisite fee which must accompany the Brief has been omitted. See 37 CFR 1.17(f).
   - C. ☐ The submitted Brief fee of ____________ is not the proper amount. The Brief fee required by 37 CFR 1.17(f) is ______________.

3. ☒ The Appeal in this application is DISMISSED because
   - A. ☐ The fee for filing the Brief as required under 37 CFR 1.17(f) was not submitted or timely submitted and the period for obtaining an extension of time to file the brief under 37 CFR 1.136 has expired.
   - B. ☒ The Brief was not filed, or was not timely filed and the period for obtaining an extension of time to file the brief under 37 CFR 1.136 has expired.

4. ☒ As the result of the dismissal in "3" above, this application:
   - A. ☒ is abandoned since there are no allowed claims.
   - B. ☐ is being returned to the examiner for disposition since it contains allowed claims. Prosecution on the merits is CLOSED.

DAVID L. LACEY
SUPERVISORY PATENT EXAMINER
GROUP 180

9/1/94

(PTOL-461, Rev. 4/89)

REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

In re Application of	
Application Number 07/715,272	Filed 6-14-91
Group Art Unit	Examiner

PROCESSED BY
OCT 19 1999
F

Paper No. #28

Assistant Commissioner for Patents
Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

___ (A) referred to in United States Patent Number 5821337, column 1.

___ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. ________ filed ________, on page ________ of paper number ________.

___ (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. ________, filed ________, or

___ (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

2001 Jefferson Davis Hwy Arl. Va. 22202
Suite #806

Brian Willingham
Signature

10/19/99
Date

Brian Willingham
Typed or printed name

FOR PTO USE ONLY
Approved by: ______ (Initials)
Unit: ______

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the [illegible]

RECEIVED
NOV 3 0 1999
File Information Unit

In re Application of	
Application Number 07/715272	Filed 6-14-91
Group Art Unit	Examiner

Paper No. #29

Assistant Commissioner for Patents
Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

✓ (A) referred to in United States Patent Number 5821337, column ____

___ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. ____, filed ____, on page ____ of paper number ____

___ (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. ____, filed ____, or

___ (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

Signature

11-30-99
Date

Typed or printed name

FOR PTO USE ONLY
Approved by: DW
(Initials)
Unit: FIU

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the indiv case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, P and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231

PTO/SB/68 (11-96)
Approved for use through 10/31/99. OMB 0651-0031
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

In re Application of

Application Number 07/715272	Filed 6/14/91
Group Art Unit	Examiner

Paper No. 30

Assistant Commissioner for Patents
Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

[✓ (A)] referred to in United States Patent Number 6054297, column ____.

____ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. ____, filed ____, on page ____ of paper number ____.

____ (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. ____, filed ____, or

____ (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

RECEIVED
01 SEP 12 AM 11:15
TECH CENTER 1600/2900

Wayne Croteau
Signature

9-10-01
Date

WAYNE CROTEAU
Typed or printed name

FOR PTO USE ONLY
Approved by: ____ (Initials)
Unit: ____

PTO/SB/68 (11-96)
Approved for use through 10/31/99. OMB 0651-0031
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

In re Application of

Application Number 07/715272	Filed 6-14-91
Group Art Unit	Examiner

Paper No. #31

Assistant Commissioner for Patents
Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

✓ (A) referred to in United States Patent Number 6054997, column ______

___ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. ______, filed ______ on page ______ of paper number ______.

___ (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. ______, filed ______, or

___ (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

______

______

______

Signature

Date 9-28-01

Typed or printed name

FOR PTO USE ONLY

Approved by: ______ (initials)

Unit: F.I.U.

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

PTO/SB/68 (04-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

In re Application of Carter	
Application Number 07/715272	Filed 6/14/91
Art Unit	Examiner

RECEIVED
MAR 11 2002
File Information Unit

Paper No. #32

Assistant Commissioner for Patents
Washington, DC 20231

1. ☐ I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

☐ (A) referred to in:

United States Patent Application Publication No. 5821337, page ____, line ____,

United States Patent Number ____________, column ____, line ____, or

an International Application which was filed on or after November 29, 2000 and which designates the United States, WIPO Pub. No. ____________, page ____, line ____.

☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No. ____________, paper No. ____, page ____, line ____.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Anita Myles
Signature

Anita Myles
Typed or printed name

3/11/02
Date

FOR PTO USE ONLY
Approved by: ________ (initials)
Unit: ________

PTO/SB/68 (04-01)
[illegible]ved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Tr[illegible]ark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

In re Application of

Application Number 07/715,272
Filed Jun. 14, 1991
Art Unit
Examiner

RECEIVED
JUN 24 2002
File Information Unit

Paper No. #33

Assistant Commissioner for Patents
Washington, DC 20231

1. ☐ I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

☑ (A) referred to in:

United States Patent Application Publication No. ________, page ____, line ____,

United States Patent Number 6,407,213, column 63, line ____, or

an International Application which was filed on or after November 29, 2000 and which designates the United States, WIPO Pub. No. ________, page ____, line ____.

☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No. ________, paper No. ____, page ____, line ____.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Chris Riley
Signature

6/24/02
Date

Chris Riley
Typed or printed name

FOR PTO USE ONLY
Approved by: A. Bryant (initials)
Unit: ________

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

PTO/SB/68 (04-01)
Approved for [illegible]hrough 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

In re Application of	
Application Number 07/715,272	Filed 6-14-91
Art Unit	Examiner

RECEIVED
AUG 21 2002
File Information Unit

Paper No. #34

Assistant Commissioner for Patents
Washington, DC 20231

1. ☐ I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

   ☐ (A) referred to in:

   United States Patent Application Publication No. ____________, page ____, line ____,

   United States Patent Number 6407213, column ______, line ______, or

   an International Application which was filed on or after November 29, 2000 and which designates the United States, WIPO Pub. No. ____________, page ____, line ____.

   ☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No.____________, paper No. ______, page ______, line ______.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Brian Willingham
Signature

8/21/02
Date

Brian Willinghan
Typed or printed name

FOR PTO USE ONLY
Approved by: ________ (initials)
Unit: ________

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

PTO/SB/56 (04-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

RECEIVED
OCT 1 8 2002
File Information Unit

In re Application of	
Application Number 07/715,272	Filed 6.14.91
Art Unit 186	Examiner Keisee

Paper No. 35

Assistant Commissioner for Patents
Washington, DC 20231

1. ☑ I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

☐(A) referred to in:

United States Patent Application Publication No. 6407213, page _____, line_______,

_______United States Patent Number_______________, column _______, line _______, or

an International Application which was filed on or after November 29, 2000 and which designates the United States, WIPO Pub. No. _______________, page _____, line_____.

☐(B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No._______________, paper No. ______, page ______, line _____.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Keisha Jenkins
Signature

10/18/02
Date

Keisha Jenkins
Typed or printed name

FOR PTO USE ONLY
Approved by: _______________. (Initials)
Unit: _______________.

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

PTO/SB/68 (04-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

RECEIVED
NOV 01 2002
File Information Unit

In re Application of 6407,213

Application Number 07/715,272

Filed 6-14-91

Art Unit

Examiner

Paper No. #36

Assistant Commissioner for Patents
Washington, DC 20231

1. ☑ I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

☑ (A) referred to in:

United States Patent Application Publication No. ________, page ____, line ____,

United States Patent Number 6407,213, column ____, line ____, or

an International Application which was filed on or after November 29, 2000 and which designates the United States, WIPO Pub. No. ________, page ____, line ____.

☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No. ________, paper No. ____, page ____, line ____.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Lucy Smith
Signature

11-1-02
Date

Lucy Smith
Typed or printed name

FOR PTO USE ONLY
Approved by: D. Bryant (initials)
Unit: FIU

PTO/SB/68 (04-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

RECEIVED
DEC 1 8 2002
File Information Unit

In re Application of	
Application Number 07-715272	Filed 06-14-91
Art Unit	Examiner

Paper No. #37

Assistant Commissioner for Patents
Washington, DC 20231

1. ☑ I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

☑ (A) referred to in:

United States Patent Application Publication No. __________, page _____, line _______,

United States Patent Number 6407213 ________, column _______, line ______, or

an International Application which was filed on or after November 29, 2000 and which designates the United States, WIPO Pub. No. __________, page _____, line _____.

☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No. __________, paper No. ______, page ______, line _____.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Shoaib Ghayour
Signature

12-18-02
Date

Shoaib Ghayour
Typed or printed name

FOR PTO USE ONLY
Approved by: ________ (initials)
Unit: ________

RECEIVED
DEC 1 8 2002
File Information Unit

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

US006407213B1

(12) **United States Patent**
Carter et al.

(10) **Patent No.:** **US 6,407,213 B1**
(45) **Date of Patent:** **Jun. 18, 2002**

---

(54) **METHOD FOR MAKING HUMANIZED ANTIBODIES**

(75) Inventors: **Paul J. Carter**; **Leonard G. Presta**, both of San Francisco, CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/146,206**

(22) PCT Filed: **Jun. 15, 1992**

(86) PCT No.: **PCT/US92/05126**

§ 371 (c)(1),
(2), (4) Date: **Nov. 17, 1993**

Related U.S. Application Data

(63) Continuation-in-part of application No. 07/715,272, filed on Jun. 14, 1991, now abandoned.

(51) **Int. Cl.**^{7} **C07K 16/00**

(52) **U.S. Cl.** **530/387.3**; 435/69.6; 435/69.7; 435/70.21; 435/91; 536/23.53; 424/133.1

(58) **Field of Search** 435/69.6, 69.7, 435/70.21, 91, 172.2, 240.1, 240.27, 252.3, 320.1, 328; 536/23.53; 424/133.1; 530/387.3

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,816,567	A	3/1989	Cabilly et al.	
4,845,198	A	7/1989	Urdal et al.	530/388.22
5,132,405	A	7/1992	Huston et al.	530/387.3
5,225,539	A	7/1993	Winter	530/389.3
5,530,101	A	6/1996	Queen et al.	530/387.3
5,558,864	A	9/1996	Bendig et al.	424/133.1
5,585,089	A	12/1996	Queen et al.	424/133.1
5,677,171	A	10/1997	Hudziak et al.	435/7.23
5,693,762	A	* 12/1997	Queen et al.	530/387.2
5,714,350	A	2/1998	Co et al.	435/69.6
5,772,997	A	6/1998	Hudziak et al.	424/130.1
5,821,337	A	10/1998	Carter et al.	530/387.3
5,834,598	A	11/1998	Lowman et al.	530/399
5,859,205	A	1/1999	Adair et al.	530/387.3

FOREIGN PATENT DOCUMENTS

AU	85058/91		3/1992	C07K/15/12
EP	120694		10/1984	
EP	125023	A1	11/1984	
EP	0 239 400		* 9/1987	C12N/15/00
EP	323806	A1	7/1989	
EP	328404	A1	8/1989	A61K/39/395
EP	338745	A1	10/1989	
EP	365209	A2	4/1990	
EP	365997	A2	5/1990	
EP	368684		5/1990	
EP	403156	A1	12/1990	
EP	438310	A2	7/1991	
EP	438312	A2	7/1991	
EP	440351	A2	8/1991	
EP	0 460 167	B1	12/1991	
EP	0 519 596	A1	12/1992	
EP	0 592 106	A1	4/1994	
EP	0 620 276		10/1994	
EP	682040	A1	11/1995	
EP	451216	B1	1/1996	C12P/21/08
EP	432249	B1	9/1996	
GB	2 188941		10/1987	
WO	WO 87/02671		5/1987	
WO	WO 88/09344		12/1988	
WO	WO 89/01783		3/1989	
WO	WO 89/06692		7/1989	
WO	WO 89/09622		10/1989	
WO	WO 90/07861		7/1990	
WO	90/07861		* 7/1990	C12P/21/00
WO	WO 91/07492		5/1991	
WO	WO 91/07500		5/1991	
WO	WO 91/09966		7/1991	C12P/21/08
WO	WO 91/09968		7/1991	C12P/21/08
WO	WO 91/09967		11/1991	
WO	WO 92/01047		1/1992	
WO	WO 92/04380		3/1992	
WO	WO 92/04381		3/1992	
WO	WO 92/05274		4/1992	
WO	WO 92/11383		7/1992	
WO	WO 92/11018		9/1992	A61K/35/14
WO	WO 92/15683		9/1992	
WO	WO 92/16562		10/1992	
WO	WO 92/22653		12/1992	
WO	WO 93/02191		2/1993	
WO	94/11509		5/1994	
WO	WO 94/12214		6/1994	

OTHER PUBLICATIONS

Riechmann et al. [Nature 332:323–327 (1988)].*

Queen et al. [Proc. Natl. Acad. Sci. 86:10029–10033 (1989)].*

Roitt [*Immunology*, published 1985, by Gower Medical Publishing Ltd. (London, England) p. 5.5].*

Tramontano et al. [J. Mol. Biol. 215:175–182 (1990)].*

"Biosym Technologies" in New Products, Chemical Design Automation 3 (Dec. 1988).

"Polygen Corporation" in New Products, Chemical Design Automation 3 (Nov. 1988).

Adair et al., "Humanization of the murine anti–human CD3 monoclonal antibody OKT3" *Hum. Antibod. Hybridomas* 5:41–47 (1994).

Chothia et al., "Principles of protein–protein recognition" *Nature* 256:705–708 (1975).

Chothia et al., "Transmission of conformational change in insulin" *Nature* 302:500–505 (1983).

Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" *J. Mol. Biol.* 235:53–60 (1994).

(List continued on next page.)

Primary Examiner—Anthony C. Caputa
Assistant Examiner—Minh-Tam Davis
(74) *Attorney, Agent, or Firm*—Wendy M. Lee

(57) **ABSTRACT**

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

82 Claims, 9 Drawing Sheets

PTO/SB/68 (04-01)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

In re Application of Carter

Application Number 07/715 272

Filed June 14, 91

Art Unit

Examiner

Paper No. 38

Assistant Commissioner for Patents
Washington, DC 20231

1. ☐ I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

☐(A) referred to in:

United States Patent Application Publication No. 6407213, page _____, line_______,

United States Patent Number________________________, column _______, line ______, or

an International Application which was filed on or after November 29, 2000 and which designates the United States, WIPO Pub. No. __________________, page _____, line_ ____.

☐(B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or 1.14(e)(2)(i), i.e., Application No.________________, paper No. ______, page ______, line ______.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Violetta Poindexter
Signature

5/20/03
Date

Violetta Poindexter
Typed or printed name

FOR PTO USE ONLY

Approved by: __________________.
(initials)

Unit: ________________________.

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

PTO/SB/68 (07-03)
Approved for use through 7/31/2003. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of

Application Number 07/715272
Filed 6-14-91

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
NOV 03 2003
File Information Unit

Paper No. #39

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ________, page, ________ line ________,

United States Patent Number 6639055 column ________, line, ________ or

WIPO Pub. No. ________, page ________, line ________.

Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

For published applications that are still pending, a member of the public may obtain a **copy** of:
- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

For unpublished applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a **copy** of:
- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
- the pending application as originally filed.

David Brown
Signature

11-3-03
Date

DAVID BROWN
Typed or printed name

Registration Number, if applicable

714182200
Telephone Number

FOR PTO USE ONLY
Approved by: PGK (initials)
Unit: FIU.

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/68 (07-03)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of
Carter

Application Number: 07/715,272 Filed: 06/14/91

Paper No. #40

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RE ED
NOV 19 2003
File Information Unit

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ______________, page, __________ line __________.

United States Patent Number 605427, column __________, line, ____________ or

WIPO Pub. No.________________, page __________, line __________.

Related Information about Access to Pending Applications (37 CFR 1.14):
Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:
For published applications that are still pending, a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.
For unpublished applications that are still pending:
(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.
(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the pending application as originally filed.

Violetta Poindexter
Signature

11/19/03
Date

Violetta Poindexter
Typed or printed name

____
Registration Number, if applicable

7/486-1150
Telephone Number

FOR PTO USE ONLY
Approved by: PK
(Initials)
Unit: FIU

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/68 (07-03)
Approved for use through 7/31/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of

Application Number	Filed
07/715272	6-14-91

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
DEC 0 4 2003
File Information Unit

Paper No. #41

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ________, page, ______ line ______,

United States Patent Number 6407213, column ______, line, ______ or

WIPO Pub. No. ________, page ______, line ______.

Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

<u>For published applications that are still pending</u>, a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.

<u>For unpublished applications that are still pending</u>:

(1) If the <u>benefit of the pending application is claimed</u> under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.

(2) If the application is <u>incorporated by reference or otherwise identified</u> in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the pending application as originally filed.

Signature

12-4-03
Date

WAYNE CROTEAU
Typed or printed name

Registration Number, if applicable

Telephone Number

FOR PTO USE ONLY

Approved by: ______ (initials)

Unit: File Information Unit

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/68 (07-03)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of

Application Number 07/715,272
Filed 6/14/91

Paper No. ~~7~~42

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
MAR 0 9 2004
File Information Unit

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ________, page ______, line ______.

United States Patent Number 6,054,297, column ______, line, ______ or

WIPO Pub. No. ________, page ______, line ______.

Related Information about Access to Pending Applications (37 CFR 1.14):
Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:
For published applications that are still pending, a member of the public may obtain a copy of:
  the file contents;
  the pending application as originally filed; or
  any document in the file of the pending application.
For unpublished applications that are still pending:
(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
  the file contents;
  the pending application as originally filed; or
  any document in the file of the pending application.
(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
  the pending application as originally filed.

Gary Wasserman
Signature

3/9/04
Date

GARY WASSERMAN
Typed or printed name

Registration Number, if applicable

(703) 931-4540
Telephone Number

RECEIVED
MAR 0 9 2004
File Information Unit

FOR PTO USE ONLY
Approved by: ______ (initials)
Unit: FIU

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS

PTO/SB/68 [illegible]
Approved for use through [illegible]. OMB 0651-[illegible]
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of
Carter et al

Application Number 07/715272
Filed 6/14/91

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1001
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

Paper No. #43

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ____________, page ________ line ________.

United States Patent Number 6639055, column ________, line, ________ or

WIPO Pub. No. ______________, page ________, line ________

Related Information about Access to Pending Applications (37 CFR 1.14):
Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows.
For published applications that are still pending, a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.
For unpublished applications that are still pending:
(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.
(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the pending application as originally filed.

Signature

4/2/04
Date

Rick Jonason
Typed or printed name

Registration Number, if applicable

703-418-2848
Telephone Number

FOR PTO USE ONLY
Approved by A. Bryant
(initials)
Unit:

RECEIVED
APR 0 2 2004
File Information Unit

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1001, 2021 South Clark Place, Arlington, VA.

If you need assistance in completing the form, call 1-800-[illegible]

PTO/SB/68

Approved for use through [illegible]. OMB 0651-[illegible]

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of

CARTOR

Application Number: 07/715272

Filed: 6/14/91

Paper No. #44

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1001
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
MAY 3 - 2004
File Information Unit

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ________, page ______, line ______,

United States Patent Number 6407213, column ______, line, ______ or

WIPO Pub. No. ________, page ______, line ______

Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

For published applications that are still pending, a member of the public may obtain a copy of:

the file contents;
the pending application as originally filed; or
any document in the file of the pending application.

For unpublished applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

the file contents;
the pending application as originally filed; or
any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

the pending application as originally filed.

Signature

5/4/03
Date

Rick Tomason
Typed or printed name

Registration Number, if applicable

703-418-2848
Telephone Number

FOR PTO USE ONLY

RECEIVED
MAY 3 - 2004
File Information Unit

Approved by: (initials)

Unit: FIU

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1001, 2021 South Clark Place, Arlington, VA.

If you need assistance in completing the form, call 1-800-[illegible]

6-45B

PTO/SB/68 (07-03)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of Carter

Application Number 07/715272
Filed 6/14/91

Paper No. #45

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
JUN 07 2004
File Information Unit

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ________, page, ________ line ________,

United States Patent Number 6407213, column ________, line, ________ or

WIPO Pub. No. ________, page ________, line ________.

Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

For published applications that are still pending, a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.

For unpublished applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the pending application as originally filed.

Anita Myles
Signature

6/7/04
Date

Anita Myles
Typed or printed name

Registration Number, if applicable

(703) 4154630
Telephone Number

FOR PTO USE ONLY
Approved by Bryant (initials)
Unit: File Information Unit

RECEIVED
JUN 07 2004
File Information Unit

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

PTO/SB/68 (07-03)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of

Application Number	Filed
07/715,272	06-14-91

Paper No. #46

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
JUN 09 2004
File Information Unit

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ______, page, ______ line ______,

United States Patent Number US 6,407,213 B1, column ______, line, ______ or

WIPO Pub. No. ______, page ______, line ______.

Related Information about Access to Pending Applications (37 CFR 1.14):
Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

For published applications that are still pending, a member of the public may obtain a copy of:
- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

For unpublished applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
- the pending application as originally filed.

Signature

06-09-04
Date

Rahel Haile
Typed or printed name

______
Registration Number, if applicable

703-486-1150
Telephone Number

FOR PTO USE ONLY
Approved by: ______ (Initials)
Unit: ______

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

PTO/SB/68 (07-03)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of Carter

Application Number 07/715272
Filed 6/14/91

Paper No. #47

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
AUG 16 2004
File Information Unit

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ______________, page, __________ line __________,

United States Patent Number 640728, column __________, line, ____________ or

WIPO Pub. No. ________________, page __________, line __________.

Related Information about Access to Pending Applications (37 CFR 1.14):
Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:
For published applications that are still pending, a member of the public may obtain a copy of:
- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

For unpublished applications that are still pending:
(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
- the pending application as originally filed.

Signature

8/16/04
Date

Violetta Poindexter
Typed or printed name

Registration Number, if applicable

4/486-1150
Telephone Number

FOR PTO USE ONLY
Approved by: (initials)
Unit:

RECEIVED
AUG 16 2004
File Information Unit

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/68 (07-03)
Approved for use through 7/31/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of

Application Number 07/715272

Filed Jun. 14, 91

Paper No. 48

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
NOV 01 2004
File Information Unit

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. 6800738, page, ______ line ______,

United States Patent Number ______, column ______, line, ______ or

WIPO Pub. No. ______, page ______, line ______.

Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

For published applications that are still pending, a member of the public may obtain a copy of:

- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

For unpublished applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

- the pending application as originally filed.

Kelvin Rodriguez
Signature

11-1-04
Date

Kelvin Rodriguez
Typed or printed name

Registration Number, if applicable

(703) 418-2772
Telephone Number

FOR PTO USE ONLY

RECEIVED
NOV 01 2004
File Information Unit

Approved (initials)

Unit: FIU

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

PTO/SB/68 (07-03)
Approved for use through 7/31/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of Carter

Application Number 07/715272

Filed 7/14/91

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

Paper No. #49

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ____________, page, ________ line ________, or

United States Patent Number 6040213, column ________, line ________, or

WIPO Pub. No.______________, page ________, line ________.

RECEIVED
NOV 29 2004
File Information Unit

Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

For published applications that are still pending, a member of the public may obtain a copy of:
- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

For unpublished applications that are still pending:
1. If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
   - the file contents;
   - the pending application as originally filed; or
   - any document in the file of the pending application.
2. If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
   - the pending application as originally filed.

Violetta Pancaker
Signature

11/29/04
Date

Violetta Pancaker
Typed or printed name

FOR PTO USE ONLY

Approved by: ________ (initials)

Unit ________

RECEIVED
NOV 29 2004
File Information Unit

____
Registration Number, if applicable

7486-1152
Telephone Number

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

PTO/SB/68 (11-04)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

Bring completed form to:
File Information Unit, Room 2E04
2900 Crystal Drive
Arlington, VA 22202-3514

Telephone: (703) 308-2733

In re Application of

Application Number 07/713272

Filed June 14, 1991

Paper No. 50

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ____________, page, ________ line ________,

United States Patent Number 6054297, column ________, line, __________ or

WIPO Pub. No.________________, page ________, line ________.

Related Information About Access to Applications Maintained in the Image File Wrapper System (IFW) and Access to Pending Applications in General

A member of the public, acting without a power to inspect, cannot order applications maintained in the IFW system through the FIU. If the member of the public is entitled to a copy of the application file, then the file is made available through the Public Patent Application Information Retrieval system (Public PAIR) on the USPTO internet web site (www.uspto.gov). Terminals that allow access to Public PAIR are available in the Public Search Room. The member of the public may also be entitled to obtain a copy of all or part of the application file upon payment of the appropriate fee. Such copies must be purchased through the **Office of Public Records** upon payment of the appropriate fee (37 CFR 1.19(b)).

For **published** applications that are still pending, a member of the public may obtain a **copy** of:
the file contents; the pending application as originally filed; or any document in the file of the pending application.

For **unpublished** applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a **copy** of: the file contents; the pending application as originally filed; or any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain **a copy** of the pending application as originally filed.

Darlene Jones
Signature

Jan. 18, 05
Date

Darlene Jones
Typed or printed name

Registration Number, if applicable

7/418·0330
Telephone Number

FOR PTO USE ONLY
JAN 18 2005
Approved by: ____________
(initials)
Unit: ____________

This collection of information is required by 37 CFR 1.11 and 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **BRING TO: File Information Unit, Room 2E04, 2900 Crystal Drive, Arlington, Virginia.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/68 (07-03)
Approved for use through 7/31/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
JAN 3 1 2005
File Information Unit

In re Application of
Carter et al

Application Number	Filed
07/715,272	6/14/91

Paper No. #51

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ____________, page, ________ line ________,

United States Patent Number 6,800,738, column 1, line, __________ or

WIPO Pub. No.______________, page ________, line ________.

Related Information about Access to Pending Applications (37 CFR 1.14):
Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:
For published applications that are still pending, a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.
For unpublished applications that are still pending:
(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.
(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the pending application as originally filed.

Rayline K. Petitt
Signature

Jan. 31, 2005
Date

Rayline K. Petitt
Typed or printed name

n/a
Registration Number, if applicable

703-415-3060
Telephone Number

FOR PTO USE ONLY
JAN 3 1 2005
Approved by: ________
(initials)
File Information Unit
Unit: ____________

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

PTO/SB/68 (07-03)
Approved for use through 7/31/2003. OMB 0651-0031
U.S. Patent and Trade[illegible]k Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of [illegible] no persons are required to respond to a collection of inform[illegible]nless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
APR 1 5 2005
File Information Unit

In re Application of

Application Number	Filed
07/715272	6-14-91

Paper No. #52

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ________, page, ________ line ________,

United States Patent Number 5821337, column ________, line, ________ or

WIPO Pub. No.________, page ________, line ________.

Related Information about Access to Pending Applications (37 CFR 1.14):
Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

For published applications that are still pending, a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.

For unpublished applications that are still pending:
(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the file contents;
the pending application as originally filed; or
any document in the file of the pending application.
(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:
the pending application as originally filed.

Signature

4-15-05
Date

WAYNE CROTEAU
Typed or printed name

____
Registration Number, if applicable

415-1077
Telephone Number

FOR PTO USE ONLY
Approved by: Bryant (initials)
Unit: ________

RECEIVED
APR 1 5 2005
File Information Unit

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

Best Available Copy

Under the Paperwork Reduction Act of 1995 no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of

Application Number 07/715272

Filed 6/4/91

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1001
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECE
SEP 12 2005
File Information Unit

Paper No. 53

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ________, page ______, line ______,

United States Patent Number 6407213, column ______, line ______, or

WIPO Pub. No. ________, page ______, line ______.

Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

For published applications that are still pending, a member of the public may obtain a copy of:

- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

For unpublished applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

- the file contents;
- the pending application as originally filed; or
- any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

- the pending application as originally filed.

Signature

RICKY GARCIA
Typed or printed name

Registration Number, if applicable

703 413 0600
Telephone Number

9/12/05
Date

FOR PTO USE ONLY

RECE

Approved by: SEP 1 9 2005
(Initials)

Unit: File Information Unit

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1001, 2021 South Clark Place, Arlington, VA.

PTO/SB/68 (07-03)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

In re Application of CARTER

Application Number 07/715272 Filed 6/15/92

Paper No. #59

Bring completed form to:
File Information Unit
Crystal Plaza Three, Room 1D01
2021 South Clark Place
Arlington, VA
Telephone: (703) 308-2733

RECEIVED
MAR 2 7 2006
File Information Unit

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. 04072/13, page, ________ line ________,

United States Patent Number ________________, column ________, line, ____________ or

WIPO Pub. No.________________, page ________, line ________.

Related Information about Access to Pending Applications (37 CFR 1.14):

Direct access to pending applications is not available to the public but copies may be available and may be purchased from the Office of Public Records upon payment of the appropriate fee (37 CFR 1.19(b)), as follows:

For published applications that are still pending, a member of the public may obtain a copy of:

the file contents;
the pending application as originally filed; or
any document in the file of the pending application.

For unpublished applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

the file contents;
the pending application as originally filed; or
any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain a copy of:

the pending application as originally filed.

Eric White
Signature

3/27/06
Date

ERIC WHITE
Typed or printed name

____
Registration Number, if applicable

____
Telephone Number

FOR PTO USE ONLY
RECEIVED
Approved by: MAR 2 7 2006
Unit: File Information Unit

This collection of information is required by 37 CFR 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. BRING TO: File Information Unit, Crystal Plaza Three, Room 1D01, 2021 South Clark Place, Arlington, VA.

PTO/SB/68 (11-04)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

Bring completed form to:
File Information Unit, Room 2E04
2900 Crystal Drive
Arlington, VA 22202-3514

Telephone: (703) 308-2733

In re Application of

Application Number 07/715272

Filed Jun 14, 1991

Paper No. #53

RECEIVED
SEP 0 1 2006
File Information Unit

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ________, page, ________ line ________,

United States Patent Number 5821337, column ________, line, ________ or

WIPO Pub. No. ________, page ________, line ________.

Related Information About Access to Applications Maintained in the Image File Wrapper System (IFW) and Access to Pending Applications in General

A member of the public, acting without a power to inspect, cannot order applications maintained in the IFW system through the FIU. If the member of the public is entitled to a copy of the application file, then the file is made available through the Public Patent Application Information Retrieval system (Public PAIR) on the USPTO internet web site (www.uspto.gov). Terminals that allow access to Public PAIR are available in the Public Search Room. The member of the public may also be entitled to obtain a copy of all or part of the application file upon payment of the appropriate fee. Such copies must be purchased through the **Office of Public Records** upon payment of the appropriate fee (37 CFR 1.19(b)).

For published applications that are still pending, a member of the public may obtain **a copy** of: the file contents; the pending application as originally filed; or any document in the file of the pending application.

For **unpublished** applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain **a copy** of: the file contents; the pending application as originally filed; or any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain **a copy** of the pending application as originally filed.

SFunes
Signature

Sal Funes
Typed or printed name

Registration Number, if applicable

703·413·3667
Telephone Number

06
Date

RECEIVED
SEP 0 1 2006
File Information Unit

FOR PTO USE ONLY
Approved by: ________ (initials)
Unit: F.I.U.

This collection of information is required by 37 CFR 1.11 and 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **BRING TO: File Information Unit, Room 2E04, 2900 Crystal Drive, Arlington, Virginia.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/68 (11-04)
Approved for use through 7/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN ABANDONED APPLICATION UNDER 37 CFR 1.14

Bring completed form to:
File Information Unit, Room 2E04
2900 Crystal Drive
Arlington, VA 22202-3514

Telephone: (703) 308-2733

In re Application of

Application Number 07/715272

Filed 6-14-91

Paper No. #54

I hereby request access under 37 CFR 1.14(a)(1)(iv) to the application file record of the above-identified ABANDONED application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and which is identified in, or to which a benefit is claimed, in the following document (as shown in the attachment):

United States Patent Application Publication No. ________, page, ________ line ________,

United States Patent Number 6407213 column ________, line, ________ or

WIPO Pub. No.________, page ________, line ________.

Related Information About Access to Applications Maintained in the Image File Wrapper System (IFW) and Access to Pending Applications in General

A member of the public, acting without a power to inspect, cannot order applications maintained in the IFW system through the FIU. If the member of the public is entitled to a copy of the application file, then the file is made available through the Public Patent Application Information Retrieval system (Public PAIR) on the USPTO internet web site (www.uspto.gov). Terminals that allow access to Public PAIR are available in the Public Search Room. The member of the public may also be entitled to obtain a copy of all or part of the application file upon payment of the appropriate fee. Such copies must be purchased through the **Office of Public Records** upon payment of the appropriate fee (37 CFR 1.19(b)).

For **published** applications that are still pending, a member of the public may obtain **a copy** of: the file contents; the pending application as originally filed; or any document in the file of the pending application.

For **unpublished** applications that are still pending:

(1) If the benefit of the pending application is claimed under 35 U.S.C. 119(e), 120, 121, or 365 in another application that has: (a) issued as a U.S. patent, or (b) published as a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain **a copy** of: the file contents; the pending application as originally filed; or any document in the file of the pending application.

(2) If the application is incorporated by reference or otherwise identified in a U.S. patent, a statutory invention registration, a U.S. patent application publication, or an international patent application publication in accordance with PCT Article 21(2), a member of the public may obtain **a copy** of the pending application as originally filed.

Signature

Date 9-1-06

WAYNE CROTEAU
Typed or printed name

Registration Number, if applicable

415-1077
Telephone Number

FOR PTO USE ONLY

Approved by: (initials)

Unit:

RECEIVED
SEP 01 2006
File Information Unit

This collection of information is required by 37 CFR 1.11 and 1.14. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **BRING TO: File Information Unit, Room 2E04, 2900 Crystal Drive, Arlington, Virginia.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

US006407213B1

(12) **United States Patent**
Carter et al.

(10) **Patent No.: US 6,407,213 B1**
(45) **Date of Patent: Jun. 18, 2002**

---

(54) **METHOD FOR MAKING HUMANIZED ANTIBODIES**

(75) Inventors: **Paul J. Carter**; **Leonard G. Presta**, both of San Francisco, CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/146,206**

(22) PCT Filed: **Jun. 15, 1992**

(86) PCT No.: **PCT/US92/05126**

§ 371 (c)(1),
(2), (4) Date: **Nov. 17, 1993**

Related U.S. Application Data

(63) Continuation-in-part of application No. 07/715,272, filed on Jun. 14, 1991, now abandoned.

(51) **Int. Cl.**^{7} **C07K 16/00**

(52) **U.S. Cl.** 530/387.3; 435/69.6; 435/69.7; 435/70.21; 435/91; 536/23.53; 424/133.1

(58) **Field of Search** 435/69.6, 69.7, 435/70.21, 91, 172.2, 240.1, 240.27, 252.3, 320.1, 328; 536/23.53; 424/133.1; 530/387.3

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,816,567 A	3/1989	Cabilly et al.		
4,845,198 A	7/1989	Urdal et al.	....	530/388.22
5,132,405 A	7/1992	Huston et al.	....	530/387.3
5,225,539 A	7/1993	Winter	....	530/389.3
5,530,101 A	6/1996	Queen et al.	....	530/387.3
5,558,864 A	9/1996	Bendig et al.	....	424/133.1
5,585,089 A	12/1996	Queen et al.	....	424/133.1
5,677,171 A	10/1997	Hudziak et al.	....	435/7.23
5,693,762 A	* 12/1997	Queen et al.	....	530/387.2
5,714,350 A	2/1998	Co et al.	....	435/69.6
5,772,997 A	6/1998	Hudziak et al.	....	424/130.1
5,821,337 A	10/1998	Carter et al.	....	530/387.3
5,834,598 A	11/1998	Lowman et al.	....	530/399
5,859,205 A	1/1999	Adair et al.	....	530/387.3

FOREIGN PATENT DOCUMENTS

AU	85058/91	3/1992	....	C07K/15/12
EP	120694	10/1984		
EP	125023 A1	11/1984		
EP	0 239 400	* 9/1987	....	C12N/15/00
EP	323806 A1	7/1989		
EP	328404 A1	8/1989	....	A61K/39/395
EP	338745 A1	10/1989		
EP	365209 A2	4/1990		
EP	365997 A2	5/1990		
EP	368684	5/1990		
EP	403156 A1	12/1990		
EP	438310 A2	7/1991		
EP	438312 A2	7/1991		
EP	440351 A2	8/1991		
EP	0 460 167 B1	12/1991		
EP	0 519 596 A1	12/1992		
EP	0 592 106 A1	4/1994		
EP	0 620 276	10/1994		
EP	682040 A1	11/1995		
EP	451216 B1	1/1996	....	C12P/21/08
EP	432249 B1	9/1996		
GB	2 188941	10/1987		
WO	WO 87/02671	5/1987		
WO	WO 88/09344	12/1988		
WO	WO 89/01783	3/1989		
WO	WO 89/06692	7/1989		
WO	WO 89/09622	10/1989		
WO	WO 90/07861	7/1990		
WO	90/07861	* 7/1990	....	C12P/21/00
WO	WO 91/07492	5/1991		
WO	WO 91/07500	5/1991		
WO	WO 91/09966	7/1991	....	C12P/21/08
WO	WO 91/09968	7/1991	....	C12P/21/08
WO	WO 91/09967	11/1991		
WO	WO 92/01047	1/1992		
WO	WO 92/04380	3/1992		
WO	WO 92/04381	3/1992		
WO	WO 92/05274	4/1992		
WO	WO 92/11383	7/1992		
WO	WO 92/11018	9/1992	....	A61K/35/14
WO	WO 92/15683	9/1992		
WO	WO 92/16562	10/1992		
WO	WO 92/22653	12/1992		
WO	WO 93/02191	2/1993		
WO	94/11509	5/1994		
WO	WO 94/12214	6/1994		

OTHER PUBLICATIONS

Riechmann et al. [Nature 332:323–327 (1988)].*

Queen et al. [Proc. Natl. Acad. Sci. 86;10029–10033 (1989)].*

Roitt [*Immunology*, published 1985, by Gower Medical Publishing Ltd. (London, England) p. 5.5].*

Tramontano et al. [J. Mol. Biol. 215:175–182 (1990)].*

"Biosym Technologies" in New Products, Chemical Design Automation 3 (Dec. 1988).

"Polygen Corporation" in New Products, Chemical Design Automation 3 (Nov. 1988).

Adair et al., "Humanization of the murine anti–human CD3 monoclonal antibody OKT3" *Hum. Antibod. Hybridomas* 5:41–47 (1994).

Chothia et al., "Principles of protein–protein recognition" *Nature* 256:705–708 (1975).

Chothia et al., "Transmission of conformational change in insulin" *Nature* 302:500–505 (1983).

Corti et al., "Idiotope Determining Regions of a Mouse Monoclonal Antibody and Its Humanized Versions" *J. Mol. Biol.* 235:53–60 (1994).

(List continued on next page.)

Primary Examiner—Anthony C. Caputa
Assistant Examiner—Minh-Tam Davis
(74) *Attorney, Agent, or Firm*—Wendy M. Lee

(57) **ABSTRACT**

Variant immunoglobulins, particularly humanized antibody polypeptides are provided, along with methods for their preparation and use. Consensus immunoglobulin sequences and structural models are also provided.

82 Claims, 9 Drawing Sheets