immunoglobulin, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is a consensus sequence of human immunoglobulin heavy chain variable region frameworks.

40. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant within about four-fold of the donor immunoglobulin, wherein said humanized immunoglobulin heavy chain comprises one or more amino acids from the donor immunoglobulin heavy chain framework outside the Kabat and Chothia CDRs, wherein the donor amino acids substitute for corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) contains an atom within a distance of 6 ANGSTROM of a CDR in said humanized immunoglobulin.

41. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variableregion frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^7 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework and comprises at least 70 amino acid residues identical to an acceptor human immunoglobulin heavy chain variable region amino acid sequence.

42. A humanized immunoglobulin according to claim 41 which is an antibody comprising two light chain/heavy chain dimers.

43. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no

greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is a consensus sequence of human immunoglobulin heavy chain variable region frameworks.

44. A pharmaceutical composition comprising a humanized immunoglobulin of claim 41 in a pharmaceutically acceptable carrier.

45. A method of producing the humanized immunoglobulin of claim 41 comprising:

introducing DNA segments encoding the humanized immunoglobulin heavy and light chains into a cell; and

expressing the DNA segments in the cell to produce the humanized immunoglobulin.

46. A method of producing a humanized immunoglobulin, comprising the steps of:

(1) comparing the sequence of a donor immunoglobulin heavy chain variable region against a collection of sequences of human heavy chain variable regions;

(2) selecting a human heavy chain variable region from the collection of human heavy chain variable regions to provide an acceptor heavy chain variable region, wherein the selected

variable region framework is at least 65% identical to the donor immunoglobulin heavy chain variable region framework;

(3) synthesizing a DNA segment encoding a humanized heavy chain variable region, comprising CDRs from the donor immunoglobulin heavy chain variable region and a variable region framework from the selected acceptor heavy chain variable region;

(4) introducing the DNA segment encoding the humanized immunoglobulin heavy chain variable region and a DNA segment encoding a humanized immunoglobulin light chain variable region into a cell; and

(5) expressing the DNA segments in the cell to produce the humanized immunoglobulin.

47. A method of producing a humanized immunoglobulin, comprising the steps of:

(1) comparing the sequence of a donor immunoglobulin light chain variable region against a collection of sequences of human light chain variable regions;

(2) selecting a human light chain variable region from the collection of human light chain variable regions to provide an acceptor light chain variable region, wherein the selected

variable region framework is at least 65% identical to the donor immunoglobulin light chain variable region framework;

(3) synthesizing a DNA segment encoding a humanized light chain variable region, comprising CDRs from the donor immunoglobulin light chain variable region and a variable region framework from the selected acceptor light chain variable region;

(4) introducing the DNA segment encoding the humanized immunoglobulin light chain variable region and a DNA segment encoding a humanized immunoglobulin heavy chain variable region into a cell; and

(5) expressing the DNA segments in the cell to produce the humanized immunoglobulin.

48. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant within about four-fold of that of the donor immunoglobulin, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is a consensus sequence of human immunoglobulin heavy chain variable region frameworks.

REMARKS

Newly added claims 32-40 have been copied from claims in Queen et al., U.S. Patent No. 5,693,761. Claims 41-48 have been copied from claims in Queen et al., U.S. Patent No. 5,693,762. Copies of both patents are enclosed. Applicants are in compliance with 35 USC §135(b) since both Queen patents were issued on December 2, 1997.

Respectfully submitted,

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Immunology

A humanized antibody that binds to the interleukin 2 receptor

(chimeric antibody/antibody affinity/autoimmune disease)

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Contributed by Thomas A. Waldmann, August 30, 1989

ABSTRACT **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 3×10^9 M^{-1}, about 1/3 that of murine anti-Tac.**

The cellular receptor for the lymphokine interleukin 2 (IL-2) plays an important role in regulation of the immune response (reviewed in ref. 1). The complete IL-2 receptor (IL-2R) consists of at least two IL-2-binding peptide chains: the p55 or Tac peptide (2, 3), and the recently discovered p75 peptide (4, 5). Identification and characterization of the p55 peptide were facilitated by the development of a monoclonal antibody, anti-Tac, which binds to human p55 (2). The p55 peptide was found to be expressed on the surface of T cells activated by an antigen or mitogen but not on resting T cells. Treatment of human T cells with anti-Tac antibody strongly inhibits their proliferative response to antigen or to IL-2 by preventing IL-2 binding (3, 6).

These results suggested that anti-IL-2R antibodies would be immunosuppressive when administered *in vivo*. Indeed, injection of an anti-IL-2R antibody into mice and rats greatly prolonged survival of heart allografts (7, 8). Anti-IL-2R was also effective in rats against experimental graft-versus-host disease (9). In animal models of autoimmune disease, an anti-IL-2R antibody alleviated insulitis in nonobese diabetic mice and lupus nephritis in NZB × NZW mice (10). Anti-Tac itself was highly effective in prolonging survival of kidney allografts in cynomolgus monkeys (11).

In human patients, the specificity of anti-Tac for activated T cells might give it an advantage as an immunosuppressive agent over OKT3 (monoclonal anti-CD3), which is effective in treating kidney transplant rejection (12), but which suppresses the entire peripheral T-cell population. In fact, in phase I clinical trials for kidney transplantation, prophylactic administration of anti-Tac significantly reduced the incidence of early rejection episodes, without associated toxicity (13). Furthermore, treatment with anti-Tac induced temporary partial or complete remission in three of nine patients with Tac-expressing adult T-cell leukemia (14). However, as a murine monoclonal antibody, anti-Tac elicits a strong human antibody response against itself, as does OKT3 (15). This response would prevent its long-term use in treating autoimmune conditions or suppressing organ transplant rejection.

The immune response against a murine monoclonal antibody may potentially be reduced by transforming it into a chimeric antibody. Such antibodies, produced by methods of genetic engineering, combine the variable (V) region binding domain of a mouse (or rat) antibody with human antibody constant (C) regions (16–18). Hence, a chimeric antibody retains the binding specificity of the original mouse antibody but contains less amino acid sequence foreign to the human immune system. Chimeric antibodies have been produced against a number of tumor-associated antigens (19–21). In some but not all cases, the chimeric antibodies have mediated human complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC) more efficiently than the mouse antibodies (21).

When the murine antibody OKT3 is used in human patients, much of the resulting antibody response is directed against the V region of OKT3 rather than the C region (15). Hence, chimeric antibodies in which the V region is still nonhuman may not have sufficient therapeutic advantages over mouse antibodies. To further reduce the immunogenicity of murine antibodies, Winter and colleagues constructed "humanized" antibodies in which only the minimum necessary parts of the mouse antibody, the complementarity-determining regions (CDRs), were combined with human V region frameworks and human C regions (22–25). We report here the construction of chimeric and humanized anti-Tac antibodies.‖ For the humanized antibody, sequence homology and molecular modeling were used to select a combination of mouse and human sequence elements that would reduce immunogenicity while retaining high binding affinity.

MATERIALS AND METHODS

Construction of Plasmids. cDNA cloning was by the method of Gubler and Hoffman (26), and sequencing was by the dideoxy method (27). The plasmid pVκ1 (Fig. 1*A*) was constructed from the following fragments: an approximately 4550-base-pair (bp) *Bam*HI–*Eco*RI fragment from the plas-

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Abbreviations: IL-2R, interleukin 2 receptor; CDR, complementarity-determining region; CDC, complement-dependent cytotoxicity; ADCC, antibody-dependent cellular cytotoxicity; V, variable; J, joining; C, constant.

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‖The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M28250 and M28251).

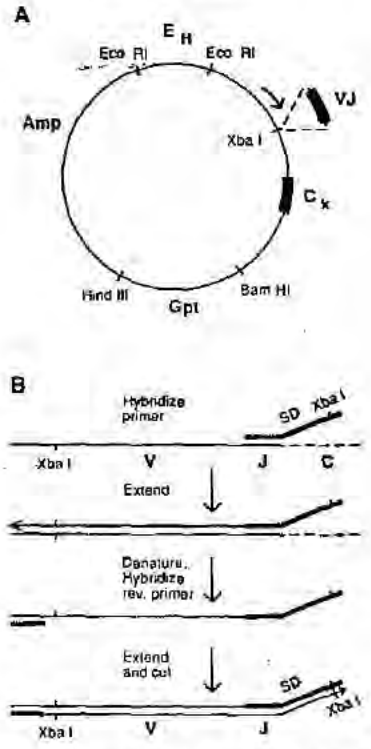

FIG. 1. (*A*) Schematic diagram of the plasmids pVκ1 and pLTac. Light chain exons are shown as boxes. An arrow indicates the direction of transcription from the κ promoter. E_H, heavy chain enhancer. Not drawn to scale. (*B*) Schematic diagram of the method used to excise the *V–J* region. SD, splice donor sequence; rev. primer, reverse primer.

mid pSV2gpt (28) containing the *amp* and *gpt* genes; an 1800-bp *Eco*RI–*Bgl* II fragment from pKcatH (29) containing the heavy chain enhancer and κ promoter; and a 1500-bp *Eco*RI–*Xba* I fragment containing the human C_κ region (30). Similarly, pVγ1 was constructed starting from a 4850-bp *Bam*HI–*Eco*RI fragment of the plasmid pSV2hph (a gift of A. Smith, A. Miyajima, and D. Strehlow, Stanford University), which is analogous to pSV2gpt except that the *gpt* gene is replaced by the *hyg* gene (31). This fragment was combined with the *Eco*RI–*Bgl* II fragment from pKcatH and a 2800-bp *Hin*dIII–*Pvu* II fragment containing the human γ1 constant region, isolated from a phage kindly provided by L. Hood (32). In each case, the fragments were combined by standard methods (ref. 33, pp. 390–401), with an *Xba* I linker inserted between the κ promoter fragment and the 5′ end of the C region fragment.

Construction of Chimeric Genes. *Eco*RI fragments containing the anti-Tac light and heavy chain cDNAs were separately inserted into the *Eco*RI site of the phage M13mp11D, a variant of M13mp11 (34) in which the *Eco*RI and *Xba* I sites of the polylinker were filled in and joined. The resulting phage, in which the 5′ ends of the cDNAs abutted the *Xba* I site, were respectively denoted M13L and M13H. The V–J (J, joining) segments of the cDNAs, followed by splice donor signals, were precisely excised from these phage, using a double-priming scheme (Fig. 1*B*). For the light chain, the following primer was synthesized (Applied Biosystems model 380B DNA synthesizer) and purified by gel electrophoresis: 5′-CCAGAATTCTAGAAAAGTGTACTTAC-GTTTCAGCTCCAGCTTGGTCCC-3′. From the 3′ end, the first 22 residues of the primer are the same as the last 22 residues of the $J_\kappa 5$ segment (noncoding strand). The next 16 nucleotides are the same as the sequence that follows $J_\kappa 5$ in mouse genomic DNA and therefore includes a splice donor signal. The final 10 nucleotides of the oligonucleotide include an *Xba* I site.

We hybridized this oligonucleotide to M13L and extended it with the Klenow fragment of DNA polymerase. The DNA was heat-denatured, hybridized with an excess of the "reverse primer" 5′-AACAGCTATGACCATG-3′, again extended with Klenow DNA polymerase, and digested with *Xba* I. The digested DNA was run on a gel, and an approximately 400-bp fragment was excised and inserted into the *Xba* I site of pVκ1. Sequencing showed that the fragment consisted of the V–J region of the light chain cDNA followed by the splice donor "tail," as expected (Fig. 1*B*), and pLTac, a clone with the appropriate orientation, was chosen. In an analogous fashion, the heavy chain V–J segment, followed by the mouse J_H2 splice donor sequence, was excised from M13H and inserted into the *Xba* I site of pVγ1 to yield pGTac.

Computer Analysis. Sequences were manipulated and homology searches were performed with the MicroGenie Sequence Analysis Software (Beckman). The molecular model of the anti-Tac V region was constructed with the ENCAD program (35) and examined with the MIDAS program (36) on an IRIS 4D-120 graphics workstation (Silicon Graphics).

Construction of Genes for Humanized Antibody. Nucleotide sequences were selected that encoded the protein sequences of the humanized light and heavy chain V regions including signal peptides (*Results*), generally utilizing codons found in the mouse anti-Tac sequence. These nucleotide sequences also included the same splice donor signals used in the chimeric genes and an *Xba* I site at each end. For the heavy chain V region, four overlapping 120- to 130-nucleotide-long oligonucleotides were synthesized that encompassed the entire sequence on alternating strands. The oligonucleotides were phosphorylated with polynucleotide kinase, annealed, extended with T4 DNA polymerase, cut with *Xba* I, and ligated into the *Xba* I site of pUC19 (34), using standard reaction conditions. An insert with the correct sequence was recloned in pVγ1. The humanized light chain V region was constructed similarly.

Transfections. For each antibody constructed, the light chain plasmid was first transfected into Sp2/0 mouse myeloma cells (ATTC CRL 1581) by electroporation (Bio-Rad Gene Pulser) and cells were selected for *gpt* expression (28). Clones secreting a maximal amount of light chain, as determined by ELISA, were transfected with the heavy chain plasmid and cells were selected for hygromycin B resistance (31). Clones secreting a maximal amount of complete antibody were detected by ELISA. The clones were used for preparation of chimeric and humanized antibodies.

Antibody Purification. Medium from confluent cells was passed over a column of staphylococcal protein A-Sepharose CL-4B (Pharmacia), and antibody was eluted with 3 M $MgCl_2$. Antibody was further purified by ion-exchange chromatography on BakerBond ABx (J. T. Baker). Final antibody concentration was determined, assuming that 1 mg/ml has an A_{280} of 1.4. Anti-Tac antibody itself was purified as described (2).

Affinity Measurements. Affinities were determined by competition binding. HuT-102 human T-lymphoma cells (ATTC TIB 162) were used as source of p55 Tac antigen. Increasing amounts of competitor antibody (anti-Tac, chimeric, or humanized) were added to 1.5 ng of radioiodinated (Pierce Iodo-Beads) tracer anti-Tac antibody (2 μCi/μg; 1 Ci = 37 GBq) and incubated with 4×10^5 HuT cells in 0.2 ml of binding buffer (RPMI 1040 medium with 10% fetal calf serum, human IgG at 100 μg/ml, 0.1% sodium azide) for 3 hr at room temperature. Cells were washed and pelleted, and their radioactivities were measured, and the concentrations of bound and free tracer antibody were calculated. The affinity of mouse anti-Tac was determined by Scatchard plot analy-

sis, using anti-Tac itself as the competitor. Then the affinities of the chimeric and humanized antibodies were each calculated according to the formula $[X] - [\text{anti-Tac}] = (1/K_x) - (1/K_a)$, where K_a is the affinity of anti-Tac ($9 \times 10^9\ M^{-1}$), K_x is the affinity of the competitor X, [ ] indicates the concentration of competitor antibody at which bound/free tracer binding is $R_0/2$, and R_0 is maximal bound/free tracer binding (37).

RESULTS

Cloning of Light and Heavy Chain cDNA. A cDNA library in λgt10 was prepared from anti-Tac hybridoma cells and screened with oligonucleotide probes for the mouse κ and γ2a constant regions. The cDNA inserts from four κ-positive and four γ2a-positive phage were subcloned in M13mp19. Partial sequencing showed that two of the κ isolates had one sequence, and the other two had another sequence. In one pair, a V_κ gene segment was joined to the $J_\kappa 2$ segment out of its reading frame. In addition, the conserved cysteine at position 23 was absent from this V segment, and the sequences of the two isolates differed slightly. Presumably, these clones were the result of an aberrant joining event in one κ allele, which continued to undergo somatic mutation after the formation of the hybridoma.

The V–J segments of the other pair of κ clones were sequenced completely and were identical. This light chain uses the $J_\kappa 5$ segment. Partial sequencing of the four γ2a clones showed they were all from the same gene. The V–J segments of two were sequenced completely and were identical. This heavy chain uses the $J_H 2$ segment and is of subgroup II (38). The DNA sequences have been deposited with GenBank; the deduced protein sequences are shown in Fig. 2. As both alleles of the κ light chain were accounted for and only one heavy chain sequence was detected, we tentatively assigned these sequences to the anti-Tac antibody genes.

Construction of Chimeric Genes. Plasmid vectors were prepared for the construction and expression of chimeric light and heavy chain genes. The plasmid pVκ1 (Fig. 1*A*) contains the human genomic C_κ segment, including 336 bp of the preceding intron and the poly(A) signal. It also contains the promoter sequence from the MOPC 41 κ gene and the heavy chain enhancer sequence, which synergize to form a very strong transcriptional unit (29). There is a unique *Xba* I site between the promoter and the intron. A similar plasmid, pVγ1, was prepared by using the human $C_\gamma 1$ region in place of the C_κ region. In that case, the region inserted between the *Xba* I and *Bam*HI sites extended from about 210 bp 5′ of the $C_H 1$ exon to beyond the $C_H 3$ exon.

Our strategy was to insert the V–J region from the anti-Tac κ cDNA, followed by a splice donor signal, at the *Xba* I site of pVκ1 to construct the plasmid pLTac. Doing so created a chimeric κ gene with a short synthetic intron between the mouse V–J and human C_κ segments (Fig. 1*A*). For this purpose, we used a form of double primer-directed mutagenesis (*Materials and Methods*; Fig. 1*B*). Similarly, the V–J region from the anti-Tac γ2a heavy chain cDNA, followed by a splice donor signal, was inserted into the *Xba* I site of pVγ1. The resulting plasmid, pGTac, contained a chimeric heavy chain gene, with a synthetic intron between the mouse V–J and human $C_\gamma 1$ segments.

Construction of a Humanized Anti-Tac Antibody. 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. The anti-Tac heavy chain sequence was therefore compared by computer with all the human heavy chain sequences in the National Biomedical Research Foundation Protein Identification Resource (release 15). The heavy chain V region of the Eu antibody (of human heavy chain subgroup I; ref. 38) was 57% identical to the anti-Tac heavy chain V region (Fig. 2*B*); all other complete V_H regions in the data bank were 30–52% identical. However, no one human light chain V region was especially homologous to the anti-Tac light chain. We therefore chose to use the Eu light chain (of human light chain subgroup I; ref. 38) together with the Eu heavy chain to supply the framework sequences for the humanized antibody. The CDRs in the humanized antibody were of course chosen to be identical to the anti-Tac CDRs (Fig. 2).

A computer program was used to construct a plausible molecular model of the anti-Tac V domain (Fig. 3), based on homology to other antibody V domains with known crystal structure and on energy minimization. Graphic manipulation shows that a number of amino acid residues outside of the CDRs are in fact close enough to them to either influence their conformation or interact directly with antigen. When these residues differ between the anti-Tac and Eu antibodies, the residue in the humanized antibody was chosen to be the anti-Tac residue rather than the Eu residue. This choice was made for residues 27, 30, 48, 67, 68, 98, and 106 in the humanized heavy chain, and for 47 and 59 in the humanized light chain (Figs. 2 and 3; amino acids shown in blue in Fig. 3), although we now consider the light chain residue 59, which was chosen on the basis of an earlier model, to be doubtful. In this way, we hoped to better preserve the precise structure of the CDRs at the cost of possibly making the humanized antibody slightly less "human."

Different human light or heavy chain V regions exhibit strong amino acid homology outside of the CDRs, within the framework regions. However, a given V region will usually

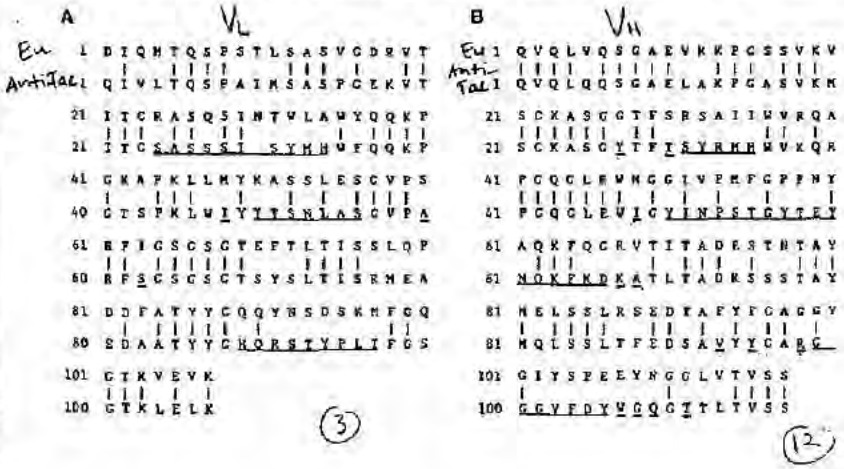

FIG. 2. Amino acid sequences of the humanized anti-Tac light (*A*) and heavy (*B*) chains. The sequences of the Eu antibody light and heavy chains (upper lines) are shown aligned above the mouse anti-Tac light and heavy chain sequences (lower lines), with a | indicating identity of amino acids. The three CDRs in each chain are underlined, and the other mouse amino acids used in the humanized antibody are double underlined. Hence, the humanized sequences are the same as the upper (Eu) sequences, except where the amino acid is underlined or double underlined.

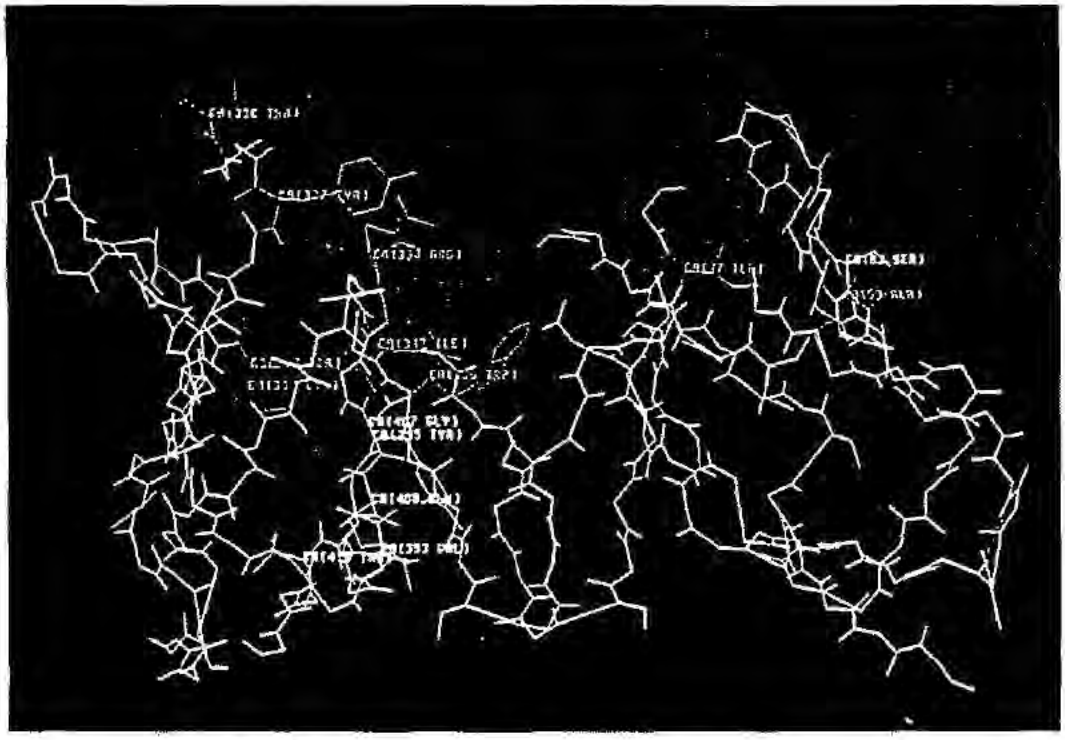

FIG. 3. Model of the mouse anti-Tac antibody V region, generated with the ENCAD program and displayed with the MIDAS program. Amino acids in the CDRs are shown in red; amino acids potentially interacting with the CDRs are shown in blue; other mouse amino acids used in the humanized antibody are shown in yellow, as described in the text. Thus, all amino acids transferred from the anti-Tac sequence to the humanized antibody are shown in red, blue, or yellow. Residue 1 is the first amino acid of V_κ; residue 301 is the first amino acid of V_H.

contain exceptional amino acids, atypical of other human V regions, at several framework positions. The Eu antibody contains such unusual residues at positions corresponding to 93, 95, 98, 106, 107, 108, and 110 of the humanized heavy chain and 47 and 62 of the light chain (Fig. 2), as determined by visual comparison of the Eu heavy and light chain V regions with other human V regions of subgroup I (38). The Eu antibody contains several other unusual residues, but at the listed positions, the murine anti-Tac antibody actually has a residue much more typical of human sequences than does Eu. At these positions, we therefore chose to use the anti-Tac residue rather than the Eu residue in the humanized antibody, to make the antibody more generically human. Some of these residues had already been selected because of their proximity to the CDRs, as described above (the remaining ones are shown in yellow in Fig. 3).

These criteria allowed the selection of all amino acids in the humanized antibody V regions as coming from either anti-Tac or Eu (Fig. 2). DNA segments encoding the desired heavy and light chain amino acid sequences were synthesized. These DNA segments also encoded typical immunoglobulin signal sequences for processing and secretion, and they contained splice donor signals at their 3′ end. The light and heavy chain segments were cloned, respectively, in pVκ1 and pVγ1 to form the plasmids pHuLTac and pHuGTac.

Properties of Chimeric and Humanized Antibodies. Sp2/0 cells, a nonproducing mouse myeloma line, were transfected sequentially with pLTac and pGTac (chimeric genes) or with pHuLTac and pHuGTac (humanized genes). Cell clones were selected first for antibiotic resistance and then for maximal antibody secretion, which reached 3 μg/10^6 cells per 24 hr. S1 nuclease mapping of RNA extracted from the cells transfected with pLTac and pGTac showed that the synthetic introns between the V and C regions (Fig. 1*A*) were correctly spliced (data not shown). Antibody was purified from the culture medium of cells producing the chimeric or humanized antibody. When analyzed by reducing SDS/polyacrylamide gel electrophoresis, the antibodies showed only two bands, having the expected molecular weights 50,000 and 25,000.

Flow cytometry showed that the chimeric and humanized antibodies bound to Hut-102 and CRII.2 cells, two human T-cell lines that express the p55 chain of the IL-2R, but not to CEM and other cell lines that do not express the IL-2R. To determine the binding affinity of the chimeric and humanized antibodies, their ability to compete with labeled mouse anti-Tac for binding to Hut-102 cells was determined. The affinity of chimeric anti-Tac was indistinguishable from that of anti-Tac (data not shown), as expected from the fact that their entire V regions are identical. The affinity of humanized anti-Tac for membrane-bound p55 was 3×10^9 M^{-1}, about 1/3 the measured affinity of 9×10^9 M^{-1} of anti-Tac itself (Fig. 4).

DISCUSSION

Because monoclonal antibodies can be produced that are highly specific for a wide variety of cellular targets, antibody therapy holds great promise for the treatment of cancer, autoimmune conditions, and other diseases. However, this promise has not been widely realized, largely because most monoclonal antibodies, which are of mouse origin, are immunogenic when used in human patients and are ineffective at recruiting human immune effector functions such as CDC and ADCC. A partial solution to this problem is the use of chimeric antibodies (16), which combine the V region binding domains of mouse antibodies with human antibody C regions. Initially, chimeric antibodies were constructed by combining genomic clones of the V and C region genes. However, this method is very time consuming because of the difficulty of genomic cloning, especially from tetraploid hybridomas.

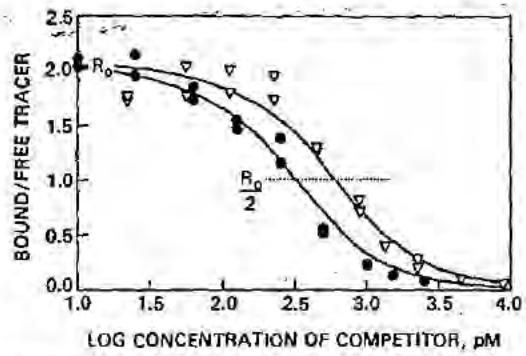

FIG. 4. Competitive binding of labeled anti-Tac tracer to Hut-102 cells. Duplicate samples are shown. ●, Mouse anti-Tac competitor; ▽, humanized anti-Tac competitor.

More recently, cDNA clones of the V and C regions have been combined, but this method is also tedious because of the need to join the V and C regions precisely (20, 21). Here we show that the V region from a readily obtainable cDNA clone can be easily joined to a human genomic C region, which need only be cloned once, by leaving a synthetic intron between the V and C regions. When linked to suitable transcriptional regulatory elements and transfected into an appropriate host cell, such chimeric genes produce antibody at a high level.

Chimeric antibodies represent an improvement over mouse antibodies for use in human patients, because they are presumably less immunogenic and sometimes mediate CDC or ADCC more effectively (21). For example, chimeric anti-Tac mediates ADCC with activated human effector cells, whereas murine anti-Tac does not (unpublished data). However, the mouse V region can itself be highly immunogenic (15). Winter and colleagues therefore took the further, innovative, step of combining the CDRs from a mouse (or rat) antibody with the framework region from a human antibody (22–25), thus reducing the xenogeneic elements in the humanized antibody to a minimum. Unfortunately, in some cases the humanized antibody had significantly less binding affinity for antigen than did the original mouse antibody. This is not surprising, because transferring the mouse CDRs from the mouse framework to the human framework could easily deform them.

In humanizing the anti-Tac antibody, which binds to the p55 chain of the human IL-2R, we have introduced two ideas that may have wider applicability. First, the human framework was chosen to be as homologous as possible to the original mouse antibody to reduce any deformation of the mouse CDRs. Second, computer modeling was used to identify several framework amino acids in the mouse antibody that might interact with the CDRs or directly with antigen, and these amino acids were transferred to the human framework along with the CDRs. The resulting humanized antibody has a high affinity, 3×10^9 M^{-1}, for its antigen. Further work is needed to determine to what extent the choice of human framework and the preservation of particular mouse amino acids in fact contributed to the affinity of the humanized antibody. The extent to which humanization eliminates immunogenicity will need to be addressed in clinical trials, where humanized anti-Tac will be administered to patients with Tac-expressing lymphomas or selected autoimmune diseases or to patients receiving organ transplants.

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United States Patent [19]

Adair et al.

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[54] **HUMANISED ANTIBODIES**

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[51] **Int. Cl.**[6] **A61K 39/395**

[52] **U.S. Cl.** **530/387.3**; 530/387.1

[58] **Field of Search** 530/387.1, 387.3, 530/388.22, 867, 864

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[57] **ABSTRACT**

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at 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)**. The CDR-grafted light chains comprise donor residues at at least one of positions **(1)** and/or **(3)** and **(46)** and/or **(47)** or at at least one of positions **(46, 48, 58)** and **(71)**. The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for in vivo therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

8 Claims, 18 Drawing Sheets

Carter Exhibit 2024
Carter v. Adair
Interference No. 105,744

```
  1  GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
 51  ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
101  gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
151  gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
201  ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
251  agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
301  caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
351  tggagtagta  accattcac gttcggctcg gggacaaagt tggaaataaa
401  ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
451  agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
501  cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
551  tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
601  gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
651  agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
701  gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
751  CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
801  CCACAAGCGC tTACCACTGT TGCGGTGCTC TAAACCTCCT CCCACCTCCT
851  TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
901  AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
(SEQ ID NO:4)
```

FIG. 1a

```
  1  MDFQVQIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
 51  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT  SYSLTISGME
101  AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP  PSSEQLTSGG
151  ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK  DSTYSMSSTL
201  TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC* (SEQ ID NO:5)
```

FIG. 1b

```
   1  GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATGGAAAGGC
  51  ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101  GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151  GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201  ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251  ATTCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301  CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351  GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401  GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451  CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501  GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551  TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601  TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651  GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701  AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 801  ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851  GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901  GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951  AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001  CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051  CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101  AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151  GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201  CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251  ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301  TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351  CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401  ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451  CAGCACCCAC AAAACTCTCA GGTCCAAAGA GAGACCCACA CTCATCTCCA
1501  TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC  (SEQ ID NO:6)
```

FIG. 2a

OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK* (SEQ ID NO:7)
```

FIG. 2b

```
                1                   23                  42
                NN      N             N       N     N
RES TYPE        SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3vl          QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI             DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQIPGK
                ? ?
                  CDR1   (LOOP)          *******
                  CDR1   (KABAT)        **********

                          56                            85
                N  NN
RES TYPE        *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3vl          SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI             APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ
ID NO:8)
                ?  ??                          ?  ?
                      *******  CDR2 (LOOP/KABAT)

                             102   108
RES TYPE        PiPIPies**iPIIsPPSPSPSS
Okt3vl          YYCQQWSSNPFTFGSGTKLEINR (SEQ ID NO:29)
REIvl           YYCQQYQSLPYTFGQGTKLQITR (SEQ ID NO:9)
                             ?    ?
                   ******        CDR3 (LOOP)
                 *********       CRD3(KABAT)
```

FIG. 3

```
          NN N                    23 26    32 35  N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePi^PIpiesss
Okt3h     QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMNHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?                  ??
                                      ******     CDR1 (LOOP)
                                         *****   CDR1 (KABAT)

                   52a   60     65    N N  N   82abc     89
RES TYPE  IleIppp^sssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh    GLEWIGYINPSRGYTNTNQKFKRKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLPPEDTGV
             ??                      ? ?  ?  ?             ?
                  ************       CDR2   (LOOP)
                  ******************* CDR2   (KABAT)


           92 N                   107    113
RES TYPE  PiPIEissssiiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS   (SEQ ID NO:30)
KOL       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS   (SEQ ID NO:10)
               ****************  CRD4 (KABAT/LOOP)
```

FIG. 4

OKT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

```
          1                          26        35  39  43
Okt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
gH341     QVQLVESGGGVVQOGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA178
gH341A    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA185

gH341E    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA198
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA207
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA209
gH341D    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA197
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA199
gH341C    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA184

gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA203
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA205
gH341B    QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA183
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA204
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA206
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA208
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
```

FIG. 5a

```
        44    50                  65                       83
□kt3vh  GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLT
gH341   GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR   JA178
gH341A  GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR   JA185

gH341E  GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKSTAFLQMDSLR   JA198
gH341*  GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTAFLQMDSLR   JA207
gH341*  GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLR   JA209
gH341D  GLEWIGYINPSRGYTNYNQKVKDRFTISTDKSKNTLFLQMDSLR   JA197
gH341*  GLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTLFLQMDSLR   JA199
gH341C  GLEWVAYINPSRGYTNYNQKFKDRFTISRDNSKNTLFLQMDSLR   JA184

gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR   JA207
gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR   JA205
gH341B  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR   JA183
gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR   JA204
gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKSTAFLQMDSLR   JA206
gH341*  GLEWIGYINPSRGYTNYN□KVKDRFTISTDKSKNTAFLQMDSLR   JA208
K□L     GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLR
```

FIG. 5b

	84 95 102 113		SEQ ID NO:
Okt3vh	SEDSAVYYCARYYDDHY.....CLDYWGQGTTLTVSS		
gH341	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA178	30
gH341A	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA185	12
gH341E	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA198	13
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA207	14
gH341D	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA197	15
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA209	16
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA199	17
gH341C	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA184	18
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA203	19
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA205	20
gH341B	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA183	21
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA204	22
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA206	23
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA208	24
KOL	PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS		10

FIG. 5c

□KT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
          1                        24        34      42
□kt3vl    QIVLTQSPA□MSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43     50    56                            85
□kt3vl    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ ID N□:8)

          86   91   96          108
□kt3vl    YYCQQWSSNPFTFGSGTKLEINR       (SEQ ID N□:29)
gL221     YYCQQWSSNPETFGQGTKLQITR       (SEQ ID N□:25)
gL221A    YYCQQWSSNPETFGQGTKLQITR       (SEQ ID N□:26)
gL221B    YYCQQWSSNPETFGQGTKLQITR       (SEQ ID N□:27)
gL221C    YYCQQWSSNPETFGQGTKLQITR       (SEQ ID N□:28)
REI       YYCQQYQSLPYTFGQGTKLQITR       (SEQ ID N□:9)
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE D□UBLE UNDERLINED

FIG. 6

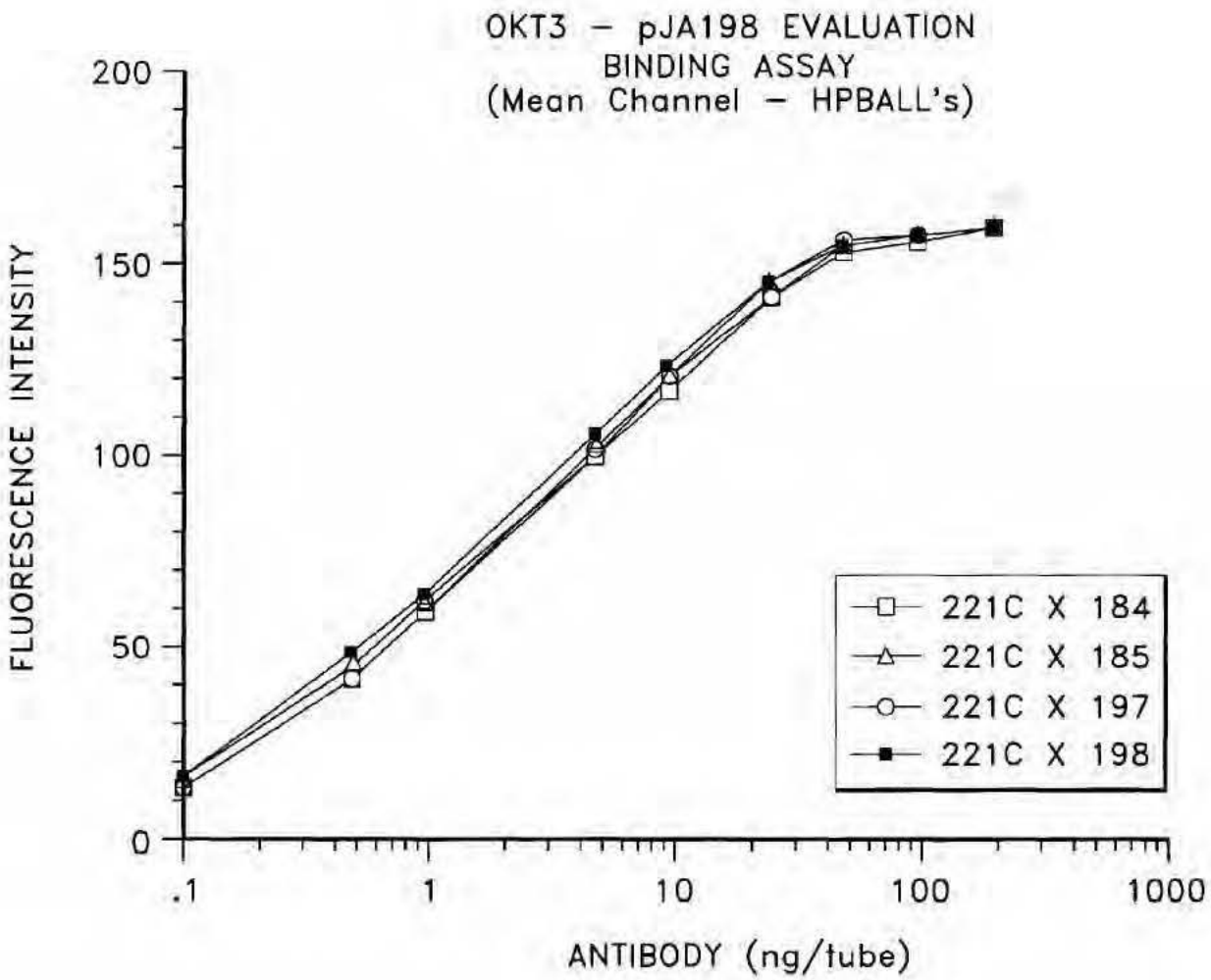
OKT3 – pJA198 EVALUATION
BINDING ASSAY
(Mean Channel – HPBALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
.1
1
10
100
1000
ANTIBODY (ng/tube)
221C X 184
221C X 185
221C X 197
221C X 198

FIG. 7

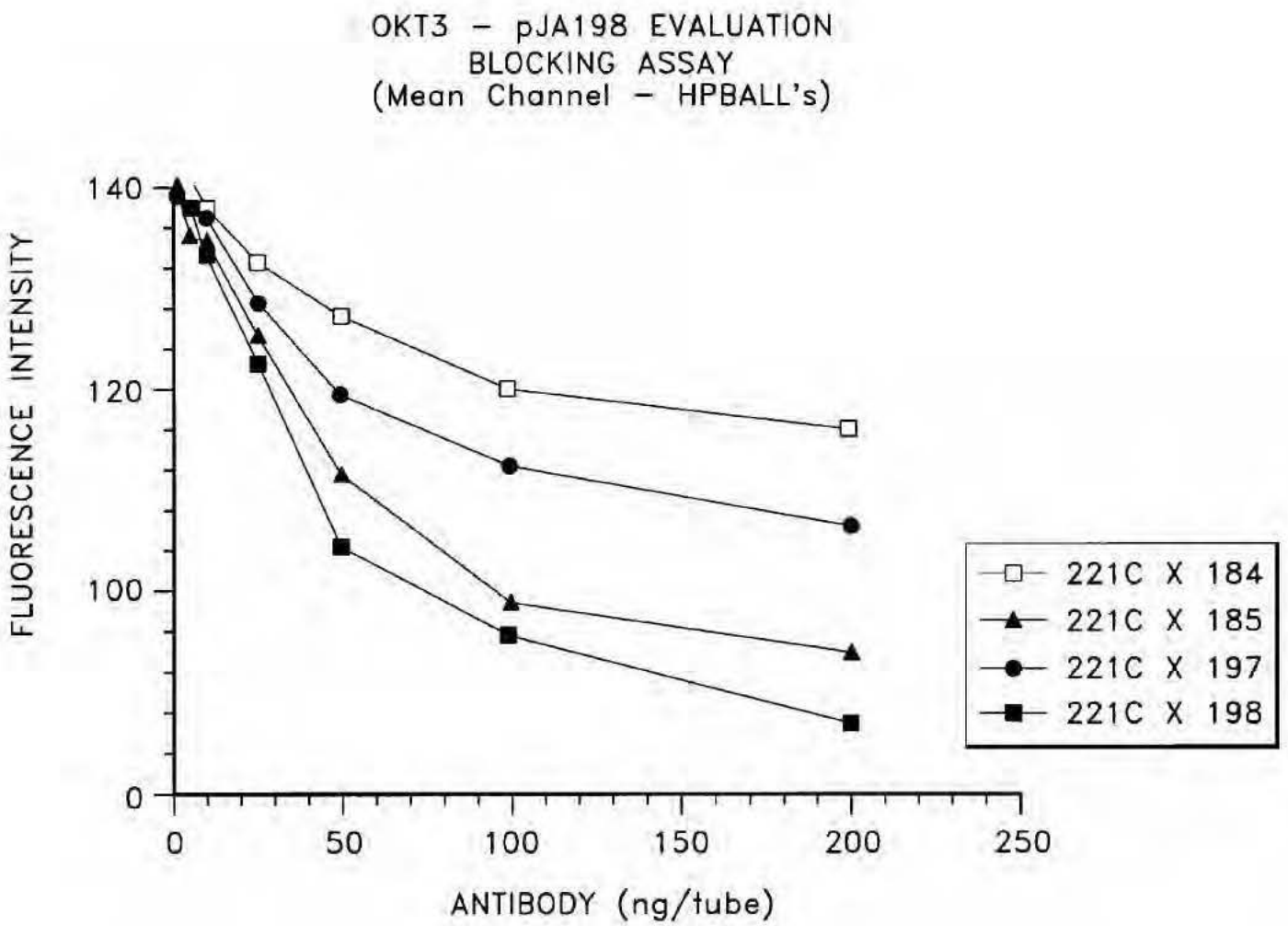
OKT3 – pJA198 EVALUATION
BLOCKING ASSAY
(Mean Channel – HPBALL's)
FLUORESCENCE INTENSITY
140
120
100
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
221C X 184
221C X 185
221C X 197
221C X 198

FIG. 8

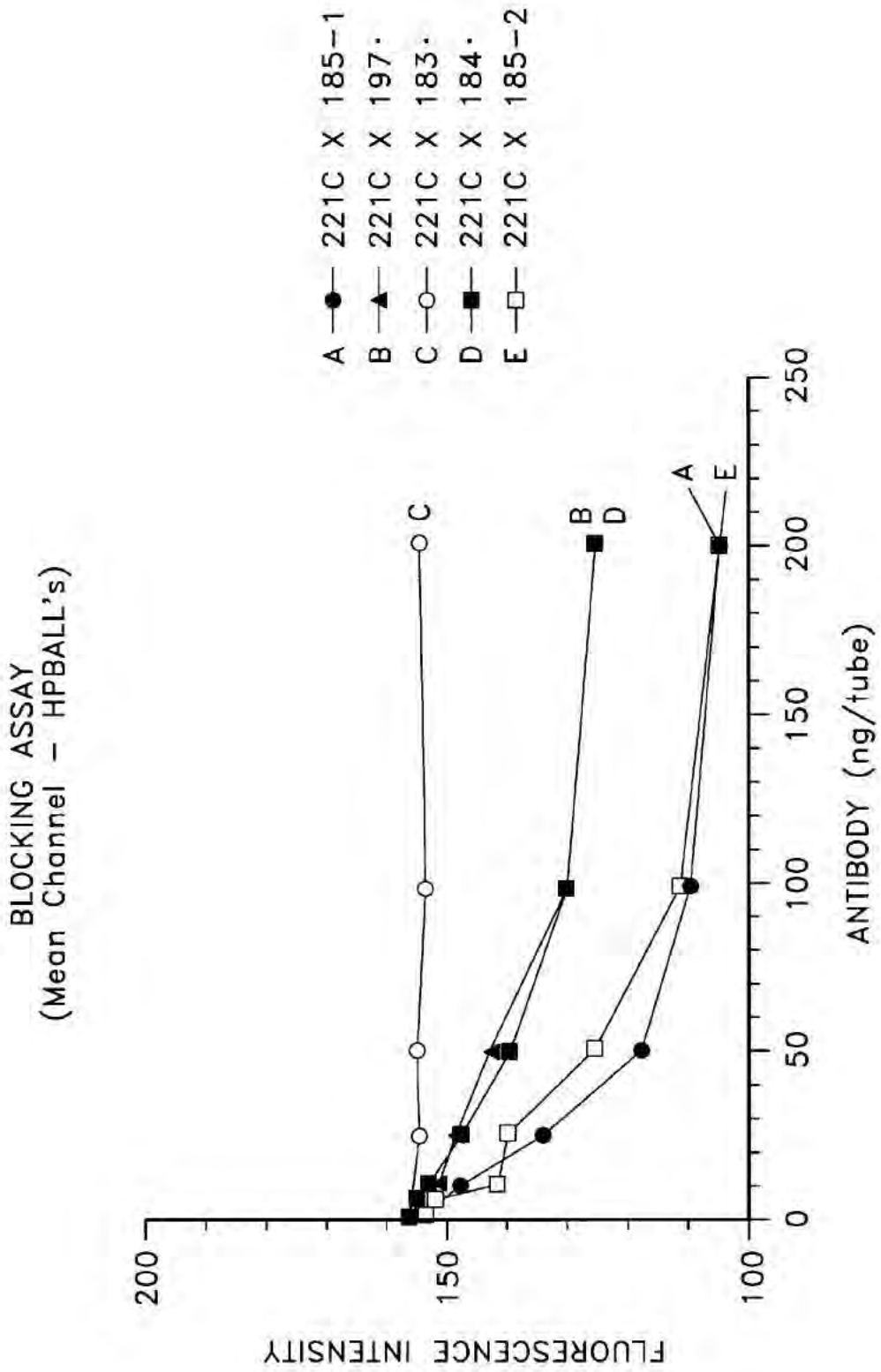
BLOCKING ASSAY
(Mean Channel – HPBALL's)
FLUORESCENCE INTENSITY
200
150
100
0
50
100
150
200
250
ANTIBODY (ng/tube)
A — 221C X 185-1
B — 221C X 197·
C — 221C X 183·
D — 221C X 184·
E — 221C X 185-2
A
B
C
D
E

FIG. 9

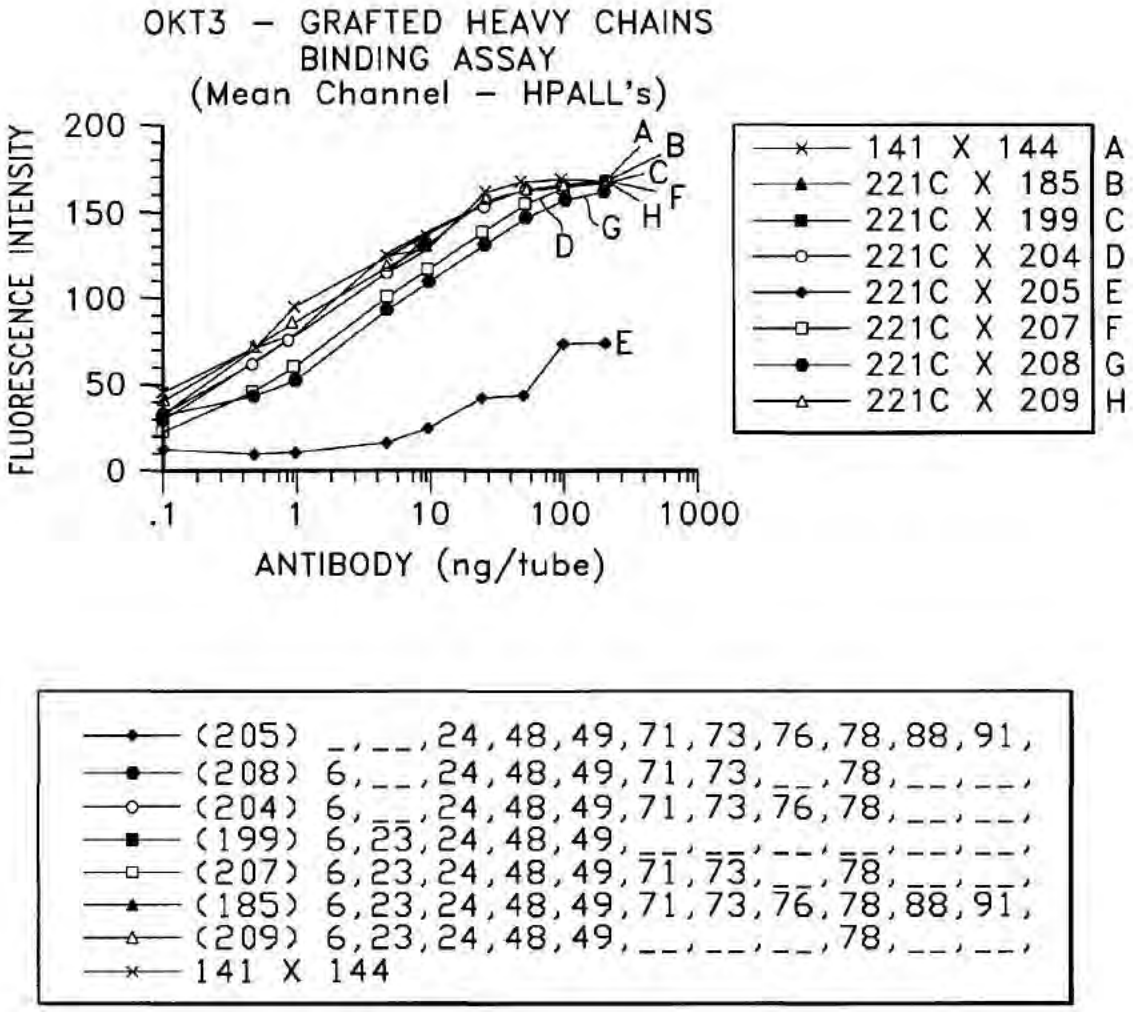
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
.1
1
10
100
1000
ANTIBODY (ng/tube)
A
B
C
F
G
H
D
E
141 X 144 A
221C X 185 B
221C X 199 C
221C X 204 D
221C X 205 E
221C X 207 F
221C X 208 G
221C X 209 H
(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

FIG. 10a

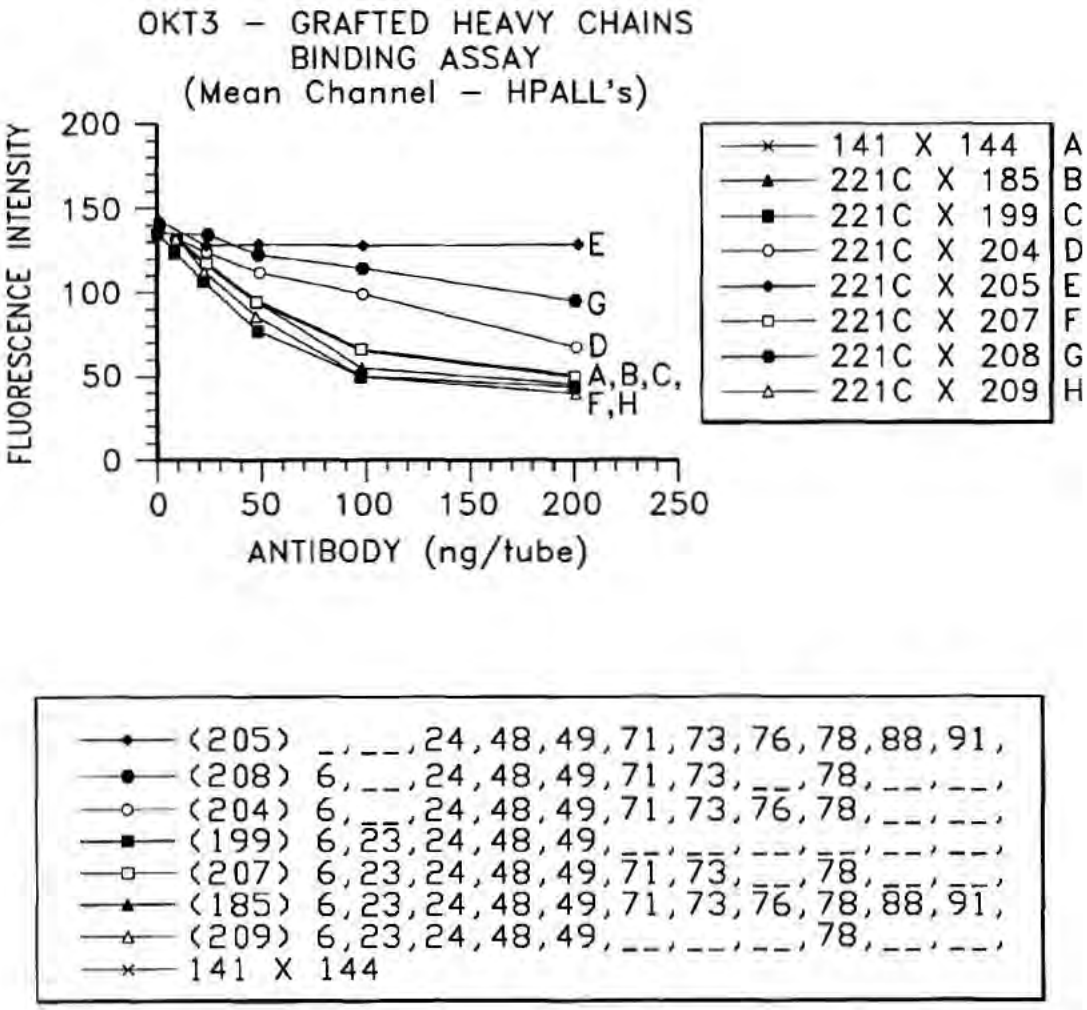
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
E
G
D
A,B,C,
F,H
141 X 144 A
221C X 185 B
221C X 199 C
221C X 204 D
221C X 205 E
221C X 207 F
221C X 208 G
221C X 209 H
(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

FIG. 10b

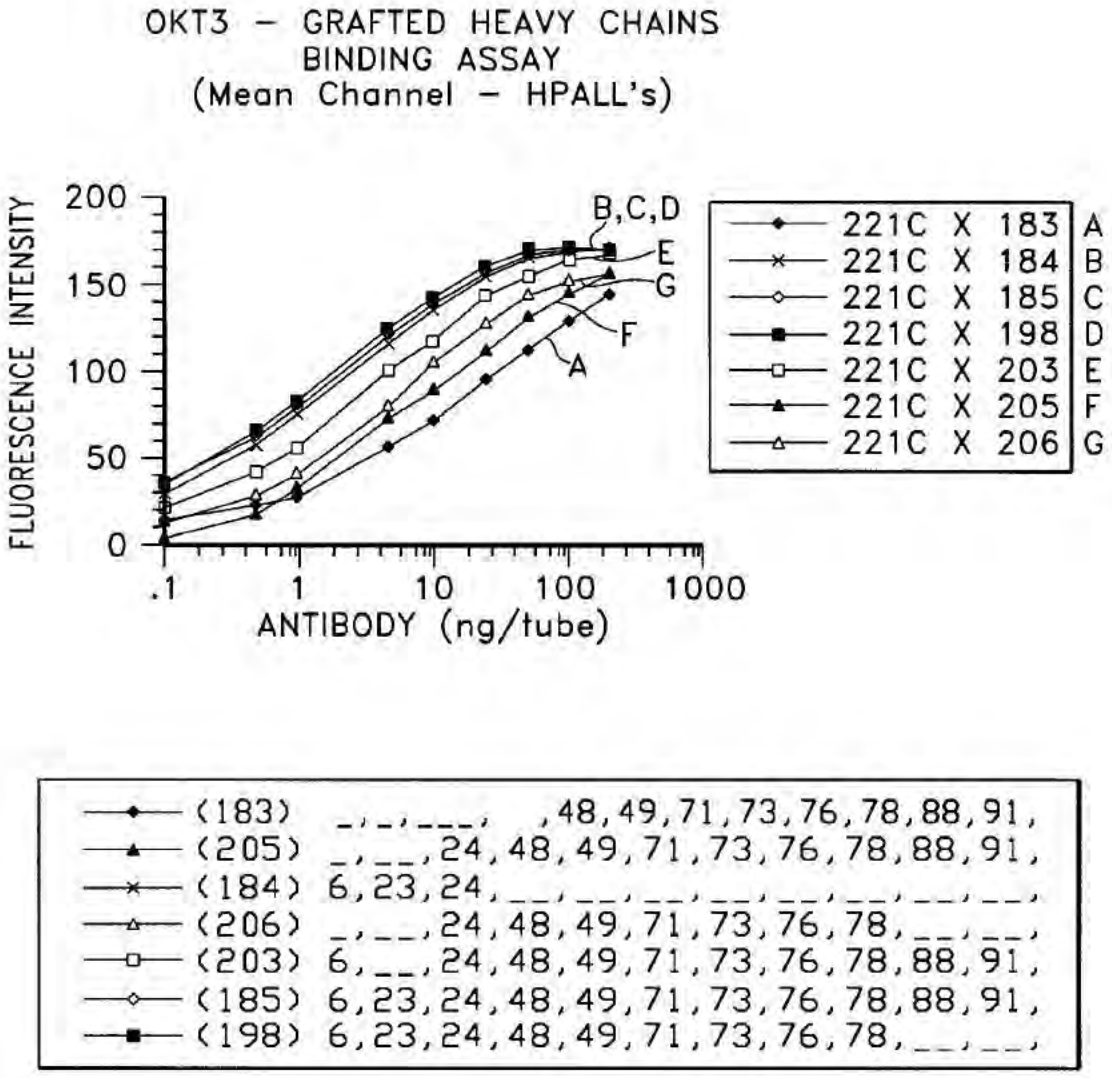
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
.1
1
10
100
1000
ANTIBODY (ng/tube)
B,C,D
E
G
F
A
221C X 183 A
221C X 184 B
221C X 185 C
221C X 198 D
221C X 203 E
221C X 205 F
221C X 206 G
(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,
(184) 6,23,24,__,__,__,__,__,__,__,__,
(206) _,__,24,48,49,71,73,76,78,__,__,
(203) 6,__,24,48,49,71,73,76,78,88,91,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(198) 6,23,24,48,49,71,73,76,78,__,__,

FIG. 11a

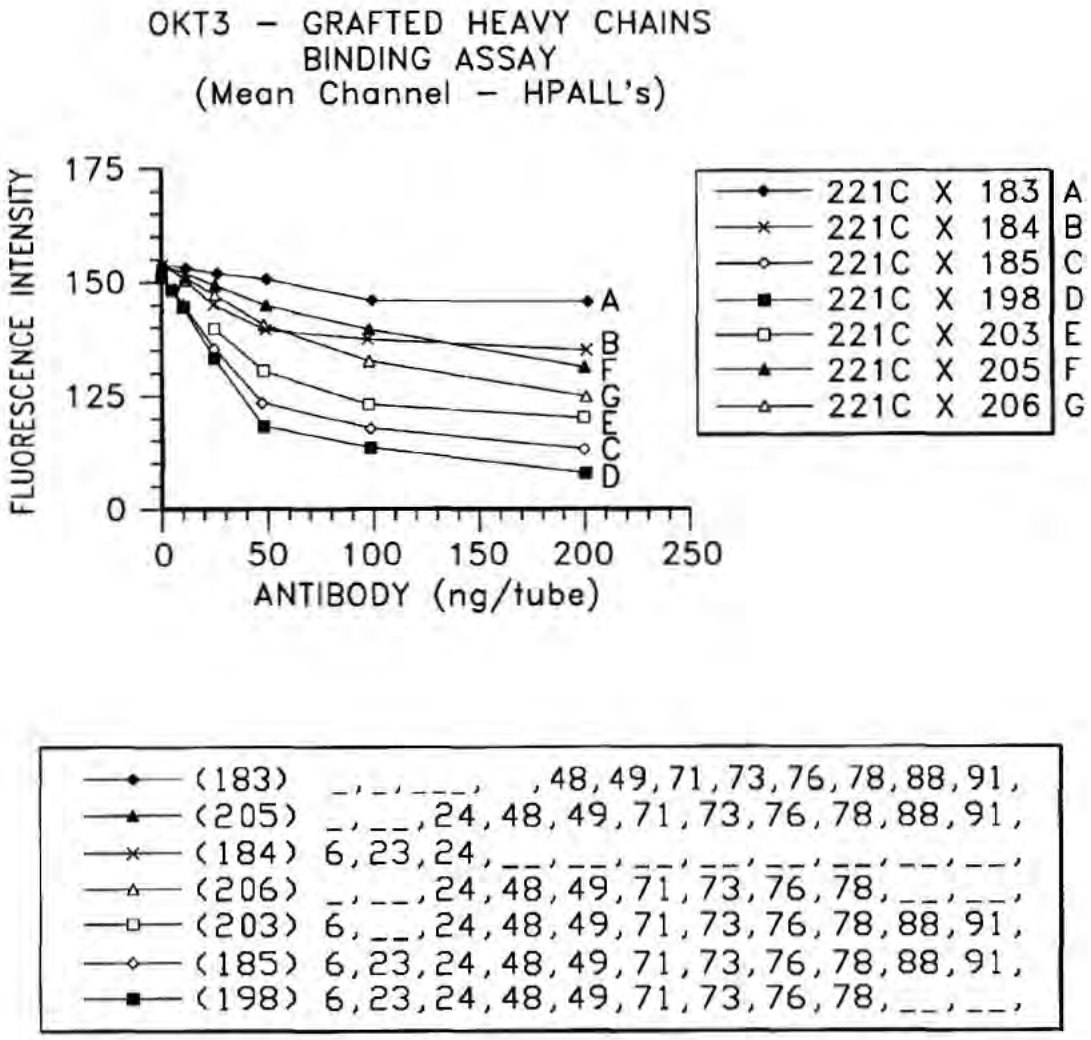
OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
175
150
125
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
A
B
F
G
E
C
D
221C X 183 A
221C X 184 B
221C X 185 C
221C X 198 D
221C X 203 E
221C X 205 F
221C X 206 G
(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,
(184) 6,23,24,__,__,__,__,__,__,__,__,
(206) _,__,24,48,49,71,73,76,78,__,__,
(203) 6,__,24,48,49,71,73,76,78,88,91,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(198) 6,23,24,48,49,71,73,76,78,__,__,

FIG. 11b

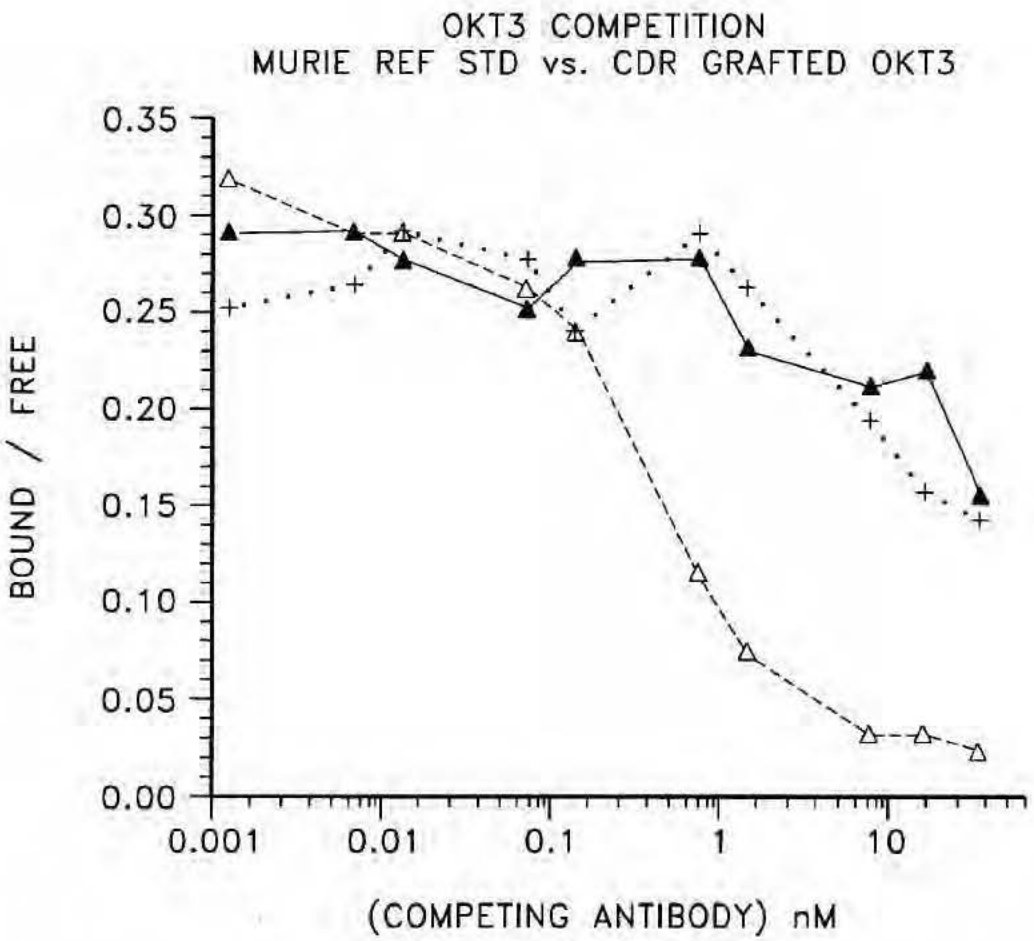
OKT3 COMPETITION
MURIE REF STD vs. CDR GRAFTED OKT3
BOUND / FREE
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.001
0.01
0.1
1
10
(COMPETING ANTIBODY) nM
MEDIA CNTRL
REF IN MEDIA
CDR 221 x 178 * #1
CDR 221 x 178 * #2
* PROTEIN CONCENTRATIONS
APPROXIMATE [ELISA]

FIG. 12

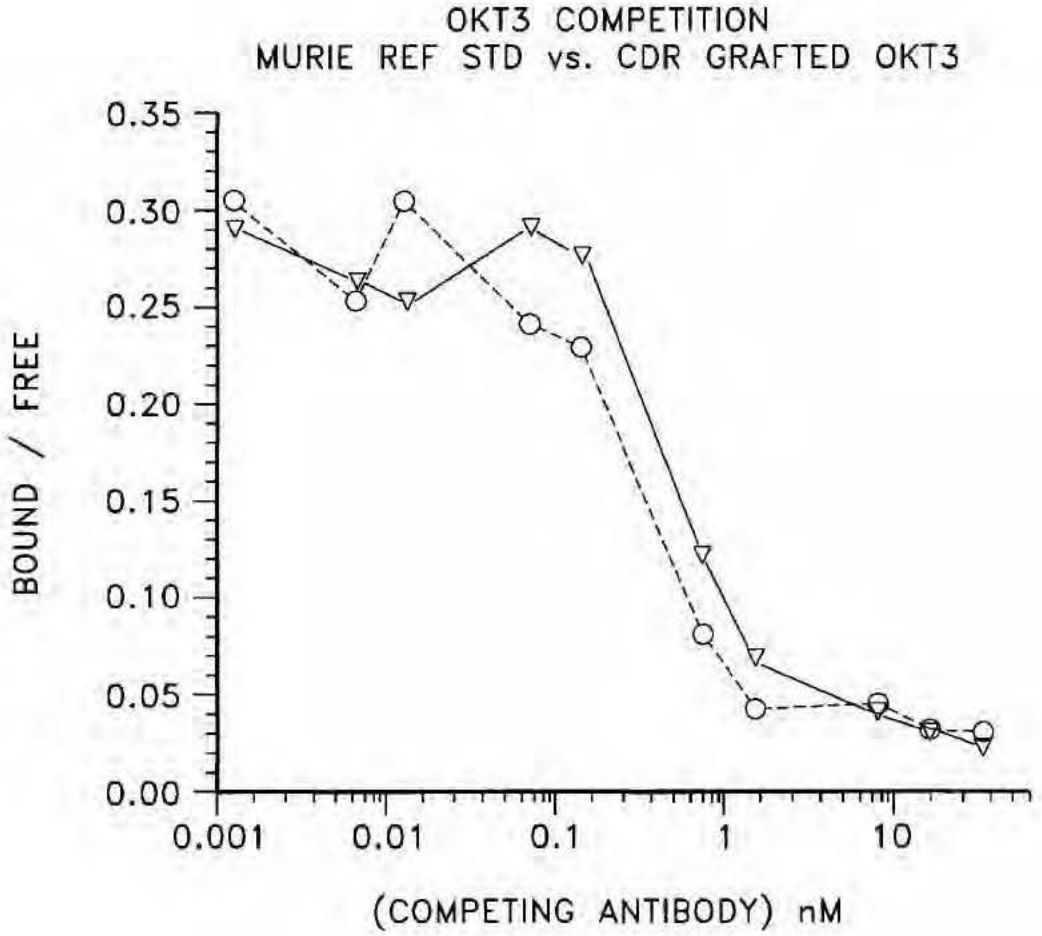
OKT3 COMPETITION
MURIE REF STD vs. CDR GRAFTED OKT3
BOUND / FREE
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.001
0.01
0.1
1
10
(COMPETING ANTIBODY) nM
REF STD
CDR 221C x 185
(PROTEIN CONCENTRATION APPROXIMATE)
* PROTEIN CONCENTRATIONS APPROXIMATE [ELISA]

FIG. 13

HUMANISED ANTIBODIES

This is a continuation of application Ser. No. 07/743,329, filed Sep. 17, 1991, now abandoned.

FIELD OF THE INVENTION

The present invention relates to humanised antibody molecules, to processes for their production using recombinant DNA technology, and to their therapeutic uses.

The term "humanised antibody molecule" is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site typically comprises complementarity determining regions (CDRS) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

BACKGROUND OF THE INVENTION

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to

diminish or abolish this undesirable HAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The present invention relates to humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted humanised antibody molecules. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain. The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen et al (5) and Riechmann et al (6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann et al/Medical Research Council it was found that transfer of the CDR regions alone [as defined by Kabat refs. (7) and (8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complete antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 A of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain SEQ ID NO:31 and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of $3\times10^9M^{-1}$, about one-third of that of the murine MAb.

We have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

SUMMARY OF THE INVENTION

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at 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.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:

1 and 3,

72 and 76,

69 (if 48 is different between donor and acceptor),

38 and 46 (if 48 is the donor residue),

80 and 20 (if 69 is the donor residue),

67,

82 and 18 (if 67 is the donor residue),

91,

88, and

any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these. Preferably, the antigen binding regions of the CDR-grafted hearty chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50–65) and CDR3 (residues 95–100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26–35).

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac

antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64–69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64–69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:

1 and 3,

60 (if 60 and 54 are able to form at potential saltbridge),

70 (if 70 and 24 are able to form a potential saltbridge),

73 and 21 (if 47 is different between donor and acceptor),

37 and 45 (if 47 is different between donor and acceptor), and

any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions; of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24–34), CDR2 (residues 50–56) and CDR3 (residues 89–97).

The invention further provides in a fourth aspect a CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The humanised antibody molecules and chains of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the heavy or light chains or humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of acceptor framework used is of the same/similar class/type as the donor antibody. Conveniently, the framework may be chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8–10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be used are KOL, NEWM, REI, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgGI and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are

well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. *E. coli,* and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and $(Fab')_2$ fragments, and especially FV fragments; and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, in a further aspect the present invention provides a process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to the first aspect of the invention;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to the second or third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.

For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for in vivo therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or call surface-specific antibodies, suitable for use in in vivo therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For example, the antibodies may have specificity for any of the following: Interferons α, β, Γ or δ IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues are substituted for acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain-CDR1: residues 26–35
-CDR2: residues 50–65
-CDR3: residues 95–102
Light chain-CDR1: residues 24–34
-CDR2: residues 50–56
-CDR3: residues 89–97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3

ii. 72, 76

iii. If 48 is different between donor and acceptor sequences, consider 69

iv. If at 48 the donor residue is chosen, consider 38 and 46

v. If at 69 the donor residue is chosen, consider 80 and then 20

vi. 67

vii. If at 67 the donor residue is chosen, consider 82 and then 18

viii. 91

ix. 88

x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64–69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3

ii. 63

iii. 60, if 60 and 54 are able to form potential saltbridge

iv. 70, if 70 and 24 are able to form potential saltbridge

v. 73, and 21 if 47 is different between donor and acceptor

vi. 37, and 45 if 47 is different between donor and acceptor

vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs

The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs,. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24–34, 50–56, 89–97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31–35, 50–65 and 95–102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26–35 to include both the loop region and the hypervariable residues 33–35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31–35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain—Key residues are 23, 71 and 73.

Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference. 2.1.2 Light Chain—Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60+54; 70+24.

2.2 Packing residues near the CDRs.

2.2.1. Heavy Chain—Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the CDR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 33 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2. Light Chain—Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3. Residues at the variable domain interface between heavy and light chains—In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1. Heavy Chain—Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2. Light Chain—Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4. Variable-Constant region interface—The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1. Heavy Chain—Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2. Light Chain—In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

The above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying FIGS. **1–13**.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1***a* and **1***b* show DNA and amino acid sequences of the OKT3 light chain (SEQ ID NO:4 and 5);

FIGS. **2***a* and **2***b* shows DNA and amino acid sequences of the OKT3 heavy chain;

FIG. **3** shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI(SEQ ID NO:29, 8 and 9);

FIG. **4** shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL(SEQ ID NO:30 and 10);

FIGS. **5***a–c* show the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts(SEQ ID NO:30 and 10–24);

FIG. **6** shows the light variable region amino acid sequences of OKT3, REI and various corresponding CDR grafts(SEQ ID NO:29, 9 and 25);

FIG. **7** shows a graph of binding assay results for various grafted OKT3 antibodies'

FIG. **8** shows a graph of blocking assay results for various grafted OKT3 antibodies;

FIG. **9** shows a similar graph of blocking assay results;

FIGS. **10***a* and *b* show similar graphs for both binding assay and blocking assay results;

FIGS. **11***a* and *b* show further similar graphs for both binding assay and blocking assay results;

FIG. **12** shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and

FIG. **13** shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Example 1

CDR-grafting of OKT3

Material and Methods

1. Incoming Cells

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882.1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20 mL of supernatant was assayed to confirm that the antibody present was OKT3.

2. Molecular Biology Procedures

Basic molecular biology procedures were as described in Maniatis et al (ref. 9) with, in some cases, minor modifications. DNA sequencing was performed as described in Sanger et al (ref. 11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al (ref. 12) and the Anglian Biotechnology Ltd. handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al (ref. 13)

3. Research Assays

3.1. Assembly Assays

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1. COS Cells Transfected With Mouse OKT3 Genes

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with $F(ab')_2$ goat anti-mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and $F(ab')_2$ goat anti-mouse IgG $F(ab')_2$ (HRPO conjugated) was then added. Substrate was added to reveal the reaction. UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2. COS and CHO Cells Transfected With Chimeric or CDR-grafted OKT3 Genes

The assembly assay for chimeric: or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:

96 well microtitre plates were coated with $F(ab')_2$ goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature. The plates were washed and $F(ab')_2$ goat anti-mouse IgG Fc (HRPO conjugated) was added. Enzyme substrate was added to reveal the reaction. Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimeric standard.

3.2. Assay for Antigen Binding Activity

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. $F(ab')_2$ goat anti-human IgG Fc (HRPO conjugated) or $F(ab')_2$ goat anti-mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction. The negative control for the cell-based assay was chimeric B72.3. The positive control was mouse

Orthomune OKT3 or chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out. In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4° C. for 1 hour with various dilutions of test antibody, positive control antibody, or negative control antibody. The cells were washed once and incubated at 4° C. for 1 hour with an FITC-labelled goat anti-human IgG (Fc-specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock-transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4° C. for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4° C., washed twice and analysed by cytofluorography.

FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 Determination of Relative Binding Affinity

The relative binding affinities of CDR-grafted anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (Fl-OKT3) of known binding affinity as a tracer antibody. The binding affinity of Fl-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of Fl-OKT3 were incubated with HPB-ALL (5×10^5) in PBS with 5% foetal calf serum for 60 min. at 4° C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, N.C.). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with Fl-OKT3 divided by the number of binding sites per bead. The amount of bound and free Fl-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of Fl-OKT3 and incubated with 5×10^5 HPB-ALL in 200 ml of PBS with 5% foetal calf serum, for 60 min at 4° C. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free Fl-OKT3 were calculated. The affinities of competing antibodies were calculated from the equation [X]–[OKT3]=(1/Kx)–(1/Ka), where Ka is the affinity of murine OKT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximal bound/free binding.

4. cDNA Library Construction

4.1. mRNA Preparation and cDNA Synthesis

OKT3 producing cells were grown as described above and 1.2×10^9 cells harvested and MRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2. Library Construction

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5' phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency *Escherichia coli* (*E.coli*) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. Screening

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:

5' TCCAGATGTTAACTGCTCAC (SEQ ID NO:1) for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5' CAGGGGCCAGTGGATGGATAGAC (SEQ ID NO: 2) for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA Sequencing

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNAs [FIGS. **1**(*a*) and **2**(*a*)(SEQ ID NO:6)] were obtained and the corresponding amino acid sequences predicted [(FIGS. **1**(*b*) and **2**(*b*)(SEQ ID NO:7]. In FIG. **1**(*a*) the untranslated DNA regions are shown in uppercase, and in both FIGS. **1** (SEQ ID NO:4 and 5) and **2** (SEQ ID NO:6 and 7) the signal sequences are underlined.

7. Construction of cDNA Expression Vectors

Celltech expression vectors are based on the plasmid pEE6hCMV (ref. 14). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamH1 cassettes in the unique BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to

insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.

The mouse sequences were excised from the M13 based vectors described above as EcoR1 fragments and cloned into either pEE6-hCMV-neo for the heavy chain and into EE6-hCMV-gpt for the light chain to yield vectors pJA136 and pJA135 respectively.

8. Expression of cDNAS in COS Cells

Plasmids pJA135 and pJA136 were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

9. Construction of Chimeric Genes

Construction of chimeric genes followed a previously described strategy [Whittle et al (ref. 13)]. A restriction site near the 3' end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

9.1. Light Chain Gene Construction

The mouse light chain cDNA sequence contains an AvaI site near the 3' end of the variable region [FIG. **1**(*a*)(SEQ ID NO:4)]. The majority of the sequence of the variable region was isolated as a 396 bp. EcoR1-AvaI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3' region of the variable region from the AvaI site and to include the 5' residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region.

A HindIII site was introduced to act as a marker for insertion of the linker.

The linker was ligated to the V_L fragment and the 413 bp EcoR1-NarI adapted fragment was purified from the ligation mixture.

The constant region was isolated as an NarI-BamH1 fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoR1/BamH1/C1P pSP65 treated vector in a three way reaction to yield plasmid JA143. Clones were isolated after transformation into *E.coli* and the linker and junction sequences were confirmed by the presence of the HindIII site and by DNA sequencing.

9.2 Light Chain Gene Construction—Version 2

The construction of the first chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the chimera junction are:

...Leu—Glu—Ile—Asn—Arg / — / Thr—Val—Ala —Ala (SEQ ID NO: 3)

VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. Therefore, a second version of the chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

An internal HindIII site was not included in this adapter, to differentiate the two chimeric light chain genes.

The variable region fragment was isolated as a 376 bp EcoR1-AvaI fragment. The oligonucleotide linker was ligated to NarI cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3. Heavy Chain Gene Construction

9.3.1. Choice of Heavy Chain Gene Isotype

The constant region isotype chosen for the heavy chain was human IgG4.

9.3.2. Gene Construction

The heavy chain cDNA sequence showed a Ban1 site near the 3' end of the variable region [FIG. **2**(*a*)(SEQ ID NO:6)]. The majority of the sequence of the variable region was isolated as a 426bp. EcoR1/C1P/Ban1 fragment. An oligonucleotide adapter was designated to replace the remainder of the 3' region of the variable region from the Ban1 site up to and including a unique HindIII site which had been previously engineered into the first two amino acids of the constant region.

The linker was ligated to the V_H fragment and the EcoR1-HindIII adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting pJA91 with EcoR1 and HindIII removing the intron fragment and replacing it with the V_H to yield pJA142. Clones were isolated after transformation into *E.coli* JM101 and the linker and junction sequences were confirmed by DNA sequencing. (N.B. The HindIII site is lost on cloning).

10. Construction of Chimeric Expression Vectors

10.1. neo and gpt Vectors

The chimeric light chain (version 1) was removed from pJA143 as an EcoR1 fragment and cloned into EcoR1/C1P treated pEE6hCMVneo expression vector to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping.

The chimeric light chain (version 2) was constructed as described above.

The chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and cloned into the EcoR1/BclI/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2. GS Separate Vectors

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sal1/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3. GS Single Vector Construction

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS and with transcription of the genes being head to tail e.g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 or pJA180 with BamH1/C1P and ligating in a Bgl11/Hind111 hCMV promoter cassette along with either the Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 or the Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181.

11. Expression of Chimeric Genes

11.1. Expression in COS Cells

The chimeric antibody plasmid pJA145 (cL) and pJA144 (cH) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin. This second version of the chimeric light chain, when expressed in association with chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

11.2 Expression in Chinese Hamster Ovary (CHO) Cells

Stable cell lines have been prepared from plasmids PJA141/pJA144 and from pJA179/pJA180, pJA181 and pJA182 by transfection into CHO cells.

12. CDR-grafting

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimeric antibodies.

12.1. Variable Region Analysis

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains. The residues chosen for transfer can be identified in a number of ways:

(a) By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.

(b) By analysis of antibody variable domain sequences regions of hypervariability [termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)]can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.

(c) Residues not identified by (a) and (b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

12.1.1. Light Chain

FIG. **3** (SEQ ID NO:29, 8 and 9) shows an alignment of sequences for the human framework region RE1 (SEQ ID NO:8and 9) and the OKT3 light variable region (SEQ ID NO:29). The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1(c). Above the sequence in FIG. **3** (SEQ ID NO:29, 8 and 9) the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N — near to CDR (From X-ray Structures)	
P — Packing	B — Buried Non-Packing
S — Surface	E — Exposed
I — Interface	* — Interface
— Packing/Part Exposed	
? — Non-CDR Residues which may require to be left as Mouse sequence.	

Residues underlined in FIG. **3** are amino acids. RE1 (SEQ ID NO:8 and 9) was chosen as the human framework because the light chain is a kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region, e.g. KOL (SEQ ID NO:10)(see below). RE1 (SEQ ID NO:8 and 9) was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

12.1.2. Heavy Chain

Similarly FIG. **4** shows an alignment of sequences for the human framework region KOL (SEQ ID NO:10) and the OKT3 (SEQ ID NO:30) heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 12.1(c). The residue type key and other indicators used in FIG. **4** are the same as those used in FIG. **3**. KOL (SEQ ID NO:10) was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also the sequence alignment of OKT3 heavy variable region (SEQ ID NO:7) showed a slightly better homology to KOL (SEQ ID NO:10) than to NEWM.

12.2. Design of Variable Genes

The variable region domains were designed with mouse variable region optimal codon usage [Grantham and Perrin (ref. 15)] and used the B72.3 signal sequences [Whittle et al (ref. 13)]. The sequences were designed to be attached to the constant region in the same way as for the chimeric genes described above. Some constructs contained the "Kozak consensus sequence" [Kozak (ref. 16)] directly linked to the 5' of the signal sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

12.3. Gene Construction

To build the variable regions, various strategies are available. The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al (ref. 17) or by

simultaneously replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al (ref. 2). Both strategies were used and a list of constructions is set out in Tables 1 and 2 and FIGS. **4** and **5***a–c*. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides.

13. Construction of Expression Vectors

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimeric genes as described above.

sion levels were raised from approximately 200 ng/ml to approximately 500 ng/ml for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) (SEQ ID NO:25) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were

TABLE 1

CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE –	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK RE1			
121	26–32, 50–56, 91–96 inclusive	SDM and gene assembly	+	n.d.
121A	26–32, 50–56, 91–96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26–32, 50–56, 91–96 inclusive +46, 47	Partial gene assembly	n.d.	+
221	24–24, 50–56, 91–96 inclusive	Partial gene assembly	+	+
221A	24–34, 50–56, 91–96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24–34, 50–56, 91–96 inclusive +1, 3	Partial gene assembly	+	+
221C	24–34, 50–56, 91–96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FREMEWORK KOL			
121	26–32, 50–56, 95–100B inclusive	Gene assembly	n.d.	+
131	26–32, 50–58, 95–100B inclusive	Gene assembly	n.d.	+
141	26–32, 50–65, 95–100B inclusive	Partial gene assembly	+	n.d.
321	26–35, 50–56, 95–100B inclusive	Partial gene assembly	+	n.d.
331	26–35, 50–58, 95–100B inclusive	Partial gene assembly Gene assembly	+	+
341	26–35, 50–65, 95–100B inclusive	SDM Partial gene assembly	+	+
341A	26–35, 50–65, 95–100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human) (SEQ ID NO: 28)	Gene assembly	n.d.	+
34B	26–35, 50–65, 95–100B inclusive +48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY
n.d. not done
SDM Site directed mutagenesis
Gene assembly Variable region assembled entirely from oligonucleotides
Partial gene assembly Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

14. Expression of CDR-grafted Genes

14.1. Production of Antibody Consisting of Grafted Light (gL) Chains With Mouse Heavy (mH) or Chimeric Heavy (cH) Chains

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2–5 fold improvement in net expression. Over an extended series of experiments expressco-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene (SEQ ID NO:27) shows little detectable binding activity in association with cH. The light chain product of gL221C(SEQ ID NO:28), in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

14.2 Production of Antibody Consisting of Grafted Heavy (gH) Chains With Mouse Light (mL) or Chimeric Light (cL) Chains

Expression of the gH genes proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appeared to have no marked effect on expression of gH genes. Expression appears to be slightly improved but not to the same degree as seen for the grafted light chain.

Also, it proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26–32) for CDR1 is used, e.g. gH121, 131, 141 and no conclusions can be drawn about these constructs.

Moreover, co-expression of the gH341 gene (SEQ ID NO:11) with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 (SEQ ID NO:11) to produce gH341(SEQ ID NO:12) and gH341B (SEQ ID NO:21) lead to improved levels of expression.

This may be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 were expressed in association with cL, antibody was produced but antibody binding activity was not detected.

When the more conservative gH341 gene (SEQ ID NO:11) was used antigen binding could be detected in association with cL or mL, but the activity was only marginally above the background level.

When further mouse residues were substituted based on the arguments in 12.1, antigen binding could be clearly demonstrated for the antibody produced when kgH341A and kgH341B were expressed in association with cL.

14.3 Production of Fully CDR-grafted Antibody

The kgL221A gene was co-expressed with kgH341, kgH341A or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression.

For the combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH was produced.

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations, although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed. In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15. Discussion of CDR-grafting Results

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. Light Chian

15.1.1. Extent of the CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and those hypervariable sequences defined by Kabat et al (refs. 4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. For CDRI the hypervariable region extends from residues 24–34 inclusive while the structural loop extends from 26–32 inclusive. In the case of OKT3 (SEQ ID NO:5) there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework RE1 has glutamine. For CDR3 the loop extends from residues 91–96 inclusive while the Kabat hypervariability extends from residues 89–97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and RE1 (FIG. **3**)(SEQ ID NO:29, 8 and 9). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. Framework Resides

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221+D1Q, Q3V, L46R, L47W, see FIG. **3** and Table 1) was made, cloned in EE6hCMVneo and co-expressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity. When the related genes gL221B (SEQ ID NO:28)(gL221+D1Q, Q3V) and gL221C (gL221+L46R, L47W) were made and similarly tested, while both genes produced antibody when co-expressed with cH, only the gL221C/cH combination showed good antigen binding. When the gL121A (gL124+D1Q, Q3V, L46R, L47W) gene was made and co-expressed with cH, antibody was produced which also bound to antigen.

15.2. Heavy Chain

15.2.1. Extent of the CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26–32 inclusive whereas the Kabat CDR extends from residues 31–35 inclusive. For CDR2 the loop region is from 50–58 inclusive while the hypervariable region covers amino acids 50–65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50–56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 e.g. gH121, gH131, gH141 very little antibody was produced in the culture supernatants. As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies.

As no net antibody was produced, analysis of these constructs was not pursued further.

When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26–35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when co-expressed with cL. Expression was gen-

erally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5' to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2–5 fold increase in net antibody production. However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated. When the kgH341 gene was co-expressed with kgL221A(SEQ ID NO:26), the net yield of antibody was too low to give a signal above the background level in the antigen binding assay.

15.2.2. Framework Residues

As in the case of the light chain the heavy chain frameworks were re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to improve domain packing. Both showed antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice.

15.3 Interim Conclusions

It has been demonstrated, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence; and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generated only weak binding activity. Therefore the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of othe other 8 mouse residues of the kgH341A gene compared to kgH341.

16. Further CDR-grafting Experiments

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and gH341A (plasmid pJA185)(SEQ ID NO:12) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light chain genes used in these further experiments were gL221 (SEQ ID NO:25), gL221A(SEQ ID NO:26), gL221B (SEQ ID NO:27) and gL221C (SEQ ID NO:28) as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341D	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199
gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S		G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL (SEQ ID NO:30, 10 AND 11–24)	E	S	S	V	A		R	N	N	L	G	F	

TABLE 2-continued

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3v1	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1 (SEQ ID NO:29, 8, 9 and 25–28)	D	Q	L	L	

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with HPB-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain (SEQ ID NO:28) are given in FIGS. **7** and **8** (for the JA184, JA185, JA197 and JA198 constructs—see Table 2), in FIG. **9** (for the JA183, JA184, JA185 and JA197 constructs) in FIG. **10***a* and *b* (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in FIG. **11***a* and *b* (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 (SEQ ID NO:25) co-expressed with gh341 (JA178)(SEQ ID NO:11), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C (SEQ ID NO:28) co-expressed with gh341A (JA185)(SEQ ID NO:12), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in FIG. **12** for the basic grafted product and in FIG. **13** for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability aLs compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-grafi a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-1 (R6-5), TAG72 (B72.3), and TNFα(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

Example 2

CDR-grafting of a Murine Anti-CD4 T Cell Receptor Antibody, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90 . . . of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 . . . is incorporated herein by reference. A number of CDR-grafted OKT4 antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

The Light Chain

The human acceptor framework used for the grafted light chains was RE1 (SEQ ID NO:8 and 9) The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human

acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64–69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

The Heavy Chain

The human acceptor framework used for the grafted heavy chains was KOL(SEQ ID NO:10).

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

Example 3

CDR-grafting of an Anti-mucin Specific Murine Antibody, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1. The regions transferred were:

CDR Number	Residues
1	24–34
2	50–56
3	90–96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL

and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that., in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and REI (SEQ ID NO:8 and 9) light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48. Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised. Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had poor homology for KOL (SEQ ID NO:10) and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27–36
2	50–63
3	93–102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody. In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~10% of B72.3 by exposure to pHs of 2–3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid=3.86 and of glutamine acid=4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.

Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain (SEQ ID NO:10), position 831 is far removed from either of the CDR loops.

Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene

On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes

In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other

All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequence reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.

Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

Example 4

CDR-graftin of a Murine Anti-ICAM-1 Monoclonal Antibody

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-1) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A (SEQ ID NO:26) and grafted heavy chain gH341D (SEQ ID NO:16) which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

Light Chain

gL221A has murine CDRs at positions 24–34 (CDR1), 50–56 (CDR2) and 89–97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87.

Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

Heavy Chain

gH341D has murine CDRs at positions 26–35 (CDR1), 50–56 (CDR2) and94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91. Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

Example 5

CDR-Grafting of Murine Anti-TNFa Antibodies

A number of murine anti-TNFa monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNF1, hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain. Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.

Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF-. The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26–35 (CDR1), 50–65 (CDR2) and 95–102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24–34 (CDR1), 50–56 (CDR2) and 89–97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3.

Based on the 61E71 CDR grafting data gL221 and gH341 (+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) (SEQ ID NO:11) and which have one or a small number of non-CDR residues at positions 73, 78 or 77–79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies are at least an order of magnitude less able to compete for TNF against the TNF receptor on 1,929 cells. Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.

It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 31

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

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(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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(2) INFORMATION FOR SEQ ID NO:3:

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(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(2) INFORMATION FOR SEQ ID NO:4:

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(A) LENGTH: 943 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 18..722

(i x) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 84..722

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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                   -22 -20             -15

CTA ATC AGT GCC TCA GTC ATA ATA TCC AGA GGA CAA ATT GTT CTC ACC         98
Leu Ile Ser Ala Ser Val Ile Ile Ser Arg Gly Gln Ile Val Leu Thr
-10                 -5                  1                   5

CAG TCT CCA GCA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG        146
Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met
            10                  15                  20

ACC TGC AGT GCC AGC TCA AGT GTA AGT TAC ATG AAC TGG TAC CAG CAG        194
Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln
        25                  30                  35

AAG TCA GGC ACC TCC CCC AAA AGA TGG ATT TAT GAC ACA TCC AAA CTG        242
Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
    40                  45                  50

GCT TCT GGA GTC CCT GCT CAC TTC AGG GGC AGT GGG TCT GGG ACC TCT        290
Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser
55                  60                  65

TAC TCT CTC ACA ATC AGC GGC ATG GAG GCT GAA GAT GCT GCC ACT TAT        338
Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr
70                  75                  80                  85

TAC TGC CAG CAG TGG AGT AGT AAC CCA TTC ACG TTC GGC TCG GGG ACA        386
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr
                90                  95                  100

AAG TTG GAA ATA AAC CGG GCT GAT ACT GCA CCA ACT GTA TCC ATC TTC        434
Lys Leu Glu Ile Asn Arg Ala Asp Thr Ala Pro Thr Val Ser Ile Phe
            105                 110                 115

CCA CCA TCC AGT GAG CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC        482
Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys
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TTC TTG AAC AAC TTC TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT          530
Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile
    135                 140                 145

GAT GGC AGT GAA CGA CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG          578
Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln
150                 155                 160                 165

GAC AGC AAA GAC AGC ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC          626
Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr
                170                 175                 180

AAG GAC GAG TAT GAA CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC          674
Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His
            185                 190                 195

AAG ACA TCA ACT TCA CCC ATT GTC AAG AGC TTC AAC AGG AAT GAG TGT          722
Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
        200                 205                 210

TAGAGACAAA GGTCCTGAGA CGCCACCACC AGCTCCCAGC TCCATCCTAT CTTCCCTTCT        782

AAGGTCTTGG AGGCTTCCCC ACAAGCGCTT ACCACTGTTG CGGTGCTCTA AACCTCCTCC        842

CACCTCCTTC TCCTCCTCCT CCCTTTCCTT GGCTTTTATC ATGCTAATAT TTGCAGAAAA        902

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 235 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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 -5                  1                   5                  10

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                15                  20                  25

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser
            30                  35                  40

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
        45                  50                  55

Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
    60                  65                  70

Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
75                  80                  85                  90

Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
                95                  100                 105

Arg Ala Asp Thr Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
            110                 115                 120

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
        125                 130                 135

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
    140                 145                 150

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
155                 160                 165                 170

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
                175                 180                 185
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Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
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(2) INFORMATION FOR SEQ ID NO:6:

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(A) LENGTH: 1570 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 41..1444

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 98..1444

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

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                                             Met Glu Arg His Trp
                                             -19                 -15

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Ile Phe Leu Leu Leu Leu Ser Val Thr Ala Gly Val His Ser Gln Val
            -10                 -5                  1

CAG CTG CAG CAG TCT GGG GCT GAA CTG GCA AGA CCT GGG GCC TCA GTG     151
Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val
    5                   10                  15

AAG ATG TCC TGC AAG GCT TCT GGC TAC ACC TTT ACT AGG TAC ACG ATG     199
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20                  25                  30

CAC TGG GTA AAA CAG AGG CCT GGA CAG GGT CTG GAA TGG ATT GGA TAC     247
His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr
35                  40                  45                  50

ATT AAT CCT AGC CGT GGT TAT ACT AAT TAC ATT CAG AAG TTC AAG GAC     295
Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp
                55                  60                  65

AAG GCC ACA TTG ACT ACA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAA     343
Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln
            70                  75                  80

CTG AGC AGC CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT GCA AGA     391
Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
        85                  90                  95

TAT TAT GAT GAT CAT TAC TGC CTT GAC TAC TGG GGC CAA GGC ACC ACT     439
Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr
    100                 105                 110

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Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu
115                 120                 125                 130

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Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys
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Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser
            150                 155                 160

GGA TCC CTG TCC AGT GGT GTG CAC ACC TTC CCA GCT GTC CTG CAG TCT     631
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Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys
                215                 220                 225

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Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val
            230                 235                 240

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        245                 250                 255

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Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp
    260                 265                 270

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275                 280                 285                 290

ACA CAA ACC CAT AGA GAG GAT TAC AAC AGT ACT CTC CGG GTG GTC AGT     1015
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                295                 300                 305

GCC CTC CCC ATC CAG CAC CAG GAC TGG ATG AGT GGC AAG GAG TTC AAA     1063
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            310                 315                 320

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Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile
        325                 330                 335

TCA AAA CCC AAA GGG TCA GTA AGA GCT CCA CAG GTA TAT GTC TTG CCT     1159
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    340                 345                 350

CCA CCA GAA GAA GAG ATG ACT AAG AAA CAG GTC ACT CTG ACC TGC ATG     1207
Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met
355                 360                 365                 370

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Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn
                375                 380                 385

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Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser
            390                 395                 400

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Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn
        405                 410                 415

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    420                 425                 430

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His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
435                 440                 445

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(2) INFORMATION FOR SEQ ID NO: 7:

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

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(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
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Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
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Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
50 55 60

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
65 70 75

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

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
95 100 105

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro
110 115 120 125

Ser Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser
130 135 140

Val Thr Leu Gly Cys Leu Val Lys Gly Thr Phe Pro Glu Pro Val Thr
145 150 155

Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro
160 165 170

Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val
175 180 185

Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His
190 195 200 205

Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro
210 215 220

Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu
225 230 235

Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu
240 245 250

Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser
255 260 265

Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu
270 275 280 285

Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr
290 295 300

Leu Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser
305 310 315

Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro
320 325 330

Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln
335 340 345

Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val
350 355 360 365

Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val
370 375 380

-continued

```
Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Gln
385                 390                 395

Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg
400                 405                 410

Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val
415                 420                 425

Val His Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg
430                 435                 440                 445

Thr Pro Gly Lys
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

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

Leu Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Thr Glu Ala Ser Asn Leu Gln Ala Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr
                85
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

```
Tyr Tyr Cys Gln Gln Tyr Glu Ser Leu Pro Tyr Thr Phe Gly Gln Gly
1               5                  10                  15

Thr Lys Leu Gln Ile Thr Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe Ser Ser Tyr
            20                  25                  30
```

-continued

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

Ala Ile Ile Trp Asp Asp Gly Ser Asp Gln His Tyr Ala Asp Ser Val
        50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Asp Gly Gly His Gly Phe Cys Ser Ser Ala Ser Cys Phe Gly
            100                 105                 110

Pro Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80
```

-continued

```
Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
```

-continued

115

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Glu Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Leu Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

-continued

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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

-continued

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60
```

-continued

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Glu Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly

-continued

100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gly Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

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

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
100 105

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid

-continued

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

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

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:27:

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

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

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45
```

-continued

```
Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
 50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
 65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Glu Trp Ser Ser Asn Pro Phe Thr
                 85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:29:

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

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
            15                  20                  25

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser
        30                  35                  40

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
    45                  50                  55

Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
60                  65                  70

Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
75                  80                  85                  90

Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
                95                 100                 105

Arg
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
            Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
             1               5                  10

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        15                  20                  25

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
    30                  35                  40                  45

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

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
65                  70                  75

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

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
            95                 100                 105
```

-continued

```
Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110                 115
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1               5                  10                  15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
            20                  25                  30

Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45

Thr Ser Tyr Arg Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
    50                  55                  60

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Thr Gly Tyr Thr Glu Tyr Asn
65                  70                  75                  80

Gln Lys Phe Lys Asp Lys Ala Thr Ile Thr Ala Asp Glu Ser Thr Asn
                85                  90                  95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Arg Gly Gly Gly Val Phe Asp Tyr Trp Gly Gln Gly
        115                 120                 125

Thr Leu Val Thr Val Ser Ser
    130                 135
```

We claim:

1. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 6 23, 24, and 49 at least are donor residues, provided that said composite heavy chain does not comprise the amino acid sequence of SEQ ID NO:31.

2. The antibody molecule of claim **1**, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

3. The antibody molecule of claim **1**, wherein amino acid residues 71, 73, and 78 in said composite heavy chain are additionally donor residues.

4. The antibody molecule of claim **1**, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

5. The antibody molecule of claim **1**, wherein at least one of amino acid residues 36, 94, 104, 106, and 107 in said composite heavy chain are additionally donor residues.

6. The antibody molecule of claim **5**, wherein at least one of amino acid residues 2, 4, 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

7. The antibody molecule of claim **1**, wherein said complementary light chain is a composite light chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain; said CDRs comprise donor residues at least at residues 24 to 34, 50 to 56, and 89 to 97; and amino acid residues 46, 48, 58, and 71 at least are donor residues.

8. The antibody molecule of claim **7**, wherein amino acid residues 1, 3, 60 (if this residue can form a salt bridge with residue 54), and 70 (if this residue can form a salt bridge with residue 24) in said composite light chain are additionally donor residues.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,859,205
DATED : January 12, 1999
INVENTOR(S) : Adair et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,

Item [30], **Foreign Application Priority Data** section thereof:
Please insert -- PCT/GB90/02017, International Filing Date: December 21, 1990 --
after "Dec. 21, 1989, [GB], United Kingdom, 8928874".

Item [56], **References Cited**, U.S. PATENT DOCUMENTS section after 4,348,376, 9/1982, Goldberg., please insert -- 5,225,539, 7/1993, Winter . --
After 5,225,539, 7/1993, Winter.,
Please insert -- 5,585,089, 12/1996, Queen et al. . --

FOREIGN PATENT DOCUMENTS section at 0239400 A2, 3/1987, European Pat. Off. .
Please delete "0239400 A2" and insert -- 0 239 400 A2 --
At A1 0323806, 7/1989, European Pat. Off. .
Please delete "Al 0323806" and insert -- 0 323 806 A1 --

OTHER PUBLICATIONS section at Chothia, Cyrus et al (Dec. 1989) *Nature*, "Conformations of Immunoglobulin Hypervariable Regions", vol. 342, pp. 877-883., it should read:

-- Chothia et al., "Conformations of Immunoglobulin Hypervariable Regions", *Nature*, 342:877-883, Dec., 1989. --

At Queen, C. et al (Dec. 1989) Proceedings of the National Academy of Sciences, "A Humanized Antibody That Binds to Interleukin 2 Receptor" vol. 86, pp. 10029-10033., it should read:

-- Queen et al., "A Humanized Antibody that Binds to the Interleukin 2 Receptor," *Proceedings of the National Academy of Sciences, USA*, 86:10029-10033, Dec., 1989. --

At Reichmann et al (Mar. 1988) Nature, "Reshaping Human Antibodies for Therapy," vol. 332, pp. 323-327., it should read:

-- Reichmann et al., "Reshaping Human Antibodies for Therapy," *Nature*, 332:323-327, Mar. 1988. --

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,859,205
DATED : January 12, 1999
INVENTOR(S) : Adair et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Item [56], **References Cited**, OTHER PUBLICATIONS section at Roberts et al. "Generation of Antibody with Enhanced Affinity and Specificity for its Antigen by Protein Engineering" Nature, 328(20):731-734, Aug., 1987., it should read:

-- Roberts et al., "Generation of Antibody with Enhanced Affinity and Specificity for its Antigen by Protein Engineering," *Nature*, 328(20):731-734, Aug., 1987. --

At Verhoeyen et al. "Reshaping Human Antibodies: Grafting an Antilysozyme Activity", Science, 239:1534-36 Mar. 25, 1988., it should read:

-- Verhoeyen et al., "Reshaping Human Antibodies: Grafting an Antilysozyme Activity", *Science*, 239:1534-36, Mar., 1988. --

At Jones et al. "Replacing the complementarity-Determining Regions in a Human Antibody with those from a Mouse", Nature, 321:522-525, 1986., it should read:

-- Jones et al., "Replacing the complementarity-Determining Regions in a Human Antibody with those from a Mouse," *Nature*, 321:522-525, May, 1986. --

At Ward et al. "Binding activities of a Repertoire of Single Immunoglobulin Variable Domains Secreted from *Escherichia coli*", *Nature*, 341:544-546, 1989., it should read:

-- Ward et al., "Binding activities of a Repertoire of Single Immunoglobulin Variable Domains Secreted from *Escherichia coli*," *Nature*, 341:544-546 Oct., 1989. --

Drawings,
Please replace Sheet 8 of 18, FIG. 5c with new Sheet 8 of 18 FIG. 5c attached.
Please replace Sheet 9 of 18, FIG. 6 with new Sheet 9 of 18 FIG. 6 attached. .

Column 2,
Line 65, "complete antigens" should read -- complex antigens --.

Column 3,
Line 59, "not: coincide" should read -- not coincide --.

Column 5,
Between lines 37 and 38, insert -- 63, --.
Line 45, "regions; of " should read -- regions of --.

Column 7,
Line 32, "FV fragments; and" should read -- FV fragments and --.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,859,205
DATED : January 12, 1999
INVENTOR(S) : Adair et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 8,
Line 23, "The the present" should read -- The present --.

Column 10,
Line 20, please make "2.1.2 Light Chain...70+24." a new paragraph.
Line 40, "with 33 and 46" should read -- with 38 and 46 --.

Column 11,
Line 29, "FIGS. **2*a*** and **2*b*** shows" should read -- FIGS. **2*a*** and **2*b*** show --.
Line 30, "heavy chain;" should read -- heavy chain (SEQ ID NO:6 and 7); --.
Line 43, "(SEQ ID NO:29, 9 and 25)" should read -- (SEQ ID NO:29, 8, 9 and 25-28) --.
Line 45, "antibodies' " should read -- antibodies; --.

Column 12,
Line 39, "chimeric: or CDR-grafted" should read -- chimeric or CDR-grafted --.

Column 13,
Line 4, please make "In this system...cytofluorography." a new paragraph.

Column 14,
Line 51, "[FIGS. **1(*a*)** and" should read -- [FIGS. **1(*a*)**(SEQ ID NO:4) and --.
Line 53, "[FIGS. **1(*b*)** and" should read -- [FIGS. **1(*b*)**(SEQ ID NO:5) and --.

Column 18,
Line 28, "Residues underlined in FIG. **3**" should read -- Residues underlined in FIG. **3** (SEQ ID NO:29, 8 and 9) --.
Line 51, "ID NO:7" should read -- ID NO:30 --.

Column 21,
Line 56, "15.1. Light Chian" should read -- 15.1. Light Chain --.

Column 22,
Line 15, "15.1.2. Framework Resides" should read -- 15.1.2. Framework Residues --.
Line 29, "gL221B (SEQ ID NO:28)(gL221+D1Q, Q3V) and gL221C" should read -- gL221B (gL221 +D1Q, Q3V) and gL221 C (SEQ ID N0:28) --.
Line 33, "When the gL121 A (gL124+D1Q, Q3V" should read -- When the gL121A (gL121+D1Q, Q3V --.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,859,205
DATED : January 12, 1999
INVENTOR(S) : Adair et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 24,
Line 16, "individual contribution of othe other 8 mouse residues of the" should read -- individual contribution of other 8 mouse residues of the --.
Table 2, on the same line as the second gH341*, "R N N A G F" should read --R N N A G F --.
Table 2, on the same line as the first gH341B, "E S S G V" should read -- E S S I G V --.
Table 2, on the same line as the sixth gH341*, "Q S A I G V" should read -- Q S A I G V --.
Table 2, on the same line as the eighth gH341*, "Q S A I G V" should read -- Q S A I G V --.

Column 25,
Line 47, "basic grafted product has neglibible binding ability aLs" should read -- basic grafted product has neglibible binding ability as --.

Column 28,
Line 55, "body. In KOL heavy chain (SEQ ID NO:10), position 831 is" should read -- body. In KOL heavy chain (SEQ ID NO:10), position 81 is --.

Column 29,
Line 17, "CDR-graftin of a Murine Anti-ICAM-1 Monoclonal" should read -- CDR-grafting of a Murine Anti-ICAM-1 Monoclonal --.
Line 49, "50-56 (CDR2) and94-100B (CDR3). In addition murine" should read -- 50-56 (CDR2) and 94-100B (CDR3). In addition murine --.
Line 57, "CDR-Grafting of Murine Anti-TNFa Antibodies" should read -- CDR-Grafting of Murine Anti-TNFα Antibodies --.
Line 58, "A number of murine anti-TNFa monoclonal antibodies" should read -- A number of murine anti-TNFα monoclonal antibodies --.

Column 30,
Line 38, "wre used at positions 24-34 (CDR1), 50-56 (CDR2) and" should read -- were used at positions 24-34 (CDR1), 50-56 (CDR2) and --.
Line 67, "receptor on L929 ells for TNF-a compared to hTNF3" should read -- receptor on L929 ells for TNF-α compared to hTNF3 --.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,859,205
DATED : January 12, 1999
INVENTOR(S) : Adair et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 31,
Line 2, "(+23, 24, 48, 49 71 and 73 as mouse) genes have been built" should read -- (+23, 24, 48, 49, 71 and 73 as mouse) genes have been built --.
Line 4, "binds well to TNF-a, but competes very poorly in the L929" should read -- binds well to TNF-α, but competes very poorly in the L929 --.
Line 11, "recognise human TNF-a. The heavy chain of this antibody" should read -- recognise human TNF-α. The heavy chain of this antibody --.
Line 23, please make "Mouse residues at other positions...assay." a new paragraph.

Column 32,
Line 22, in the REFERENCES section "13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1934, Nucl. Acids. Res. 12, 9441" should read -- 13. Kramer, W., Drutsa, V., Jansen, H.-W., Kramer, B., Plugfelder, M., Fritz, H.-J., 1984, Nucl. Acids. Res. 12, 9441 --

IN THE SEQUENCE LISTING:
Please replace the Sequence Listing with the attached Sequence Listing.

Column 63,
Line 52, "residues 6 23, 24, and 49 at least are donor residues." should read -- residues 6, 23, 24, and 49 at least are donor residues. --.

Signed and Sealed this

Twelfth Day of November, 2002

Attest:

Attesting Officer

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Adair, John R.
Athwal, Diljeet S.
Emtage, John S.

(ii) TITLE OF INVENTION: Humanised Antibodies

(iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz & Norris
(B) STREET: One Liberty Place - 46th Floor
(C) CITY: Philadelphia
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19103

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/303,569
(B) FILING DATE: 07-SEP-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Trujillo, Doreen Yatko
(B) REGISTRATION NUMBER: 35,719
(C) REFERENCE/DOCKET NUMBER: CARP-0032

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (215) 568-3100
(B) TELEFAX: (215) 568-3439

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

TCCAGATGTT AACTGCTCAC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

CAGGGGCCAG TGGATGGATA GAC 23

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Leu Glu Ile Asn Arg Thr Val Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 943 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

-69-

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 18..722

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 84..722

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

```
GAATTCCCAA AGACAAA ATG GAT TTT CAA GTG CAG ATT TTC AGC TTC CTG          50
                   Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu
                   -22     -20                 -15

CTA ATC AGT GCC TCA GTC ATA ATA TCC AGA GGA CAA ATT GTT CTC ACC          98
Leu Ile Ser Ala Ser Val Ile Ile Ser Arg Gly Gln Ile Val Leu Thr
 -10                 -5                  1                   5

CAG TCT CCA GCA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG         146
Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met
            10                  15                  20

ACC TGC AGT GCC AGC TCA AGT GTA AGT TAC ATG AAC TGG TAC CAG CAG         194
Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln
        25                  30                  35

AAG TCA GGC ACC TCC CCC AAA AGA TGG ATT TAT GAC ACA TCC AAA CTG         242
Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
    40                  45                  50

GCT TCT GGA GTC CCT GCT CAC TTC AGG GGC AGT GGG TCT GGG ACC TCT         290
Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser
 55                  60                  65

TAC TCT CTC ACA ATC AGC GGC ATG GAG GCT GAA GAT GCT GCC ACT TAT         338
Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr
70                  75                  80                  85

TAC TGC CAG CAG TGG AGT AGT AAC CCA TTC ACG TTC GGC TCG GGG ACA         386
Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr
                90                  95                 100

AAG TTG GAA ATA AAC CGG GCT GAT ACT GCA CCA ACT GTA TCC ATC TTC         434
Lys Leu Glu Ile Asn Arg Ala Asp Thr Ala Pro Thr Val Ser Ile Phe
            105                 110                 115

CCA CCA TCC AGT GAG CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC         482
Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys
        120                 125                 130
```

```
TTC TTG AAC AAC TTC TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT          530
Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile
    135                 140                 145

GAT GGC AGT GAA CGA CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG          578
Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln
150                 155                 160                 165

GAC AGC AAA GAC AGC ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC          626
Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr
                170                 175                 180

AAG GAC GAG TAT GAA CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC          674
Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His
            185                 190                 195

AAG ACA TCA ACT TCA CCC ATT GTC AAG AGC TTC AAC AGG AAT GAG TGT          722
Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
        200                 205                 210

TAGAGACAAA GGTCCTGAGA CGCCACCACC AGCTCCCAGC TCCATCCTAT CTTCCCTTCT        782

AAGGTCTTGG AGGCTTCCCC ACAAGCGCTT ACCACTGTTG CGGTGCTCTA AACCTCCTCC        842

CACCTCCTTC TCCTCCTCCT CCCTTTCCTT GGCTTTTATC ATGCTAATAT TTGCAGAAAA        902

TATTCAATAA AGTGAGTCTT TGCCTTGAAA AAAAAAAAAA A                            943
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 235 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
-22      -20                 -15                 -10

Val Ile Ile Ser Arg Gly Gln Ile Val Leu Thr Gln Ser Pro Ala Ile
     -5                  1                   5                  10

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
                15                  20                  25
```

```
Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser
            30                  35                  40

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
        45                  50                  55

Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
    60                  65                  70

Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
75                  80                  85                  90

Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
                95                  100                 105

Arg Ala Asp Thr Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
            110                 115                 120

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
        125                 130                 135

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
    140                 145                 150

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
155                 160                 165                 170

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
                175                 180                 185

Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
            190                 195                 200

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
        205                 210
```

(2) INFORMATION FOR SEQ ID NO:6:

SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1570 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 41..1444

-72-

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 98..1444

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

```
GAATTCCCCT CTCCACAGAC ACTGAAAACT CTGACTCAAC ATG GAA AGG CAC TGG          55
                                            Met Glu Arg His Trp
                                            -19             -15

ATC TTT CTA CTC CTG TTG TCA GTA ACT GCA GGT GTC CAC TCC CAG GTC          103
Ile Phe Leu Leu Leu Leu Ser Val Thr Ala Gly Val His Ser Gln Val
               -10                 -5                   1

CAG CTG CAG CAG TCT GGG GCT GAA CTG GCA AGA CCT GGG GCC TCA GTG          151
Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val
          5                  10                  15

AAG ATG TCC TGC AAG GCT TCT GGC TAC ACC TTT ACT AGG TAC ACG ATG          199
Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met
     20                  25                  30

CAC TGG GTA AAA CAG AGG CCT GGA CAG GGT CTG GAA TGG ATT GGA TAC          247
His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr
 35                  40                  45                  50

ATT AAT CCT AGC CGT GGT TAT ACT AAT TAC ATT CAG AAG TTC AAG GAC          295
Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp
                55                  60                  65

AAG GCC ACA TTG ACT ACA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAA          343
Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln
           70                  75                  80

CTG AGC AGC CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT GCA AGA          391
Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
      85                  90                  95

TAT TAT GAT GAT CAT TAC TGC CTT GAC TAC TGG GGC CAA GGC ACC ACT          439
Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr
 100                 105                 110

CTC ACA GTC TCC TCA GCC AAA ACA ACA GCC CCA TCG GTC TAT CCA CTG          487
Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu
115                 120                 125                 130
```

```
GCC CCT GTG TGT GGA GAT ACA ACT GGC TCC TCG GTG ACT CTA GGA TGC          535
Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys
                135                 140                 145

CTG GTC AAG GGT TAT TTC CCT GAG CCA GTG ACC TTG ACC TGG AAC TCT          583
Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser
            150                 155                 160

GGA TCC CTG TCC AGT GGT GTG CAC ACC TTC CCA GCT GTC CTG CAG TCT          631
Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
        165                 170                 175

GAC CTC TAC ACC CTC AGC AGC TCA GTG ACT GTA ACC TCG AGC ACC TGG          679
Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp
    180                 185                 190

CCC AGC CAG TCC ATC ACC TGC AAT GTG GCC CAC CCG GCA AGC AGC ACC          727
Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr
195                 200                 205                 210

AAG GTG GAC AAG AAA ATT GAG CCC AGA GGG CCC ACA ATC AAG CCC TGT          775
Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys
                215                 220                 225

CCT CCA TGC AAA TGC CCA GCA CCT AAC CTC TTG GGT GGA CCA TCC GTC          823
Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val
            230                 235                 240

TTC ATC TTC CCT CCA AAG ATC AAG GAT GTA CTC ATG ATC TCC CTG AGC          871
Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser
        245                 250                 255

CCC ATA GTC ACA TGT GTG GTG GTG GAT GTG AGC GAG GAT GAC CCA GAT          919
Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp
    260                 265                 270

GTC CAG ATC AGC TGG TTT GTG AAC AAC GTG GAA GTA CAC ACA GCT CAG          967
Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln
275                 280                 285                 290

ACA CAA ACC CAT AGA GAG GAT TAC AAC AGT ACT CTC CGG GTG GTC AGT         1015
Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser
                295                 300                 305

GCC CTC CCC ATC CAG CAC CAG GAC TGG ATG AGT GGC AAG GAG TTC AAA         1063
Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys
            310                 315                 320

TGC AAG GTC AAC AAC AAA GAC CTC CCA GCG CCC ATC GAG AGA ACC ATC         1111
Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile
        325                 330                 335
```

```
TCA AAA CCC AAA GGG TCA GTA AGA GCT CCA CAG GTA TAT GTC TTG CCT          1159
Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro
    340                 345                 350

CCA CCA GAA GAA GAG ATG ACT AAG AAA CAG GTC ACT CTG ACC TGC ATG          1207
Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met
355                 360                 365                 370

GTC ACA GAC TTC ATG CCT GAA GAC ATT TAC GTG GAG TGG ACC AAC AAC          1255
Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn
                375                 380                 385

GGG AAA ACA GAG CTA AAC TAC AAG AAC ACT GAA CCA GTC CTG GAC TCT          1303
Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser
            390                 395                 400

GAT GGT TCT TAC TTC ATG TAC AGC AAG CTG AGA GTG GAA AAG AAG AAC          1351
Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn
        405                 410                 415


TGG GTG GAA AGA AAT AGC TAC TCC TGT TCA GTG GTC CAC GAG GGT CTG          1399
Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu
    420                 425                 430


CAC AAT CAC CAC ACG ACT AAG AGC TTC TCC CGG ACT CCG GGT AAA              1444
His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
435                 440                 445

TGAGCTCAGC ACCCACAAAA CTCTCAGGTC CAAAGAGACA CCCACACTCA TCTCCATGCT       1504

TCCCTTGTAT AAATAAAGCA CCCAGCAATG CCTGGGACCA TGTAAAAAAA AAAAAAAAAG       1564

GAATTC                                                                  1570
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Glu Arg His Trp Ile Phe Leu Leu Leu Leu Ser Val Thr Ala Gly
-19                 -15                 -10                 -5
```

```
Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
                1               5                  10

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
    15                  20                  25

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
30                  35                  40                  45

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

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
            65                  70                  75

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

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
    95                  100                 105

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro
110                 115                 120                 125

Ser Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser
                130                 135                 140

Val Thr Leu Gly Cys Leu Val Lys Gly Thr Phe Pro Glu Pro Val Thr
            145                 150                 155

Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro
        160                 165                 170

Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val
    175                 180                 185

Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His
190                 195                 200                 205

Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro
                210                 215                 220

Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu
            225                 230                 235

Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu
        240                 245                 250

Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser
    255                 260                 265
```

```
Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu
270                 275                 280                 285

Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr
            290                 295                 300

Leu Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser
        305                 310                 315

Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro
    320                 325                 310

Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln
335                 340                 345

Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val
350                 355                 360                 365

Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val
            370                 375                 380

Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu
        385                 390                 395

Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg
    400                 405                 410

Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val
415                 420                 425

Val His Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg
430                 435                 440                 445

Thr Pro Gly Lys
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

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

Leu Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Thr Glu Ala Ser Asn Leu Gln Ala Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr
85

2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Tyr Tyr Cys Gln Gln Tyr Gln Ser Leu Pro Tyr Thr Phe Gly Gln Gly
1 5 10 15

Thr Lys Leu Gln Ile Thr Arg
20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

```
Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe Ser Ser Tyr
            20                  25                  30

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

Ala Ile Ile Trp Asp Asp Gly Ser Asp Gln His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Thy Phe Cys
                85                  90                  95

Ala Arg Asp Gly Gly His Gly Phe Cys Ser Ser Ala Ser Cys Phe Gly
            100                 105                 110

Pro Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Try Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95
```

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60
```

```
Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95
```

```
Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60
```

```
Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Ash Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110
```

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

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

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

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CIARACTERISTICS:
(A) LENGTI: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLDGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gly Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60
```

```
Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTI: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

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

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

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

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTI: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

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

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30

```
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
1               5                  10

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
     15                  20                  25

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
 30                  35                  40                  45

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

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
            65                  70                  75

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

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
     95                  100                 105

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110                 115
```

LH 4-19-99 Amdt 17/H

DOCKET NO.: CARP-0057 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Reeves**

For: **Humanised Antibodies**

I, **Doreen Yatko Trujillo**, Registration No. **35,179** certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On April 9, 1999
Doreen Yatko Trujillo
Doreen Yatko Trujillo Reg. No. 35,179

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

Pursuant to 37 C.F.R. § 1.115, please amend the above-identified application as a follows.

In the specification:

Page 1, after "September 7, 1994," please insert -- now U.S. Patent No. 5.859,205, --.

Page 1, after "September 17, 1991," please insert -- abandoned, --.

In the claims:

24. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least

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10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence or contributes to antigen binding as determined by X-ray crystallography.

28. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence or contributes to antigen binding as determined by X-ray crystallography.

29. (Amended) A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity [equivalent to that of a chimeric antibody formed from] similar to that of said donor immunoglobulin.

REMARKS

This paper is being filed in response to the Office Action dated November 16, 1998. A petition for a two-month extension of time and the appropriate fee accompany this response.

Claims 24-31 are pending. In the Office Action, all pending claims were rejected. In view of the foregoing amendments and the arguments that follow, Applicants

respectfully submit that allowable subject matter has been identified and request that the interference be declared.

Preliminarily, as requested by the Examiner, the specification has been amended to update the status of parent applications.

Additionally, the Examiner stated that the Information Disclosure Statements filed in the parent cases will not be considered unless they are filed with the present case and the references have been submitted. This appears to be contrary to MPEP § 609, page 600-103, specifically. As stated therein, information that has been considered by the Office in a parent application of a FWC filed prior to December 1, 1997 will be part of the file and need not be resubmitted to have the information considered. Likewise, an Examiner will consider information that has been considered by the Office in a parent application when examining a continuation under 36 C.F.R. § 1.60. The present application is a continuation under 37 C.F.R. § 1.60 of prior Application Serial No. 08/303,569, filed September 7, 1994, which is a continuation under 37 C.F.R.§ 1.62 (i.e., FWC) of Application Serial No. 07/7443,329, filed September 17, 1991. According to MPEP § 609, then, information considered in both parent applications is to be considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 24-31 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection for the reasons that follow. For the Examiner's convenience, the paragraphs are designated to correspond to the Examiner's paragraphs under this section.

a. The Examiner rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 have been amended herein to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. Support for these amendments can be found, *inter alia*, on page 38, lines 1-

12, and lines 23 through 38, of the application as filed. As is clear therefrom, the contribution to antigen binding can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing, i.e., the residues are spatially near a CDR. On page 17, lines 9-11, of the application as filed, the extents of the heavy chain CDRs are taught. On page 6, lines 25-35, it is indicated that the heavy chain "framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49...." Residue 49 is clearly adjacent a CDR. As evident from Figure 4, residues 6, 23, 24, and 48 contribute to antigen binding, as determined by X-ray crystallography. Applicants respectfully request that this rejection be withdrawn.

b. The Examiner rejected claims 27, 30, and 31, seeking evidence that CD3 is the same as "OKT3" and that CD4 is the same as "OKT4." Actually, one term refers to the antibody, while the other refers to the antigen bound. Specifically, OKT3 refers to a monoclonal antibody that recognizes the CD3 antigen and OKT4 refers to a monoclonal antibody that recognizes the CD4 antigen. Consistent therewith, on page 28, lines 19-22, of the application as filed, the testing of the ability of CDR-grafted OKT3 light chain to bind to CD3 positive cells is disclosed, and on page 52, line 29, of the application as filed, the reference "CD4 (OKT4)" is made. Applicants respectfully request that this rejection be withdrawn.

Claim 29 was rejected under 35 U.S.C. § 112, first paragraph, in view of the phrase "which specifically binds to an antigen with a binding affinity equivalent to that of a chimeric antibody formed from said donor immunoglobulin." The Examiner requested that Applicants point to support in the specification for the phrase. Claim 29 has been amended herein to recite that the binding affinity is "similar to that of" the donor. Support for this amendment can be found, *inter alia*, on page 48, lines 24-27 and page 51, lines 27-31 of the application as filed. Applicants respectfully request that this rejection be withdrawn.

Rejection Under 35 U.S.C. § 102(e)

Claims 24-31 were rejected under 35 U.S.C. § 102(e) in view of U.S. Patent No. 5,585,089 ("Queen et al."). Applicants respectfully traverse this rejection.

The Examiner observed that Queen et al. is entitled to priority back to "at least

12/28/90." (It is assumed that the Examiner meant 12/19/90, the filing date of the latest application designated as a continuation-in-part in the series of Queen et al. applications.) Although seeming to recognize that Queen et al. may not be entitled to a priority date earlier than 12/19/90, the Examiner, nonetheless, proceeded to argue that limitations recited in claims 24-31 are found in the earlier Queen et al. applications. The relevant inquiry for Queen et al. to be an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that the limitation "outside the Kabat and Chothia CDRs" is not found in, nor supported by, the priority documents.

This limitation requires that the framework residues to be replaced be outside both the Kabat and Chothia CDRs. As submitted in the Preliminary Amendment filed concurrently with the present application, however, the earliest Queen et al. applications do not teach, either explicitly or implicitly, that the framework residues to be replaced by donor **must be** outside **both** the Kabat and Chothia CDRs. Indeed, in the only example found in these early applications, and even in the specification of the Queen et al. patent as issued, changes were made to residues inside what Queen et al. denotes as CDRH1 of Chothia, i.e., inside a Chothia CDR. Considering that this limitation was required for patentability, Queen et al. cannot be entitled to a priority date earlier than the filing date of the application in which this limitation was first introduced, i.e., 12/19/90. Queen et al., thus, fails as a reference under 102(e) because, as also submitted in the Preliminary Amendment filed concurrently with the present application, Applicants are entitled to their GB priority date of 12/21/89.

Applicants respectfully request that this rejection be withdrawn.

Presentation of a Revised Proposed Count

Applicants present in Appendix A attached hereto a revised "Proposed Count." In compliance with 37 CFR §1.606, the revised proposed Count 1 is broader than any of claims 1-4, the broadest claims in the Queen patent, and as broad as any one of claims 24-31 being entered into the instant application.

The proposed count contains disjunctive or alternative language to cover the claim terminology of the two parties. Such counts were expressly approved by the Board in *Hsing v. Myers*, 2 USPQ2d 1861 (Bd, Pat,. App. & Int. 1987). It is clear, however, that both alternatives are directed to the same invention as that claimed in the Queen patent.

(c) Identification of Claims Corresponding to the Count

Applicants identify all of the Queen patent claims 1-11 and applicant's claims 24-31 as corresponding to the Count and as being directed to the same patentable invention.

(d) Application of the Terms of Applicants' Disclosure to the Copied Claims

In attached Appendix B, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24 substantially copied from Queen claims 1, 5, 9 and 10. There is, of course, additional support in applicants' application omitted for the sake of brevity.

(e) Applicants' Effective Filing Date

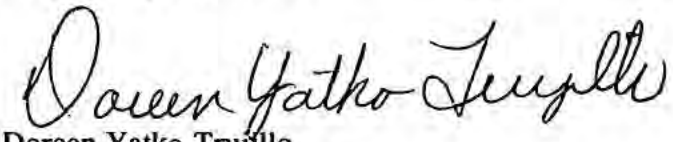
Applicants' present application, being a Rule 60 continuation, has the identical specification and drawings as that originally filed in U.S. application Serial No. 08/303.569, filed September 7, 1994, which is a U.S. national phase application stemming from PCT/GB-90/02017, filed December 21, 1990. The latter PCT application claimed priority benefit of GB national application Serial No. 89/28874.0, filed December 21, 1989.

In attached Appendix C is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claims 28 and 29 which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner advise them as soon as possible whether the Examiner intends to declare an interference between the present application and

Queen et al. Specifically, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,



Doreen Yatko Trujillo
Registration No. 35,719

Date: April 9, 1999

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APPENDIX A

PROPOSED COUNT FOR INTERFERENCE

Count 1:

A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with:

(i) an effective antigen binding activity, or

(ii) an affinity constant of at least 10^7 M^{-1} and no greater than about four-fold that of the donor immunoglobulin,

wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside:

(a) the Kabat and Chothia CDRs, or

(b) both the Kabat CDRs and the structural loop CDRs of the variable regions,

wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) (a) contains an atom within a distance of 4 of or (b) is spatially close to a CDR in said humanized immunoglobulin .

APPENDIX B

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1},	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.
and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence	See page 11, lines 16-20, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At page 6, lines 25-35, it is indicated that the heavy chain "framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49...." In the heavy chain, Kabat CDR2 together with [Chothia] structural loop H2 extends from residues 50 to 65. Thus, residue 49 is immediately adjacent the beginning of this CDR2/H2 region.

or contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 6, 23,24, and 48 are identified in Figure 4.

APPENDIX C

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.
and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence	See page 7, lines 11-14, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At p.5, l. 9-16, reference is made to heavy chain "framework comprises donor at at least one of residues 6, 23 and/or 24, 48 and/or 49...." Residue 49 is immediately adjacent CDR2/H2 loop region.
or contributes to antigen binding as determined by X-ray crystallography.	Page 18, lines 11-17, and lines 33-37, and Figs. 20-21 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 6, 23,24, and 48 are identified in Figure 21.

29. A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity similar to that of said donor immunoglobulin.	Page 23, lines 1-10.

AF

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75962

DATE: November 4, 1999

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DOCKET NO.: CARP-0037 **PATENT**

RESPONSE UNDER 37 CFR 1.116
EXPEDITED PROCEDURE
EXAMINING GROUP NO. 1642

21 II
D.G.J
11/9/99
(NE)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Burke**

For: **Humanised Antibodies**

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being transmitted by facsimile to Examiner Burke of the U.S. Patent and Trademark Office, Washington, D.C. 20231.

On November 3, 1999

Doreen Yatko Trujillo
Doreen Yatko Trujillo Reg. No. 35,719

Do not enter
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Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

11/09/1999 LPENDER 00000003 23 [illegible]
01 FC:117 [illegible]

Pursuant to 37 C.F.R. § 1.116, please amend the above-identified application as a follows.

In the claims:

24. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the

variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

28. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

Please add the following claim:

32. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks at residues 48, 49, 71, 73, 76, 78, 88, and 91.

REMARKS

This paper is being filed in response to the Final Rejection dated May 28, 1999. No extension of time is believed necessary for responding to the Final Rejection. To the extent this belief is in error, Applicants hereby request the necessary extension and the undersigned authorizes charging any such fee to Deposit Account 23-3050.

Claims 24-31 were pending. In the Final Rejection, all pending claims were rejected. Claim 32 has been added herein. In view of the foregoing amendments and the arguments that follow, Applicants respectfully submit that allowable subject matter has been identified and request that the interference be declared.

The Examiner stated that the Information Disclosure Statements filed in the parent cases will be considered once the references are submitted. To the extent the Examiner is requiring that Applicants resubmit references already submitted, this appears to be contrary to MPEP § 609, page 600-103. Applicants are not required to resubmit references to get them considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 24-31 were again rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection for the reasons that follow. For the Examiner's convenience, the paragraphs are designated to correspond to the Examiner's paragraphs for the rejection remaining under this section.

a. and b. The Examiner again rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 were previously amended to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. During a telephone conference between the Examiner and the undersigned, the Examiner indicated that removal of the "adjacent to a CDR" language would

obviate this rejection. Although Applicants disagree with the Examiner's reasoning, the claims have been amended herein to remove the recitation "adjacent to a CDR in the donor immunoglobulin sequence." As Applicants made clear in the previous response, the contribution to antigen binding need not be direct and, indeed, can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing. Naturally, even for an indirect effect, the residues must be spatially near the CDR.

Applicants respectfully request that this rejection be withdrawn.

As the Examiner is aware, Applicants desire to provoke an interference between the present application and the Queen patent (U.S. Patent No. 5,585,089). Although Applicants are confident that the present claims are directed to the same invention as the Queen patent, new claim 32 is submitted herewith. New claim 32 recites the residues changed in example g341B disclosed in Applicants' specification as filed and, indeed, in GB8928874. Of the residues recited, all are either adjacent a CDR (49), or contribute to antigen binding as determined by X-ray crystallography (48, 71, 73, 76, 78, 88, and 91). Claim 32 is clearly allowable and clearly directed to the same invention as claim 1 of the Queen patent.

> If at least one of the presented claims is not rejectable on any [ ] ground and is claiming the same invention as at least one claim of the patent, the examiner should proceed to initiate an interference.

MPEP 2307.02.

Applicants respectfully request that an interference between the present application and the Queen patent be declared.

Rejection Under 35 U.S.C. § 102(e)

Claims 24-31 were again rejected under 35 U.S.C. § 102(e) in view of the Queen patent. Applicants respectfully traverse this rejection. Again, the relevant inquiry as to whether the Queen patent is an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that the limitation "outside the

Kabat and Chothia CDRs" is not found in, nor supported by, the priority documents.

This limitation requires that framework residues be changed outside both the Kabat and Chothia CDRs. This limitation is significant because the "CDRs" as defined by Kabat and Chothia differ.[1] Kabat defines CDR1 of the heavy chain as amino acids 31-35. Chothia defines the first hypervariable loop of the heavy chain as residues 26-32. As submitted in the Preliminary Amendment filed concurrently with the present application, the earliest Queen patent applications do not teach, either explicitly or implicitly, that the framework residues to be replaced by donor must be outside both the Kabat and Chothia CDRs. Indeed, in the specification of the Queen patent as issued, changes were made to residues inside what the Queen patent denotes as CDRH1 of Chothia, i.e., inside a Chothia CDR. Considering that the "outside the Kabat and Chothia CDRs" limitation was required for patentability, the Queen patent cannot be entitled to a priority date earlier than the filing date of the application in which this limitation was first introduced, i.e., 12/19/90.

The Examiner argued in the Final Rejection that the limitation is taught, for example, on page 9, lines 1-5 of Queen priority Application Serial No. 07/290,975 ("Queen '975") and page 13, lines 1-8 of Queen priority Application Serial No. 08/310,252 ("Queen '252"). The passages cited by the Examiner, however, do not support the Examiner's position.

The passage on page 9, lines 1-5, of Queen '975, contains a background discussion of the hypervariable regions, which it is therein stated are also called the CDRs. References by Kabat and Chothia are cited, and incorporated by reference. This is the only in passage in Queen '975 linking the Chothia reference to the term "CDRs." Other passages specifically referring to the CDRs as encompassed by the invention of Queen '975 make it clear that the CDRs are as defined by Kabat. For example, on page 10, line 2, the framework regions are defined in terms of Kabat. If the framework regions are defined in terms of Kabat,

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[1]Notably, the Chothia reference refers to loops and carefully distinguishes these loops from the Kabat CDRs.

the CDRs must be as well. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 19-22, residues which fall in positions within a CDR "as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106" are to be donor. In lines 28-30, amino acid 30 is listed as a position immediately adjacent to a CDR to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but within the heavy chain Chothia "CDR" as that term is used in Queen '975. The description of Figure 1 of Queen '975 indicates that it refers to the heavy chains and that the three CDRs are underlined (page 6, lines 1-6). In Figure 1, amino acids 31-35 are underlined for CDR1. Clearly, all specific references to CDRs were to Kabat CDRs only.

Further, in Figure 1, framework amino acids changed to donor are indicated by asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '975 teaches changing only one or two amino acids, and that both can be within the Chothia CDR. There is no support in Queen '975 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Neither is there support for the limitation in Queen '252. In this instance, the passage relied upon by the Examiner for referring to Chothia is in the context of computer programs for computer models. There is no reference to CDRs. Contrastingly, the specific references to CDRs make it clear that the CDRs are as defined by Kabat. On page 8, lines 22-26, Queen '252 reports that the extents of the framework region and CDRs have been "precisely defined" by Kabat. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 20-22, residues which fall in positions within a CDR "as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106" are to be donor. In lines 27-29, amino acid 30 is listed as a position immediately adjacent to a CDR to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but within the heavy chain Chothia "CDR" as that term is used in Queen '975.

Again, in Figure 1, framework amino acids changed to donor are indicated by

asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '252 teaches changing only one or two amino acids to donor, and that both can be within the Chothia CDR. There is no support in Queen '252 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Applicants respectfully request that this rejection be withdrawn.

The Proposed Count is the same as that submitted with the Amendment filed April 9, 1999. Applicants again identify all of the Queen patent claims 1-11 and Applicants' claims 24-32 as corresponding to the Proposed Count.

In attached Appendix A, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24 and new claim 32. There is, of course, additional support in applicants' application omitted for the sake of brevity.

In attached Appendix B is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claim 28 and new claim 38 which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner declare an interference between the

present application and the Queen patent. Specifically, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo
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Date: November 3, 1999

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APPENDIX A

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are so identified in Figure 4.
32. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 41.

APPENDIX B

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 18, lines 11-17, and lines 33-37, and Figs. 20-21 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are identified in Figure 21.
32. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 20.

DOCKET NO.: CARP-0057 **PATENT**

~~RESPONSE UNDER 37 CFR 1.116~~
~~EXPEDITED PROCEDURE~~
~~EXAMINING GROUP NO. 1642~~

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Burke**

For: **Humanised Antibodies**

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24 J ME
60
2-200

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being transmitted by facsimile to Examiner Burke of the U.S. Patent and Trademark Office, Washington, D.C. 20231,

On January 19, 2000
Doreen Yatko Trujillo
Doreen Yatko Trujillo Reg No. 35,719

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Please do not enter 2/3/00 QB

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

Pursuant to 37 C.F.R. § 1.116, please amend the above-identified application as a follows.

In the claims:

24. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^6 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the

Carter Exhibit 2027
Carter v. Adair
Interference No. 105,744

variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

28. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

Please add the following claim:

49. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy chain framework at residues 48, 49, 71, 73, 76, 78, 88, and 91.

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REMARKS

This paper is being filed following the Advisory Action dated December 2, 1999. A Notice of Appeal was filed November 29, 1999. Accordingly, it is Applicants' belief that no extension of time or accompanying fee is required. If Applicants' belief is erroneous, this serves to request the requisite extension of time and authorizes the charging of any fee to Deposit Account 23-3050.

Claims 24-31 were pending. In the Final Rejection, all pending claims were rejected. An Amendment and Request for Reconsideration ("Amendment") was filed November 3, 1999 in response to the Final Rejection. The Amendment was not entered in view of what the Examiner considered new matter in a newly submitted claim. The Advisory Action, however, indicated that the Amendment would have overcome the then outstanding rejections under 112 and for new matter of claims 24 and 28. The previous amendments to claims 24 and 28 are resubmitted herein. Their entry is earnestly requested.

New claim 49 has been added herein. New claim 49 refers to specific replacements in the heavy chain. In that regard, the Examiner is directed to Table 1 of the application as filed, specifically to the "Heavy Chain" designated as 341 b. Applicants respectfully submit that new claim 49 does not contain new matter and does not raise new 35 U.S.C. § 112, first and second paragraph issues, nor does it raise new 102/103 issues. Claim 49 is submitted herein in an abundance of caution in view of the removal of the phrase "adjacent to a CDR in the donor immunoglobulin sequence" from claims 24 and 28 as suggested by the Examiner in the Final Rejection. Claim 49 recites a specific residue that is adjacent a CDR, i.e., residue 49. If, however, Applicants' submission of claim 49 is all that stands between the application being in condition for interference , Applicants respectfully request that the Examiner so advise the undersigned at (215) 564-8352.

In view of the foregoing amendments and the arguments that follow, Applicants respectfully submit that allowable subject matter has been identified and request that the interference be declared.

The Examiner stated that the Information Disclosure Statements filed in the

parent cases will be considered once the references are submitted. To the extent the Examiner is requiring that Applicants resubmit references already submitted, this appears to be contrary to MPEP § 609, page 600-103. Applicants are not required to resubmit references to get them considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 24-31 were again rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection for the reasons that follow. For the Examiner's convenience, the paragraphs are designated to correspond to the Examiner's paragraphs for the rejection remaining under this section.

a. and b. The Examiner again rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 were previously amended to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. During a telephone conference between the Examiner and the undersigned, the Examiner indicated that removal of the "adjacent to a CDR" language would obviate this rejection. Although Applicants disagree with the Examiner's reasoning, the claims have been amended herein to remove the recitation "adjacent to a CDR in the donor immunoglobulin sequence." As Applicants made clear in the previous response, the contribution to antigen binding need not be direct and, indeed, can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing. Naturally, even for an indirect effect, the residues must be spatially near the CDR.

Applicants respectfully request that this rejection be withdrawn.

As the Examiner is aware, Applicants desire to provoke an interference between the present application and the Queen patent (U.S. Patent No. 5,585,089). Although Applicants are confident that the present claims are directed to the same invention as the

Queen patent, new claim 49 is submitted herewith. New claim 49 recites the residues changed in example g341B disclosed in Applicants' specification as filed and, indeed, in GB8928874. Of the residues recited, all are either adjacent a CDR (49), or contribute to antigen binding as determined by X-ray crystallography (48, 71, 73, 76, 78, 88, and 91). Claim 49 is clearly allowable and clearly directed to the same invention as claim 1 of the Queen patent.

> If at least one of the presented claims is not rejectable on any [ ] ground and is claiming the same invention as at least one claim of the patent, the examiner should proceed to initiate an interference.

MPEP 2307.02.

Applicants respectfully request that an interference between the present application and the Queen patent be declared.

Rejection Under 35 U.S.C. § 102(e)

Claims 24-31 were again rejected under 35 U.S.C. § 102(e) in view of the Queen patent. Applicants respectfully traverse this rejection. Again, the relevant inquiry as to whether the Queen patent is an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that the limitation "outside the Kabat and Chothia CDRs" is not found in, nor supported by, the priority documents.

This limitation requires that framework residues be changed outside both the Kabat and Chothia CDRs. This limitation is significant because the "CDRs" as defined by Kabat and Chothia differ.[1] Kabat defines CDR1 of the heavy chain as amino acids 31-35. Chothia defines the first hypervariable loop of the heavy chain as residues 26-32. As submitted in the Preliminary Amendment filed concurrently with the present application, the earliest Queen patent applications do not teach, either explicitly or implicitly, that the

[1]Notably, the Chothia reference refers to loops and carefully distinguishes these loops from the Kabat CDRs.

framework residues to be replaced by donor **must be outside both** the Kabat and Chothia CDRs. Indeed, in the specification of the Queen patent as issued, changes were made to residues inside what the Queen patent denotes as CDRH1 of Chothia, i.e., inside a Chothia CDR. Considering that the "outside the Kabat and Chothia CDRs" limitation was required for patentability, the Queen patent cannot be entitled to a priority date earlier than the filing date of the application in which this limitation was first introduced, i.e., 12/19/90.

The Examiner argued in the Final Rejection that the limitation is taught, for example, on page 9, lines 1-5 of Queen priority Application Serial No. 07/290,975 ("Queen '975") and page 13, lines 1-8 of Queen priority Application Serial No. 08/310,252 ("Queen '252"). The passages cited by the Examiner, however, do not support the Examiner's position.

The passage on page 9, lines 1-5, of Queen '975, contains a background discussion of the hypervariable regions, which it is therein stated are also called the CDRs. References by Kabat and Chothia are cited, and incorporated by reference. This is the only in passage in Queen '975 linking the Chothia reference to the term "CDRs." Other passages specifically referring to the CDRs as encompassed by the invention of Queen '975 make it clear that the CDRs are as defined by Kabat. For example, on page 10, line 2, the framework regions are defined in terms of Kabat. If the framework regions are defined in terms of Kabat, the CDRs must be as well. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 19-22, residues which fall in positions within a CDR "**as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106**" are to be donor. In lines 28-30, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but **within** the heavy chain Chothia "CDR" as that term is used in Queen '975. The description of Figure 1 of Queen '975 indicates that it refers to the heavy chains and that the three CDRs are underlined (page 6, lines 1-6). In Figure 1, amino acids 31-35 are underlined for CDR1. Clearly, all specific references to CDRs were to Kabat CDRs only.

Further, in Figure 1, framework amino acids changed to donor are indicated by

asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '975 teaches changing only one or two amino acids, and that both can be within the Chothia CDR. There is no support in Queen '975 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Neither is there support for the limitation in Queen '252. In this instance, the passage relied upon by the Examiner for referring to Chothia is in the context of computer programs for computer models. There is no reference to CDRs. Contrastingly, the specific references to CDRs make it clear that the CDRs are as defined by Kabat. On page 8, lines 22-26, Queen '252 reports that the extents of the framework region and CDRs have been "precisely defined" by Kabat. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 20-22, residues which fall in positions within a CDR "**as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106**" are to be donor. In lines 27-29, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but **within** the heavy chain Chothia "CDR" as that term is used in Queen '975.

Again, in Figure 1, framework amino acids changed to donor are indicated by asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '252 teaches changing only one or two amino acids to donor, and that both can be within the Chothia CDR. There is no support in Queen '252 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Applicants respectfully request that this rejection be withdrawn.

The Proposed Count is the same as that submitted with the Amendment filed April 9, 1999. Applicants again identify all of the Queen patent claims 1-11 and Applicants' claims 24-31 and 49 as corresponding to the Proposed Count.

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In attached Appendix A, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24 and new claim 49. There is, of course, additional support in applicants' application omitted for the sake of brevity.

In attached Appendix B is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claim 28 and new claim 49 which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner declare an interference between the present application and the Queen patent. Specifically, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo

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Registration No. 35,719

Date: January 19, 2000

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