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ISSUE CLASSIFICATION

Class

...LITY ...RIAL ...MBER	PATENT DATE	PATENT NUMBER
08/146206	JUN 18 2002	[redacted]

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER

APPLICANTS

Foreign priority claimed 35 USC 119 conditions met	yes / no	yes / no	Verified and Acknowledged	Examiner's Initials	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
	☐	☑						52	5		

ADDRESS

TITLE

U.S. DEPT. OF COMM./ PAT. & TM—PTO-436L (Rev.12-94)

PARTS OF APPLICATION FILED SEPARATELY

Applications Examiner

NOTICE OF ALLOWANCE MAILED	Assistant Examiner	CLAIMS ALLOWED	
		Total Claims	Print Claim
		50	1

ISSUE FEE		Primary Examiner	DRAWING		
Amount Due	Date Paid		Sheets Drwg.	Figs. Drwg.	Print Fig.
			01	8	1

Label Area

PREPARED FOR ISSUE

ISSUE BATCH NUMBER

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

Form PTO-436A (Rev. 8/92)

(FACE)

PFIZER EX. 1502

PATENT NUMBER

APPLICATION SERIAL NUMBER
08/146 206

APPLICANT'S NAME (PLEASE PRINT)
CARTER et al

RE-ISSUE, ORIGINAL PATENT NUMBER

ORIGINAL CLASSIFICATION

CLASS	SUBCLASS
530	387.3

CROSS REFERENCE(S)

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)			
435	69.6	69.7	70.21	91
536	23.53			
424	133.1			

INTERNATIONAL CLASSIFICATION

C	07	K		16	/00
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					/

GROUP ART UNIT: 1642

ASSISTANT EXAMINER (PLEASE STAMP OR PRINT FULL NAME): MINH-TAM DAVIS

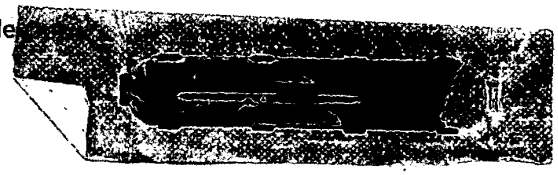
PRIMARY EXAMINER (PLEASE STAMP OR PRINT FULL NAME): Anthony C. Caputa

PTO 270
(REV. 5-91)

ISSUE CLASSIFICATION SLIP

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

Best Available



SEARCHED

Class	Sub.	Date	Exmr.
435	69.6	12/5/94	GA
	69.7		
	70.21		
	91		
	1722		
	240.1		
	240.27		
	252.3		
	320.1		
536	23.53		
Updated		11/13/95	GA
updated		12-16-96	PN
updated		12-20-97	PN
updated		3/25/99	YR
424	133.1		
435	328		
530	387.3		
updated		10/13/00	TDC
updated		12/10/01	TDC

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.
530	387.3	12/10/01	TDC
435	69.6		
	69.7		
	70.21		
	91		
536	23.53		
424	133.1		
435	328		

34. After Issue Clerk 10/8/02
35. Clerical 3rd 12/2
36. Change of Address
37. Int. Summary 8-13-98
38. Appeal Brief(s) 8-10-98
39. Amdt & (Rule 1.129(a)) 08-26-98
40. Declaration Suppl. Dec 08-26-98
41. Interview Summary 11/16/98
42. Suppl. Amdt I 11/16/98
43. Interview Summary 1/7/99
44. Suppl. Amdt J 1/15/99
45. Req. For PTE application (156) 8-25-06
46. PTE Eligibility STP 6-15-07

SEARCH NOTES

	Date	Exmr.
UPDATED	12/16/96	PN
updated	12/20/97	PN
Received 07/715,272 -updated	3/25/99	YR
discussed case w/ Lila Feisee & SPE Paula Hutzell	17Nov99	DB
updated	10/13/00	TDC
consultation w/ Paula Hutzell, Technical specialist on 112, 1st and 103 issues, Toni Scott, SPE	07/19/01	TDC
updated	12/10/01	TDC

(RIGHT [illegible] IDE)

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POSITION		ID NO.	DATE
CLASSIFIER			
EXAMINER		112	
TYPIST		339	5/9/94
VERIFIER			
CORPS CORR.			
SPEC. HAND			
FILE MAINT.			
DRAFTING			

ISSUE CLASSIFICATION

Class

SERIAL NUMBER 08/14620

APPLICANTS

Foreign priority claimed ☐
35 USC 119 conditions met ☐
Verified and Acknowledged Exa

ADDRESS

TITLE

PARTS OF APPLICATION FILED SEPARATELY

NOTICE OF ALLOWANCE MAILED

12/18/01

ISSUE FEE

Amount Due	Date Paid

Label Area

Form PTO-436A
(Rev. 8/92)

INDEX OF CLAIMS

Final	Original
	1
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	3
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SYMBOLS

✓ .... Rejected
= .... Allowed
- (Through numberal) Canceled
+ .... Restricted
N .... Non-elected
I .... Interference
A .... Appeal
O .... Objected

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INDEX OF CLAIMS

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

Claim Final	Claim Original	3/15/99	10/13/00	12/10/01
59	1	=	✓	=
60	2			=
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62	4			=
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64	13		✓	=
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66	15			=
67	16			=
68	17			=
69	18			=
70	19			=
71	20			=
72	21			=
73	22			=
74	23			=
75	24			=
76	25			=
77	26			=
78	27			=
79	28		✓	=
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81	30			=
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If more than 150 claims or 10 actions staple additional sheet here

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08/146206 US

11 Rec'd PCT/PTO 17 NOV 1993

EXPRESS MAIL: HB2147593580US
MAILED: 17 NOVEMBER 1993

FORM PTO-1390 (REV 11-92) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER: 709P1

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US92/05126	15 June 1992 (15.06.92)	14 June 1991 (14.06.91)

TITLE OF INVENTION: METHOD FOR MAKING HUMANIZED ANTIBODIES

APPLICANT(S) FOR DO/EO/US: Paul J. CARTER, Leonard G. PRESTA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. [X] This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).
2. [X] The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees as follows:

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
	TOTAL CLAIMS	23 -20 =	3	X $22.00	$ 66.00
	INDEPENDENT CLAIMS	10 -3 =	7	X $74.00	518.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $230.00	230.00

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

- [ ] For filing with EPO or JPO search report (37CFR 1.492(a)(5))..... $830.00
- [ ] International preliminary examination fee paid to USPTO (37 CFR 1.482) $640.00
- [ ] No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))..$710.00
- [X] Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37CFR 1.445(a)(2)) paid to USPTO..... $950.00

950.00

- [ ] International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Articles 33(2)-33(4)... $90.00

Surcharge of $130.00 for furnishing the National fee or oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

TOTAL OF ABOVE CALCULATIONS	= 1,764.00
Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (Note 37 CFR 1.9, 1.27, 1.28).	
SUBTOTAL	+
Processing fee of $130.00 for furnishing the English translation later the [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	
TOTAL NATIONAL FEE	$1,764.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)).	+ 40.00
TOTAL FEES ENCLOSED	$1,804.00

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.
b. [X] Please charge my Deposit Account No. 07-0630 in the amount of $1,804.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 07-0630. A duplicate copy of this sheet is enclosed.

(January 1993)

ATTORNEY'S DOCKET NUMBER
709P1

3. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International bureau).
   b. ☒ is not required, as the application was filed in the United States receiving Office (RO/US).
   c. ☐ has been transmitted by the International Bureau.

4. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

5. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.

6. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

7. ☒ An oath or declaration of the inventor (35 U.S.C. 371(c)(4)).

8. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Other document(s) or information included:

9. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

10. ☒ An assignment document for recording.
    PLEASE MAIL THE RECORDED ASSIGNMENT DOCUMENT TO :
    a. ☒ the person whose signature, name and address appears at the bottom of this page.
    b. ☐ the following:

11. The above checked items are being transmited:
    a. ☐ before the eighteenth (18) month publication.
    b. ☐ after publication of the Article 20 communication but before twenty (20) months from the priority date.
    c. ☐ after twenty (20) months but before twenty-two (22) months (surcharge and/or processing fee included).
    d. ☐ after twenty-two (22) months (surcharge and /or processing fee included).
    NOTE: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 22 months *and NO proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.*
    e. ☒ by thirty (30) months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
    f. ☐ after thirty (30) months but before thirty-two (32) months and a proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date (surcharge and/or processing fee included).
    g. ☐ after thirty-two (32) months (surcharge and/or processing fee included).
    NOTE: Petition to revive (37 CFR 1.137(a) or (b)) is necessary if 35 U.S.C. 371 requirements submitted after 32 months *and a proper demand for International Preliminary Examination was made by 19 months from the earliest claimed priority date.*

12. At the time of transmittal, the time limit for amending claims under Article 19:
    a. ☒ has expired and no amendments were made.
    b. ☐ has not yet expired.

13. ☐ Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____, namely:

14. Submitted herewith are: Sequence Diskette, Sequence Listing, Preliminary Amendment

NAME Janet E. Hasak
ADDRESS Genentech, Inc.
460 Point San Bruno Boulevard
South San Francisco, CA 94080-4990

SIGNATURE Janet E. Hasak
REGISTRATION NUMBER 28,616

Form PTO-1390 (REV 11-92)

CERTIFICATION UNDER 37 CFR 1.10

HB214759358US : Express Mail Number **17 NOVEMBER 1993** : Date of Deposit

I hereby certify that this request to initial national processing, including: TRANSMITTAL LETTER, PRELIMINARY AMENDMENT, SEQUENCE LISTING & DISKETTE, COMBINED DECLARATION & POWER OF ATTORNEY, ASSIGNMENT and COPY OF PRELIMINARY EXAMINATION REPORT is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Carol Koehler

FIGURE 1A: V_L DOMAIN

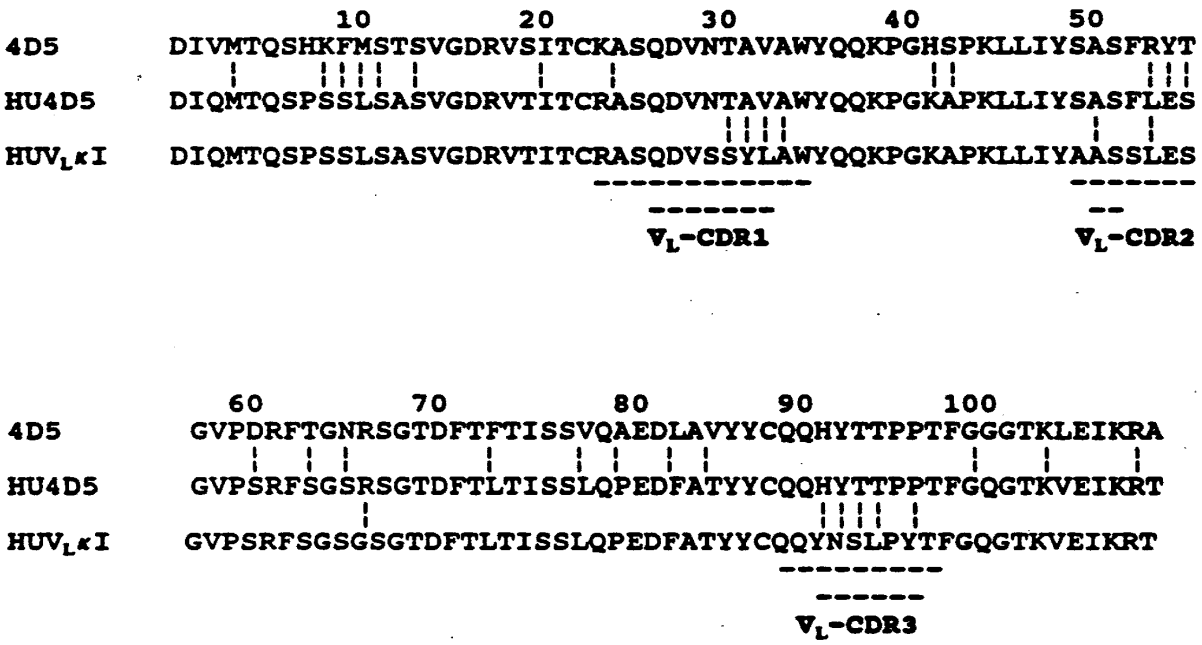

FIGURE 1B: V_H DOMAIN

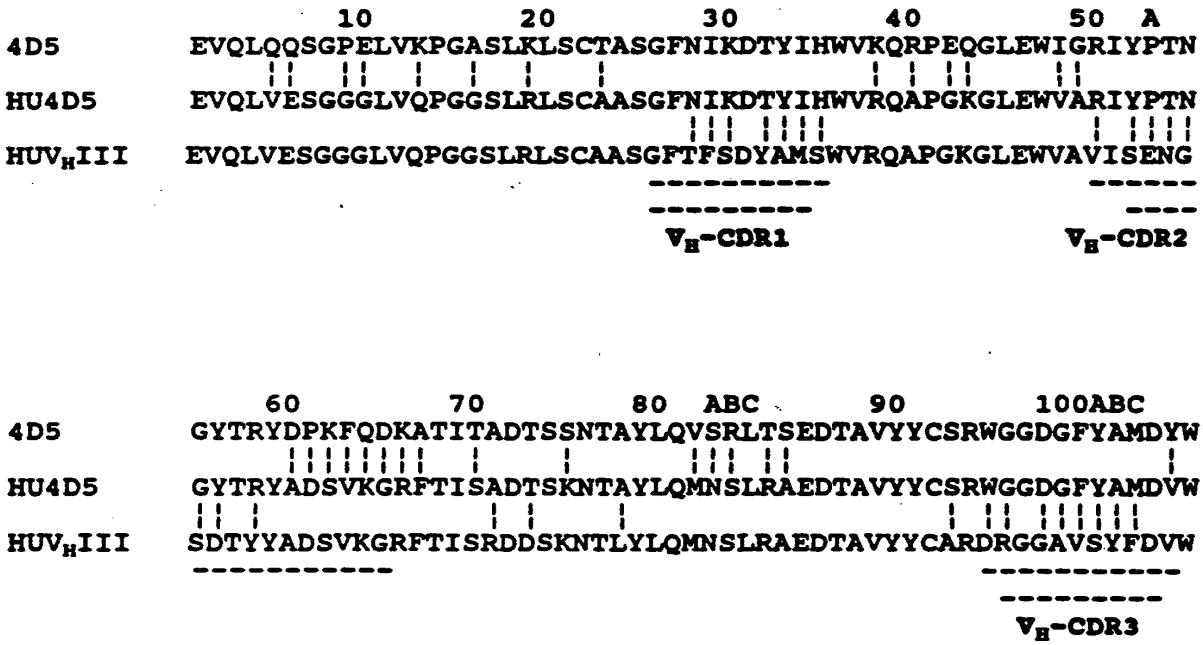

FIGURE 2

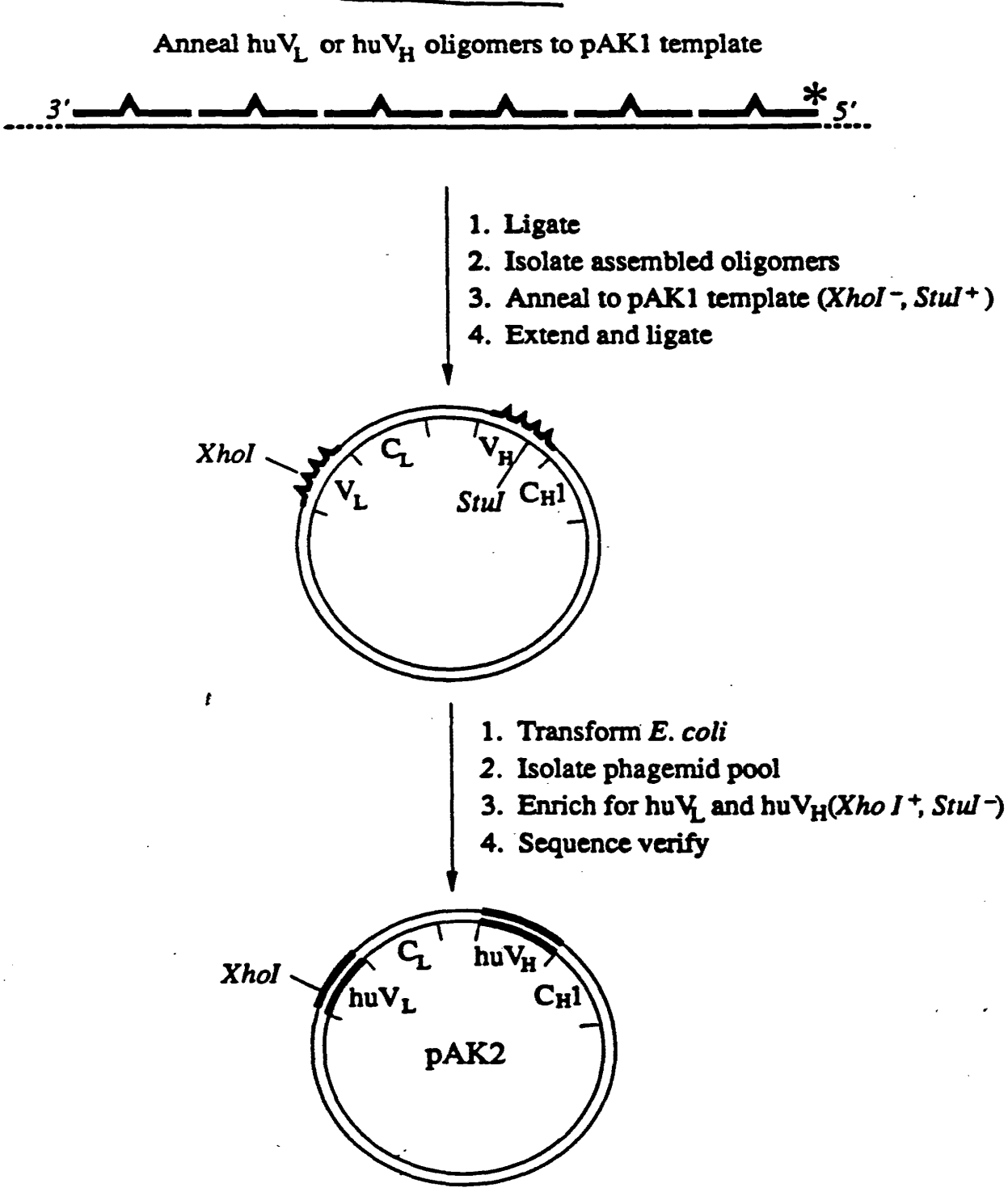
Anneal huV$_L$ or huV$_H$ oligomers to pAK1 template
3'
*
5'
1. Ligate
2. Isolate assembled oligomers
3. Anneal to pAK1 template (*XhoI*$^-$, *StuI*$^+$)
4. Extend and ligate
XhoI
C$_L$
V$_H$
V$_L$
StuI
C$_H$1
1. Transform *E. coli*
2. Isolate phagemid pool
3. Enrich for huV$_L$ and huV$_H$(*Xho I*$^+$,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

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FIGURE 3

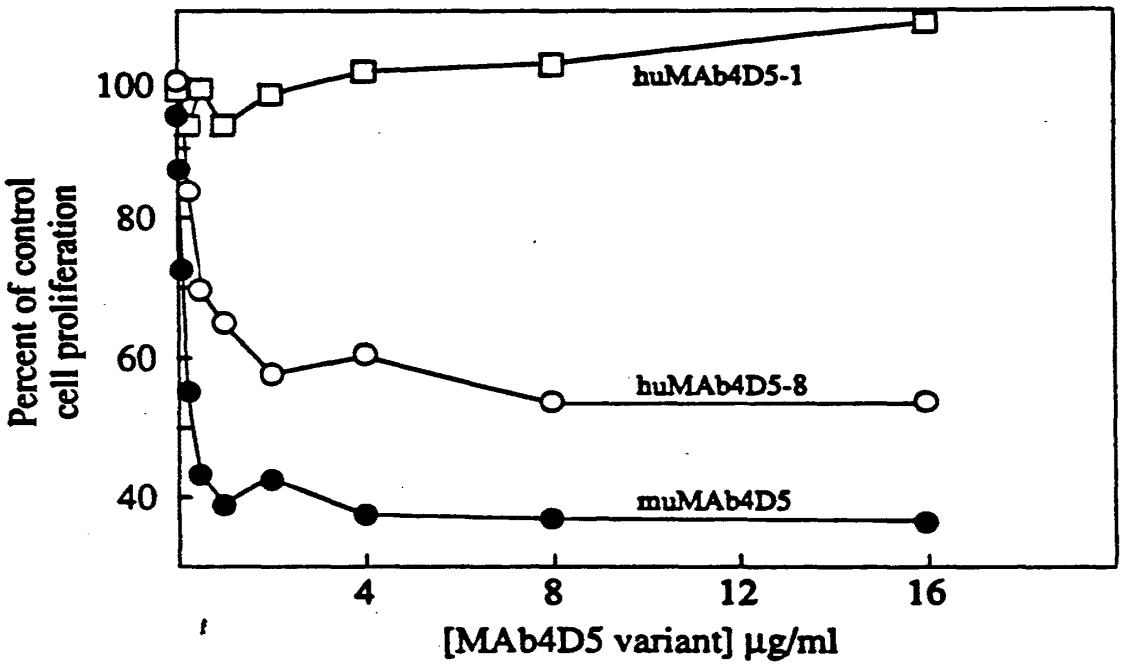
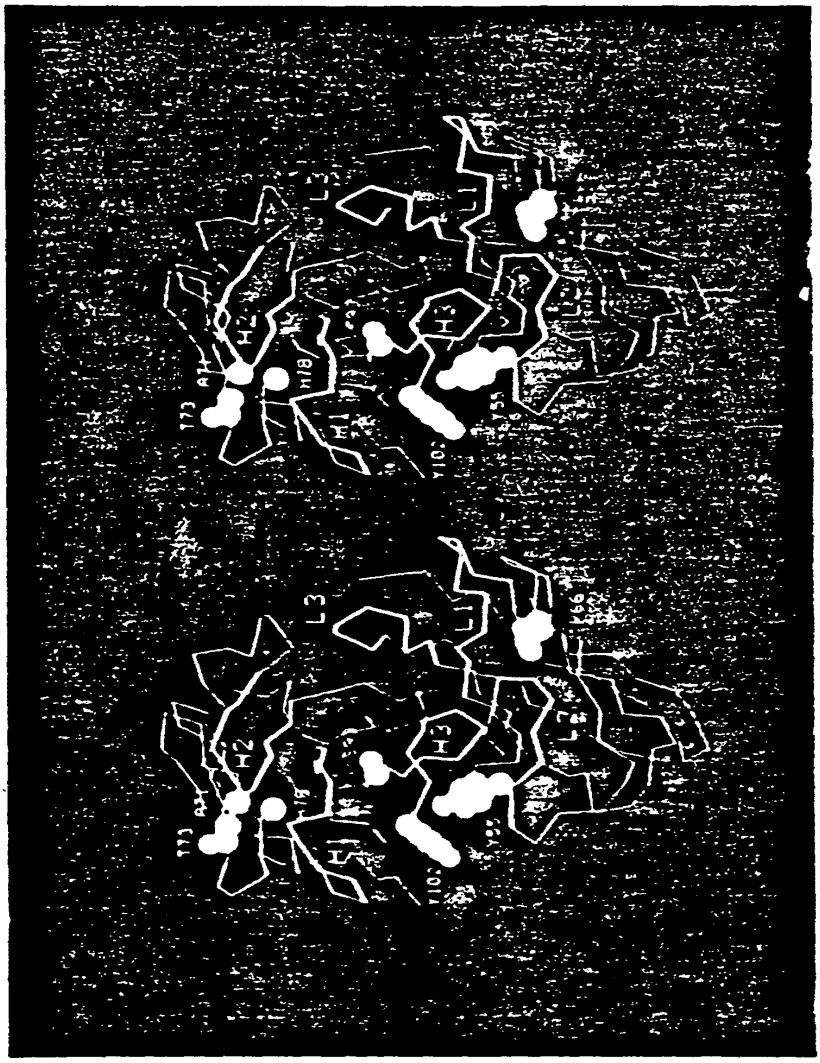

Percent of control cell proliferation
100
80
60
40
huMAb4D5-1
huMAb4D5-8
muMAb4D5
4
8
12
16
[MAb4D5 variant] µg/ml

FIGURE 4



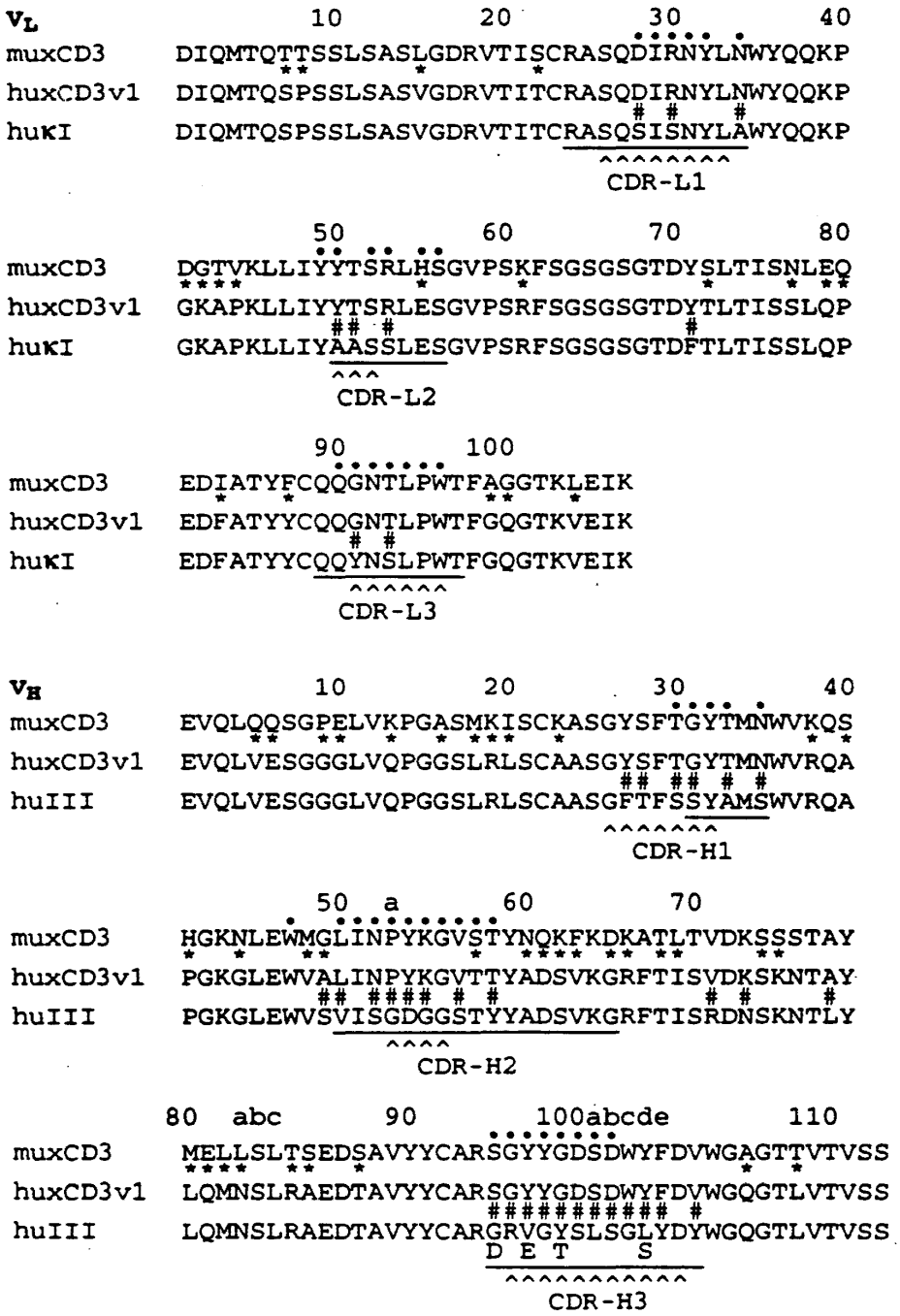

V_L
10 20 30 40
muxCD3 DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLNWYQQKP
huxCD3v1 DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKP
huκI DIQMTQSPSSLSASVGDRVTITCRASQSISNYLAWYQQKP
CDR-L1
50 60 70 80
muxCD3 DGTVKLLIYYTSRLHSGVPSKFSGSGSGTDYSLTISNLEQ
huxCD3v1 GKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQP
huκI GKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQP
CDR-L2
90 100
muxCD3 EDIATYFCQQGNTLPWTFAGGTKLEIK
huxCD3v1 EDFATYYCQQGNTLPWTFGQGTKVEIK
huκI EDFATYYCQQYNSLPWTFGQGTKVEIK
CDR-L3
V_H
10 20 30 40
muxCD3 EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQS
huxCD3v1 EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQA
huIII EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA
CDR-H1
50 a 60 70
muxCD3 HGKNLEWMGLINPYKGVSTYNQKFKDKATLTVDKSSSTAY
huxCD3v1 PGKGLEWVALINPYKGVTTYADSVKGRFTISVDKSKNTAY
huIII PGKGLEWVSVISGDGGSTYYADSVKGRFTISRDNSKNTLY
CDR-H2
80 abc 90 100abcde 110
muxCD3 MELLSLTSEDSAVYYCARSGYYGDSDWYFDVWGAGTTVTVSS
huxCD3v1 LQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSS
huIII LQMNSLRAEDTAVYYCARGRVGYSLSGLYDYWGQGTLVTVSS
D E T S
CDR-H3

FIGURE 5

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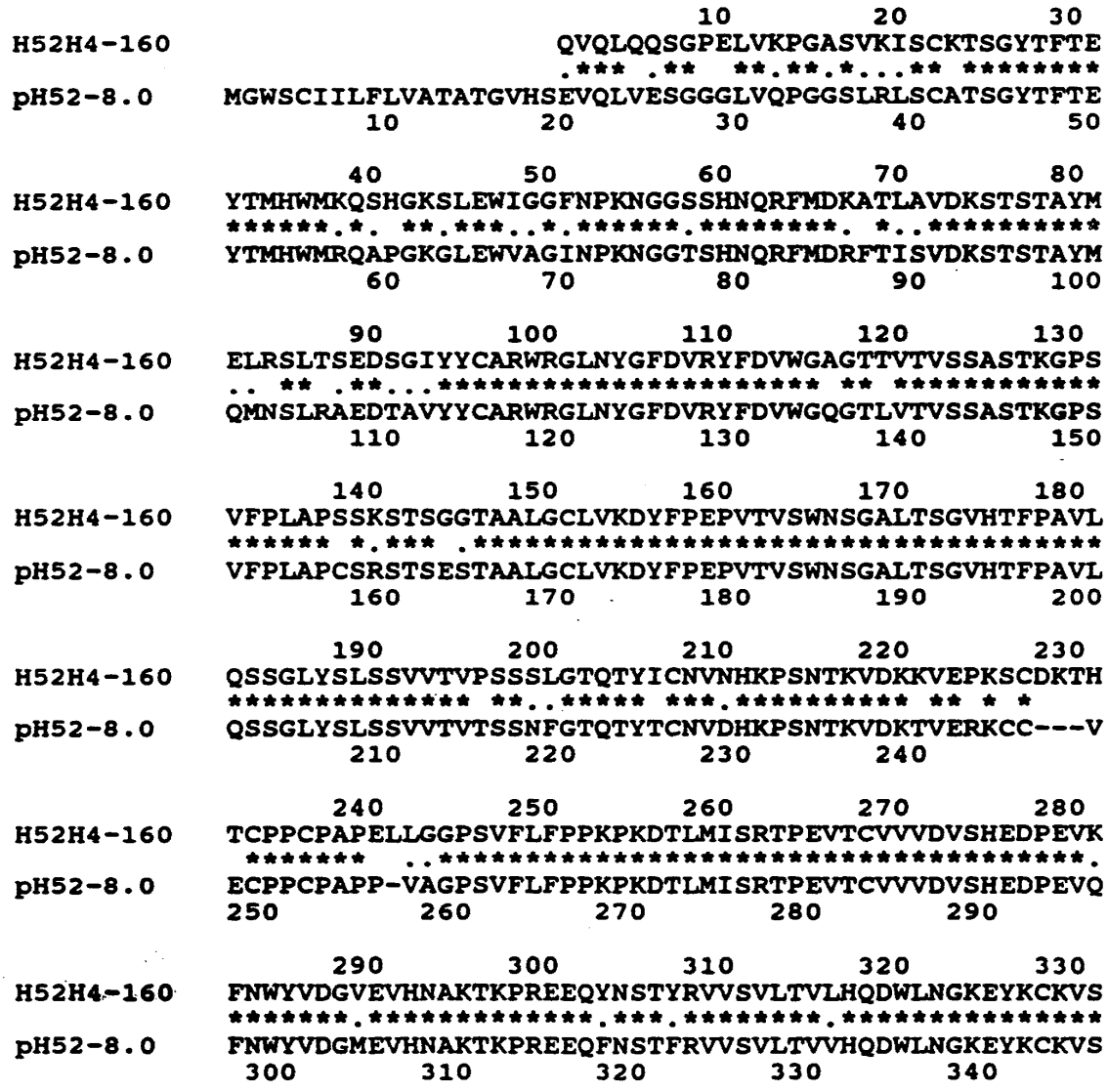
FIGURE 6A


H52H4-160 QVQLQQSGPELVKPGASVKISCKTSGYTFTE
pH52-8.0 MGWSCIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCATSGYTFTE
H52H4-160 YTMHWMKQSHGKSLEWIGGFNPKNGGSSHNQRFMDKATLAVDKSTSTAYM
pH52-8.0 YTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISVDKSTSTAYM
H52H4-160 ELRSLTSEDSGIYYCARWRGLNYGFDVRYFDVWGAGTTVTVSSASTKGPS
pH52-8.0 QMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPS
H52H4-160 VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
pH52-8.0 VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
H52H4-160 QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
pH52-8.0 QSSGLYSLSSVVTVTSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC---V
H52H4-160 TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
pH52-8.0 ECPPCPAPP-VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ
H52H4-160 FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
pH52-8.0 FNWYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVS

```
                340        350        360        370        380
H52H4-160       NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                **.**********.**********************************
pH52-8.0        NKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
                 350        360        370        380        390

                390        400        410        420        430
H52H4-160       SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
                **********************.******************************
pH52-8.0        SDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
                 400        410        420        430        440

                440        450
H52H4-160       CSVMHEALHNHYTQKSLSLSPGK
                ***********************
pH52-8.0        CSVMHEALHNHYTQKSLSLSPGK
                 450        460
```

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FIGURE 6B

```
                                        10        20        30
H52L6-158                          DVQMTQTTSSLSASLGDRVTINCRASQDINN
                                   *.****. ******.****** *********
pH52-9.0    MGWSCIILFLVATATGVHSDIQMTQSPSSLSASVGDRVTITCRASQDINN
                      10        20        30        40        50

                    40        50        60        70        80
H52L6-158   YLNWYQQKPNGTVKLLIYYTSTLHSGVPSRFSGSGSGTDYSLTISNLDQE
            ********* . ****************************.****.*. *
pH52-9.0    YLNWYQQKPGKAPKLLIYYTSTLHSGVPSRFSGSGSGTDYTLTISSLQPE
                      60        70        80        90        100

                    90       100       110       120       130
H52L6-158   DIATYFCQQGNTLPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
            *.***.*********** **********************************
pH52-9.0    DFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
                      110       120       130       140       150

                   140       150       160       170       180
H52L6-158   VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
            **************************************************
pH52-9.0    VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
                      160       170       180       190       200

                   190       200       210
H52L6-158    SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
             ********************************
pH52-9.0     SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
                      210       220       230
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IMMUNOGLOBULIN VARIANTS

Field of the Invention

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

Background of the Invention

Naturally occurring antibodies (immunoglobulins) comprise two heavy chains linked together by disulfide bonds and two light chains, one light chain being linked to each of the heavy chains by disulfide bonds. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain (V_L) at one end and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains, see e.g. Chothia *et al., J. Mol. Biol.* 186:651-663 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci. USA* 82:4592-4596 (1985).

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

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to the formation of the antigen binding site.

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

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

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

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

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

The therapeutic promise of this approach is supported by the clinical efficacy of a humanized antibody specific for the CAMPATH-1 antigen with two non-Hodgkin lymphoma patients, one of whom had previously developed an anti-globulin response to the parental rat antibody (Riechmann, L. *et al.*, *Nature* **332**:323-327 (1988); Hale, G. *et al.*, *Lancet* i:1394-1399 (1988)). A murine antibody to the interleukin 2 receptor has also recently been humanized (Queen, C. *et al.*, *Proc. Natl. Acad. Sci. USA* **86**:10029-10033 (1989)) as a potential immunosuppressive reagent. Additional references related to humanization of antibodies include Co *et al.*, *Proc. Natl. Acad. Sci. USA* 88:2869-2873 (1991); Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4181-4185 (1991); Daugherty *et al.*, *Nucleic Acids Research* 19(9):2471-2476 (1991); Brown *et al.*, *Proc. Natl. Acad. Sci. USA* 88:2663-2667 (1991); Junghans *et al.*, *Cancer Research* 50:1495-1502 (1990).

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

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

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

The three-dimensional structure of immunoglobulin chains has been studied, and crystal

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

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

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

The murine monoclonal antibody known as muMAb4D5 (Fendly, B. M. *et al., Cancer Res.* **50**:1550-1558 (1990)), directed against the extracellular domain (ECD) of $p185^{HER2}$,

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

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

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

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

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

Summary of the Invention

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

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

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

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

d. aligning the amino acid sequences of a Framework Region (FR) of the import

antibody and the corresponding FR of the consensus antibody;

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

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

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L - V_H interface; and

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

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

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

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

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

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

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

b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

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

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

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

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

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLESGVP SRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLV TVSS

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

SEQ. ID NO. 3 (light chain):

DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLESGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT, and

SEQ. ID NO. 4 (heavy chain):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENGGYT RYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTL VTVSS

Brief Description of the Drawings

FIGURE 1A shows the comparison of the V_L domain amino acid residues of muMAb4D5, huMAb4D5, and a consensus sequence (Fig. 1A, SEQ.ID NO. 5, SEQ. ID NO. 1 and SEQ. ID NO. 3, respectively). FIGURE 1B shows the comparison between the V_H domain

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

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

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

FIGURE 4 shows a stereo view of α-carbon tracing for a model of huMAb4D5-8 V_L and V_H. The CDR residues (Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) are shown in bold and side chains of V_H residues A71, T73, A78, S93, Y102 and V_L residues Y55 plus R66 (see Table 3) are shown.

FIGURE 5 shows an amino acid sequence comparison of V_L (top panel) and V_H (lower panel) domains of the murine anti-CD3 monoclonal Ab UCHT1 (muxCD3, Shalaby *et al., J. Exp. Med.* **175**, 217-225 (1992) with a humanized variant of this antibody (huxCD3v9). Also shown are consensus sequences (most commonly occurring residue or pair of residues) of the most abundant human subgroups, namely V_L κ 1 and V_H III upon which the humanized sequences are based (Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest*, 5th edition, National Institutes of Health, Bethesda, MD, USA (1991)). The light chain sequences--muxCD3, huxCD3v9 and huκI--correspond to SEQ.ID.NOs 16, 17, and 18, respectively. The heavy chain sequences--muxCD3, huxCD3v9 and huκI--correspond to SEQ.ID.NOs 19, 20, and 21, respectively. Residues which differ between muxCD3 and huxCD3v9 are identified by an asterisk (*), whereas those which differ between humanized and consensus sequences are identified by a sharp sign (#). A bullet (•) denotes that a residue at this position has been found to contact antigen in one or more crystallographic structures of antibody/antigen

complexes (Kabat *et al.*, 1991; Mian, I. S. *et al.*, *J. Mol. Biol.* 217, 133-151 (1991)). The location of CDR residues according to a sequence definition (Kabat *et al.*, 1991) and a structural definition (Chothia and Lesk, *supra* 1987) are shown by a line and carats (^) beneath the sequences, respectively.

FIGURE 6A compares murine and humanized amino acid sequences for the heavy chain of an anti-CD18 antibody. H52H4-160 (SEQ. ID. NO. 22) is the murine sequence, and pH52-8.0 (SEQ. ID. NO. 23) is the humanized heavy chain sequence. pH52-8.0 residue 143S is the final amino acid in the variable heavy chain domain V_H, and residue 144A is the first amino acid in the constant heavy chain domain C_{H1}.

FIGURE 6B compares murine and humanized amino acid sequences for the light chain of an anti-CD18 antibody. H52L6-158 (SEQ. ID. NO. 24) is the murine sequence, and pH52-9.0 (SEQ. ID. NO. 25) is the humanized light chain sequence. pH52-9.0 residue 128T is the final amino acid in the light chain variable domain V_L, and residue 129V is the first amino acid in the light chain constant domain C_L.

Detailed Description of the Invention

Definitions

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

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

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

Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are referred to herein as "import" residues, which are typically taken from an "import" antibody domain,

particularly a variable domain. An import residue, sequence, or antibody has a desired affinity and/or specificity, or other desirable antibody biological activity as discussed herein.

In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain.

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

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

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

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

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

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

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

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

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

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

Throughout this description, reference is made to the numbering scheme from Kabat, E. A., *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health,

Bethesda, MD (1987) and (1991). In these compendiums, Kabat lists many amino acid sequences for antibodies for each subclass, and lists the most commonly occurring amino acid for each residue position in that subclass. Kabat uses a method for assigning a residue number to each amino acid in a listed sequence, and this method for assigning residue numbers has become standard in the field. The Kabat numbering scheme is followed in this description.

For purposes of this invention, to assign residue numbers to a candidate antibody amino acid sequence which is not included in the Kabat compendium, one follows the following steps. Generally, the candidate sequence is aligned with any immunoglobulin sequence or any consensus sequence in Kabat. Alignment may be done by hand, or by computer using commonly accepted computer programs; an example of such a program is the Align 2 program discussed in this description. Alignment may be facilitated by using some amino acid residues which are common to most Fab sequences. For example, the light and heavy chains each typically have two cysteines which have the same residue numbers; in V_L domain the two cysteines are typically at residue numbers 23 and 88, and in the V_H domain the two cysteine residues are typically numbered 22 and 92. Framework residues generally, but not always, have approximately the same number of residues, however the CDRs will vary in size. For example, in the case of a CDR from a candidate sequence which is longer than the CDR in the sequence in Kabat to which it is aligned, typically suffixes are added to the residue number to indicate the insertion of additional residues (see, e.g. residues 100abcde in Fig. 5). For candidate sequences which, for example, align with a Kabat sequence for residues 34 and 36 but have no residue between them to align with residue 35, the number 35 is simply not assigned to a residue.

Thus, in humanization of an import variable sequence, where one cuts out an entire human or consensus CDR and replaces it with an import CDR sequence, (a) the exact number of residues may be swapped, leaving the numbering the same, (b) fewer import amino acid residues may be introduced than are cut, in which case there will be a gap in the residue numbers, or (c) a larger number of amino acid residues may be introduced then were cut, in which case the numbering will involve the use of suffixes such as 100abcde.

The terms "consensus sequence" and "consensus antibody" as used herein refers to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all immunoglobulins of any particular subclass or subunit structure. The consensus sequence may be based on immunoglobulins of a particular species or of many species. A "consensus" sequence, structure, or antibody is understood to encompass a consensus human sequence as described in certain embodiments of this invention, and to refer

to an amino acid sequence which comprises the most frequently occurring amino acid residues at each location in all human immunoglobulins of any particular subclass or subunit structure. This invention provides consensus human structures and consensus structures which consider other species in addition to human.

The subunit structures of the five immunoglobulin classes in humans are as follows:

Class	Heavy Chain	Subclasses	Light Chain	Molecular Formula
IgG	γ	$\gamma 1$, $\gamma 2$, $\gamma 3$, $\gamma 4$	κ or λ	$(\gamma_2\kappa_2)$, $(\gamma_2\lambda_2)$
IgA	α	$\alpha 1$, $\alpha 2$	κ or λ	$(\alpha_2\kappa_2)_n^*$, $(\alpha_2\lambda_2)_n^*$
IgM	μ	none	κ or λ	$(\mu_2\kappa_2)_5$, $(\mu_2\lambda_2)_5$
IgD	δ	none	κ or λ	$(\delta_2\kappa_2)$, $(\delta_2\lambda_2)$
IgE	ϵ	none	κ or λ	$(\epsilon_2\kappa_2)$, $(\epsilon_2\lambda_2)$

($^*_n$ may equal 1, 2, or 3)

In preferred embodiments of an IgGγ1 human consensus sequence, the consensus variable domain sequences are derived from the most abundant subclasses in the sequence compilation of Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda MD (1987), namely V_L κ subgroup I and V_H group III. In such preferred embodiments, the V_L consensus domain has the amino acid sequence: DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKAPKLLIYAASSLESGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQYNSLPYTFGQGTKVEIKRT (SEQ. ID NO. 3); the V_H consensus domain has the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENGGYTRYAD SVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS (SEQ. ID NO. 4).

These sequences include consensus CDRs as well as consensus FR residues (see for example in Fig. 1).

While not wishing to be limited to any particular theories, it may be that these preferred embodiments are less likely to be immunogenic in an individual than less abundant subclasses. However, in other embodiments, the consensus sequence is derived from other subclasses of human immunoglobulin variable domains. In yet other embodiments, the consensus sequence is derived from human constant domains.

Identity or homology with respect to a specified amino acid sequence of this invention is defined herein as the percentage of amino acid residues in a candidate sequence that are

identical with the specified residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal or internal extensions, deletions, or insertions into the specified sequence shall be construed as affecting homology. All sequence alignments called for in this invention are such maximal homology alignments. While such alignments may be done by hand using conventional methods, a suitable computer program is the "Align 2" program for which protection is being sought from the U.S. Register of Copyrights (Align 2, by Genentech, Inc., application filed 9 December 1991).

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

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

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLESGVP SRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRT (SEQ. ID NO. 1, which is the light chain variable domain of huMAb4D5); or

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDVWGQGTLV TVSS (SEQ. ID NO. 2, which is the heavy chain variable domain of huMAb4D5).

"Biological property", as relates for example to anti-p185^{HER2}, for the purposes herein means an *in vivo* effector or antigen-binding function or activity that is directly or indirectly performed by huMAb4D5 (whether in its native or denatured conformation). Effector functions include p185^{HER2} binding, any hormonal or hormonal antagonist activity, any mitogenic or agonist or antagonist activity, any cytotoxic activity. An antigenic function means possession

of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against the polypeptide sequence of huMAb4D5.

Biologically active huMAb4D5 is defined herein as a polypeptide that shares an effector function of huMAb4D5. A principal known effector function of huMAb4D5 is its ability to bind to $p185^{HER2}$.

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

An "isolated" polypeptide means polypeptide which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, for example, a polypeptide product comprising huMAb4D5 will be purified from a cell culture or other synthetic environment (1) to greater than 95% by weight of protein as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a gas- or liquid-phase sequenator (such as a commercially available Applied Biosystems sequenator Model 470, 477, or 473), or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated huMAb4D5 includes huMAb4D5 <u>in situ</u> within recombinant

cells since at least one component of the huMAb4D5 natural environment will not be present. Ordinarily, however, isolated huMAb4D5 will be prepared by at least one purification step.

In accordance with this invention, huMAb4D5 nucleic acid is RNA or DNA containing greater than ten bases that encodes a biologically or antigenically active huMAb4D5, is complementary to nucleic acid sequence encoding such huMAb4D5, or hybridizes to nucleic acid sequence encoding such huMAb4D5 and remains stably bound to it under stringent conditions, and comprises nucleic acid from a muMAb4D5 CDR and a human FR region.

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

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

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

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally,

"operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading phase. However enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, then synthetic oligonucleotide adaptors or linkers are used in accord with conventional practice.

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

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

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

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

utilizes a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid or to amplify or generate a specific piece of nucleic acid which is complementary to a particular nucleic acid.

Suitable Methods for Practicing the Invention

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

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

Molecular Modeling

An integral step in our approach to antibody humanization is construction of computer graphics models of the import and humanized antibodies. These models are used to determine if the six complementarity-determining regions (CDRs) can be successfully transplanted from the import framework to a human one and to determine which framework residues from the import antibody, if any, need to be incorporated into the humanized antibody in order to maintain CDR conformation. In addition, analysis of the sequences of the import and humanized antibodies and reference to the models can help to discern which framework residues are unusual and thereby might be involved in antigen binding or maintenance of proper antibody structure.

All of the humanized antibody models of this invention are based on a single three-dimensional computer graphics structure hereafter referred to as the consensus structure. This consensus structure is a key distinction from the approach of previous workers in the field, who typically begin by selecting a human antibody structure which has an amino acid sequence which is similar to the sequence of their import antibody.

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The consensus structure of one embodiment of this invention was built in five steps as described below.

Step 1: Seven Fab X-ray crystal structures from the Brookhaven Protein Data Bank were used (entries 2FB4, 2RHE, 3FAB, and 1REI which are human structures, and 2MCP, 1FBJ, and 2HFL which are murine structures). For each structure, protein mainchain geometry and hydrogen bonding patterns were used to assign each residue to one of three secondary structure types: alpha-helix, beta-strand or other (i.e. non-helix and non-strand). The immunoglobulin residues used in superpositioning and those included in the consensus structure are shown in Table 1.

Table I
Immunoglobulin Residues Used in Superpositioning and Those Included in the Consensus Structure

				$V_L\kappa$ domain				
Ig[a]	2FB4	2RHE	2MCP	3FAB	1FBJ	2HFL	1REI	Consensus[b]
								2-11
	18-24	18-24	19-25	18-24	19-25	19-25	19-25	16-27
	32-37	34-39	39-44	32-37	32-37	32-37	33-38	33-39
								41-49
	60-66	62-68	67-72	53-66	60-65	60-65	61-66	59-77
	69-74	71-76	76-81	69-74	69-74	69-74	70-75	
	84-88	86-90	91-95	84-88	84-88	84-88	85-89	82-91
								101-105
RMS[c]		0.40	0.60	0.53	0.54	0.48	0.50	

			V_H domain			
Ig[a]	2FB4	2MCP	3FAB	1FBJ	2HFL	Consensus[b]
						3-8
	18-25	18-25	18-25	18-25	18-25	17-23
	34-39	34-39	34-39	34-39	34-39	33-41
	46-52	46-52	46-52	46-52	46-52	45-51
	57-61	59-63	56-60	57-61	57-61	57-61
	68-71	70-73	67-70	68-71	68-71	66-71
	78-84	80-86	77-83	78-84	78-84	75-82
	92-99	94-101	91-98	92-99	92-99	88-94
						102-108
RMS[c]		0.43	0.85	0.62	0.91	
RMS[d]	0.91	0.73	0.77	0.92		

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a Four-letter code for Protein Data Bank file.
b Residue numbers for the crystal structures are taken from the Protein Data Bank files. Residue numbers for the consensus structure are according to Kabat et al.
c Root-mean-square deviation in Å for (N,Cα,C) atoms superimposed on 2FB4.
d Root-mean-square deviation in Å for (N,Cα,C) atoms superimposed on 2HFL.

Step 2: Having identified the alpha-helices and beta-strands in each of the seven structures, the structures were superimposed on one another using the INSIGHT computer program (Biosym Technologies, San Diego, CA) as follows: The 2FB4 structure was arbitrarily chosen as the template (or reference) structure. The 2FB4 was held fixed in space and the other six structures rotated and translated in space so that their common secondary structural elements (i.e. alpha-helices and beta-strands) were oriented such that these common elements were as close in position to one another as possible. (This superpositioning was performed using accepted mathematical formulae rather than actually physically moving the structures by hand.)

Step 3: With the seven structures thus superimposed, for each residue in the template (2FB4) Fab one calculates the distance from the template alpha-carbon atom (Cα) to the analogous Cα atom in each of the other six superimposed structures. This results in a table of Cα-Cα distances for each residue position in the sequence. Such a table is necessary in order to determine which residue positions will be included in the consensus model. Generally, if all Cα-Cα distances for a given residue position were $\leq$ 1.0Å, that position was included in the consensus structure. If for a given position only one Fab crystal structure was > 1.0Å, the position was included but the outlying crystal structure was not included in the next step (for this position only). In general, the seven β-strands were included in the consensus structure while some of the loops connecting the β-strands, e.g. complementarity-determining regions (CDRs), were not included in view of Cα divergence.

Step 4: For each residue which was included in the consensus structure after step 3, the average of the coordinates for individual mainchain N, Cα, C, O and Cβ atoms were calculated. Due to the averaging procedure, as well as variation in bond length, bond angle and dihedral angle among the crystal structures, this "average" structure contained some bond lengths and angles which deviated from standard geometry. For purposes of this invention, "standard geometry" is understood to include geometries commonly accepted as typical, such as the compilation of bond lengths and angles from small molecule structures in Weiner, S.J. *et. al., J. Amer. Chem. Soc.*, **106**: 765-784 (1984).

Step 5: In order to correct these deviations, the final step was to subject the "average" structure to 50 cycles of energy minimization (DISCOVER program, Biosym Technologies) using the AMBER (Weiner, S.J. *et. al., J. Amer. Chem. Soc.*, **106**: 765-784 (1984)) parameter set with only the Cα coordinates fixed (i.e. all other atoms are allowed to move) (energy minimization is described below). This allowed any deviant bond lengths and angles to assume a standard (chemically acceptable) geometry. See Table II.

Table II
Average Bond Lengths and Angles for "Average" (Before) and Energy-Minimized Consensus (After 50 Cycles) Structures

	V_{LK} before (Å)	V_{LK} after (Å)	V_H before (Å)	V_H after (Å)	Standard Geometry (Å)
N-Cα	1.459(0.012)	1.451(0.004)	1.451(0.023)	1.452(0.004)	1.449
Cα-C	1.515(0.012)	1.523(0.005)	1.507(0.033)	1.542(0.005)	1.522
O=C	1.208(0.062)	1.229(0.003)	1.160(0.177)	1.231(0.003)	1.229
C-N	1.288(0.049)	1.337(0.002)	1.282(0.065)	1.335(0.004)	1.335
Cα-Cβ	1.508(0.026)	1.530(0.002)	1.499(0.039)	1.530(0.002)	1.526
	(°)	(°)	(°)	(°)	(°)
C-N-Cα	123.5(4.2)	123.8(1.1)	125.3(4.6)	124.0(1.1)	121.9
N-Cα-C	110.0(4.0)	109.5(1.9)	110.3(2.8)	109.5(1.6)	110.1
Cα-C-N	116.6(4.0)	116.6(1.2)	117.6(5.2)	116.6(0.8)	116.6
O=C-N	123.1(4.1)	123.4(0.6)	122.2(4.9)	123.3(0.4)	122.9
N-Cα-Cβ	110.3(2.1)	109.8(0.7)	110.6(2.5)	109.8(0.6)	109.5
Cβ-Cα-C	111.4(2.4)	111.1(0.7)	111.2(2.2)	111.1(0.6)	111.1

Values in parentheses are standard deviations. Note that while some bond length and angle averages did not change appreciably after energy-minimization, the corresponding standard deviations are reduced due to deviant geometries assuming standard values after energy-minimization. Standard geometry values are from the AMBER forcefield as implemented in DISCOVER (Biosym Technologies).

The consensus structure might conceivably be dependent upon which crystal structure was chosen as the template on which the others were superimposed. As a test, the entire procedure was repeated using the crystal structure with the worst superposition versus 2FB4, i.e. the 2HFL Fab structure, as the new template (reference). The two consensus structures compare favorably (root-mean-squared deviation of 0.11Å for all N, Cα and C atoms).

Note that the consensus structure only includes mainchain (N, Cα, C, O, Cβ atoms) coordinates for only those residues which are part of a conformation *common* to all seven X-ray crystal structures. For the Fab structures, these include the common β-strands (which comprise two β-sheets) and a few non-CDR loops which connect these β-strands. The consensus structure does *not* include CDRs or sidechains, both of which vary in their conformation among the seven structures. Also, note that the consensus structure includes only the VL and VH domains.

This consensus structure is used as the archetype. It is not particular to any species, and has only the basic shape without side chains. Starting with this consensus structure the model of *any* import, human, or humanized Fab can be constructed as follows. Using the amino acid sequence of the particular antibody VL and VH domains of interest, a computer graphics program (such as INSIGHT, Biosym Technologies) is used to add sidechains and CDRs to the consensus structure. When a sidechain is added, its conformation is chosen on the basis of known Fab crystal structures (see the Background section for publications of such crystal structures) and rotamer libraries (Ponder, J.W. & Richards, F. M., *J. Mol. Biol.* **193**: 775-791 (1987)). The model also is constructed so that the atoms of the sidechain are positioned so as to not collide with other atoms in the Fab.

CDRs are added to the model (now having the backbone plus side chains) as follows. The size (i.e. number of amino acids) of each import CDR is compared to canonical CDR structures tabulated by Chothia *et al., Nature*, **342**:877-883 (1989)) and which were derived from Fab crystals. Each CDR sequence is also reviewed for the presence or absence of certain specific amino acid residues which are identified by Chothia as structurally important: e.g. light chain residues 29 (CDR1) and 95 (CDR3), and heavy chain residues 26, 27, 29 (CDR1) and 55 (CDR2). For light chain CDR2, and heavy chain CDR3, only the size of the CDR is compared to the Chothia canonical structure. If the size and sequence (i.e. inclusion of the specific, structurally important residues as denoted by Chothia *et al.*) of the import CDR agrees in size and has the same structurally important residues as those of a canonical CDR, then the mainchain conformation of the import CDR in the model is taken to be the same as that of the canonical CDR. This means that the import sequence is assigned the structural configuration

of the canonical CDR, which is then incorporated in the evolving model.

However, if no matching canonical CDR can be assigned for the import CDR, then one of two options can be exercised. First, using a program such as INSIGHT (Biosym Technologies), the Brookhaven Protein Data Bank can be searched for loops with a similar size to that of the import CDR and these loops can be evaluated as possible conformations for the import CDR in the model. Minimally, such loops must exhibit a conformation in which no loop atom overlaps with other protein atoms. Second, one can use available programs which calculate possible loop conformations, assuming a given loop size, using methods such as described by Bruccoleri *et al., Nature* **335**: 564-568 (1988).

When all CDRs and sidechains have been added to the consensus structure to give the final model (import, human or humanized), the model is preferably subjected to energy minimization using programs which are available commercially (e.g. DISCOVER, Biosym Technologies). This technique uses complex mathematical formulae to refine the model by performing such tasks as checking that all atoms are within appropriate distances from one another and checking that bond lengths and angles are within chemically acceptable limits.

Models of a humanized, import or human antibody sequence are used in the practice of this invention to understand the impact of selected amino acid residues of the activity of the sequence being modeled. For example, such a model can show residues which may be important in antigen binding, or for maintaining the conformation of the antibody, as discussed in more detail below. Modeling can also be used to explore the potential impact of changing any amino acid residue in the antibody sequence.

Methods for Obtaining a Humanized Antibody Sequence

In the practice of this invention, the first step in humanizing an import antibody is deriving a consensus amino acid sequence into which to incorporate the import sequences. Next a model is generated for these sequences using the methods described above. In certain embodiments of this invention, the consensus human sequences are derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)), namely V_L κ subgroup I and V_H group III, and have the sequences indicated in the definitions above.

While these steps may be taken in different order, typically a structure for the candidate humanized antibody is created by transferring the at least one CDR from the non-human, import sequence into the consensus human structure, after the entire corresponding human

CDR has been removed. The humanized antibody may contain human replacements of the non-human import residues at positions within CDRs as defined by sequence variability (Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) or as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* **196**:901-917 (1987)). For example, huMAb4D5 contains human replacements of the muMAb4D5 residues at three positions within CDRs as defined by sequence variability (Kabat, E. A. *et al., Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) but not as defined by structural variability (Chothia, C. & Lesk, A. M., *J. Mol. Biol.* **196**:901-917 (1987)): V_L-CDR1 K24R, V_L-CDR2 R54L and V_L-CDR2 T56S.

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

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

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

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

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

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

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

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

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

g. for any non-homologous import antibody amino acid residue which is reasonably

expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence.

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

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

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

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

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

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

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

b. (in the FR of the variable domain of the heavy chain) 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

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

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

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

Antibodies

Certain aspects of this invention are directed to natural antibodies and to monoclonal antibodies, as illustrated in the Examples below and by antibody hybridomas deposited with the ATCC (as described below). Thus, the references throughout this description to the use

of monoclonal antibodies are intended to include the use of natural or native antibodies as well as humanized and chimeric antibodies. As used herein, the term "antibody" includes the antibody variable domain and other separable antibody domains unless specifically excluded.

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

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

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

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

After immunization, monoclonal antibodies are prepared by recovering immune lymphoid cells--typically spleen cells or lymphocytes from lymph node tissue--from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by

Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Kohler and Milstein, *Eur. J. Immunol.* 6:511 (1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

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

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

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

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules (known as Fab fragments) which bypass the generation of monoclonal antibodies are encompassed within the practice of this invention. One extracts antibody-specific messenger RNA molecules from immune system cells taken from an immunized animal,

transcribes these into complementary DNA (cDNA), and clones the cDNA into a bacterial expressions system. One example of such a technique suitable for the practice of this invention was developed by researchers at Scripps/Stratagene, and incorporates a proprietary bacteriophage lambda vector system which contains a leader sequence that causes the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional FAb fragments for those which bind the antigen. Such FAb fragments with specificity for the antigen are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

Amino Acid Sequence Variants

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

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

A useful method for identification of certain residues or regions of the target polypeptide that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (Science, 244: 1081-1085 [1989]).

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

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

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

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

Other insertional variants of the target polypeptide include the fusion to the N- or C-terminus of the target polypeptide of immunogenic polypeptides, e.g., bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli trp* locus, or yeast protein, and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant

regions (or other immunoglobulin regions), albumin, or ferritin, as described in WO 89/02922 published 6 April 1989.

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

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

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

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

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

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

of the molecule and prevent aberrant crosslinking.

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

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

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

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

For alteration of the native DNA sequence (to generate amino acid sequence variants, for example), the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of the target polypeptide, and the other strand (the original template) encodes the native, unaltered sequence of the target polypeptide. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such

as *E. coli* JM101. After the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabeled with 32-phosphate to identify the bacterial colonies that contain the mutated DNA. The mutated region is then removed and placed in an appropriate vector for protein production, generally an expression vector of the type typically employed for transformation of an appropriate host.

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

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

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

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

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

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

If the ratio of template to product material is extremely low, the vast majority of product DNA fragments incorporate the desired mutation(s). This product material is used to replace the corresponding region in the plasmid that served as PCR template using standard DNA technology. Mutations at separate positions can be introduced simultaneously by either using a mutant second primer, or performing a second PCR with different mutant primers and ligating the two resulting PCR fragments simultaneously to the vector fragment in a three (or more)-part ligation.

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

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

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

Insertion of DNA into a Cloning Vehicle

The cDNA or genomic DNA encoding the target polypeptide is inserted into a replicable

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

(a) Signal Sequence Component

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

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

(b) Origin of Replication Component

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal

DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

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

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

(c) Selection Gene Component

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

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern *et al.*, J. Molec. Appl. Genet., 1: 327 [1982]),

mycophenolic acid (Mulligan *et al.*, Science, 209: 1422 [1980]) or hygromycin (Sugden *et al.*, Mol. Cell. Biol., 5: 410-413 [1985]). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid), or hygromycin, respectively.

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

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

A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, Nature, 282: 39 [1979]; Kingsman *et al.*, Gene, 7: 141 [1979]; or

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

(d) Promoter Component

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

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

sequence operably linked to the DNA encoding the target polypeptide.

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

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

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

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

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209: 1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78: 7398-7402 (1981). The immediate early promoter of the human

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

(e) Enhancer Element Component

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

(f) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal,

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

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

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

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

Other methods, vectors, and host cells suitable for adaptation to the synthesis of the target polypeptide in recombinant vertebrate cell culture are described in Gething *et al.*, Nature, 293: 620-625 [1981]; Mantei *et al.*, Nature, 281: 40-46 [1979]; Levinson *et al.*; EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of the target polypeptide is pRK5 (EP pub. no. 307,247) or pSVI6B.

Selection and Transformation of Host Cells

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

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

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

available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the target polypeptide DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding target polypeptide is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the target polypeptide DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1: 561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. See EP 321,196 published 21 June 1989.

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

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

Transfection refers to the taking up of an expression vector by a host cell whether or

not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

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

Culturing the Host Cells

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

The mammalian host cells used to produce the target polypeptide of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58: 44 (1979), Barnes and Sato, Anal. Biochem., 102: 255 (1980), U.S. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Pat. Re. 30,985, may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements

(defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

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

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

Detecting Gene Amplification/Expression

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

Gene expression, alternatively, may be measured by immunological methods, such as

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

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

Purification of The Target polypeptide

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

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

Target polypeptide variants in which residues have been deleted, inserted or substituted are recovered in the same fashion, taking account of any substantial changes in properties occasioned by the variation. For example, preparation of a target polypeptide fusion with another protein or polypeptide, e.g. a bacterial or viral antigen, facilitates purification; an

immunoaffinity column containing antibody to the antigen (or containing antigen, where the target polypeptide is an antibody) can be used to adsorb the fusion. Immunoaffinity columns such as a rabbit polyclonal anti-target polypeptide column can be employed to absorb the target polypeptide variant by binding it to at least one remaining immune epitope. A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. One skilled in the art will appreciate that purification methods suitable for native target polypeptide may require modification to account for changes in the character of the target polypeptide or its variants upon expression in recombinant cell culture.

Covalent Modifications of Target Polypeptides

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

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

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

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include

imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

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

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

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

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

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

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

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

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

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

Another means of increasing the number of carbohydrate moieties on the target polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- and O- linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free

carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published 11 September 1987, and in Aplin and Wriston (CRC Crit. Rev. Biochem., pp. 259-306 [1981]).

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

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

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

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

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

Since it is often difficult to predict in advance the characteristics of a variant target polypeptide, it will be appreciated that some screening of the recovered variant will be needed

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

Diagnostic and Related Uses of the Antibodies

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

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

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

The label used (and this is also useful to label antigen nucleic acid for use as a probe) is any detectable functionality that does not interfere with the binding of analyte and its binding partner. Numerous labels are known for use in immunoassay, examples including moieties that may be detected directly, such as fluorochrome, chemiluminescent, and radioactive labels, as well as moieties, such as enzymes, that must be reacted or derivatized

to be detected. Examples of such labels include the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

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

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

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

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

Competitive assays rely on the ability of a tracer analogue to compete with the test sample analyte for a limited number of binding sites on a common binding partner. The binding

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

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

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

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

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

above.

Immunotoxins

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

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

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

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

Advantageously, monoclonal antibodies specifically binding the domain of the antigen which is exposed on the infected cell surface, are conjugated to ricin A chain. Most

advantageously the ricin A chain is deglycosylated and produced through recombinant means. An advantageous method of making the ricin immunotoxin is described in Vitetta *et al.*, *Science* 238:1098 (1987).

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

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

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

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

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

Antibody Dependent Cellular Cytotoxicity

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

Biological activity of antibodies is known to be determined, to a large extent, by the constant domains or Fc region of the antibody molecule (Uananue and Benacerraf, *Textbook of Immunology*, 2nd Edition, Williams & Wilkins, p. 218 (1984)). This includes their ability to activate complement and to mediate antibody-dependent cellular cytotoxicity (ADCC) as effected by leukocytes. Antibodies of different classes and subclasses differ in this respect,

as do antibodies from the same subclass but different species; according to the present invention, antibodies of those classes having the desired biological activity are prepared. Preparation of these antibodies involves the selection of antibody constant domains are their incorporation in the humanized antibody by known technique. For example, mouse immunoglobulins of the IgG3 and IgG2a class are capable of activating serum complement upon binding to the target cells which express the cognate antigen, and therefore humanized antibodies which incorporate IgG3 and IgG2a effector functions are desirable for certain therapeutic applications.

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

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

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

Therapeutic and Other Uses of the Antibodies

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

administration of tumor necrosis factor, interferon or other cytoprotective or immunomodulatory agent.

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

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

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

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

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

Deposit of Materials

As described above, cultures of the muMAb4D5 have been deposited with the American Type Culture Collection, ~~12301 Parklawn Drive, Rockville, MD~~ 10801 University Blvd, Manassas, VA, USA (ATCC).

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

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

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

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

skilled in the art from the foregoing description and fall within the scope of the appended claims.

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

EXAMPLES

EXAMPLE 1. HUMANIZATION OF muMAb4D5

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

MATERIALS and METHODS

Cloning of Variable Region Genes. The muMAb4D5 V_H and V_L genes were isolated by polymerase chain reaction (PCR) amplification of mRNA from the corresponding hybridoma (Fendly, B. M. *et al.*, *Cancer Res.* **50**:1550-1558 (1990)) as described by Orlandi et al. (Orlandi, R. *et al.*, *Proc. Natl. Acad. Sci. USA* **86**:3833-3837 (1989)). Amino terminal sequencing of muMAb4D5 V_L and V_H was used to design the sense strand PCR primers, whereas the anti-sense PCR primers were based upon consensus sequences of murine framework residues (Orlandi, R. *et al.*, *Proc. Natl. Acad. Sci. USA* **86**:3833-3837 (1989); Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987)) incorporating restriction sites for directional cloning shown by underlining and listed after the sequences: V_L sense, 5'-TCCGATATCCAGCTGACCCAGTCTCCA-3' (SEQ. ID NO. 7), *Eco*RV; V_L anti-sense, 5'-

GTTTGATCTCCAGCTTGGTACCHSCDCCGAA-3' (SEQ. ID NO. 8), *Asp*718; V_H sense, 5'-AGGTSMARCTGCAGSAGTCWGG-3' (SEQ. ID NO. 9), *Pst*I and V_H anti-sense, 5'-TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG-3' (SEQ. ID NO. 10), *Bst*EII; where H = A or C or T, S = C or G, D = A or G or T, M = A or C, R = A or G and W = A or T. The PCR products were cloned into pUC119 (Vieira, J. & Messing, J., *Methods Enzymol.* **153**:3-11 (1987)) and five clones for each variable domain sequenced by the dideoxy method (Sanger, F. *et al., Proc. Natl. Acad. Sci. USA* **74**:5463-5467 (1977)).

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

In humanizing muMAb4D5, consensus human sequences were first derived from the most abundant subclasses in the sequence compilation of Kabat et al. (Kabat, E. A. *et al.,*

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

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

reduce the risk of anti-allotype antibodies interfering with therapy.

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

Expression and Purification of MAb4D5 Variants. Appropriate MAb4D5 light and heavy chain cDNA expression vectors were co-transfected into an adenovirus transformed human

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

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

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

RESULTS

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

variants (Table 3) were constructed by site-directed mutagenesis of huMAb4D5-5.

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

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

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

The anti-proliferative activity of huMAb4D5 variants against $p185^{HER2}$ overexpressing SK-BR-3 cells is not simply correlated with their binding affinity for the $p185^{HER2}$ ECD. For

example, installation of three murine residues into the V_H domain of huMAb4D5-2 (D73T, L78A and A93S) to create huMAb4D5-3 does not change the antigen binding affinity but does confer significant anti-proliferative activity (Table 3).

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

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

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

Secondary Immune Function of huMAb4D5-8. MuMAb4D5 inhibits the growth of human breast tumor cells which overexpress $p185^{HER2}$ (Hudziak, R. M. *et al.*, *Molec. Cell. Biol.* **9**:1165-1172 (1989)). The antibody, however, does not offer the possibility of direct tumor cytotoxic effects. This possibility does arise in huMAb4D5-8 as a result of its high affinity (K_d = 0.1 μM) and its human IgG_1 subtype. Table 4 compares the ADCC mediated by huMAb4D5-8 with muMAb4D5 on a normal lung epithelial cell line, WI-38, which expresses a low level of $p185^{HER2}$ and on SK-BR-3, which expresses a high level of $p185^{HER2}$. The results demonstrate that: (1) huMAb4D5 has a greatly enhanced ability to carry out ADCC as compared with its murine parent; and (2) that this activity may be selective for cell types

which overexpress $p185^{HER2}$.

DISCUSSION

MuMAb4D5 is potentially useful for human therapy since it is cytostatic towards human breast and ovarian tumor lines overexpressing the *HER2*-encoded $p185^{HER2}$ receptor-like tyrosine kinase. Since both breast and ovarian carcinomas are chronic diseases it is anticipated that the optimal MAb4D5 variant molecule for therapy will have low immunogenicity and will be cytotoxic rather than solely cytostatic in effect. Humanization of muMAb4D5 should accomplish these goals. We have identified 5 different huMAb4D5 variants which bind tightly to $p185^{HER2}$ ECD ($K_d \leq 1$ nM) and which have significant anti-proliferative activity (Table 3). Furthermore huMAb4D5-8 but not muMAb4D5 mediates ADCC against human tumor cell lines overexpressing $p185^{HER2}$ in the presence of human effector cells (Table 4) as anticipated for a human $\gamma 1$ isotype (Brüggemann, M. *et al., J. Exp. Med.* **166**:1351-1361 (1987); Riechmann, L. *et al., Nature* **332**:323-327 (1988)).

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

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

simple function of their binding affinity for $p185^{HER2}$ ECD. For example the huMAb4D5-8 variant binds $p185^{HER2}$ 3-fold more tightly than muMAb4D5 but the humanized variant is slightly less potent in blocking the proliferation of SK-BR-3 cells. Additional huMAb4D5 variants are currently being constructed in an attempt to identify residues triggering the anti-proliferative activity and in an attempt to enhance this activity.

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

Table 3. $p185^{HER2}$ ECD binding affinity and anti-proliferative activities of MAb4D5 variants

	V_H Residue*					V_L Residue*			
MAb4D5 cell Variant proliferation‡	71 FR3	73 FR3	78 FR3	93 FR3	102 CDR3	55 CDR2	66 FR3	K_d† nM	Relative
huMAb4D5-1	R	D	L	A	V	E	G	25	102
huMAb4D5-2	Ala	D	L	A	V	E	G	4.7	101
huMAb4D5-3	Ala	Thr	Ala	Ser	V	E	G	4.4	66
huMAb4D5-4	Ala	Thr	L	Ser	V	E	Arg	0.82	56
huMAb4D5-5	Ala	Thr	Ala	Ser	V	E	Arg	1.1	48
huMAb4D5-6	Ala	Thr	Ala	Ser	V	Tyr	Arg	0.22	51
huMAb4D5-7	Ala	Thr	Ala	Ser	Tyr	E	Arg	0.62	53
huMAb4D5-8	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.10	54
muMAb4D5	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.30	37

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

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

‡ Proliferation of SK-BR-3 cells incubated for 96 hr with MAb4D5 variants shown as a percentage of the untreated control as described (Hudziak, R. M. *et al., Molec. Cell. Biol.* 9:1165-1172 (1989)). Data represent the maximal anti-proliferative effect for each variant (see Fig. 3A) calculated as the mean of triplicate determinations at a MAb4D5 concentration of 8 μg/ml. Data are all taken from the same experiment with an estimated standard error of

To730

≤ ± 15%.

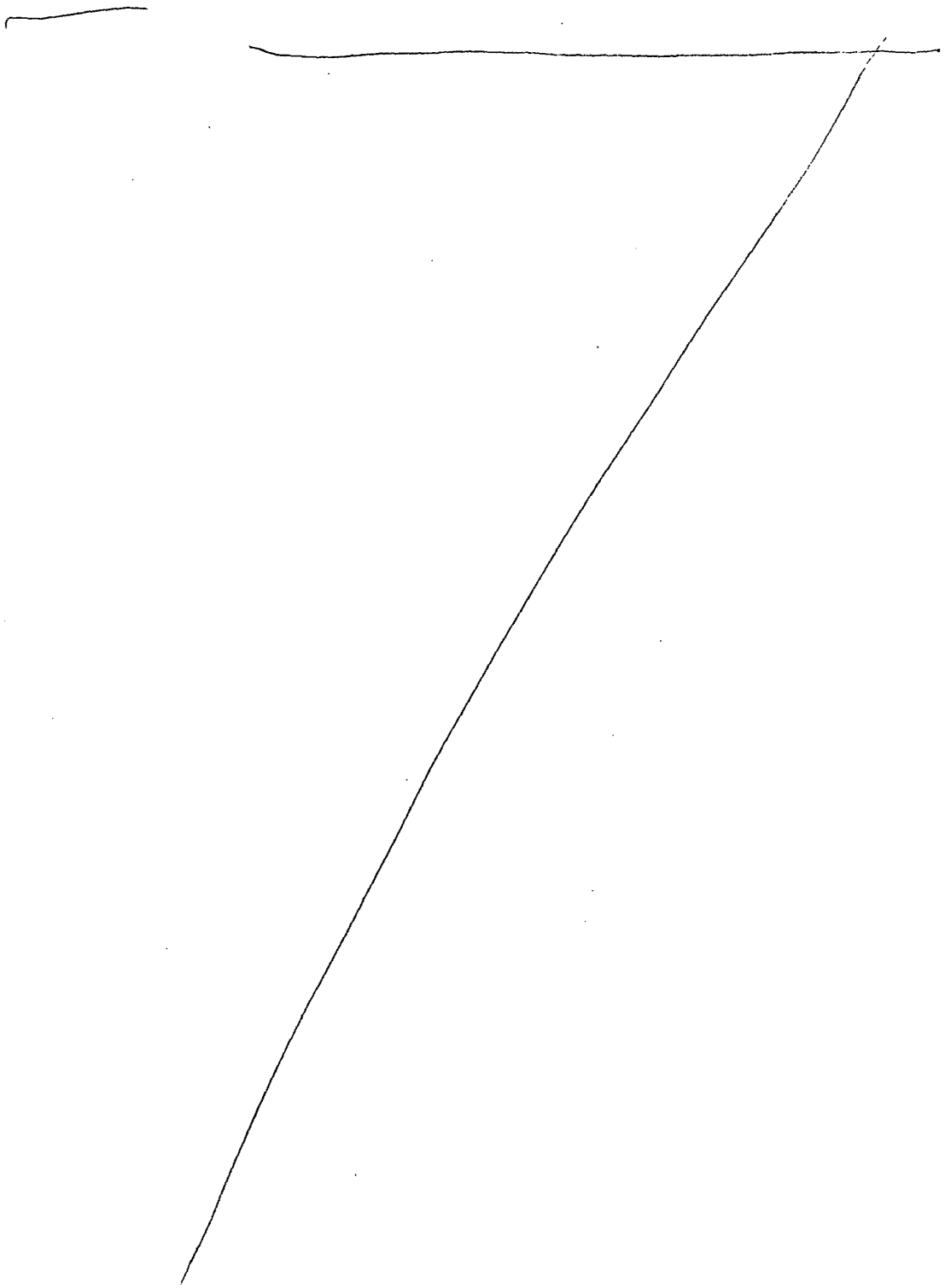

Table 4. Selectivity of antibody dependent tumor cell cytotoxicity mediated by huMAb4D5-8

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

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

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

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

EXAMPLE 2. Schematic Method for Humanizing an Antibody Sequence

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

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

activity of the CDRs by considering 1) could it bind antigen directly and 2) could it affect the conformation of the CDR. If the conclusion is that an affect on the CDRs is likely, substitute the import residue. If the conclusion is that a CDR affect is unlikely, leave the humanized residue unchanged.

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

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

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

7. Search the import sequence, the consensus sequence and the humanized sequence for glycosylation sites outside the CDRs, and determine if this glycosylation site is likely to have any affect on

antigen binding and/or biological activity. If no effect is likely, use the human sequence at that site; if some affect is likely, eliminate the glycosylation site or use the import sequence at that site.

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

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

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

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

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

      i. Variable light domain:

         a) CDR-1 (residues 24L-34L): **2L**, 4L, 66L-69L, **71L**

         b) CDR-2 (residues 50L-56L): 35L, 46L, *47L*, **48L**, *49L*, 58L, 62L, **64L**-66L, 71L, 73L

         c) CDR-3 (residues 89L-97L): 2L, 4L, 36L, 98L, 37H, 45H, 47H, 58H, 60H

      ii. Variable heavy domain:

a) CDR-1 (residues 26H-35H): 2H, 4H, 24H, 36H, 71H, 73H, 76H, 78H, 92H, ***94H***

b) CDR-2 (residues 50H-55H): 49H, 69H, 69H, **71H**, 73H, 78H

c) CDR-3 (residues 95H-102H): examine all residues as possible interaction partners with this loop, because this loop varies in size and conformation much more than the other CDRs.

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

EXAMPLE 3. Engineering a Humanized Bispecific F(ab')$_2$ Fragment

This example demonstrates the construction of a humanized bispecific antibody (BsF(ab')$_2$v1 by separate *E. coli* expression of each Fab' arm followed by directed chemical coupling *in vitro*. BsF(ab')$_2$ v1 (anti-CD3 / anti-p185^{HER2}) was demonstrated to retarget the cytotoxic activity of human CD3$^+$ CTL *in vitro* against the human breast tumor cell line, SK-BR-3, which overexpresses the p185^{HER2} product of the protooncogene *HER2*. This example demonstrates the minimalistic humanization strategy of installing as few murine residues as possible into a human antibody in order to recruit antigen-binding affinity and biological properties comparable to that of the murine parent antibody. This strategy proved very successful for the anti-p185^{HER2}arm of BsF(ab')$_2$v1. In contrast BsF(ab')$_2$ v1 binds to T cells *via* its anti-CD3 arm much less efficiently than does the chimeric BsF(ab')$_2$ which contains the variable domains of the murine parent anti-CD3 antibody. Here we have constructed additional BsF(ab')$_2$ fragments containing variant anti-CD3 arms with selected murine residues restored in an attempt to improve antibody binding to T cells. One such variant, Bs F(ab')$_2$ v9, was created by replacing six residues in the second hypervariable loop of the anti-CD3 heavy chain variable domain of BsF(ab')$_2$ v1 with their counterparts from the murine parent anti-CD3 antibody. BsF(ab')$_2$ v9 binds to T cells (Jurkat) much more efficiently than does BsF(ab')$_2$ v1 and almost as efficiently as the chimeric BsF(ab')$_2$. This improvement in the efficiency of T cell binding of the humanized BsF(ab')$_2$ is an important step in its development as a potential therapeutic agent for the treatment of p185^{HER2}-overexpressing cancers.

Bispecific antibodies (BsAbs) with specificities for tumor-associated antigens and surface markers on immune effector cells have proved effective for retargeting effector cells to kill tumor targets both *in vitro* and *in vivo* (reviewed by Fanger, M. W. *et al.*, *Immunol. Today* **10**: 92-99 (1989); Fanger, M. W. *et al.*, *Immunol. Today* **12**: 51-54 (1991); and Nelson, H., *Cancer Cells* **3**: 163-172 (1991)). BsF(ab')$_2$ fragments have often been used in preference to intact BsAbs in retargeted cellular cytotoxicity to avoid the risk of killing innocent bystander cells binding to the Fc region of the antibody. An additional advantage of BsF(ab')$_2$ over intact BsAbs is that they

are generally much simpler to prepare free of contaminating monospecific molecules (reviewed by Songsivilai, S. and Lachmann, P. J., *Clin. Exp. Immunol.* **79**: 315-321 (1990) and Nolan, O. and O'Kennedy, R., *Biochim. Biophys. Acta* **1040**: 1-11 (1990)).

$BsF(ab')_2$ fragments are traditionally constructed by directed chemical coupling of Fab' fragments obtained by limited proteolysis plus mild reduction of the parent rodent monoclonal Ab (Brennan, M. *et al., Science* **229**, 81-83 (1985) and *Glennie, M. J. et al., J. Immunol.* **139**: 2367-2375 (1987)). One such $BsF(ab')_2$ fragment (anti-glioma associated antigen / anti-CD3) was found to have clinical efficacy in glioma patients (Nitta, T. *et al., Lancet* **335**: 368-371 (1990) and another $BsF(ab')_2$ (anti-indium chelate / anti-carcinoembryonic antigen) allowed clinical imaging of colorectal carcinoma (Stickney, D. R. *et al., Antibody, Immunoconj. Radiopharm.* **2**: 1-13 (1989)). Future $BsF(ab')_2$ destined for clinical applications are likely to be constructed from antibodies which are either human or at least "humanized" (Riechmann, L. *et al., Nature* **332**: 323-327 (1988) to reduce their immunogenicity (Hale, G. *et al., Lancet* **i**: 1394-1399 (1988)).

Recently a facile route to a fully humanized $BsF(ab')_2$ fragment designed for tumor immunotherapy has been demonstrated (Shalaby, M. R. *et al., J. Exp. Med.* **175**: 217-225 (1992)). This approach involves separate *E. coli* expression of each Fab' arm followed by traditional directed chemical coupling *in vitro* to form the $BsF(ab')_2$. One arm of the $BsF(ab')_2$ was a humanized version (Carter, P. *et al., Proc. Natl. Acad. Sci. USA* (1992a) and Carter, P., *et al., Bio/Technology* **10**: 163-167 (1992b)) of the murine monoclonal Ab 4D5 which is directed against the $p185^{HER2}$ product of the protooncogene *HER2* (c-*erbB*-2) (Fendly, B. M. *et al.. Cancer Res.* **50**: 1550-1558 (1989)). The humanization of the antibody 4D5 is shown in Example 1 of this application. The second arm was a minimalistically humanized anti-CD3 antibody (Shalaby *et al. supra*) which was created by installing the CDR loops from the variable domains of the murine parent monoclonal Ab UCHT1 (Beverley, P. C. L. and Callard, R. E., *Eur. J. Immunol.* **11**: 329-334 (1981)) into the humanized anti-$p185^{HER2}$ antibody. The $BsF(ab')_2$ fragment containing the most potent humanized anti-CD3 variant (v1) was demonstrated by flow cytometry to bind specifically to a tumor target

overexpressing $p185^{HER2}$ and to human peripheral blood mononuclear cells carrying CD3. In addition, Bs F(ab')$_2$ v1 enhanced the cytotoxic effects of activated human CTL 4-fold against SK-BR-3 tumor cells overexpressing $p185^{HER2}$. The example descries efforts to improve the antigen binding affinity of the humanized anti-CD3 arm by the judicious recruitment of a small number of additional murine residues into the minimalistically humanized anti-CD3 variable domains.

MATERIALS AND METHODS

Construction of mutations in the anti-CD3 variable region genes.

The construction of genes encoding humanized anti-CD3 variant 1 (v1) variable light (V_L) and heavy (V_H) chain domains in phagemid pUC119 has been described (Shalaby *et al. supra*). Additional anti-CD3 variants were generated using an efficient site-directed mutagenesis method (Carter, P., *Mutagenesis: a practical approach*, (M. J. McPherson, Ed.), Chapter 1, IRL Press, Oxford, UK (1991)) using mismatched oligonucleotides which either install or remove unique restriction sites. Oligonucleotides used are listed below using lowercase to indicate the targeted mutations. Corresponding coding changes are denoted by the starting amino acid in one letter code followed by the residue numbered according to Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest*, 5th edition, National Institutes of Health, Bethesda, MD, USA (1991), then the replacement amino acid and finally the identity of the anti-CD3 variant:

HX11, 5' GTAGATAAATCCtctAACACAGCCTAtCTGCAAATG 3' (SEQ.ID. NO. 11) V_H K75S, v6;

HX12, 5' GTAGATAAATCCAAAtctACAGCCTAtCTGCAAATG 3' (SEQ.ID. NO. 12) V_H N76S, v7;

HX13, 5' GTAGATAAATCCtcttctACAGCCTAtCTGCAAATG 3' (SEQ.ID. NO. 13) V_H K75S:N76S, v8;

X14, 5' CTTATAAAGGTGTTtCcACCTATaaCcAgAaatTCAA GGatCGTTTCAC**gATAtc**CGTAGATAAATCC 3' (SEQ.ID.NO. 14) V_H T57S:A60N:D61Q:S62K:V63F:G65D, v9;

LX6, 5' CTATACCTCCCGTCTgcatTCTGGAGTCCC 3' (SEQ.ID. NO. 15)

V_L E55H, v11.

Oligonucleotides HX11, HX12 and HX13 each remove a site for BspMI, whereas LX6 removes a site for XhoI and HX14 installs a site for EcoRV (bold). Anti-CD3 variant v10 was constructed from v9 by site-directed mutagenesis using oligonucleotide HX13. Mutants were verified by dideoxynucleotide sequencing (Sanger, F. *et al., Proc. Natl. Acad. Sci. USA* **74**: 5463-5467 (1977)).

E. coli expression of Fab' fragments

The expression plasmid, pAK19, for the co-secretion of light chain and heavy chain Fd' fragment of the most preferred humanized anti-p185^{HER2} variant, HuMAb4D5-8, is described in Carter *et al.*, 1992b, *supra*. Briefly, the Fab' expression unit is bicistronic with both chains under the transcriptional control of the *phoA* promoter. Genes encoding humanized V_L and V_H domains are precisely fused on their 5' side to a gene segment encoding the heat-stable enterotoxin II signal sequence and on their 3' side to human k_1 C_L and IgG1 C_H1 constant domain genes, respectively. The C_H1 gene is immediately followed by a sequence encoding the hinge sequence CysAlaAla and followed by a bacteriophage λ t_0 transcriptional terminator. Fab' expression plasmids for chimeric and humanized anti-CD3 variants (v1 to v4, Shalaby *et al.*,*supra*; v6 to v12, this study) were created from pAK19 by precisely replacing anti-p185^{HER2} V_L and V_H gene segments with those encoding murine and corresponding humanized variants of the anti-CD3 antibody, respectively, by sub-cloning and site-directed mutagenesis. The Fab' expression plasmid for the most potent humanized anti-CD3 variant identified in this study (v9) is designated pAK22. The anti-p185^{HER2} Fab' fragment was secreted from *E. coli* K12 strain 25F2 containing plasmid pAK19 grown for 32 to 40 hr at 37°C in an aerated 10 liter fermentor. The final cell density was 120-150 OD_{550} and the titer of soluble and functional anti-p185^{HER2} Fab' was 1-2 g/liter as judged by antigen binding ELISA (Carter *et al.*, 1992b, *supra*). Anti-CD3 Fab' variants were secreted from *E. coli* containing corresponding expression plasmids using very similar fermentation protocols. The highest expression titers of chimeric and

humanized anti-CD3 variants were 200 mg/liter and 700 mg/liter, respectively, as judged by total immunoglobulin ELISA.

Construction of BsF(ab')$_2$ fragments

Fab' fragments were directly recovered from *E. coli* fermentation pastes in the free thiol form (Fab'-SH) by affinity purification on Streptococcal protein G at pH 5 in the presence of EDTA (Carter *et al.*, 1992b *supra*). Thioether linked BsF(ab')$_2$ fragments (anti-p185^{HER2} / anti-CD3) were constructed by the procedure of Glennie *et al. supra* with the following modifications. Anti-p185^{HER2} Fab'-SH in 100 mM Tris acetate, 5 mM EDTA (pH 5.0) was reacted with 0.1 vol of 40 mM N,N'-1,2-phenylenedimalemide (*o*-PDM) in dimethyl formamide for ~1.5 hr at 20 °C. Excess *o*-PDM was removed by protein G purification of the Fab' maleimide derivative (Fab'-mal) followed by buffer exchange into 20 mM sodium acetate, 5 mM EDTA (pH 5.3) (coupling buffer) using centriprep-30 concentrators (Amicon). The total concentration of Fab' variants was estimated from the measured absorbance at 280 nm (HuMAb4D5-8 Fab' $e^{0.1\%}$ = 1.56, Carter *et al.*, 1992b, *supra*). The free thiol content of Fab' preparations was estimated by reaction with 5, 5'-dithiobis(2-nitrobenzoic acid) as described by Creighton, T. E., *Protein structure: a practical approach*, (T. E. Creighton, Ed.), Chapter 7, IRL Press, Oxford, UK (1990). Equimolar amounts of anti-p185^{HER2} Fab'-mal (assuming quantitative reaction of Fab'-SH with *o*-PDM) and each anti-CD3 Fab'-SH variant were coupled together at a combined concentration of 1 to 2.5 mg/ml in the coupling buffer for 14 to 48 hr at 4 °C. The coupling reaction was adjusted to 4 mM cysteine at pH 7.0 and incubated for 15 min at 20 °C to reduce any unwanted disulfide-linked F(ab')$_2$ formed. These reduction conditions are sufficient to reduce inter-heavy chain disulfide bonds with virtually no reduction of the disulfide between light and heavy chains. Any free thiols generated were then blocked with 50 mM iodoacetamide. BsF(ab')$_2$ was isolated from the coupling reaction by S100-HR (Pharmacia) size exclusion chromatography (2.5 cm x 100 cm) in the presence of PBS. The BsF(ab')$_2$ samples were passed through a 0.2 mm filter flash frozen in liquid nitrogen and stored at −70 °C.

Flow cytometric analysis of F(ab')$_2$ binding to Jurkat cells

The Jurkat human acute T cell leukemia cell line was purchased from the American Type Culture Collection (~~Rockville, MD~~ Manassas, VA) (ATCC TIB 152) and grown as recommended by the ATCC.[1] Aliquots of 10^6 Jurkat cells were incubated with appropriate concentrations of BsF(ab')$_2$ (anti-p185^{HER2} / anti-CD3 variant) or control mono-specific anti-p185^{HER2} F(ab')$_2$ in PBS plus 0.1% (w/v) bovine serum albumin and 10 mM sodium azide for 45 min at 4 °C. The cells were washed and then incubated with fluorescein-conjugated goat anti-human F(ab')$_2$ (Organon Teknika, West Chester, PA) for 45 min at 4 °C. Cells were washed and analyzed on a FACScan® (Becton Dickinson and Co., Mountain View, CA). Cells (8 x 10^3) were acquired by list mode and gated by forward light scatter *versus* side light scatter excluding dead cells and debris.

RESULTS

Design of humanized anti-CD3 variants

The most potent humanized anti-CD3 variant previously identified, v1, differs from the murine parent antibody, UCHT1 at 19 out of 107 amino acid residues within V_L and at 37 out of 122 positions within V_H (Shalaby *et al.*,*supra*) 1992). Here we recruited back additional murine residues into anti-CD3 v1 in an attempt to improve the binding affinity for CD3. The strategy chosen was a compromise between minimizing both the number of additional murine residues recruited and the number of anti-CD3 variants to be analyzed. We focused our attentions on a few CDR residues which were originally kept as human sequences in our minimalistic humanization regime. Thus human residues in V_H CDR2 of anti-CD3 v1 were replaced *en bloc* with their murine counterparts to give anti-CD3 v9: T57S:A60N:D61Q:S62K:V63F:G65D (SEQ ID NO:20) ~~(Fig. 5)~~. Similarly, the human residue E55 in V_L CDR2 of anti-CD3 v1 was replaced with histidine from the murine anti-CD3 antibody to generate anti-CD3 v11. In addition, V_H framework region (FR) residues 75 and 76 in anti-CD3 v1 were also replaced with their murine counterparts to create anti-CD3 v8: K75S:N76S. V_H residues 75 and 76 are located in a loop close to V_H CDR1 and CDR2 and therefore might

influence antigen binding. Additional variants created by combining mutations at these three sites are described below.

Preparation of BsF(ab')$_2$ fragments

Soluble and functional anti-p185^{HER2} and anti-CD3 Fab' fragments were recovered directly from corresponding *E. coli* fermentation pastes with the single hinge cysteine predominantly in the free thiol form (75-100 % Fab'-SH) by affinity purification on Streptococcal protein G at pH 5 in the presence of EDTA (Carter *et al.*, 1992b, *supra*). Thioether-linked BsF(ab')$_2$ fragments were then constructed by directed coupling using *o*-PDM as described by Glennie *et al.*, *supra*. One arm was always the most potent humanized anti-p185^{HER2} variant, HuMAb4D5-8 (Carter *et al.*, 1992a, *supra*) and the other either a chimeric or humanized variant of the anti-CD3 antibody. Anti-p185^{HER2} Fab'-SH was reacted with *o*-PDM to form the maleimide derivative (Fab'-mal) and then coupled to the Fab'-SH for each anti-CD3 variant. F(ab')$_2$ was then purified away from unreacted Fab' by size exclusion chromatography as shown for a representative preparation (BsF(ab')$_2$ v8) in data not shown. The F(ab')$_2$ fragment represents ~54% of the total amount of antibody fragments (by mass) as judged by integration of the chromatograph peaks.

SDS-PAGE analysis of this BsF(ab')$_2$ v8 preparation under non-reducing conditions gave one major band with the expected mobility (M_r ~ 96 kD) as well as several very minor bands (data not shown). Amino-terminal sequence analysis of the major band after electroblotting on to polyvinylidene difluoride membrane Matsudaira, P., *J. Biol. Chem.* **262**: 10035-10038 (1987) gave the expected mixed sequence from a stoichiometric 1:1 mixture of light and heavy chains (V_L / V_H: D/E, I/V, Q/Q, M/L, T/V, Q/E, S/S) expected for BsF(ab')$_2$. The amino terminal region of both light chains are identical as are both heavy chains and correspond to consensus human FR sequences. We have previously demonstrated that F(ab')$_2$ constructed by directed chemical coupling carry both anti-p185^{HER2} and anti-CD3 antigen specificities (Shalaby *et al.*, *supra*). The level of contamination of the BsF(ab')$_2$ with monospecific F(ab')$_2$ is likely to be very low since mock coupling reactions with either anti-p185^{HER2} Fab'-mal or anti-CD3 Fab'-SH alone did not yield detectable

quantities of F(ab')$_2$. Furthermore the coupling reaction was subjected to a mild reduction step followed by alkylation to remove trace amounts of disulfide-linked F(ab')$_2$ that might be present. SDS-PAGE of the purified F(ab')$_2$ under reducing conditions gave two major bands with electrophoretic mobility and amino terminal sequence anticipated for free light chain and thioether-linked heavy chain dimers.

Scanning LASER densitometry of a *o*-PDM coupled F(ab')$_2$ preparation suggest that the minor species together represent ~10% of the protein. These minor contaminants were characterized by amino terminal sequence analysis and were tentatively identified on the basis of stoichiometry of light and heavy chain sequences and their electrophoretic mobility (data not shown). These data are consistent with the minor contaminants including imperfect F(ab')$_2$ in which the disulfide bond between light and heavy chains is missing in one or both arms, trace amounts of Fab' and heavy chain thioether-linked to light chain.

Binding of BsF(ab')$_2$ to Jurkat cells

Binding of BsF(ab')$_2$ containing different anti-CD3 variants to Jurkat cells (human acute T cell leukemia) was investigated by flow cytometry (data not shown). BsF(ab')$_2$ v9 binds much more efficiently to Jurkat cells than does our starting molecule, BsF(ab')$_2$ v1, and almost as efficiently as the chimeric BsF(ab')$_2$. Installation of additional murine residues into anti-CD3 v9 to create v10 (V_H K75S:N76S) and v12 (V_H K75S:N76S plus V_L E55H) did not further improve binding of corresponding BsF(ab')$_2$ to Jurkat cells. Nor did recruitment of these murine residues into anti-CD3 v1 improve Jurkat binding: V_H K75S (v6), V_H N76S (v7), V_H K75S:N76S (v8), V_L E55H (v11) (not shown). BsF(ab')$_2$ v9 was chosen for future study since it is amongst the most efficient variants in binding to Jurkat cells and contains fewest murine residues in the humanized anti-CD3 arm. A monospecific anti-p185^{HER2} F(ab')$_2$ did not show significant binding to Jurkat cells consistent with the interaction being mediated through the anti-CD3 arm.

DISCUSSION

A minimalistic strategy was chosen to humanize the anti-p185^{HER2}

(Carter *et al.*, 1992a, *supra*) and anti-CD3 arms (Shalaby *et al.*, *supra*) of the $BsF(ab')_2$ in this study in an attempt to minimize the potential immunogenicity of the resulting humanized antibody in the clinic. Thus we tried to install the minimum number of murine CDR and FR residues into the context of consensus human variable domain sequences as required to recruit antigen-binding affinity and biological properties comparable to the murine parent antibody. Molecular modeling was used firstly to predict the murine FR residues which might be important to antigen binding and secondly to predict the murine CDR residues that might *not* be required. A small number of humanized variants were then constructed to test these predictions.

Our humanization strategy was very successful for the anti-$p185^{HER2}$ antibody where one out of eight humanized variants (HuMAb4D5-8, IgG1) was identified that bound the $p185^{HER2}$ antigen ~3-fold more tightly than the parent murine antibody (Carter *et al.*, 1992a, *supra*). HuMAb4D5-8 contains a total of five murine FR residues and nine murine CDR residues, including V_H CDR2 residues 60-65, were discarded in favor of human counterparts. In contrast, $BsF(ab')_2$ v1 containing the most potent humanized anti-CD3 variant out of four originally constructed (Shalaby *et al.*, *supra*) binds J6 cells with an affinity (K_d) of 140 nM which is ~70-fold weaker than that of the corresponding chimeric $BsF(ab')_2$.

Here we have restored T cell binding of the humanized anti-CD3 close to that of the chimeric variant by replacing six human residues in V_H CDR2 with their murine counterparts: T57S:A60N:D61Q:S62K:V63F:G65D (anti-CD3 v9, Fig. 5). It appears more likely that these murine residues enhance antigen binding *indirectly* by influencing the conformation of residues in the N-terminal part of V_H CDR2 rather than by *directly* contacting antigen. Firstly, only N-terminal residues in V_H CDR2 (50-58) have been found to contact antigen in one or more of eight crystallographic structures of antibody/antigen complexes (Kabat *et al.*, *supra*; and Mian, I. S. *et al.*, *J. Mol. Biol.* **217**: 133-151 (1991), Fig. 5). Secondly, molecular modeling suggests that residues in the C-terminal part of V_H CDR2 are at least partially buried (Fig. 5). $BsF(ab')_2$ v9 binds to SK-BR-3 breast tumor cells with equal efficiency to $BsF(ab')_2$ v1 and chimeric $BsF(ab')_2$ as anticipated since the anti-$p185^{HER2}$ arm is identical in all of these molecules (Shalaby *et al.*, *supra*, not

shown).

Our novel approach to the construction of BsF(ab')$_2$ fragments exploits an *E. coli* expression system which secretes humanized Fab' fragments at gram per liter titers and permits their direct recovery as Fab'-SH (Carter *et al.*, 1992b, *supra*). Traditional directed chemical coupling of Fab'-SH fragments is then used to form BsF(ab')$_2$ *in vitro* (Brennan *et al.*,*supra*; and Glennie *et al.*, *supra*). This route to Fab'-SH obviates problems which are inherent in their generation from intact antibodies: differences in susceptibility to proteolysis and nonspecific cleavage resulting in heterogeneity, low yield as well as partial reduction that is not completely selective for the hinge disulfide bonds. The strategy of using *E. coli*-derived Fab'-SH containing a single hinge cysteine abolishes some sources of heterogeneity in BsF(ab')$_2$ preparation such as intra-hinge disulfide formation and contamination with intact parent antibody whilst greatly diminishes others, eg. formation of F(ab')$_3$ fragments.

BsF(ab')2 fragments constructed here were thioether-linked as originally described by Glennie *et al.*, *supra* with future *in vivo* testing of these molecules in mind. Thioether bonds, unlike disulfide bonds, are not susceptible to cleavage by trace amounts of thiol, which led to the proposal that thioether-linked F(ab')$_2$ may be more stable than disulfide-linked F(ab')$_2$ *in vivo* (Glennie *et al.*, *supra*). This hypothesis is supported by our preliminary pharmacokinetic experiments in normal mice which suggest that thioether-linked BsF(ab')$_2$ v1 has a 3- fold longer plasma residence time than BsF(ab')$_2$ v1 linked by a single disulfide bond. Disulfide and thioether-linked chimeric BsF(ab')$_2$ were found to be indistinguishable in their efficiency of cell binding and in their retargeting of CTL cytotoxicity, which suggests that *o*-PDM directed coupling does not compromise binding of the BsF(ab')$_2$ to either antigen (not shown). Nevertheless the nature of the linkage appears not to be critical since a disulfide-linked BsF(ab')$_2$ (murine anti-p185^{HER2} / murine anti-CD3) was recently shown by others (Nishimura *et al.*, *Int. J. Cancer* 50: 800-804 (1992) to have potent anti-tumor activity in nude mice. Our previous study (Shalaby *et al.*, *supra*) together with this one and that of Nishimura, T. *et al.*, *supra* improve the potential for using BsF(ab')$_2$ in targeted immunotherapy of p185^{HER2}-overexpressing cancers in humans.

EXAMPLE 4. Humanization of an anti-CD18 antibody

A murine antibody directed against the leukocyte adhesion receptor β-chain (known as the H52 antibody) was humanized following the methods described above. Figures 6A and 6B provide amino acid sequence comparisons for the murine and humanized antibody light chains and heavy chains.

CLAIMS

WE CLAIM:

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

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

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

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

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

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

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

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

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

2. The method of claim 1, having an additional step of determining if any such non-homologous residues are exposed on the surface of the domain or buried within it, and if the residue is exposed, retaining the consensus residue.

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

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

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

6. The method of claim 1, wherein the corresponding consensus antibody residues are selected from the group consisting of 4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

7. A method comprising providing at least a portion of an import, non-human antibody variable domain amino acid sequence having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR,

substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:

4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 67H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

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

9. The method of claim 1 ~~or 7~~, wherein the consensus human variable domain is a consensus based on human variable domains and additionally variable domains from species other than human.

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

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

12. The humanized antibody variable domain of claim 10, wherein no human FR residue other than those set forth in the group has been substituted.

13. A polypeptide comprising the amino acid sequence:
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLI
YSASFLESGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTF
GQGTKVEIKRT

14. A polypeptide comprising the sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWV
ARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYC
SRWGGDGFYAMDVWGQGTLVTVSS

15. A method for engineering a humanized antibody comprising introducing amino acid residues from an import antibody variable domain into an amino acid sequence representing a consensus of mammalian antibody variable domain sequences.

16. A computer comprising the sequence data of the following amino acid sequence:
    a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKA
    PKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
    QYNSLPYTFGQGTKVEIKRT, or
    b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGK
    GLEWVAVISENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLR
    AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS

17. A computer representation of the following amino acid sequence:
    a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKA
    PKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
    QYNSLPYTFGQGTKVEIKRT, or
    b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGK
    GLEWVAVISENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLR
    AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS

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

a. DIQMTQSPSSLSASVGDRVTITCRASQDVSSYLAWYQQKPGKA
PKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QYNSLPYTFGQGTKVEIKRT, or

b. EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGK
GLEWVAVISENGGYTRYADSVKGRFTISADTSKNTAYLQMNSLR
AEDTAVYYCSRWGGDGFYAMDVWGQGTLVTVSS

add E6 7

add J2 7

add J10 7 & O3

Abstract

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

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Genentech, Inc.

(ii) TITLE OF INVENTION: Immunoglobulin Variants

(iii) NUMBER OF SEQUENCES: 25

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

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

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/715272
(B) APPLICATION DATE: 14-JUN-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Adler, Carolyn R.
(B) REGISTRATION NUMBER: 32,324
(C) REFERENCE/DOCKET NUMBER: 709P1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415/225-2614
(B) TELEFAX: 415/952-9881
(C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

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

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

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

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

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

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

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

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

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

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:2:

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

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

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

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

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

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

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

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

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

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

(2) INFORMATION FOR SEQ ID NO:3:

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

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

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

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

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

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

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

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

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

Ile Lys Arg Thr
109

(2) INFORMATION FOR SEQ ID NO:4:

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

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

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

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

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

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

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

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

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

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

(2) INFORMATION FOR SEQ ID NO:5:

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

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

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

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

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

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

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

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

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

Ile Lys Arg Ala
109

(2) INFORMATION FOR SEQ ID NO:6:

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

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

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

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

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

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

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

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

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

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

(2) INFORMATION FOR SEQ ID NO:7:

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

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

TCCGATATCC AGCTGACCCA GTCTCCA 27

(2) INFORMATION FOR SEQ ID NO:8:

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

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

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

GTTTGATCTC CAGCTTGGTA CCXXCXCCGA A 31

(2) INFORMATION FOR SEQ ID NO:9:

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

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

AGGTXXAXCT GCAGXAGTCX GG 22

(2) INFORMATION FOR SEQ ID NO:10:

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

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

TGAGGAGACG GTGACCGTGG TCCCTTGGCC CCAG 34

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTAGATAAAT CCTCTAACAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTAGATAAAT CCAAATCTAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTAGATAAAT CCTCTTCTAC AGCCTATCTG CAAATG 36

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTATAAAGG TGTTTCCACC TATAACCAGA AATTCAAGGA TCGTTTCACG 50

ATATCCGTAG ATAAATCC 68

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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

CTATACCTCC CGTCTGCATT CTGGAGTCCC 30

(2) INFORMATION FOR SEQ ID NO:16:

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

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

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

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

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys
35 40 45

Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser
50 55 60

Lys Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
65 70 75

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

Gly Asn Thr Leu Pro Trp Thr Phe Ala Gly Gly Thr Lys Leu Glu
95 100 105

Ile Lys
107

(2) INFORMATION FOR SEQ ID NO:17:

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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

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

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

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

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

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

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

Ile Lys
107

(2) INFORMATION FOR SEQ ID NO:18:

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

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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

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

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

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

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

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

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

Ile Lys
107

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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

Ala Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
20 25 30

Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu
35 40 45

Glu Trp Met Gly Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr
50 55 60

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

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

Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
95 100 105

Ser Gly Tyr Tyr Gly Asp Ser Asp Trp Tyr Phe Asp Val Trp Gly
110 115 120

Ala Gly Thr Thr Val Thr Val Ser Ser
125 129

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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

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

Gly Tyr Thr Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

100

Glu Trp Val Ala Leu Ile Asn Pro Tyr Lys Gly Val Ser Thr Tyr
50 55 60

Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Val Asp Lys Ser
65 70 75

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

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

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser
122

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

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

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

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

Glu Trp Val Ser Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
50 55 60

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

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

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Val Gly Tyr Ser Leu
95 100 105

Ser Gly Leu Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser
122

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

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

Ala Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr
20 25 30

Glu Tyr Thr Met His Trp Met Lys Gln Ser His Gly Lys Ser Leu
35 40 45

Glu Trp Ile Gly Gly Phe Asn Pro Lys Asn Gly Gly Ser Ser His
50 55 60

Asn Gln Arg Phe Met Asp Lys Ala Thr Leu Ala Val Asp Lys Ser
65 70 75

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

Ser Gly Ile Tyr Tyr Cys Ala Arg Trp Arg Gly Leu Asn Tyr Gly
95 100 105

Phe Asp Val Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val
110 115 120

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
125 130 135

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
140 145 150

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
155 160 165

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
170 175 180

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
185 190 195

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
200 205 210

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
215 220 225

```
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
                230                 235                 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                245                 250                 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                260                 265                 270

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
                275                 280                 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                290                 295                 300

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
                305                 310                 315

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
                320                 325                 330

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                335                 340                 345

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
                350                 355                 360

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
                365                 370                 375

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                380                 385                 390

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
                395                 400                 405

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                410                 415                 420

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                425                 430                 435

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                440                 445                 450

Ser Pro Gly Lys
            454
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 557 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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

Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr
            20                  25                  30

Phe Thr Glu Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala
        35                  40                  45

Thr Ala Thr Gly Val His Ser Glu Val Gln Leu Val Glu Ser Gly
    50                  55                  60

Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
65                  70                  75

Thr Ser Gly Tyr Thr Phe Thr Glu Tyr Thr Met His Trp Met Arg
            80                  85                  90

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Gly Ile Asn Pro
        95                  100                 105

Lys Asn Gly Gly Thr Ser His Asn Gln Arg Phe Met Asp Arg Phe
    110                 115                 120

Thr Ile Ser Val Asp Lys Ser Thr Ser Thr Ala Tyr Met Gln Met
125                 130                 135

Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
            140                 145                 150

Trp Arg Gly Leu Asn Tyr Gly Phe Asp Val Arg Tyr Phe Asp Val
        155                 160                 165

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
    170                 175                 180

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
185                 190                 195

Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
            200                 205                 210

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
        215                 220                 225

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    230                 235                 240

Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln
245                 250                 255
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Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val
                260                 265                 270

Asp Lys Thr Val Glu Arg Lys Cys Cys Val Thr Cys Pro Pro Cys
                275                 280                 285

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
                290                 295                 300

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
                305                 310                 315

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
                320                 325                 330

Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
                335                 340                 345

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                350                 355                 360

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
                365                 370                 375

Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu Val His
                380                 385                 390

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
                395                 400                 405

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn
                410                 415                 420

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala
                425                 430                 435

Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
                440                 445                 450

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
                455                 460                 465

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
                470                 475                 480

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
                485                 490                 495

Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe
                500                 505                 510

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                515                 520                 525
```

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
530 535 540

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
545 550 555

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 214 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

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

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

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

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

Leu Leu Ile Tyr Tyr Thr Ser Thr Leu His Ser Gly Val Pro Ser
50 55 60

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

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

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

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
140 145 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
155 160 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
170 175 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
185 190 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
200 205 210

Arg Gly Glu Cys
214

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

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

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

Gly Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
20 25 30

Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
35 40 45

Gln Asp Ile Asn Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly
50 55 60

Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Thr Leu His Ser
65 70 75

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
80 85 90

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
95 100 105

Tyr Cys Gln Gln Gly Asn Thr Leu Pro Pro Thr Phe Gly Gln Gly
110 115 120

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
125 130 135

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
140 145 150

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
155 160 165

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
170 175 180

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
185 190 195

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
200 205 210

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
215 220 225

Lys Ser Phe Asn Arg Gly Glu Cys
230 233

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APPLICATION TITLES:
METHOD FOR MAKING HUMANIZED ANTIBODIES

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APPLICANT

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CONTINUING DATA********
VERIFIED THIS APPLN IS A 371 OF /US92/05126 06/15/92

FOREIGN/PCT APPLICATIONS********
VERIFIED PCT PCT/US92/05126 06/15/92

STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	ATTORNEY DOCKET NO.
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TITLE

IMMUNOGLOBULIN VARIANTS

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By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

Date Certifying Officer

PATENT APPLICATION SERIAL NO. 08/146206

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

DS20071

OK Refund 172.0

MR1

PTO-1556
(5/87)

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1992

Application or Docket Number: 08/146,206

CLAIMS AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
BASIC FEE				~~$355.00~~ 475	OR		~~$710.00~~ 950
TOTAL CLAIMS	24 minus 20 =	* 4	x$11=		OR	x$22=	
INDEPENDENT CLAIMS	9 minus 3 =	* 6	x 37=		OR	x 74=	
MULTIPLE DEPENDENT CLAIM PRESENT			+115=		OR	+230=	
			TOTAL		OR	TOTAL	

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

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total	*	Minus	**	=	x$11=		OR	x$22=	
Independent	*	Minus	***	=	x 37=		OR	x 74=	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 115=		OR	+230=	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total	*	Minus	**	=	x$11=		OR	x$22=	
Independent	*	Minus	***	=	x 37=		OR	x 74=	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+ 115=		OR	+ 230=	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
Total	*	Minus	**	=	x$11=		OR	x$22=	
Independent	*	Minus	***	=	x 37=		OR	x 74=	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					+115=		OR	+230=	
					TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

FORM PTO-875
(Rev.10-92)

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

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

(FOR USE WITH FORM PTO-875)

SERIAL NO.

FILING DATE

APPLICANT(S)

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT			*		*		*	
	IND.	DEP.	IND.	DEP.	IND.	DEP.		IND.	DEP.	IND.	DEP.	IND.	DEP.
1	1						51						
2		1					52						
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4		1					54						
5		1					55						
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7	1						57						
8		1					58						
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10	1						60						
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43							93						
44							94						
45							95						
46							96						
47							97						
48							98						
49							99						
50							100						
TOTAL IND.	9						TOTAL IND.						
TOTAL DEP.	15						TOTAL DEP.						
TOTAL CLAIMS	24						TOTAL CLAIMS						

PTO-1360 (3-78)

*MAY BE USED FOR ADDITIONAL CLAIMS OR AMENDMENTS

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(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC (European patent), MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU+,TD (OAPI patent), TG (OAPI patent), US.

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(54) Title: RECOMBINANT ANTIBODIES SPECIFIC FOR TNFα

(57) Abstract

Recombinant, in particular humanised, e.g. humanised chimeric and CDR-grafted humanised, antibody molecules having specificity for human TNFα, are provided for use in diagnosis and therapy. In particular the antibody molecules have antigen binding sites derived from murine monoclonal antibodies CB0006, CB0010, hTNF3 or 101.4. Preferred CDR-grafted humanised anti-hTNFα antibodies comprise variable region domains comprising human acceptor framework and donor antigen binding regions and wherein the frameworks comprise donor residues at specific positions. The antibody molecules may be used for therapeutic treatment of human patients suffering from or at risk of disorders associated with undesirably high levels of TNF, in particular for treatment of immunoregulatory and inflammatory disorders or of septic, endotoxic or cardiovascular shock.

+ See back of page

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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RECOMBINANT ANTIBODIES SPECIFIC FOR TNF-α

Field of the Invention

This invention relates to recombinant, in particular humanised, antibody molecules having specificity for antigenic determinants of tumour necrosis factor alpha (TNF-α), to processes for their production using recombinant DNA technology, and to their therapeutic uses.

For the purposes of the present description the term "recombinant antibody molecule" is used to describe an antibody molecule produced by any process involving the use of recombinant DNA technology, including any analogues of natural immunoglobulins or their fragments.

Also for the purposes of the present description 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. Thus humanised antibody molecules include humanised chimeric antibody molecules comprising complete non-human heavy and/or light chain variable region domains linked to human constant region domains. Humanised antibody molecules also comprise CDR-grafted humanised antibody molecules comprising one or more CDRs from a non-human antibody grafted into a heavy and/or light chain human variable region framework.

The antigen binding specificity of antibodies is determined by their complementarily determining regions (CDRs) which are relatively short peptide sequences carried on the framework regions of the variable domains. There are 3CDRs, (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

The abbreviation "MAb" is used to indicate a monoclonal antibody. In the present description reference is made to a number of publications by number, and these 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, Fv, $(Fab')_2$ 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 reproducible 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 if the MAb is administered to a human. 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 such 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). These prior patent applications generally disclose processes for

preparing antibody molecules 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. 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 heavy chain 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 indicated that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1 of the heavy chain, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Recently Queen et al (9) have described the preparation of a humanised antibody that binds to an 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 WO90/07861 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 Å 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 the second, third or fourth criteria may be applied in addition or alternatively to the first criterion, and may be applied singly or in any combination.

WO90/07861 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 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 3×10^9 M^{-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 overlaps but does not coincide with the residues identified by Queen et al (9). Our copending International patent application WO91/09967 describes this protocol for the preparation of CDR-grafted, in particular humanised, antibody heavy and light chains and complete molecules of any desired specificity. The full disclosure of International patent application WO/91/09967 is incorporated in the present description by reference.

Tempest et al (10) have very recently described the preparation of a reshaped human monoclonal antibody for use in inhibiting human respiratory syncytial virus (RSV) infection in vivo. This reshaped antibody was prepared by grafting synthetic oligo nucleotides coding for the CDRs of a murine MAb, which neutralises RSV infection, by site - directed mutagenesis into DNA coding for the frameworks of a human IgG1, monoclonal antibody. However the simple reshaped antibody in which the CDRs alone had been transferred between mouse and human antibodies had only very poor binding for RSV which was not significantly above background. In order to partially restore binding ability it proved necessary to additionally convert human residues to mouse residues in a framework region adjacent to CDR3 of the heavy chain. Tempest et al did not convert human residues to mouse residues at important positions identified in the protocol of WO91/09967.

TNFα is a cytokine which is released by and interacts with cells of the immune system. Thus TNFα is released by macrophages which have been activated by lipopolysaccharide (LPS) of gram negative bacteria. As such TNFα appears to be an endogenous mediator of central importance involved in the development and pathogenesis of endotoxic shock associated with bacterial sepsis. Antibodies to TNFα has been proposed for the prophylaxis and treatment of endotoxic shock (Beutler et al (11)). However the antibodies to TNFα currently available for use in such treatment are typically murine MAbs. As such these murine MAbs are of only limited use for treatment of humans in view of the undesirable HAMA (Human Anti-Mouse Antibody) response which they can elicit if used for more than one or a few treatments. It is thus a highly desirable objective to prepare humanised anti-TNFα products for use in human therapy.

Our co-pending International patent application WO91/09967 describes, among other things, the preparation of humanised CDR-grafted antibody products which have specificity for TNFα. In particular WO91/09967 describes, in Example 5, preparation of specific humanised CDR grafted antibodies to human TNFα derived from the murine anti-human TNFα MAbs identified as 61E71 (alternatively known as CB0006), hTNF1 (alternatively known as CB0010), hTNF3 and 101.4. The present application relates specifically to recombinant, in particular humanised antibodies to human TNFα, including those described in WO91/09967 and further improved humanised CDR-grafted antibodies to human TNFα based upon the hTNF1 (CB0010) and 101.4 murine MAbs. Further studies of various anti-human TNFα murine MAbs have revealed that hTNF1 and 101.4 have particularly desirable properties for use in anti-TNF therapy.

Summary of the Invention

Accordingly the present invention provides recombinant antibody molecules which have specificity for human TNFα.

The recombinant antibody molecules of the invention are preferably TNF neutralising, i.e. are capable of reducing or inhibiting a biological activity of human TNFα as measured by an in vitro or in vivo test.

Preferably the invention provides recombinant antibody molecules having antigen binding sites derived from the murine MAbs CB0006. CB0010, hTNF3 or 101.4, especially from the murine MAbs CB0010 or 101.4.

Preferably the recombinant antibody molecules of the invention are humanised antibody molecules including both chimeric humanised antibody molecules and CDR-grafted humanised antibody molecules.

For the purposes of the present description a "chimeric humanised antibody molecule" comprises complete non-human (e.g. murine MAb) variable domains linked to human constant domains, and a "CDR-grafted humanised antibody molecule" comprises an antibody heavy and/or light chain containing one or more CDRs from a non-human antibody (e.g. a murine MAb) grafted into a human heavy and/or light chain variable region framework.

The CDR-grafted humanised anti-TNFα antibody products of this invention include anti-human TNFα antibody heavy and light chain and molecule products as defined in the first, second, third and fourth aspects of the invention described in WO91/09967.

Detailed Description of the Invention

Thus in first preferred embodiments, the invention provides a CDR-grafted humanised anti-hTNFα antibody heavy chain having a variable region domain comprising human 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.

Preferably in these first 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.

Especially in these first 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 present description, typically the donor antibody is a non-human anti-hTNFα antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the CDR-grafted humanised anti-hTNFα antibodies 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 heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-102) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35). These preferred CDR designations are preferably used for the CDR-grafted heavy chains of the first preferred embodiments, i.e. residues 26-30 are included within CDR1.

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.

It will be appreciated that when the CDR-grafted humanised antibody molecule embodiments of the invention, as described above and elsewhere in the present description, are applied to a particular donor/acceptor antibody pair, in some cases the donor and acceptor amino acid residues may be identical at a particular position identified for change to the donor residue, and thus no change or acceptor framework residue is required.

The invention also provides in second preferred embodiments a CDR-grafted humanised anti-hTNFα antibody light chain having a variable region domain comprising human 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 preferred embodiment comprises donor residues at positions 46 and/or 47.

The invention also provides in third preferred embodiments a CDR-grafted humanised anti-hTNFα antibody light chain having a variable region domain comprising human 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 the third preferred embodiments, the framework preferably comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third preferred embodiments, 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 human 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 preferred embodiments optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
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, including those of the second and third preferred embodiments described above, 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 preferred embodiment 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 preferred embodiments of the invention.

In a first particularly preferred embodiment, however, the invention provides a CDR-grafted humanised antibody heavy chain having a variable region domain comprising human acceptor framework (especially EU human acceptor framework) and hTNF1 donor antigen binding regions wherein the framework comprises hTNF1 donor residues at positions 12, 27, 30, 38, 46, 48, 66, 67, 69, 71, 73, 76, 83, 89, 91 and 94.

The EU heavy chain framework has residues in framework 4 (FR4) of the heavy chain which are anomalous for human heavy chain frameworks. Thus preferably human consensus residues are used in place of EU residues in FR4 of the heavy chain. In particular, the human consensus residue threonine (T) may be used at position 108. Fortuitously the murine hTNF1 residue at position 108 is also threonine.

In a second particularly preferred embodiment the invention provides a CDR-grafted humanised antibody light chain having a variable domain comprising human acceptor framework (especially EU human acceptor framework) and hTNF1 donor antigen binding regions wherein the framework comprises hTNF1 donor residues at positions 3, 42 and 49.

When the EU human framework is used for the light chain it is also desirable to change residues from EU residues at positions 48, 83, 106 and 108, as the EU residues at these positions are anomalous for human antibodies. Thus the human consensus residues may be used at some or preferably all of these residues, i.e. isoleucine (I) at position 48,

valine (V) at position 83, isoleucine (I) at position 106 and arginine (R) at position 108. Fortuitously the murine hTNF1 residues are the same as the human consensus residues at positions 48 (I), 106 (I) and 108 (R). However, the human consensus residue valine (V) at position 83 differs from both the EU residue (F) and the hTNF1 residue (L) at this position.

Especially the invention includes CDR-grafted humanised antibody molecules comprising at least one CDR-grafted humanised heavy chain according to the first particularly preferred embodiment and at least one CDR-grafted humanised light chain according to the second particularly preferred embodiment.

Also in a third particularly preferred embodiment the invention provides a CDR-grafted humanised antibody heavy chain having a variable region domain comprising human acceptor framework (especially KOL human acceptor framework) and 101.4 donor antigen binding regions wherein the framework comprises 101.4 donor residues at positions 4, 11, 23, 24, 28, 73, 77, 78, 79, 91, 93 and 94.

The KOL residue proline (P) at position 108 of the heavy chain is anomalous for human antibodies. Thus preferably the human consensus residue leucine (L) is at this position if KOL is used as the human acceptor framework. Fortuitously the murine 101.4 antibody has the human consensus residue (L) at this position.

Moreover in a fourth particularly preferred embodiment the invention provides a CDR-grafted humanised antibody light chain having a variable region domain comprising human acceptor framework (especially REI human acceptor framework) and 101.4 donor residues at positions 1, 3, 4 and 73.

The REI light chain human framework has residues which are anomalous for human antibodies at positions 39 (threonine, T), 104 (leucine, L), 105 (glutamine, Q), and 107 (threonine, T). Thus when REI is used as the light chain framework, human consensus residues are used at positions 39 (lysine, K), 104 (valine, V), 105 (glutamic acid, E) and 107 (lysine, K). Fortuitously the murine 101.4 residues are the same as the human consensus residues at positions 39 (K), 105 (E) and 107 (K). However, the human consensus residue at position 104 (V) differs from the leucine (L) REI and murine 101.4 residues at this position.

Especially also the invention includes CDR grafted humanised antibody molecules comprising at least one CDR-grafted humanised heavy chain according to the third particularly preferred embodiment and at least one CDR-grafted humanised light chain according to the fourth particularly preferred embodiment.

Preferably the Kabat CDRs are used for all of the CDRs (CDR1, CDR2 and CDR3) of both the heavy and light chains of the first, second, third and fourth particularly preferred embodiments described above.

The recombinant and 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', $F(ab')_2$ 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 recombinant, chimeric or CDR-grafted molecule with the same specificity as the original donor antibodies. Similarly the heavy and light chain variable region may be combined with other antibody domains as appropriate.

Also the heavy or light chains or recombinant or humanised complete 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.

The amino acid sequences of the heavy and light chain variable domains of the CB0010, 101.4, CB0006 and hTNF3 murine MAbs, CDR-grafted variants thereof and human acceptor antibodies are given in the accompanying diagrams Figures 1, 2, 3 and 4 respectively. The recombinant and humanised antibody products of the invention may be prepared using recombinant DNA techniques, for instance substantially as described in WO91/09967.

Any appropriate human 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 human 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 critical 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 anti-hTNFα donor and human acceptor antibodies irrespective of the level of homology between their sequences. Examples of human frameworks which may be used are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (refs. 7 and 8) 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 IgG1 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 TNF 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.

In further aspects the invention also includes DNA sequences coding for the recombinant and humanised antibody, e.g. 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 recombinant and humanised, e.g. 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. Such methods are shown, for instance, in references 12 and 13.

The DNA sequences which encode the anti-hTNFα antibody molecule amino acid sequences may be obtained by methods well known in the art. For example the anti-TNF coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable anti-hTNFα producing 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 sequence coding for part or all of the antibody heavy and light chains may be synthetised as desired from the determined DNA sequence or on the basis of the corresponding amino acid sequence.

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, or may be readily synthetised on the basis of their known amino acid sequences (see refs. 7 & 8).

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the chimeric and CDR-grafted humanised antibody 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. 14) may be used. Also oligonucleotide directed mutagenesis of a pre-existing 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 recombinant, chimeric and CDR-grafted humanised antibody 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 $F(ab')_2$ 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, and/or if glycosylated products are required. 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 recombinant or humanised anti-hTNFα antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an anti-hTNFα antibody heavy chain;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary anti-hTNFα antibody light chain;

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

(d) culturing the transfected cell line to produce the recombinant anti-hTNFα antibody product.

The recombinant or humanised anti-hTNFα 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, a first vector containing an operon encoding a light chain-derived polypeptide and a 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.

The invention also includes therapeutic and diagnostic compositions comprising the recombinant and humanised antibody products of the invention and the uses of these products and the compositions in therapy and diagnosis.

Thus in a further aspect the invention provides a therapeutic or diagnostic composition comprising a recombinant or humanised antibody according to the invention in combination with a pharmaceutically acceptable excipient, diluent or carrier.

The invention also provides a process for the preparation of a therapeutic or diagnostic composition comprising admixing a recombinant or humanised antibody according to the invention together with a pharmaceutically acceptable excipient, diluent or carrier.

The recombinant or humanised antibody may be the sole active ingredient in the therapeutic or diagnostic composition or may be accompanied by one or more other active ingredients including other antibody ingredients, e.g. anti-T cell, anti-IFNγ or anti-LPS antibodies, or non-antibody ingredients such as xanthines. The therapeutic and diagnostic compositions may be in unit dosage form, in which case each unit dose comprises an effective amount of the recombinant or humanised antibody of the invention.

Furthermore, the invention also provides methods of therapy and diagnosis comprising administering an effective amount of a recombinant or humanised antibody according to the invention to a human or animal subject.

The antibodies and compositions may be utilised in any therapy where it is desired to reduce the level of TNF present in the human or animal body. The TNF may be in circulation in the body or present in an undesirably high level localised at a particular site in the body.

For example, elevated levels of TNF are implicated in immunoregulatory and inflammatory disorders and in septic, or endotoxic, and cardiovascular shock. The antibody or composition may be utilised in therapy of conditions which include sepsis, septic or endotoxic shock, cachexia, adult respiratory distress syndrome, AIDS, allergies, psoriasis, T.B., inflammatory bone disorders, blood coagulation disorders, burns, rejection episodes following organ or tissue transplant and autoimmune disease e.g. organ specific disease such as thyroiditis or non-specific organ diseases such as rheumatoid and osteo-arthritis.

Additionally, the antibody or composition may be used to ameliorate side effects associated with TNF generation during neoplastic therapy and also to eliminate or ameliorate shock related symptoms associated with the treatment or prevention of graft rejection by use of an antilymphocyte antibody, or may be used for treating multi-organ failure (MOF).

The recombinant and humanised antibodies and compositions of the invention are preferably for treatment of sepsis or septic/endotoxic shock.

The antibodies and compositions may be for administration in any appropriate form and amount according to the therapy in which they are employed. This may be for prophylactic use, for example where circumstances are such that an elevation in the level of TNF might be expected or alternatively, they may be for use in reducing the level of TNF after it has reached an undesirably high level or as the level is rising.

The therapeutic or diagnostic composition may take any suitable form for administration, and, preferably is in a form suitable for parenteral administration e.g. by

injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents such as suspending, preservative, stabilising and/or dispersing agents.

Alternatively, the antibody or composition may be in dry form, for reconstitution before use with an appropriate sterile liquid.

If the antibody or composition is suitable for oral administration, e.g. in the case of antibody fragments, the formulation may contain, in addition to the active ingredient, additives such as: starch - e.g. potato, maize or wheat starch or cellulose - or starch derivatives such as microcrystalline cellulose; silica; various sugars such as lactose; magnesium carbonate and/or calcium phosphate. It is desirable that, if the formulation is for oral administration it will be well tolerated by the patient's digestive system. To this end, it may be desirable to include in the formulation mucus formers and resins. It may also be desirable to improve tolerance by formulating the antibody or compositions in a capsule which is insoluble in the gastric juices. It may also be preferable to include the antibody or composition in a controlled release formulation.

In a still further aspect of the invention, there is provided a method of treatment of a human or animal subject suffering from or at risk of a disorder associated with an undesirably high level of TNF, the method comprising administering to the subject an effective amount of the antibody or composition of the invention. In particular, the human or animal subject may be suffering from, or at risk from, sepsis, or septic or endotoxic shock.

The dose at which the antibody is administered depends on the nature of the condition to be treated, the degree to which the TNF to be neutralised is, or is expected to be, raised above a desirable level, and on whether the antibody is being used prophylactically or to treat an existing condition. The dose will also be selected according to the age and conditions of the patient.

Thus, for example, where the product is for treatment or prophylaxis of septic shock suitable doses of antibody to TNF lie in the range 0.001-30mg/kg/day, preferably 0.01-10mg/kg/day and particularly preferably 0.1-2mg/kg/day.

The antibody products may be used in diagnosis e.g. in in vivo diagnosis and imaging of disease states involving elevated TNF levels.

The invention is further described by way of illustration only in the following Examples which refers to the accompanying diagrams, Figures 1 - 6.

Brief Description of the Figures

Figure 1 shows amino acid sequences for the variable domains of the heavy and light chains for the human acceptor antibody EU (1EU), the murine MAb CB0010 (*h t n f 1*) and humanised CDR grafted light (gEU) and heavy (2hEUg) chains;

Figure 2 shows amino acid sequences for the variable region domains of the human acceptor antibodies REI (*1 r e i*) for the light chain and KOL (KOL) for the heavy chain, of the heavy and light chains of the murine MAb 101.4 (101/4) and humanised grafted light and heavy chains (both designated g1014);

Figure 3 shows amino acid sequences for the variable region domains of the human acceptor antibodies REI (REI) for the light chain and KOL (KOL) for the heavy chain, of the heavy and light chains of the murine MAb CB0006 (CB6) and humanised grafted light and heavy chains (both designated gCB6);

Figure 4 shows amino acid sequences for the variable region domains of the human acceptor antibodies REI (REI) for the light chain and KOL (KOL) for the heavy chain, of the heavy (HTNF3) and light (hTNF3) chains of the murine MAb HTNF3 and humanised grafted light (gHTNF3) and heavy (ghTNF3) chains;

Figure 5 shows a graph comparing the ability of murine CB0010 (hTNF1) and CDR-grafted CB0010 (GrhTNF1; CDP571) to compete with HRP-conjugated murine HTNF1 for binding to recombinant human TNFα, and

Figure 6 shows a graph comparing the ability of murine HTNF1 (CB0010) and CDR-grafted HTNF1 (CP571) to neutralise recombinant TNFα in the L929 bioassay.

Detailed Description of Embodiments of the Invention

Example 1

CDR-Grafting of murine anti-TNFα antibodies

A number of murine anti-human TNFα MAbs were CDR-grafted substantially as described in detail in WO91/09967 for the CDR-grafting of the murine anti-CD3 antibody OKT3. In this and subsequent Examples, the chimeric and CDR-grafted humanised antibodies were prepared using human IgG4 constant region domains, substantially as described for preparation of γ4 chimeric and CDR-grafted OKT3 antibodies in WO91/09967. It will be appreciated, however, that human constant region domains of other types and isotypes, e.g. IgG1, IgG2 and IgG3, could also have been used without significantly altering the procedures described.

These anti-hTNFα antibodies included the murine MAbs designated CB0006 (also known as 61E71), CB0010 (also known as hTNF1), hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

CB0006

A similar analysis as described in Example 1, Section 12.1. of WO91/09967 was carried out for CB0006 and for the heavy chain 10 framework residues were identified at positions 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. The amino acid sequences of the murine CB0006 (CB6) (heavy and Light) REI (REI) light and KOL (KOL) heavy chain variable domains are given in Figure 3.

Three genes were built, the first of which coded for amino acid residues 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The amino acid sequence of the variable

domain coded by this first gene is shown as gCB6 in the heavy chain summary in Figure 3. The second gene also had amino acid residues 75 and 88 as murine residues [gH341(8)] while the third gene additionally had amino acid residues 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only) shown as gCB6 in the heavy chain summary in Figure 3. 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 CB0006.

CB0010 (also known as hTNF1)

CB0010 is a monoclonal antibody which recognises an epitope of human TNF-α. The EU human framework was used for CDR-grafting of both the heavy and light variable domains. The amino acid sequences of the heavy and light variable domains of EU (EU), CB0010 (*h t n f 1*) and grafted versions of CB0010 (gEU, light; 2hEUg, heavy) are shown in Figure 1.

Heavy Chain

In the CDR-grafted heavy chain 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 mouse CDRs were 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 CB0010 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. However when the grafted heavy chain/grafted light chain product was assayed in the L929 assay (see Example 4), it was found to have an activity only half that of the chimeric product. Thus further CDR-grafting experiments were carried out as described in Example 2.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to CB0006 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 chain variable domain amino acid sequences of hTNF3 (Htnf3, light;

hTNF3, heavy), CDR-grafted hTNF3 (gHTNF3, light; ghTNF3, heavy) and REI (REI, light) and KOL (KOL, heavy) are shown in Figure 4. The light and heavy chains of the CB0006 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However CB0006 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 CB0006 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. The gL221 gene codes for the gHTNF3 and the gH341 etc. gene codes for the ghTNF3 variable domain sequences as shown in Figure 4. It is likely that in this case other framework residues may need to be changed to improve the competitive binding ability of this antibody.

101.4

101.4 is a further murine MAb able to recognise human TNF-α. 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. The heavy and light variable domain amino acid sequences of 101.4 (101/4) and a CDR-grafted version of 101.4 (g1014) and the REI light chain (*l r e i*) and KOL heavy chain (KOL) variable domains are given in Figure 2. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) 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 the chimeric light chain or the Kabat CDR-grafted light chain. 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 L929 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.

Example 2

Further CDR-Grafting of Murine anti-human TNFα Antibodies CB0010 and 101.4

Murine anti-human TNFα monoclonal antibodies CB0010 and 101.4 were further CDR-grafted substantially as described in WO91/09667.

CB0010

CB0010 is a monoclonal antibody which recognises an epitope on human TNF-α. The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

The amino acid sequences of the heavy and light chain variable domains of the EU acceptor, CB0010 (*h t n f 1*) murine donor and CDR-grafted (gEU, light chain and 2hEUg, heavy chain) antibodies are given in Figure 1.

Heavy chain

In the CDR-grafted heavy chain (2hEUg), mouse CDRs were used at positions 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3).

Mouse residues were also used in the frameworks at positions 12, 27, 30, 38, 46, 48, 66, 67, 69, 71, 73, 76, 83, 89, 91, 94 and 108. Comparison of the CB0010 mouse and EU human heavy chain residues reveals that these are identical as positions 23, 24, 29 and 78.

Light chain

In the CDR-grafted light chain (gEU) mouse CDRs were used at positions 24-34 (CDR1), 50-65 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 106 and 108. The human consensus residue (valine) was used at position 83. Comparison of the CB0010 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted CB0010 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. The specific combination of grafted light chain (gEU) and grafted heavy chain (2hEUg), as shown in Figure 1, provides the antibody known as CDP571. The murine CB0010 (CB0010), chimeric CB0010 (chimeric CB0010) and the grafted heavy chain/grafted light chain product(CDP571) were compared for binding to human TNFα in a standard assay. The results obtained are given in the table below in terms of the K_D (pM) measured for each antibody.

Antibody	K_D (pM)
CB0010	80
Chimeric CB0010	81
CDP571	87

The fully grafted antibody product (CDP571) is currently in pre-clinical development for treatment of sepsis syndrome and acute transplant rejection.

101.4

101.4 is a further murine MAb able to recognise human TNF-α. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on REI for the light chain and KOL for the heavy chain. An improved CDR-grafted product has been prepared. Variable

domain amino acid sequences for REI (rei, light chain), KOL (KOL, heavy chain) murine 101.4 (101/4, heavy and light chain) and fully grafted antibody (g1014, heavy and light chain) are shown in Figure 2.

Heavy chain

In the CDR-grafted heavy chain (g1014) mouse CDRs were used at position 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the framework at positions 4, 11, 23, 24, 28, 73, 77, 78, 79, 91, 93, 94 and 108.

Light chain

In the CDR-grafted light chain (g1014) mouse CDRs were used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the framework at positions 1, 3, 4, 39, 73, 105 and 107. The human consensus residue (valine) was used at position 104.

The fully grafted heavy and light chain (g1014) were co-expressed and their binding to TNF compared with murine and chimeric 101.4 and also the fully grafted (gEU/2hEUg, CDP571) CB0010 antibody. The fully grafted 101.4 antibody was found to having binding properties for human TNFα similar to the murine, chimeric, and grafted CB0010 antibodies.

Example 3
In vitro comparison of Murine and CDR-grafted Antibodies

A. Affinity Measurements for Murine CB0010 and CDP571

Materials and Methods
Materials:

PBS/BSA: Dulbeccos PBS + 1% (w/v) bovine serum albumin.
TNF: 50nM rec. human TNF-alpha (Bissendorf Biochemicals), 0.85μg/ml in PBS/BSA.
Stock ^{125}I-TNF: 5μCi, 185kBq (Amersham International) dissolved in 500μl water and stored at -70°C.
Working Solution ^{125}I-TNF: -62pM for titration curve and 124pM for Scatchard analysis, in PBS/BSA.
Antibodies: Purified murine CB0010 (mHTNF1) and CDP571 were quantified by A280nm ($E^{1mg/ml}_{280nm} = 1.4$), and diluted to a concentration of 1μg/ml for titration, or 200ng/ml for Scatchard analysis.
Immunobeads: Goat anti-murine IgG whole molecule-agarose or goat anti-human IgG whole molecule-agarose (Sigma) were used undiluted.

Method:
Antibody titration: mHTNF1 and CDP571 were titrated in doubling dilutions (100μl each) to give a total of 16 samples and ^{125}I-TNF (100μl, 62pM) was added. The final top concentration of antibody was 500ng/ml and ^{125}I-TNF was 31pM. Control tubes (8) contained ^{125}I-TNF and PBS/BSA only. The samples were left to equilibrate overnight at room temperature, with shaking. After equilibration, 25μl goat anti-mouse-agarose was added to the mHTNF1 samples, and 50μl goat anti-human beads were added to the CDP571 samples except for the total ^{125}I-TNF controls. Non-specific absorption of ^{125}I-TNF to the agarose beads was corrected for by adding beads to

4 of the controls and comparing supernatant counts for these samples with those containing PBS/BSA instead of beads. After 1 hour equilibration at room temperature PBS/BSA (0.5ml) was added and the samples were centrifuged at 1500rpm for 10 mins at 20°C. The supernatant (0.5ml) was removed and radioactivity was counted in a gamma counter.

Confirmation that ^{125}I-TNF behaved similarly to the unlabelled material in this assay was made by performing the antibody titration in the presence of mixtures of ^{125}I-TNF and unlabelled TNF (at 25% and 75% ^{125}I-TNF) at the same total concentration.

Scatchard analysis: For both antibodies, unlabelled TNF (100µl, 50nM) was titrated in duplicate, in 13 doubling dilutions. One sample containing PBS/BSA in place of TNF was included for each antibody. ^{125}I-TNF (50µl, 124pM) was added to each sample. A constant amount of antibody, determined from the titration curve (50µl, 200ng/ml) was then added.

This gave the following final concentrations: antibody, 50ng/ml; TNF, 25nM top concentration; ^{125}I-TNF, 31pM. The samples were left to equilibrate overnight and then treated exactly as for the antibody titration samples.

Calculations

Titration Curves

Bound ^{125}I-TNF cpm = NSB cpm - supernatant cpm

$$\frac{\text{Bound } ^{125}\text{I-TNF cpm}}{\text{Total } ^{125}\text{I-TNF}} = B/T$$

NSB = non-specific absorption blank, supernatant cpm

Total = total counts for ^{125}I-TNF only

B/T was plotted against antibody concentration and the appropriate antibody concentration for use in Scatchard analyses was chosen at B/T = 0.6

Scatchard analysis

The mean of duplicate determination was used throughout

NSB = Total cpm-NSB supernatant cpm

Free cpm = sample cpm + NSB

Proportion of free TNF = Free/Total (F/T) =

$$\frac{\text{sample cpm + NSB cpm}}{\text{Total cpm}} = B/F = \frac{1-F/T}{F/T}$$

B/F was plotted against Bound TNF to give a slope of $-1/K_d$ from which K_d was calculated

RESULTS

Dissociation constants for murine CB0010 and CDP571

Antibody	K_d.M
Murine HTNF1	1.3×10^{-10}
CDP571	1.4×10^{-10}

B. Competition of murine CB0010 (Muhtnf1) and CDP571 (GrhTNF1) with HRP-conjugated in Murine CB0010 for binding to rhuTNF

Method

A 96 well microtitre plate (Nunc, Maxisorb) was coated with 100μl/well TNF at 0.5μg/ml.
Serial dilutions of murine or grafted antibody were prepared using PBS/1% BSA diluent, from 200μg/ml to 0.01μg/ml. 50μl of antibody was added to each well followed by 50μl HRP-murine CB0010 at 3 concentrations (0.625, 0.315 and 0.16μg/ml). Plates were incubated for 2 hours at room temperature with agitation, washed 4 times with PBS and 100μl of TMB substrate added.
Optical Density was measured and OD plotted against antibody concentration.

Conclusions

Curves for both murine antibody (MuhTNF1) and grafted antibody (GrhTNF1) are superimposable, indicating both antibodies compete with similar affinity for binding to TNF (see Figure 5).

Example 4

Comparison of Murine CB0010 and CDR-grafted CDP571 Antibodies in Bioassay and Animal Model Experiments

A. Neutralisation of TNF by CB0010 and CDP571 in the L929 Assay

The ability of the parent murine antibody CB0010 (hTNF1) and the CDR-grafted antibody CDP571 to neutralise recombinant human TNF was determined using the L929 bioassay. The assay uses the L929 mouse fibroblastoid cell line which is killed by TNF. The assay is performed in the presence of 1 ug/ml actinomycin D which renders the cells more sensitive to TNF. Serial dilution of the two antibodies were mixed with a constant amount of recombinant human TNF (100 pg/ml) and added to a L929 monolayer in 96 well flat bottomed plates. After a 16 hour incubation the cells which had not been killed by TNF were revealed using the stain crystal violet. The apparent amount of TNF not neutralised (residual TNF) was determined by comparison with a recombinant TNF standard curve. Results from a representative experiment where residual TNF is plotted against antibody concentration are shown in Figure 6. It can be seen that CB0010 and CDP571 have similar neutralisation activities.

B. Effect of CDP571 in Baboon Sepsis Model

In this study the effect of the prior treatment with CDP571 on the physiological consequences of severe sepsis (including death) was assessed. Baboons were chosen as a relevant species to study since CDP571 is known to neutralise baboon TNF.

Male adult baboons, Papio ursinus, weighting 20-25 kg were anaesthetised with ketamine hydrochloride and sodium pentabarbitone and instrumented for the measurement of blood pressure, cardiac index (by thermodilution), ECG and right atrial filling pressures. An infusion of either

saline only or antibody was then given for 120 min at a rate of 2.5 ml/kg/h following which they were given a further 120 min infusion of live E.coli at the same infusion rate. The bacterial strain used was Hinshaw's strain B7 ([086a:61], ATCC 33985) administered whilst in the log growth phase at a dose of $2x10^9$ CFU/kg giving a plasma concentration of $2-2.5x10^5$ CFU/ml at the end of the infusion. Following a further 120 min, animals were returned to their home cages, given free access to food and water and monitored for cardiovascular changes twice a day for 3 days. All animals were given constant fluid replacement infusion of 5 ml/kg/h which was adjusted, where necessary, to maintain adequate right heart filling pressures. Baboons that had died during treatment or that had survived the 72h experimental period, and then killed were post-mortemed. All major organs were assessed for gross macro-pathalogical damage according to semi-quantitative scale (+++ being the most severe).

Animals were randomly assigned to one of 4 treatment groups;

-saline only
-CDP571 0.1 mg/kg
-CDP571 1.0 mg/kg
-CB0010 0.1 mg/kg (parent murine antibody)

The survival and cumulative organ damage scores are shown in table 1. CDP571 at 1.0 mg/kg prevented death and significantly ($P<0.005$) reduced the incidence of organ damage in this model; furthermore, these effects were dose-related ($P<0.005$). In addition, the survival rate and organ damage score seen with CB0010 were similar to those seen with CDP571 at the same dose, indicating a maintained in vivo potency of CDP571 compared to its parent murine antibody.

BABOON SEPSIS STUDY

Table 1

SURVIVAL FOLLOWING ADMINISTRATION OF $2X10^9$ CFU E.Coli GIVEN IV 2H AFTER SALINE OR CDP 571

TREATMENT	No	DEAD	SURVIVED	PERCENT SURVIVAL	ORGAN PATHOL.
SALINE	8	7	1	13	+++
CDP571 0.1 mg/kg	6	2	4	67	++
CDP571 1.0 mg/kg	6	0	6	100	+/-
CB0010 0.1 mg/kg	4	1	3	75	++

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CLAIMS

1. A recombinant antibody molecule which has specificity for human TNFα.

2. A recombinant antibody molecule according to Claim 1 having an antigen binding site derived from the murine monoclonal antibody CB0006 (alternatively known as 61E71), CB0010 (alternatively known as hTNF1), hTNF3 or 101.4.

3. A recombinant antibody molecule according to Claim 1 or 2 which is a humanised antibody molecule.

4. A humanised chimeric antibody molecule according to Claim 3.

5. A CDR-grafted humanised antibody according to Claim 3.

6. A CDR-grafted humanised antibody heavy chain according to Claim 5 having a variable region domain comprising human 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.

7. A CDR-grafted humanised heavy chain according to Claim 6 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

8. A CDR-grafted humanised heavy chain according to Claim 6 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

9. A CDR-grafted humanised heavy chain according to Claim 7 or 8, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

10. A CDR-grafted humanised heavy chain according to any of Claims 5-9 comprising donor CDRs at positions 26-35, 50-65 and 95-100.

11. A CDR-grafted humanised antibody light chain according to Claim 5 having a variable region domain comprising human 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.

12. A CDR-grafted light chain according to Claim 11 comprising donor residues at positions 46 and 47.

13. A CDR-grafted humanised antibody light chain according to Claim 5 having a variable region domain comprising human acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

14. A CDR-grafted light chain according to Claim 13 comprising donor residues at positions 46, 48, 58 and 71.

15. A CDR-grafted light chain according to Claim 11 or 13, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

16. A CDR-grafted light chain according to Claim 15, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a 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 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

17. A CDR-grafted light chain according to any one of Claims 11-16, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

18. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of Claims 6-10 and at least one CDR-grafted light chain according to any one of Claims 11-17.

19. A CDR-grafted humanised antibody heavy chain having a variable region domain comprising human acceptor framework (especially EU human acceptor framework) and hTNF1 donor antigen binding regions wherein the framework comprises hTNF1 donor residues at positions 12, 27, 30, 38, 46, 48, 66, 67, 69, 71, 73, 76, 83, 89, 91 and 94.

20. A CDR-grafted humanised antibody light chain having a variable domain comprising human acceptor framework (especially EU human acceptor framework) and hTNF1 donor antigen binding regions wherein the framework comprises hTNF1 donor residues at positions 3, 42 and 49.

21. A CDR-grafted humanised antibody molecule comprising at least one CDR-grafted humanised heavy chain according to Claim 19 and at least one CDR-grafted humanised light chain according to Claim 20.

22. A CDR-grafted humanised antibody heavy chain having a variable region domain comprising human acceptor framework (especially KOL human acceptor framework) and 101.4 donor antigen binding regions wherein the framework comprises 101.4 donor residues at positions 4, 11, 23, 24, 28, 73, 77, 78, 79, 91, 93 and 94.

23. A CDR-grafted humanised antibody light chain having a variable region domain comprising human acceptor framework (especially REI human acceptor framework) and 101.4 donor residues at positions 1, 3, 4 and 73.

24. A CDR-grafted humanised antibody molecule comprising at least one CDR-grafted humanised heavy chain according to Claim 22 and at least one CDR-grafted humanised light chain according to Claim 23.

25. A DNA sequence which codes for a heavy or light chain antibody molecule which has specificity for human TNFα.

26. A DNA sequence which codes for a CDR-grafted heavy chain according to any one of Claims 6-10, 19 or 22, or a CDR-grafted light chain according to any one of Claims 11-17, 20 or 23.

27. A cloning or expression vector containing a DNA sequence according to Claim 26.

28. A host cell transformed with a DNA sequence according to Claim 27.

29. A process for the production of a CDR-grafted antibody comprising expressing a DNA sequence according to Claim 25 or Claim 26 in a transformed host cell.

30. A process for producing a recombinant or humanised anti-hTNFα antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an anti-hTNFα antibody heavy chain.

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary anti-hTNFα antibody light chain.

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

(d) culturing the transfected cell line to produce the anti-hTNFα antibody product.

31. A therapeutic or diagnostic composition comprising a recombinant antibody molecule according to Claim 1 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

32. A process for the preparation of a therapeutic or diagnostic composition comprising admixing a recombinant antibody molecule according to Claim 1 together with a pharmaceutically acceptable excipient, diluent or carrier.

33. A method of therapy or diagnosis comprising administering an effective amount of a recombinant antibody molecule according to Claim 1 to a human or animal subject.

34. A recombinant antibody molecule according to Claim 1 or a therapeutic composition according to Claim 31 for use in the amelioration of side effects associated with TNF generation during neoplastic therapy.

35. A recombinant antibody molecule according to Claim 1 or a therapeutic composition according to Claim 31 for use in the elimination or amelioration of shock related symptoms associated with antilymphocyte therapy.

36. A recombinant antibody according to Claim 1 or a therapeutic composition according to Claim 31 for use in the treatment of multi organ failure.

37. A recombinant antibody according to Claim 1 or a therapeutic composition according to Claim 31 for use in the treatment of sepsis or septic/endotoxic shock.

38. A method of treatment of a human or animal subject, suffering from or at risk of a disorder associated with an undesirably high level of TNF, comprising administering to the subject an effective amount of a recombinant antibody according to Claim 1.

39. A method according to Claim 33 or 38 comprising administering doses of anti-TNF antibody product in the range 0.001-30mg/kg/day, preferably 0.01-10mg/kg/day, or particularly preferably 0.1-2mg/kg/day.

CDR GRAFTING OF hTNF-1

Light Chain Data

```
1 Eu    DIQMTQSPST LSASVGDRVT ITCRASQSI. ....NTWLA WYQQKPGKAPK
htnf1   DIMMSQSPSS LAVSVGEKVTMS CKSSQSLLYSNNQKNYLA WYQQKPGQSPK
g Eu    DIMMTQSPST LSASVGDRVTIT CKSSQSLLYSNNQKNYLA WYQQKPGQAPK

Eu      LLMYKASSLE SGVPSRFIGS GSGTEFTLTI SSLQPDDFAT YYCQQYNSDS
htnf1   LLISWASTRES GVPDRFTGS GSGTDFTLTI SSVKAEDLAV YYCQQYYDYP
gEu     LLISWASTRES GVPSRFIGS GSGTEFTLTI SSLQPDDVAT YYCQQYYDYP

3 Eu    KMFGQG TKVEVKG..(KAPPA)
htnf1   WTFGGG SKLEIK.....anti human TNF seq from
g Eu    WTFGQG TKVEIKR..(KAPPA)

framework residues changed (# = Kabat)

chgs 3/42/48/49/83/106/108
```

Heavy Chain Data

```
Eu      QVQLVQSGAE VKKPGSSVKV SCKASGGTFSRSAII WVRQA PGQGLEWMGG
htnf1   EVLLQQSGPE LVKPGASVKI PCKASGYTFTDYNVD WVKQS HGKSLQWIGN
2hEug   QVQLVQSGAE VVKPGSSVKV SCKASGYTFTDYNVD WVKQA PGQGLQWIGN

Eu      IVPMFGPPNYAQKFQG  RVTITADESTNTAYMELSSLRSED  TAFYFCAGGY
htnf1   INPNNGGTIYNQKFKG  KGTLTVDKSSSTAYMELRSLTSED  TAVYYCARSA
2hEug   INPNNGGTIYNQKFKG  KGTLTVDKSTSTAYMELSSLTSED  TAVYYCARSA

Eu      GIYSPE          WGQGTLVTVSS.grp 1kabat cdr chg frwk4
htnf1   FYNNYEYFDV      WGAGTTVTVSS
2hEug   FYNNYEYFDV      WGQGTTVTVSS

framework residues changed (# = kabat)

chgs 12/27/30/38/46/48/66/67/69/71/73/76/83/89/91/94/108
```

Fig. 1

CDR GRAFTING OF 101-4

LIGHT CHAIN SUMMARY

```
1 rei  DIQMTQSPSS LSASVGDRVT ITCQASQDI. ....IKYLNW YQQTPGKAPK
101/4  QIVLTQSPPI MSASPGEKVT MTCSASSSVSFMY       W YQQKPGSSPR
g1014  QIVLTQSPSS LSASVGDRVT ITCSASSSVSFMY       W YQQKPGKAPK

2 rei  LLIYEASNLQA GVPSRFSGS GSGTDYTFTI SSLQPEDIAT YYCQQYQSLP
101/4  LLIYDASILAS GVPVRFSGS GSGTSYSLTI SRMEAEDVAT YYCQQWSDYS
g1014  LLIYDASILAS GVPSRFSGS GSGTDYTLTI SSLQPEDIAT YYCQQWSDYS

3 rei   YTFGQGTKLQ ITR..celltech rei
101/4  PRTFGGGTKLE IKR.....THIS IS MOUSE(INSERTION IN CDR3)JSE
g1014  PRTFGQGTKVE IKR..celltech rei
```

framework residues changed (# = Kabat)

1/3/4/39/73/104/105/107

HEAVY CHAIN SUMMARY

```
                                  23                              48
KOL    QVQLVESGGG VVQPGRSLRL SCSSSGFIFSSYAMY WVRQA PGKGLEWVAI
101/4  EVKIEESGGG WVQPGGSMKL SCIASGFTFSNYWMN WVRQS PEKGLEWVAE
g1014  QVQIVESGGG WVQPGRSLRL SCIASGFTFSNYWMN WVRQA PGKGLEWVAE

                                71                          88
KOL    IWDDGSDQHYADSVKG  RFTISRDNSKNTLFLQMDSLRPED TGVYFCARDG
101/4  VRLQSDNFTTHYAESVKGRFTISRDDSKSGVYLQMNNLGAED TGIYYCTPFA
g1014  VRLQSDNFTTHYAESVKGRFTISRDDSKNGVYLQMDSLRPED TGVYYCTPFA

KOL    GHGFCSSASCFGPDY WGQGTPVTVSS....HUMAN(kabat CDR defn)
101/4                Y WGQGTLVTVSP...MOUSE seq
g1014                Y WGQGTLVTVSS
```

framework residues changed (# = Kabat)

4/11/23/24/28/73/77/78/79/91/93/94/108

Fig. 2

61E71

LIGHT CHAIN SUMMARY

```
REI    DIQMTQSPSS LSASVGDRVT ITCQASQDI. ....IKYLN WYQQTPGKAPK
CB6    SIVMTQTPKF LLVSAGDRVT ITCKASQSVS NDVA      WYQQKSGQSPK
gCB6   DIQMTQSPSS LSASVGDRVT ITCKASQSVS NDVA      WYQQTPGKAPK

REI    LLIYEASNLQA GVPSRFSGS GSGTDYTFTI SSLQPEDIAT YYCQQYQSLP
CB6    VLIYHVSNRYT GVPDRFTGS GYGTDFTFTI TTVQAEDLAV YFCQQDYSSP
gCB6   LLIYHVSNRYT GVPSRFSGS GSGTDYTFTI SSLQPEDIAT YYCQQDYSSP

REI    YTFGQGTKLQ ITR... celltech rei(KAPPA)
CB6     WTFGGGTKLE IK.... MOUSE AB... REI (NO FWRKS)
gCB6   WTFGQGTKLQ ITR... grafted AB sequence
```

HEAVY CHAIN SUMMARY

```
KOL    QVQLVESGGG VVQPGRSLRL SCSSSGFIFSSYAMY WVRQA PGKGLEWVAI
CB6    QIQLVQSGPD LKKPGETVKI SCKASGYTFTNYGMN WVKQT PGKGLKWMGW
gCB6   QVQLVESGGG VVQPGRSLRL SCKASGYTFTNYGMN WVRQA PGKGLEWMGW

KOL    IWDDGSDQHYADSVKG RFTISRDNSKNTLFLQMDSLRPED TGVYFCARDG
CB6    INTYTGEPTYDDDFKG RPAFSLEASASTAYLQINNLKNED MATFFCARQE
gCB6   INTYTGEPTYDDDFKG RFTISLDASKNTLFLQMDSLRPED TGVYFCARQE

KOL    GHGFCSSASCFGPDY WGQGTPVTVS.HUMAN grp3(kabat CDR defn)
CB6            GFYAMDY WGQGTSLTVSS..MOUSE ANTI-TNF sequence
gCB6           GFYAMDY WGQGTPVTVS.grafted AB sequence
```

Fig. 3

hTNF3

LIGHT CHAIN SUMMARY

```
REI     DIQMTQSPSS LSASVGDRVT ITCQASQDI. ....IKYLN WYQQTPGKAPK
HTNF3   NIVMTQTPKF LLVSAGDRIT ITCKASQSVS NDVA      WYQQKPGQSPR
gHTNF3  DIQMTQSPSS LSASVGDRVT ITCKASQSVS NDVA      WYQQTPGKAPK

REI     LLIYEASNLQA GVPSRFSGS GSGTDYTFTI SSLQPEDIAT YYCQQYQSLP
HTNF3   LLIYYVSNRYT GVPDRFTGS GYGTDFTFTI NTVQAEDLAY YFCQQDYSSP
gHTNF3  LLIYYVSNRYT GVPSRFSGS GSGTDYTFTI SSLQPEDIAT YYCQQDYSSP

REI     YTFGQGTKLQ ITR.. (KAPPA)
HTNF3   YTFGGGTRLE VK.... MOUSE AB sequence
gHTNF3  YTFGQGTKLQ ITR..grafted sequence
```

HEAVY CHAIN SUMMARY

```
KOL     QVQLVESGGG VVQPGRSLRL SCSSSGFIFSSYAMY WVRQA PGKGLEWVAI
hTNF3   RIQLVQSGPE LKKPGETVKI SCKASGYTFTNYGMN WVTQA PGKGLKWMGW
ghTNF3  QVQLVESGGG VVQPGRSLRL SCKASGYTFTNYGMN WVRQA PGKGLEWMGW

KOL     IWDDGSDQHYADSVKG RFTISRDNSKNTLFLQMDSLRPED TGVYFCARDG
hTNF3   INTYTGEPTYADDFKG RFAFSLETSASTAYLQINNLKNED TATYFCARKE
ghTNF3  INTYTGEPTYADDFKG RFTISLDTSKNTLFLQMDSLRPED TGVYFCARKE

KOL     GHGFCSSASCFGPDY WGQGTPVTVS.HUMAN grp3
hTNF3           GFYAMDY WGQGTSVTVSS...MOUSE.ANTI-TNF sequence
ghTNF3          GFYAMDY WGQGTPVTVS.grafted AB sequence
```

Fig. 4

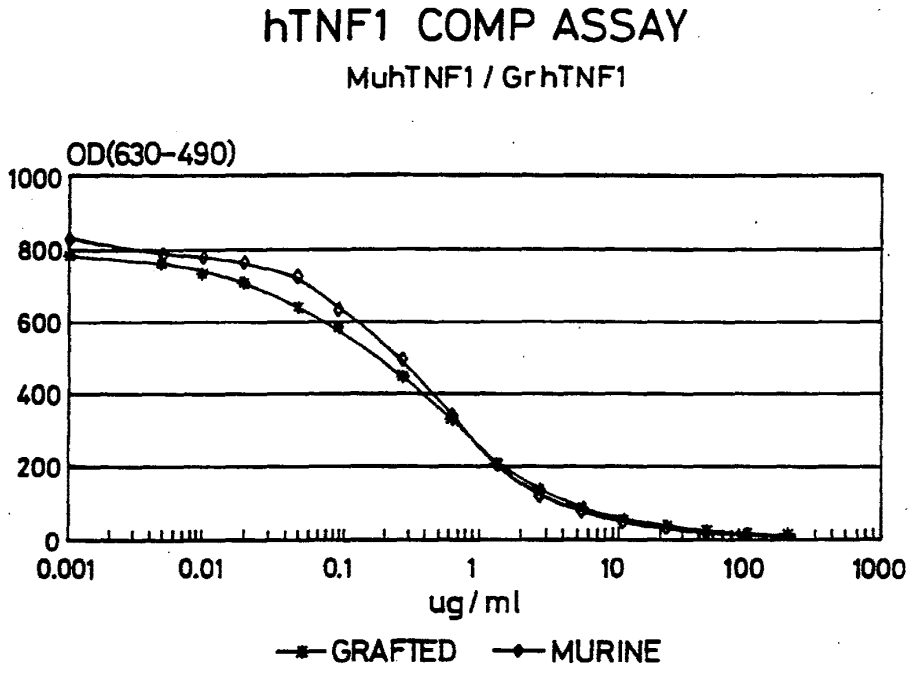
hTNF1 COMP ASSAY
MuhTNF1 / GrhTNF1
OD(630-490)
1000
800
600
400
200
0
0.001
0.01
0.1
1
10
100
1000
ug/ml
GRAFTED
MURINE

Fig. 5

SUBSTITUTE SHEET

NEUTRALISATION OF 100 pg / ml RECOMBINANT TNF BY CB0010 AND CDP571

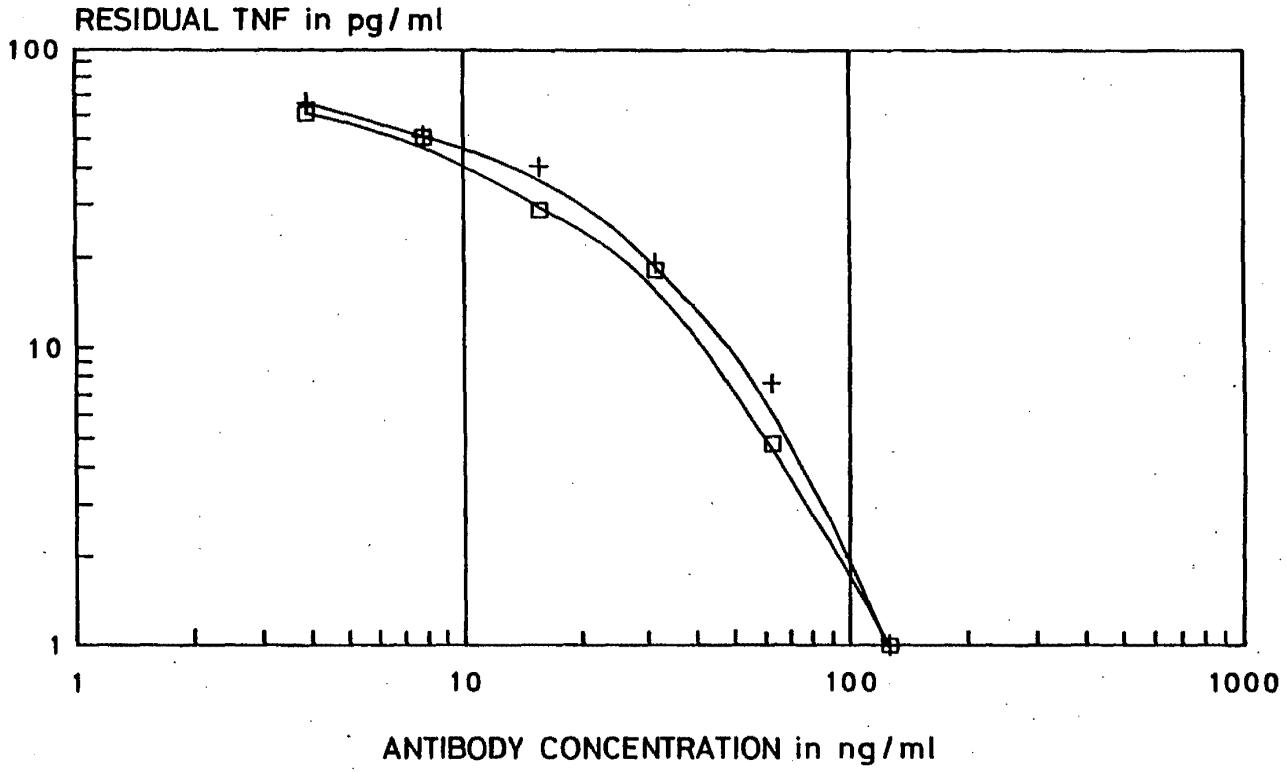

Fig. 6

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/02300

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

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

Int.Cl.5 C 12 P 21/08 C 07 K 15/28 C 12 N 15/13
C 12 N 15/62 A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 12 P C 12 N A 61 K

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

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EP,A,0355067 (CELLTECH LTD) 21 February 1990, see the whole document	1,2,31-38
Y	---	3-30
Y	EP,A,0403156 (GENZYME CORP.) 19 December 1990, see example 12	3-30
X	--- Journal of Infectious Diseases, vol. 161, no. 6, June 1990, (Chicago, US), S. OPAL et al.: "Efficacy of a monoclonal antibody directed against tumor necrosis factor in protecting neutropenic rats from lethal infection with Pseudomonas aeruginosa", pages 1148-1152, see the whole article	1,2,31, 32,37, 38
Y	---	3-30,33-36
	--- -/-	

[10] Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26-03-1992	23 APR 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	

Form PCT/ISA/210 (second sheet) (January 1985)

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

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	Proceedings of the National Academy of Sciences of USA, vol. 86, no. 24, December 1989, (Washington, US), C. QUEEN et al.: "A humanized antibody that binds to the interleukin 2 receptor", pages 10029-10033, see page 10033, left-hand column, lines 10-45 (cited in the application) ---	3-30,33 -36
X,P L	WO,A,9109967 (CELLTECH LTD) 11 July 1990, see the whole document (cited in the application) (cited as L document since it may throw doubt on a priority claim) -----	1-38

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

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

1. ☒ Claim numbers * because they relate to subject matter not required to be searched by this Authority, namely:

Remark: although claims 33 and 38 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

* claim not searched: 39 See PCT-Rule 39.1 (iv)

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

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

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

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

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

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

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

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

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

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9102300 SA 54719

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/04/92
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0355067	21-02-90	AU-A- 4182289	23-03-90
		WO-A- 9001950	08-03-90
EP-A- 0403156	19-12-90	CA-A- 2018248	07-12-90
		JP-A- 3219896	27-09-91
WO-A- 9109967	11-07-91	AU-A- 6974091	24-07-91
		AU-A- 7033091	24-07-91
		AU-A- 7048691	24-07-91
		EP-A- 0460167	11-12-91
		EP-A- 0460171	11-12-91
		EP-A- 0460178	11-12-91
		WO-A- 9109966	11-07-91
		WO-A- 9109968	11-07-91
		GB-A- 2246781	12-02-92
		GB-A- 2246570	05-02-92

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

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(54) Title: HUMANIZED ANTIBODY AGAINST CD18

(57) Abstract

A humanized antibody having all or part of the CDRs as defined and capable of binding to the human CD-18 antigen. The antibody is of use in therapy in treating leukocyte mediated conditions such as inhibiting ingress of leukocytes into the lung and other organs and treatment of inflammation.

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HUMANIZED ANTIBODY AGAINST CD18

The present invention relates to an antibody which binds to the CD18 antigen, to the preparation of such an antibody and to a pharmaceutical composition which contains the antibody.

Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. CDRs and framework regions of antibodies may be determined by reference to Kabat et al ("Sequences of proteins of immunological interest" US Dept. of Health and Human Services, US Government Printing Office, 1987).

The preparation of an altered antibody in which the CDRs are derived from a different species than the framework of the antibody's variable domains is disclosed

in EP-A-0239400. The CDRs may be derived from a rat or mouse monoclonal antibody. The framework of the variable domains, and the constant domains, of the altered antibody may be derived from a human antibody. Such a humanised antibody elicits a negligible immune response when administered to a human compared to the immune response mounted by a human against a rat or mouse antibody. Humanised CAMPATH-1 antibody is disclosed in EP-A-0328404 (Campath is a Trade Mark of The Wellcome group of companies).

According to one aspect of the present invention, there is provided a humanised antibody in which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to the human CD18 antigen:

light chain: CDR1 (SEQ ID NOS: 3 and 4)
CDR2 (SEQ ID NOS: 5 and 6)
CDR3 (SEQ ID NOS: 7 and 8)
heavy chain: CDR1 (SEQ ID NOS: 11 and 12)
CDR2 (SEQ ID NOS: 13 and 14)
CDR3 (SEQ ID NOS: 15 and 16)

According to another aspect the invention provides a DNA molecule encoding a humanised antibody in which sufficient of the amino acid sequence of each CDR shown above is provided such that the antibody is capable of binding to the human CD-18 antigen.

The antibody preferably has the structure of a natural antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a $(Fab')_2$ fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. The antibody may be an IgG such as IgG1, IgG2, IgG3 or IgG4; or IgM, IgA, IgE or IgD. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain.

The antibody may be a chimeric antibody of the type

described in WO 86/01533. A chimeric antibody according to WO 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain and/or heavy chain variable domain. Typically the chimeric antibody comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused to the C-terminus of the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from a protein having known binding specificity, from a protein toxin or indeed from any protein expressed by a gene. The non-immunoglobulin region may be a carbohydrate region. The two regions of the chimeric antibody may be connected via a cleavable linker sequence.

The light chain CDRs 1 to 3 and heavy chain CDRs 1 to 3 of SEQ ID NOS: 3 to 8 and SEQ ID NOS: 11 to 16 respectively are the CDRs of the YFC51.1.1 rat antibody which is a CD18 antibody. The specificity of a humanised antibody for the human CD18 antigen can be determined by flow cytometry, monocyte adhesion and/or by T-cell proliferation assays as follows:

Monocyte (MNC) Adhesion

MNC's are treated with the phorbol diester PDBu (10^{-9} M) in the presence and absence of antibody (20μl) for 5 minutes. These cells are then transferred to bovine aortic endothelial cell (BAEC) monolayers and incubated for 30 minutes in a humidified atmosphere of 95% air, 5% CO_2 at 37°C. Non-adherent cells are removed by washing in phosphate buffered saline (PBS) three times. The adherent cells are then lysed in situ with 50μl, 0.5% hexadecyltrimethyl ammonium bromide. Dianisidine dihydrochloride (0.63mM) containing 0.4mM hydrogen peroxide is added (250μl) to each well and incubated for a further 10 minutes. Enzyme activity is then assessed using the presence of monocyte-specific myeloperoxidase, recorded as

an increase in absorbance. The optical density of the samples can then be recorded at 450nm using a multi-well plate reader (Anthos series, Lab Teck instruments). Comparisons can then be made between treated and untreated samples (Bath et al, J. Immunol. Meth., 118, 59-65, (1989)).

Flow cytometry

Surface labelling of rat, rabbit, guinea-pig and human monocytes with antibody is carried out according to the method of Gladwin et al., (Biochim. Biophys. Acta., 1052, 166-172 (1990)). Briefly, 1 ml aliquots of cells suspension ($5x10^6$) are incubated with the appropriate antibody, monodispersed and incubated on melting ice for 30 minutes. The cells are twice washed in PBS and incubated for a further 30 minutes with a 1:200 dilution of rabbit anti-rat $F(ab')_2$ FITC conjugate on melting ice. The cells are finally washed three times in PBS and fixed in 0.1% para-formaldehyde. Analysis of surface labelling can be performed using an Epics Elite flow cytometer (Coulter cytometry, Hialhea, FL) using standard computer, electronics and optics. The Elite is configured with a 15mW 488nm Argon-ion laser (Cyonics model 2201, San Jose, CA). Monocyte and lymphocyte populations are separated by forward angle light scatter and side scatter. Green fluorescence data for 2 x 10^4 monocytes is collected using bit-map gating and collected on a three decade log scale. Green fluorescence data for 2 x 10^4 neutrophils is collected in a similar manner. For each sample, mean fluorescence intensity in the presence of the primary mAb is compared with cells incubated with rabbit anti-rat $F(ab')_2$ FITC fragments alone and the percentage labelling of the cells determined. Samples can be labelled in triplicate and repeat experiments can be performed on three separate occasions.

T-cell proliferation assay

Human mononuclear cells are prepared from defibrinated blood using density gradient separation over Ficoll-paque. Lymphocytes (2 x 10^5 cells) are cultured in each well of a flat bottomed 96-well microtitre plate (Nunclon, Roskild, Denmark), in RPMI 1640 supplemented with 10% autologous serum, 2mM glutamine and 100iU penicillin/ – 100μg ml^{-1} streptomycin. Triplicate cultures are set up with the medium alone or with antigen (Tetanus Toxoid, 3μg ml^{-1}) or mitogen (PHA, 1μg ml^{-1}), in the presence or absence of different concentrations of monoclonal antibodies. Cells are cultured at 37°C in a humidified atmosphere of 95% air, 5% CO_2 for five days. Wells are then pulsed with 1μCi [methyl^{3}H] thymidine (2Ci $mmol^{-1}$, Amersham), harvested 18 hours later and radioactivity counted by liquid scintillation using a B counter (LKB, Betaplate, Sweden). The results are expressed as mean +/- SEM.

Suitably, the CDRs of a humanised antibody are the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 above. The amino acid sequences of these CDRs may be changed, however. The amino acid sequence of each CDR may be changed by up to 40% by amino acid substitutions, insertions and/or deletions, for example by up to 30%, up to 20% or up to 10%.

Each CDR may therefore include one or two amino acid substitutions, insertions and/or deletions. There may be up to three amino acid substitutions, insertions, and/or deletions in light chain CDR. Up to four amino acid substitutions, insertions and/or deletions may be present in light chain CDR1 or heavy chain CDR3. Up to six amino acid substitutions, insertions and/or deletions may be present in heavy chain CDR2. Preferably the amino acid sequence of each CDR is substantially homologous to that of each CDR of YFC 51.1.1.

The framework and the constant domains of the antibody are human framework and human constant domains.

Preferably the framework of the variable region of the antibody heavy chain is substantially homologous to the corresponding framework of the human protein NEWM (Saul et al, J. Biol. Chem. 25, 585-597, (1987)). Homology in respect of the framework is generally 80% or more with respect to NEWM, for example 90% or more or 95% or more. A number of amino acid substitutions, insertions and/or deletions may be present. Candidate framework changes that may be made to restore binding include changes of amino acid residues 27, 30, 48, 66, 67, 71, 91, 93 and 94. The amino acid numbering is according to Kabat et al.

The framework of the variable region of the antibody light chain is typically substantially homologous to the variable domain framework of the protein REI (Epp et al, Eur. J. Biochem. 45, 513-524, (1974)). Homology in respect of the framework is generally 80% or more with respect to REI, for example 90% or more or 95% or more. A number of amino acid substitutions, insertions and/or deletions may be present, for example at amino acid residue 71 according to the numbering of Kabat et al.

A humanised antibody is prepared according to the invention by a process which comprises maintaining a host transformed with a first expression vector which encodes the light chain of the humanised antibody and with a second expression vector which encodes the heavy chain of the humanised antibody under such conditions that each chain is expressed and isolating the humanised antibody formed by assembly of the thus-expressed chains.

The first and second expression vectors may be the same vector. The invention further provides:

- a DNA sequence encoding the light chain or the heavy chain of the humanised antibody;
- an expression vector which incorporates a said DNA sequence; and
- a host transformed with a said expression vector.

Each chain of the antibody may be prepared by CDR

replacement. The CDRs of a variable region of a light or heavy chain of a human antibody are replaced by sufficient of the amino acid sequence of each CDR of the rat anti-human-CD18 antibody YFC51.1.1 such that the resulting antibody is capable of binding to the CD18 antigen. The CDR-encoding regions of DNA encoding a hypervariable region of a human antibody chain are replaced by DNA encoding the desired CDRs. If appropriate, this altered DNA is linked to DNA encoding a constant domain for the antibody chain. The DNA is cloned into an expression vector. The expression vector is introduced into a compatible host cell which is cultured under such conditions that the antibody chain is expressed. Complementary antibody chains which are co-expressed in this way may then assemble to form the humanised antibody.

The present invention is described herein with particular reference to the production of a humanised antibody having CDRs derived directly or indirectly from the rat antibody YFC51.1.1. However the techniques described herein can equally be used to derive other humanised anti CD-18 antibodies. According to a further aspect, the present invention provides a humanised (CDR grafted) anti CD-18 antibody.

There are four general steps to humanise a monoclonal antibody. These are:

(1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains;

(2) designing the humanised antibody, i.e. deciding which antibody framework region to use during the humanising process;

(3) the actual humanising methodologies/techniques; and

(4) the transfection and expression of the humanised antibody.

Step 1: Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

To humanise an antibody only the amino acid sequence of antibody's heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant because these do not contribute to the reshaping strategy. The simplest method of determining an antibody's variable domain amino acid sequence is from cloned cDNA encoding the heavy and light chain variable domain.

There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs: (1) via a conventional cDNA library, or (2) via the polymerase chain reaction (PCR). Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains. In the present instance, the nucleotide sequence and predicted amino acid sequence of the light and heavy chains of the rodent YFC51.1.1 antibody are shown in SEQ ID NOS: 1 and 2 and SEQ ID NOS: 9 and 10.

Step 2: Designing the humanised antibody

There are several factors to consider in deciding which human antibody sequence to use during the humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is basically similar for each.

This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper spatial orientation to recognize antigen. Thus the substitution of rodent CDRs into a human

variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework is highly homologous to the rodent variable domain from which they originated. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s). A suitable human antibody variable domain sequence can be selected as follows:

1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if only human immunoglobulin sequences are included.
2. List the human antibody variable domain sequences and compare for homology. Primarily the comparison is performed on length of CDRs, except CDR3 of the heavy chain which is quite variable. Human heavy chains and Kappa and Lambda light chains are divided into subgroups; Heavy chain 3 subgroups, Kappa chain 4 subgroups, Lambda chain 6 subgroups. The CDR sizes within each subgroup are similar but vary between subgroups. It is usually possible to match a rodent antibody CDR to one of the human subgroups as a first approximation of homology. Antibodies bearing CDRs of similar length are then compared for amino acid sequence homology, especially within the CDRs, but also in the surrounding framework regions. The human variable domain which is most homologous is chosen as the framework for humanisation.

Step 3: The actual humanising methodologies/techniques

An antibody may be humanised by grafting the desired CDRs onto a human framework according to EP-A-0239400. A DNA sequence encoding the desired reshaped antibody can therefore be made beginning with the human DNA whose CDRs it is wished to reshape. The rodent variable domain amino acid sequence containing the desired CDRs is compared to that of the chosen human antibody variable domain sequence. The residues in the human variable domain are marked that need to be changed to the corresponding residue in the rodent to make the human variable region incorporate the rodent CDRs. There may also be residues that need substituting in, adding to or deleting from the human sequence.

Oligonucleotides are synthesized that can be used to mutagenize the human variable domain framework to contain the desired residues. Those oligonucleotides can be of any convenient size. One is normally only limited in length by the capabilities of the particular synthesizer one has available. The method of oligonucleotide-directed in vitro mutagenesis is well known.

Alternatively, humanisation may be achieved using the recombinant polymerase chain reaction (PCR) methodology of WO 92/07075. Using this methodology, a CDR may be spliced between the framework regions of a human antibody.

In general, the technique of WO 92/07075 can be performed using a template comprising two human framework regions, AB and CD, and between them, the CDR which is to be replaced by a donor CDR. Primers A and B are used to amplify the framework region AB, and primers C and D used to amplify the framework region CD. However, the primers B and C each also contain, at their 5′ ends, an additional sequence corresponding to all or at least part of the donor CDR sequence. Primers B and C overlap by a length sufficient to permit annealing of their 5′ ends to each other under conditions which allow a PCR to be performed.

Thus, the amplified regions AB and CD may undergo gene splicing by overlap extension to produce the humanised product in a single reaction.

Step 4: The transfection and expression of the reshaped antibody

Following the mutagenesis reactions to reshape the antibody, the mutagenised DNAs can be linked to an appropriate DNA encoding a light or heavy chain constant region, cloned into an expression vector, and transfected into host cells, preferably mammalian cells. These steps can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising:

(a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a human antibody and the CDRs required for the humanised antibody of the invention;

(b) preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

(c) transforming a cell line with the first or both prepared vectors; and

(d) culturing said transformed cell line to produce said altered antibody.

Preferably the DNA sequence in step (a) encodes both the variable domain and the or each constant domain of the human antibody chain. The humanised antibody can be prepared using any suitable recombinant expression system. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may

also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

The CHO cells used for expression of the antibodies according to the invention may be dihydrofolate reductase (dhfr) deficient and so dependent on thymidine and hypoxanthine for growth (Urlaub et al., Proc. Natl. Acac. Sci. U.S.A., 77 4216-4220 (1980)). The parental dhfr CHO cell line is transfected with the DNA encloding the antibody and dhfr which enables selection of CHO cell transformants of dhfr positive phenotype. Selection is carried out by culturing the colonies on media devoid of thymidine and hypoxanthine, the absence of which prevents untransformed cells from growing and transformed cells from resalvaging the folate pathway and thus bypassing the selection system. These transformants usually express low levels of the DNA of interest by virtue of co-integration of transfected DNA of interest and DNA encoding dhfr. The expression levels of the DNA encoding the antibody may be increased by amplification using methotrexate (MTX). This drug is a direct inhibitor of the enzyme dhfr and allows isolation of resistant colonies which amplify their dhfr gene copy number sufficiently to survive under these conditions. Since the DNA sequences encoding dhfr and the antibody are closely linked in the original transformants, there is usually concomitant amplification, and therefore increased expression of the desired antibody.

Another preferred expression system for use with CHO or myeloma cells is the glutamine synthetase (GS) amplification system described in WO 87/04462. This system involves the transfection of a cell with DNA encoding the enzyme GS and with DNA encoding the desired antibody. Cells are then selected which grow in glutamine free medium and can thus be assumed to have integrated the DNA encoding

GS. These selected clones are then subjected to inhibition of the enzyme GS using methionine sulphoximine (Msx). The cells, in order to survive, will amplify the DNA encoding GS with concomitant amplification of the DNA encoding the antibody.

Although the cell line used to produce the humanised antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. In particular, it is envisaged that E. coli - derived bacterial strains could be used. The antibody obtained is checked for functionality. If functionality is lost, it is necessary to return to step (2) and alter the framework of the antibody.

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be recovered and purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (See, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, a humanised antibody may then be used therapeutically or in developing and performing assay procedures, immunofluorescent stainings, and the like (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The humanised CD18 antibodies can be used for example in the treatment of leukocyte mediated conditions. The humanised CD18 antibodies typically find use in inhibiting influx of leukocytes into the lungs and other organs during sepsis or other infectious or non-infectious trauma. The

humanised CD18 antibody can therefore be used for inhibiting the ingress of leukocytes into the lung and other organs in patients having endotoxic shock or adult respiratory distress syndrome. The antibody can be used to treat asthma or leukocyte-mediated reperfusion damage post thrombolytic therapy, to treat inflammation in the lung and other organs in patients having an inflammation caused by sepsis or other infectious or non-infectious trauma, to eliminate or reduce inflammation in a patient being administered with an anti-infective agent or to assist in the administration of a therapeutic drug to a patient during chemotherapy (EP-A-0346078).

The humanised antibodies of the present invention may also be used in combination with other antibodies, particularly human monoclonal antibodies reactive with other markers on cells responsible for the disease. For example, suitable T-cell markers can include those grouped into the so-called "Clusters of Differentiation" as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard, et al., Eds., Springer-Verlag, N.Y. (1984).

The antibodies can also be used as separatelyadministered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

An antibody of the present invention may form part of an immunotoxin. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle", provides a means for delivering the toxic agent

to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet", Thorpe et al, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982).

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). See, generally, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25, 335-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985).

The delivery component of the immunotoxin is a humanised antibody according to the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgA, IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The invention further provides a pharmaceutical composition comprising a pharmaceutically acceptable

carrier or diluent and, as active ingredient, a humanised antibody according to the invention. The composition may comprise an immunotoxin according to the invention. The humanised antibody, immunotoxin and pharmaceutical compositions thereof of this invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously.

The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th

ed., Mack Publishing Company, Easton, Pennsylvania (1980).

The antibodies of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g., with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

The compositions containing the present human-like antibodies or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest or alleviate the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the infection and the general state of the patient's own immune system, but generally range from about 1 to about 200 mg of antibody per dose, with dosages of from 5 to 25 mg per patient being more commonly used. It must be kept in mind that the materials of the invention may generally be employed in serious disease states, that is life-threatening or potentially life-threatening situations. In such cases, in view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by the present human-like antibodies of this invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these antibodies.

In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are

administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose". In this use, the precise amounts again depend upon the patient's state of health and general level of immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per patient. A preferred prophylactic use is for the prevention of kidney transplant rejection.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the patient.

Human-like antibodies of the present invention can further find a wide variety of utilities in vitro. By way of example, the exemplary antibodies can be utilized for T-cell typing, for isolating specific CD18 antigen-bearing cells or fragments of the receptor, for vaccine preparation, or the like.

For diagnostic purposes, the antibodies may either be labelled or unlabelled. Unlabelled antibodies can be used in combination with other labelled antibodies (second antibodies) that are reactive with the humanised antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labelled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly haptens), etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of a cellular activity or for the presence of a selected antigen. Thus, a humanised antibody of the present

invention may be provided, usually in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for the desired cell type. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above. The kit will generally also include a set of instructions for use.

The following Example illustrates the invention.

EXAMPLE

Cloning and sequencing of the YFC51.1.1 rat anti-human-CD18 heavy and light chains

Total RNA was isolated from 2.5 x 10^7 YFC51.1.1 expressing cells following the method of Chomczynski and Sacchi (Anal. Biochem., 162, 156-159, (1987)), using 1ml of extraction solution per 1 x 10^7 cells. The resulting RNA pellet was redissolved in ⁄50µl diethyl pyrocarbonate (DEPC)-treated distilled water, and spectrophotometrically determined to be at a concentration of 4µg/µl. Dynabeads Oligo $(dT)_{25}$ (Dynal) was used to extract mRNA from 75µg total RNA employing the manufacturer's protocol.

cDNA was synthesized from the isolated mRNA and cloned into the plasmid pSPORT-1 using the SUPERSCRIPT Plasmid System for cDNA Synthesis and Plasmid Cloning kit (BRL) following the method recommended by the manufacturer. Escherichia coli MAX EFFICIENCY DH5α Competent Cells (BRL) were transformed with the resulting cDNA/pSPORT-1 ligation. Approximately 5000 colonies were lifted onto Hybond-N nylon filters (Amersham) and lysed, denatured and fixed following the method of Buluwela et al (Nucleic Acids Res., 17, 452, (1989)). The filters were treated with proteinase K (50μg//ml) in 0.2 x SSC, 0.1% SDS at 55°C for 30 min, and then excess debris removed with a tissue.

(i) Heavy chain

An oligonucleotide as shown in SEQ ID NO: 17 complementary to a portion of rat gamma-CH1 constant region (bases 496-515) was end-labelled and used to screen the filters for YFC51.1.1 heavy chain following the standard protocols. Approximately 50 potential positive colonies were detected, and 20 selected for further analysis. Plasmid DNA was prepared using the method of Del Sal et al (Nucleic Acids Res., 16, 9878, (1988)) and 12 of the 20 contained inserts of the expected size for rat immunoglobulin heavy chain cDNA. A clone, p51H.6, was selected, and the variable region sequenced in both directions by plasmid priming following the dideoxy chain termination method (Sanger et al, (Proc. Natl. Acad. Sci., USA, 74, 5463-5467, (1977)), according to the Sequenase kit (USB) protocol. The sequence of the variable region is shown in SEQ ID NOS: 9 and 10.

(ii) Light Chain

A clone of the rat myeloma Y3-Ag 1.2.3 light chain (Crowe et al, Nucleic Acid Res., 17, 7992, (1989)) was labelled with digoxigenin-11-dUTP using the Nonradioactive DNA labelling and Detection Kit (Boehringer Mannheim) and used to screen the filters for the YFC51.1.1 light chain,

following the manufacturer's protocol. Approximately 40 potential positive colonies were detected, and 24 selected for further analysis. Plasmid DNA was prepared as described above. Both Y3-Ag 1.2.3 and YFC51.1.1 light chains were isolated (Y3 cell line being hybridoma fusion partner) but were distinguishable by having different restriction patterns. One clone, p51L.4, containing the YFC51.1.1 light chain was chosen and sequenced as described for the heavy chain. The sequence of the variable region is shown in SEQ ID NOS: 1 and 2.

Designing the humanised antibody

Using the selection procedure described in Step (2) above, the human variable domain frameworks of the NEWM heavy chain and REI light chain (Kabat et al, 1987) were chosen for the humanisation process.

Construction of the humanised heavy and light chain genes

The humanised heavy and light chains were constructed following the method of Lewis and Crowe (Gene, 101, 297-302, (1991)).

(i) Light Chain

Light chain oligonucleotide primers:

A_L: SEQ ID NO: 18:
B_L: SEQ ID NO: 19:
C_L: SEQ ID NO: 20:
D_L: SEQ ID NO: 21:
E_L: SEQ ID NO: 22:
F_L: SEQ ID NO: 23:
G_L: SEQ ID NO: 24:
H_L: SEQ ID NO: 25:

PCR reactions (Saiki et al., Science 239, 487-491, (1988)) were performed in a programmable heating block (Hybaid) using 20 rounds of temperature cycling (94°C for 1 min, 50°C for 2 min, and 72°C for 3 min) followed by a

final 10 min step at 72°C. 1μg of each primer, a specified amount of template, and 2.5 units of Taq polymerase (Perkin Elmer Cetus) were used in a final volume of 100 μl with the reaction buffer as recommended by the manufacturer.

The initial template for the PCR was CAMPATH-1H light chain (humanised CAMPATH-1 on RE1 framework; Page and Sydenham, Biotechnology, 9, 64-68, (1991)). Four initial PCR reactions were carried out, with 10ng of template per reaction, using the primer pairs A_L with B_L, C_L with D_L, E_L with F_L, and G_L with H_L respectively. The products of these PCR reactions, fragments AB_L, CD_L, EF_L and GH_L respectively, were purified using Prep-A-Gene (Bio-Rad) following the protocol recommended by the manufacturer. Fragments AB_L with CD_L, and EF_L with GH_L were combined using a quarter of each purified product, and subjected to recombinant PCR reactions with primers A_L plus D_L, and E_L plus H_L respectively. The products of these reactions, fragments AD_L and EH_L, were purified as above, and a quarter of each combined in a recombinant PCR reaction using primers A_L and H_L. The final humanised light chain recombinant PCR product, AH_L, was cloned into the HindIII site of pUC-18 (BRL) following the method of Crowe et al., Nucleic Acids Res., 19, 184, (1991), utilising the HindIII sites in primers A_L and H_L. Plasmid isolates were sequenced by the dideoxy chain termination method, and clones of the correct sequence chosen.

(ii) Heavy Chain

Heavy chain oligonucleotide primers:

A_H: . SEQ ID NO: 26:

B_H: . SEQ ID NO: 27:

C_H: . SEQ ID NO: 28:

D_H: . SEQ ID NO: 29:

E_H: . SEQ ID NO: 30:

F_H: . SEQ ID NO: 31:

G_H: . SEQ ID NO: 32:

H_H: . SEQ ID NO: 33:

The initial template for the PCR was CAMPATH-1H heavy chain. The rodent CDR's were grafted on to the template following the recombinant PCR method as described above, but using oligonucleotide primers A_H to H_H. The final PCR, i.e. fragments AD_H and EH_H with primers A_H and H_H, did not give a high yield of product so a fragment AF_H was generated (from fragments AD_H and EF_H) and used with fragment EH_H in a PCR with primers A_H and H_H. Oligonucleotides A_H and H_H were designed with HindIII and EcoRI sites respectively to enable initial cloning of the humanised variable region, and a SpeI site was introduced into the NEWM framework 4 (FR4) region of oligonucleotide G_H to facilitate subsequent cloning of the variable region with a suitable constant region of choice. The SpeI site was chosen so as not to alter the leucine residue at position 109 (numbering according to Kabat et al, 1987) of the humanised heavy chain template. Four out of the six human heavy J-minigenes possess a leucine at this position (Kabat et al, 1987). Thus the use of the engineered SPeI site should be generally applicable.

The humanised heavy chain variable region recombinant PCR product was cloned into HindIII/EcoRI-cut pUC-18 (BRL), and plasmid isolates of the correct sequence were chosen. The FR4 and γ1 constant regions of CAMPATH-1H heavy chain were PCR cloned into pUC-18 (BRL) using oligonucleotide primers X_H (SEQ ID NO: 34) and Y_H (SEQ ID NO: 35). Primer X_H contains SpeI and HindIII sites, and Y_H an EcoRI site. The HindIII and ECoRI sites were used to clone the PCR product into pUC-18, and plasmid isolates of the correct sequence were selected. The complete heavy chain was subsequently reconstituted from the humanised variable region and γ1 constant region clones using the engineered FR4 Spel site.

Transient expression in COS cells

DNA encoding the humanised heavy and light chains were cloned into the vectors pEE6.hCMV and pEE12 respectively, see Stephens & Cockett, Nucleic Acids Res., 17, 7110 (1989); Bebbington et al., Biotechnology, 10, 169 (1992); and Bebbington and Hentschel in Glover ed., DNA Cloning Volume III, Academic Press (1987). The vector pEE12 is a pBR322 - based vector containing the h-CMV-MEI promoter and the hamster glutamine synthetase (GS) cDNA under control of the SV40 early region promoter. The vector pEE12 corresponds to pEE6 (see EP-A-0338841) with the GS cDNA expression cassette driven by the SV40 promoter transcribing in the same direction as the h-CMV-MEI promoter. Cells transfected with the vectors pEE6, hCMV and pEE12 are capable of growth in glutamine free medium because of the presence of the GS cDNA. As the selection is only on the pEE12 plasmid, effective expression relies upon co-integration of both plasmids.

The recombinant plasmids (5μg of each) were transfected into $5x10^5$ COS-1 cells using the Transfectam reagent (Promega, Southampton, U.K.) under the conditions recommended by the manufacturer. Stock COS-1 cells (source ECACC, Porton Down, U.K.) were maintained in DMEM medium (Flow, Irvine, U.K.) supplemental with 10% foetal calf serum (APP, Dudley, U.K.). COS cell transfections were carried out in DMEM medium (Flow, Irvine, U.K.). Growth media from COS-1 cells four days post transfection were assayed by a sandwich ELISA assay using flexible microtitre plates (Falcon, Becton-Dickinson, Plymouth, U.K.) coated with polyclonal anti-human IgG (Sigma, Poole, U.K.) as capture antibody. The assay sample was added and detection performed with an anti-human IgG · γ chain-specific peroxidase conjugate (Seralab, Crawley Down, U.K.) and orthophenylene dimine-HCl (Sigma, Poole U.K.) as substrate.

The humanised antibody was shown to be expressed transiently in the COS cells by using the spent COS cell supernatant to surface label MF-14 (a T-cell clone) cells

for FACS analysis according to the method of Gladwin et al, Biochem. Biophs. Acta, 1052, 166-172 (1990). Briefly 100µl aliquots of a cell suspension (10^5) were incubated with the appropriate antibody (spent COS cell supernatant) and incubated on melting ice for 30 minutes. The cells were washed twice in PBS and incubated for a further 30 minutes with the appropriate second antibody (see below). The cells were washed again and 1:50 dilutions of anti-rat Ig-FITC or anti-human Ig-FITC conjugates were added on melting ice. Finally, the cells were washed three times in PBS and fixed in 0.1% paraformaldehyde. Analysis of surface labelling was performed using a Becton-Dickenson FACScan using standard computer, electronics and optics.

The humanised antibody in the COS cell supernatant was shown to bind MF-14 cells as well as inhibiting the binding of the rat YFC51.1.1 monoclonal antibody. Since the humanised antibody was shown to have retained binding for CD18 by blocking the binding of the rat monoclonal antibody, stable NSO transfactants were generated.

Stable expression in NSO cells

A single expression vector for generating stable transfectants of NSO cells was generated by cloning the complete heavy chain expression cassette from pEE6 into the BamHI site of the pEE12 - light chain plasmid. Thus both heavy and light chain coding sequences are transcribed in the same direction from the same vector. 40µg of plasmid for transfection was linearised by digestion with SalI restriction enzyme that has a recognition sequence within the bacterial plasmid sequence. The linearised DNA was precipitated from solution using ethanol, washed in 70% ethanol, dried and resuspended in sterile water.

Exponentially growing NSO cells (a Human mycloma cell line; see Jarvis, Methods in Enzymology, 73B, 3 (1981); source ECACC , Porton Down, U.K.) were maintained in non-selective DMEM medium (i.e. without glutamine and ferric

nitrate but with sodium pyruvate at 110 mg/l (GIBCO/BRL, Paisley, U.K.) supplemented with 1X non-essential amino acids (Flow, Irvine, U.K.) 2mM glutamine (GIBCO) and 10% foetal calf serum (APP, Dudley, U.K.). NSO cells were centrifuged, washed and re-suspended in cold PBS, such that after the addition of the DNA the cells would be at a concentration of 10^7 cells/ml. The linearised plasmid DNA, 40μg, was added to 10^7 cells in an electroporation cuvette on ice. The cells and DNA were mixed gently so as to avoid generating bubbles and the mixture was left on ice for 5 minutes. The outside of the cuvette was wiped dry and two consecutive pulses at 1500V, 3mF were delivered using a Gene Pulser (Bio-Rad). The cuvette was returned to ice for 5 minutes.

Transfected cells were transferred to 96 well plates at densities of 3×10^5, 7.5×10^4 and 1.5×10^4 cells/ml in 50μl of non-selective medium and incubated at 37°C for 24 hours. Subsequently 100 μl of selective DMEM medium (i.e. without glutamine and ferric nitrate but with sodium pyruvate at 100 mg/l (GIBCO/BRL, Paisley, U.K.) supplemented with glutamate (60 mg/ml), asparagine (60 mg/ml; Sigma, Poole, U.K.), 1X non-essential amino acids, 7 mg/l of adenosine, cytidine, guanosine and uridine, 2.4 mg/l of thymidine (Sigma, Poole, U.K.) and 10% dialysed foetal calf serum (APP, Dudley U.K.)) was added to selected clones which had integrated the transfected plasmid. The plates were returned to the incubator and left until substantial cell death had occurred and discrete surviving colonies had appeared. Once colonies of glutamine-independent transfectants could be seen, wells with single colonies were selected and spent tissue culture supernatants were collected and assayed for human IgG secretion.

Wells with single colonies that were positive for IgG secretion were then expanded in culture using selective medium. The cells were distributed in 96 well plates at 10^4

cells/well in 100μl of medium and incubated overnight. 100μl of selective medium containing a concentration of L-methionine sulphoximine (MSX) was added. MSX is a toxic glutamine analogue that allows for selection of vector amplification. Each 96-well plate had a different final concentration of MSX, ranging from 200μM down to 12.5μM. Individual colonies were isolated from each independent transfectant at the highest MSX concentration at which MSX resistance occurred. The colonies were expanded and antibody secretion rate (in $\mu g/10^6$ cells/day) was compared with the unamplified rate. Clones were obtained that expressed the humanised antibody at 1 to 3 $\mu g/10^6$ cells/day.

The humanised antibody was purified from spent tissue culture supernatant by affinity chromatography over a Superose protein-G column (Pharmacia) and used in T-cell proliferation assays and C1q binding studies.

T-cell Proliferation

Peripheral human mono-nuclear cells were isolated from defibrinated whole human blood using Lymphoprep (Nycomed, Oslo, Norway) and following the manufacturer's protocol. Triplicate cultures were set up in 96 well flat bottomed microtitre plates (Nunclon, Roskild, Denmark) with the medium clone (RPMI 1640 supplemented with 10% autologous serum, 2mM glutamine and 100 IU/ml penicillin, 100μg/ml streptomycin) or with medium and antigen (Tetanus toxoid, 5μg/ml) or medium and mitogen (PHA, 5μg/ml), in the presence or absence ofYFC 51.1.1 or the humanised antibody. Cells were cultured at 37°C in a humidified atmosphere of 95% air, 5% CO_2 for five days. Wells were then pulsed with 1μCi [methyl ^{3}H]thymidine (2Ci/mmol, Amersham), harvested 4 hours later and radioactivity counted by liquid scintillation using a ß counter (LKB, Betaplate, Sweden).

Both the rat YFC51.1.1 monoclonal antibody and the humanised antibody strongly inhibited the antigen specific

T-cell response but had little effect on the PHA induced proliferation. However, at high levels of antibody (50μg/ml) and low levels of PHA (2.5μg/ml) up to 80% inhibition could be obtained.

Complement binding

Human mononuclear cells (prepared as above) were stimulated with PHA at 5μg/ml and incubated at 37°C for 3 days. The PHA was removed by washing the cells in PBS. The cells were then incubated with 10μg/ml of test antibody for 20 minutes on ice, cells washed in ice cold PBS and incubated with ice cold human serum for 20 minutes. The human serum was removed by washing in ice cold PBS. The cells were then incubated for 20 minutes with a fluoreceinated polyclonal sheep anti-human C1q. Unbound anti-C1q was removed by washing cells in PBS and cells were analysed on a Becton-Dickenson FACScan. YFC51.1.1 was found to bind human C1q weakly and no binding was detected for the humanised antibody. Potential therapeutic uses for anti-CD18 antibodies rely on transient inhibition of CD18-mediated adherence of leukocytes rather than depletion of CD18 positive cells. Accordingly the inability of the humanised antibody to fix human complement on CD18 positive cells is an advantage since it suggests that in vivo the antibody will not deplete using complement but will function as a blocking antibody.

FACS analysis

A CD18 positive T-cell clone (MF14) was used to determine the binding of humanised compared with rat antibody. Cells were incubated with rat or humanised antibody for 30 minutes on ice. Unbound antibody was removed by washing and the second antibody was added (i.e. rat antibody was added to cells pre-incubated with humanised antibody and vice versa) and incubated for 30 minutes on ice. Cells were washed to remove unbound

antibody and a FITC-labelled anti-human or anti-rat antibody added. Unbound label was removed by washing and the cells were analysed on a Becton-Dickenson FACScan. Pre-incubation of MF14 cells with 10μg/ml of YFC51.1.1 antibody completely blocked the binding of 0.1μg/ml of humanised antibody. In the reciprocal experiment, pre-incubation with 10μg/ml of humanised antibody completely blocked the binding of 0.1μg/ml YFC51.1.1. In both cases use of 1.0 and 0.1μg/ml of the first antibody led to a titration of blocking.

SEQUENCE LISTING

(1) INFORMATION FOR SEQ ID NO : 1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 375 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..375
(D) OTHER INFORMATION : /product= "Variable region light chain"
/standard_name= "YFC51.1.1"

(ix) FEATURE:

(A) NAME/KEY : misc_signal
(B) LOCATION : 1..60
(D) OTHER INFORMATION : /function= "Signal Sequence"

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 130..162

(D) OTHER INFORMATION : /function= "CDR 1"

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 208..228
(D) OTHER INFORMATION : /function= "CDR 2"

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 325..351
(D) OTHER INFORMATION : /function= "CDR 3"

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

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  1               5                  10                  15

GGT GCC CAG TGT GAT GTC CAG ATG ACC CAG TCT CCG TCT TAT CTT GCT    96
Gly Ala Gln Cys Asp Val Gln Met Thr Gln Ser Pro Ser Tyr Leu Ala
             20                  25                  30

GCG TCT CCT GGA GAA AGT GTT TCC ATC AGT TGC AAG GCA AGT AAG AGC   144
Ala Ser Pro Gly Glu Ser Val Ser Ile Ser Cys Lys Ala Ser Lys Ser
         35                  40                  45

ATT AGC AAT TAT TTA GCC TGG TAT CAA CAG AAA CCT GGG GAA GCA AAT   192
Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Glu Ala Asn
     50                  55                  60

AAA CTT CTT GTC TAT TAT GGG TCA ACT TTG CGA TCT GGA ATT CCA TCG   240
Lys Leu Leu Val Tyr Tyr Gly Ser Thr Leu Arg Ser Gly Ile Pro Ser
 65                  70                  75                  80

AGG TTC AGT GGC AGT GGA TCT GGT ACA GAT TTC ACT CTC ACC ATC AGA   288
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Arg
                 85                  90                  95
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AAC CTG GAG CCT GCA GAT TTT GCA GTC TAC TAC TGT CAA CAG TAT TAT    336
Asn Leu Glu Pro Ala Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
            100                 105                 110

GAA AGA CCG CTC ACG TTC GGT TCT GGG ACC AAG CTG GAG                375
Glu Arg Pro Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu
        115                 120                 125
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(2) INFORMATION FOR SEQ ID NO : 2

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 125 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

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

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Met Arg Val Gln Val Gln Phe Leu Gly Leu Leu Leu Trp Thr Ser
 1               5                  10                  15

Gly Ala Gln Cys Asp Val Gln Met Thr Gln Ser Pro Ser Tyr Leu Ala
            20                  25                  30

Ala Ser Pro Gly Glu Ser Val Ser Ile Ser Cys Lys Ala Ser Lys Ser
        35                  40                  45

Ile Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Glu Ala Asn
    50                  55                  60

Lys Leu Leu Val Tyr Tyr Gly Ser Thr Leu Arg Ser Gly Ile Pro Ser
65                  70                  75                  80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Arg
                85                  90                  95

Asn Leu Glu Pro Ala Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
            100                 105                 110
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Glu Arg Pro Leu Thr Phe Gly Ser Gly Thr Lys Leu Glu
115 120 125

(3) INFORMATION FOR SEQ ID NO : 3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 33 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 1..33
(D) OTHER INFORMATION : /function= "CDR 1"

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..33

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

AAG GCA AGT AAG AGC ATT AGC AAT TAT TTA GCC 33
Lys Ala Ser Lys Ser Ile Ser Asn Tyr Leu Ala
1 5 10

(4) INFORMATION FOR SEQ ID NO : 4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 11 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

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

Lys Ala Ser Lys Ser Ile Ser Asn Tyr Leu Ala
1 5 10

(5) INFORMATION FOR SEQ ID NO : 5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 21 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 1..21
(D) OTHER INFORMATION : /function= "CDR 2"

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..21

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

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TAT GGG TCA ACT TTG CGA TCT                                    21
Tyr Gly Ser Thr Leu Arg Ser
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(6) INFORMATION FOR SEQ ID NO : 6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 7 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

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

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Tyr Gly Ser Thr Leu Arg Ser
 1               5
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(7) INFORMATION FOR SEQ ID NO : 7

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 27 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 1..27
(D) OTHER INFORMATION : /function= "CDR 3"

(ix) FEATURE

(A) NAME/KEY : CDS

(B) LOCATION : 1..27

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

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CAA CAG TAT TAT GAA AGA CCG CTC ACG                                    27
Gln Gln Tyr Tyr Glu Arg Pro Leu Thr
  1               5
```

(8) INFORMATION FOR SEQ ID NO : 8

(i) SEQUENCE CHARACTERISTICS:

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

(ii) MOLECULE TYPE : protein

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

```
Gln Gln Tyr Tyr Glu Arg Pro Leu Thr
  1               5
```

(9) INFORMATION FOR SEQ ID NO : 9

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 417 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..417
(D) OTHER INFORMATION : /product= "Heavy chain variable region with signal sequence"
/standard_name "YFC51.1.1"

(ix) FEATURE:

(A) NAME/KEY : misc_signal
(B) LOCATION : 1..57
(D) OTHER INFORMATION : /function= "Signal sequence"

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 148..162
(D) OTHER INFORMATION : /function= "CDR 1"

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 205..255

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 352..384

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

```
ATG AAA TGC AGC TGG ATC AAC CTC TTC TTG ATG GCA CTA GCT TCA GGG     48
Met Lys Cys Ser Trp Ile Asn Leu Phe Leu Met Ala Leu Ala Ser Gly
 1               5                  10                  15

GTC TAC GCA GAA GTG CAG CTG CAA CAG TCT GGG CCC GAG CTT CGG AGA     96
Val Tyr Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Arg Arg
            20                  25                  30

CCT GGG TCC TCA GTC AAG TTG TCT TGT AAG ACT TCT GGC TAC AGC ATT    144
Pro Gly Ser Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Ser Ile
        35                  40                  45

AAA GAT TAC CTT CTG CAC TGG GTA AAA CAT AGG CCA GAA TAC GGC CTG    192
Lys Asp Tyr Leu Leu His Trp Val Lys His Arg Pro Glu Tyr Gly Leu
    50                  55                  60

GAA TGG ATA GGA TGG ATT GAT CCT GAG GAT GGT GAA ACA AAG TAT GGT    240
Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly
65                  70                  75                  80

CAG AAG TTT CAA AGC AGG GCC ACA CTC ACT GCA GAT ACA TCC TCC AAC    288
Gln Lys Phe Gln Ser Arg Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn
                85                  90                  95

ACA GCC TAC ATG CAA CTC AGC AGC CTG ACG TCT GAC GAC ACA GCA ACC    336
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Asp Asp Thr Ala Thr
            100                 105                 110

TAT TTT TGT ACT AGA GGC GAA TAT AGA TAC AAC TCG TGG TTT GAT TAC    384
```

```
Tyr Phe Cys Thr Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
        115                 120                 125

TGG GGC CAA GGC ACT CTG GTC ACT GTC TCT TCA                     417
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
    130                 135
```

(10) INFORMATION FOR SEQ ID NO : 10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 139 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

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

```
Met Lys Cys Ser Trp Ile Asn Leu Phe Leu Met Ala Leu Ala Ser Gly
  1               5                  10                  15

Val Tyr Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Arg Arg
             20                  25                  30

Pro Gly Ser Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Ser Ile
         35                  40                  45

Lys Asp Tyr Leu Leu His Trp Val Lys His Arg Pro Glu Tyr Gly Leu
     50                  55                  60

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly
 65                  70                  75                  80

Gln Lys Phe Gln Ser Arg Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn
                 85                  90                  95

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Asp Asp Thr Ala Thr
            100                 105                 110

Tyr Phe Cys Thr Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
```

115 120 125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135

(11) INFORMATION FOR SEQ ID NO : 11

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 15 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 1..15
(D) OTHER INFORMATION : /function= "CDR 1"

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..15

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 11:

GAT TAC CTT CTG CAC 15
Asp Tyr Leu Leu His
1 5

(12) INFORMATION FOR SEQ ID NO : 12

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 5 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 12:

Asp Tyr Leu Leu His
1 5

(13) INFORMATION FOR SEQ ID NO : 13

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 51 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 1..51
(D) OTHER INFORMATION : /function= "CDR 2"

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..51

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 13:

```
TGG ATT GAT CCT GAG GAT GGT GAA ACA AAG TAT GGT CAG AAG TTT CAA    48
Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly Gln Lys Phe Gln
 1               5                  10                  15

AGC                                                              51
Ser
```

(14) INFORMATION FOR SEQ ID NO : 14

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 17 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 14:

```
Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly Gln Lys Phe Gln
 1               5                  10                  15

Ser
```

(15) INFORMATION FOR SEQ ID NO : 15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 33 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM : Rattus rattus

(ix) FEATURE:

(A) NAME/KEY : misc_feature
(B) LOCATION : 1..33
(D) OTHER INFORMATION : /function= "CDR 3"

(ix) FEATURE:

(A) NAME/KEY : CDS
(B) LOCATION : 1..33

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

```
GGC GAA TAT AGA TAC AAC TCG TGG TTT GAT TAC                    33
Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
 1               5                  10
```

(16) INFORMATION FOR SEQ ID NO : 16

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 11 amino acids
(B) TYPE : amino acid
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein

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

Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
1 5 10

(17) INFORMATION FOR SEQ ID NO : 17

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 20 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(vi) ORIGINAL SOURCE

(A) ORGANISM : Rattus Rattus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 17:

AGTGGATAGA CAGATGGGGC 20

(18) INFORMATION FOR SEQ ID NO : 18

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 30 bases

(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 18:

GATCAAGCTT CTCTACAGTT ACTGAGCACA 30

(19) INFORMATION FOR SEQ ID NO : 19

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 43 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 19:

GCTAAATAAT TGCTAATGCT CTTACTTGCT TTACAGGTGA TGG 43

(20) INFORMATION FOR SEQ ID NO : 20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 43 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 20:

AGAGCATTAG CAATTATTTA GCCTGGTACC AGCAGAAGCC AGG 43

(21) INFORMATION FOR SEQ ID NO : 21

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 41 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 21:

AGATCGCAAA GTTGACCCAT AGTAGATCAG CAGCTTTGGA G 41

(22) INFORMATION FOR SEQ ID NO : 22

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 41 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 22:

TATGGGTCAA CTTTGCGATC TGGTGTGCCA AGCAGATTCA G 41

(23) INFORMATION FOR SEQ ID NO : 23

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 47 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 23:

CGTGAGCGGT CTTTCATAAT ACTGTTGGCA GTAGTAGGTG GCGATGT 47

(24) INFORMATION FOR SEQ ID NO : 24

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 47 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

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

CAACAGTATT ATGAAAGACC GCTCACGTTC GGCCAAGGGA CCAAGGT 47

(25) INFORMATION FOR SEQ ID NO : 25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 30 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

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

GATCAAGCTT CTAACACTCT CCCCTGTTGA 30

(26) INFORMATION FOR SEQ ID NO : 26

(i) SEQUENCE CHARACTERISTICS:

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

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 26:

TGGGATCGAT CAAGCTTTAC AGTTACTGAG C 31

(27) INFORMATION FOR SEQ ID NO : 27

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 27:

GTGCAGAAGG TAATCGGTGA AGGTGAAGCC AGACAC 36

(28) INFORMATION FOR SEQ ID NO : 28

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 28:

GATTACCTTC TGCACTGGGT GAGACAGCCA CCTGGA 36

(29) INFORMATION FOR SEQ ID NO : 29

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 54 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 29:

ATACTTTGTT TCACCATCCT CAGGATCAAT CCATCCAATC CACTCAAGAC CTCG 54

(30) INFORMATION FOR SEQ ID NO : 30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 54 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 30:

GGTGAAACAA AGTATGGTCA GAAGTTTCAA AGCAGAGTGA CAATGCTGGT AGAC 54

(31) INFORMATION FOR SEQ ID NO : 31

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 45 bases
(B) TYPE : nucleic acid

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

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 31:

CCACGAGTTG TATCTATATT CGCCTCTTGC ACAATAATAG ACCGC 45

(32) INFORMATION FOR SEQ ID NO : 32

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 54 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 32:

AGATACAACT CGTGGTTTGA TTACTGGGGT CAAGGCTCAC TAGTCACAGT CTCC 54

(33) INFORMATION FOR SEQ ID NO : 33

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 36 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 33:

TAGAGTCCTG AGGGAATTCG GACAGCCGGG AAGGTG 36

(34) INFORMATION FOR SEQ ID NO : 34

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 48 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : NO

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 34:

GCTGCTCCTT TTAAGCTTTG GGGTCAAGGC TCACTAGTCA CAGTCTCC 48

(35) INFORMATION FOR SEQ ID NO : 35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 33 bases
(B) TYPE : nucleic acid
(C) STRANDEDNESS : single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : ssDNA

(iii) HYPOTHETICAL : NO

(iv) ANTI-SENSE : YES

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 35:

AAGCTTCCGT CGAATTCATT TACCCGGAGA CAG 33

CLAIMS:

1. A humanised antibody in which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to the human CD-18 antigen:

light chain: CDR1 (SEQ ID NOS: 3 and 4)
CDR2 (SEQ ID NOS: 5 and 6)
CDR3 (SEQ ID NOS: 7 and 8)
heavy chain: CDR1 (SEQ ID NOS: 11 and 12)
CDR2 (SEQ ID NOS: 13 and 14)
CDR3 (SEQ ID NOS: 15 and 16).

2. An antibody as claimed in claim 1, in which the variable domain framework of the light chain is or is substantially homologous to the variable domain framework of the protein REI.

3. An antibody as claimed in claim 1 or 2, in which the variable domain framework of the heavy chain is or is substantially homologous to the variable domain framework of the protein NEWM.

4. An antibody as claimed in any one of claims 1 to 3 in which the CDRs are the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 specified in claim 1.

5. A process for the preparation of a humanised antibody as defined in any of claims 1 to 4, which process comprises providing a host transformed with either (i) a first expression vector which encodes the light chain of the humanised antibody and a second expression vector which encodes the heavy chain of the humanised antibody; or (ii) a single expression vector which encodes both the light chain and the heavy chain of the humanised antibody; and maintaining said host under such conditions that each chain is expressed and isolating the humanised antibody formed by assembly of the thus-expressed chains.

6. A DNA molecule encoding a humanised antibody in

which sufficient of the amino acid sequence of each CDR shown below is provided such that the antibody is capable of binding to the human CD-18 antigen:

light chain: CDR1 (SEQ ID NOS: 3 and 4)
CDR2 (SEQ ID NOS: 5 and 6)
CDR3 (SEQ ID NOS: 7 and 8)

heavy chain: CDR1 (SEQ ID NOS: 11 and 12)
CDR2 (SEQ ID NOS: 13 and 14)
CDR3 (SEQ ID NOS: 15 and 16).

7. A DNA molecule as claimed in claim 6, in which the variable domain framework of the light chain is or is substantially homologous to the variable domain framework of the protein REI.

8. A DNA molecule as claimed in claim 6 or 7, in which the variable domain framework of the heavy chain is or is substantially homogenous to the variable domain framework of the protein NEWM.

9. A DNA molecule as claimed in any one of claims 6 to 8 in which the CDRs are the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 specified in claim 6.

10. A DNA molecule as claimed in any of claims 6 to 9 in the form of an expression vector.

11. A host transformed with an expression vector as claimed in claim 10.

12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, a humanised antibody as defined in any of claims 1 to 4.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/01289

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

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

Int.Cl. 5 C12N15/13; C12P21/08; C07K15/28; C12N5/10
A61K39/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C12P ; C07K ; A61K

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

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[6]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	NUCLEIC ACIDS RESEARCH vol. 19, no. 9, 11 May 1991, LONDON, GB pages 2471 - 2476 B. DAUGHERTY ET AL. 'Polymerase chain reaction facilitates the cloning, CDR-grafting, and rapid expression of a murine monoclonal antibody directed against the CD18 component of leukocyte integrins.' see the whole document --- -/--	1-12

[6] Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 SEPTEMBER 1992	19. 10. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NOOIJ F.J.M.

Form PCT/ISA/210 (second sheet) (January 1985)

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Page 254

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

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	GENE vol. 101, no. 2, 30 May 1991, AMSTERDAM, THE NETHERLANDS pages 297 - 302 A. LEWIS ET AL. 'Immunoglobulin complementarity-determining region grafting by recombinant polymerase chain reaction to generate humanised monoclonal antibodies.' cited in the application see the whole document	1-12
Y	EP,A,0 346 078 (THE ROCKEFELLER UNIVERSITY) 13 December 1989 cited in the application see claims	1-12
Y	(EDS. W. KNAPP ET AL.) 'Leukocyte Typing IV. White cell differentiation antigens' 1989 , OXFORD UNIVERSITY PRESS , OXFORD see page 1079	1-12
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88, no. 10, 15 May 1991, WASHINGTON DC, US pages 4181 - 4185 S. GORMAN ET AL. 'Reshaping a therapeutic CD4 antibody.' see abstract	1-12
P,X	EP,A,0 438 312 (MERCK & CO., INC.) 24 July 1991 see claims	1-12

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9201289 SA 62146

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 30/09/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0346078	13-12-89	AU-B- 620100	13-02-92
		AU-A- 3608489	14-12-89
		JP-A- 2104534	17-04-90
EP-A-0438312	24-07-91	AU-A- 6984391	25-07-91
		CA-A- 2034574	20-07-91
		EP-A- 0440351	07-08-91

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

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 338 745 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

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(51) Int. Cl.6: **C12N 15/00**, C12P 21/00, A61K 39/395

(21) Application number: **89303767.1**

(22) Date of filing: **17.04.89**

The file contains technical information submitted after the application was filed and not included in this specification

(54) Method for producing recombinant DNA proteins.

(30) Priority: **16.04.88 GB 8809050**
13.05.88 GB 8811342
26.08.88 GB 8820284

(43) Date of publication of application:
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AT BE CH DE ES FR GB GR IT LI LU NL SE

(56) References cited:
EP-A- 0 088 994
WO-A-88/01649

GENE, vol. 43, 1986, pages 319-324, Elsevier Science Publishers B.V.; G.T. WILLIAMS et al.: "Production of antibody-tagged enzymes by myeloma cells: application to DNA polymerase I Klenow fragment"

NATURE, vol. 312, 13th December 1984, pages 604-608; M.S. NEUBERGER et al.: "Recombinant antibodies possessing novel effector functions"

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Rank Xerox (UK) Business Services
(3.10/3.09/3.3.3)

EP 0 338 745 B1

BIOESSAYS, vol. 8, no. 2, February/March 1988, pages 74-78; M. VERHOEYEN et al.: "Engineering of antibodies"

A. Klausner, Biotechnology, 4(1986), 12, 1041-1043

C. R. Wood et al., Nature, 1985, 314(4), 446-449

L. Hendershot et al., Immunology Today, 1987, 8(4), 111-114

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Description

The present invention relates to a method for producing recombinant DNA proteins, and in particular to a method for producing antibody Fv fragments by recombinant DNA technology.

In the following description, various publications are referred to. These are indicated by a name and a number in square brackets. The full references to the publications are given in numerical order at the end of the description.

The structure of natural antibody molecules is well known. It comprises four chains, two heavy chains and two light chains, the N-terminal ends of which are aligned. Each chain of the antibody forms a number of domains linked to the other domains by relatively flexible amino acid sequences. The light chains consist of an N-terminal variable domain linked to a C-terminal constant domain. The heavy chain consists of an N- terminal variable domain followed by three or more constant domains. The light and heavy chain variable domains in each pair co-operate to form an antigen binding region.

It has been reported in a few isolated instances that it is possible, by careful proteolytic digestion, to separate the antigen binding (or variable) regions of an antibody, each comprising a light and a heavy chain variable domain, from the remainder. This separated region is generally known as the Fv region. It is also known that isolated heavy or light chain variable domains can dimerise to give pseudo-Fv regions.

It has been suggested that Fv or pseudo-Fv regions (hereinafter collectively referred to as Fv fragments) could be of use as diagnostic or therapeutic agents. For instance, an Fv fragment which has been radiolabelled could be used to locate tumours in the body. An Fv structure to which is linked a cytotoxic agent may be used as a chemotherapeutic agent. It has been conjectured that, since Fv fragments are relatively small compared to whole antibody molecules or Fab or F-$(ab')_2$ fragments, they will be able readily to reach their targets, bind in large numbers on the targets, and be cleared rapidly from the patient.

Producing Fv regions by proteolytic cleavage is difficult to achieve, even under laboratory conditions, and could not practically be achieved in an industrial context. It has been proposed by Moore and Zaffaroni [1] that Fv fragments could be produced in microorganisms by use of recombinant DNA technology. However, it has been found that, although a microorganism transformed so that it contains the genetic information necessary to produce an Fv fragment can synthesise the required proteins, it is extremely difficult to obtain active Fv fragments. The synthesised protein is usually found in insoluble inclusions in the microorganism. In order to obtain the Fv fragments, it is necessary to disrupt the microorganism structure, isolate the protein and then renature the protein, as described by Field et al. [2]. However, yields of Fv fragments made by such methods are low.

Skerra and Pluckthun [3] confirm that in general it was believed that it was not possible to produce functional Fv fragments by recombinant DNA technology using bacterial host cells. However, they devised a particular expression system which enabled them to produce a functional anti-phosphorylcholine Fv fragment in E. Coli. Only small amounts of protein (0.2 mg/l of culture) were produced by this expression system. Thus, it remains to be seen whether the system they have developed can produce commercially viable quantities of product. It also remains to be seen whether the particular expression system can be used to produce Fv fragments derived from other antibodies in other bacterial systems.

It is therefore desirable to be able to produce Fv fragments in good yields by recombinant DNA technology.

According to the present invention, there is provided a method for producing Fv fragments comprising:

transforming a eukaryotic host cell with a eukaryotic expression vector comprising an operon having a DNA sequence which encodes the variable domain only of an antibody light or heavy chain,

culturing the transformed host cell under conditions which cause the protein encoded by the DNA sequence to be synthesised; and

collecting the synthesised protein.

Preferably, the chains of the Fv fragment are secreted and correctly assembled by the host cell such that fully functional Fv fragments are produced in the culture supernatant.

In one preferred alternative, the host cell is also transformed with a second eukaryotic expression vector comprising an operon having a DNA sequence encoding a complementary heavy or light chain variable domain respectively. In a second preferred alternative, the first expression vector has a second operon comprising a DNA sequence encoding a complementary heavy or light chain variable domain respectively. In either case, the translation product will be an Fv fragment comprising a dimer of a light and a heavy chain variable domain.

If desired, the or each variable domain may be expressed as a fusion protein having an effector protein, such as an enzyme, ligand or toxin, fused to its C- terminal end. The effector protein may be used to bind radioactive or fluorescent molecules for imaging, or cytotoxic agents for therapeutic use). In this case, the or each operon will include a

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DNA sequence encoding the effector protein linked in reading frame to the 3′ end of the variable domain encoding sequence.

Where the or each variable domain is translated separately, the Fv fragments produced will be held together only by non-covalent bonding. Thus, the Fv fragment may be susceptible to disassociation, for instance by lowering of pH. In order to improve the stability of the Fv fragment, the or each DNA coding sequence may be altered towards its 3′ end so that one or more cysteine residues are produced towards the C- terminal end of each variable domain. If such an alteration is made, the variable domains in the dimer may become linked together by disulphide bonding. This may also promote assembly of the Fv fragment.

Alternatively, the Fv fragment may be stabilised by use of a vector having in the operon a first DNA sequence encoding a first variable domain and a second DNA sequence encoding a second variable domain, the first and second sequences being linked by a third DNA sequence which encodes a joining peptide sequence. A similar proposal to this is disclosed by Ladner and Bird [4]. The domain encoding sequences may be arranged so that, in the translated product, the first variable domain has its C- terminus linked by the joining peptide sequence to the normal N-terminal of the second variable domain. In this case, the joining peptide sequence will need to be reasonably long and flexible.

The first and second domain encoding sequences may encode the same domain, but preferably encode complementary light and heavy chain domains. In this case, only one effector protein, if desired, could be fused to the Fv fragment.

The DNA coding sequence(s) may comprise cDNA, genomic DNA or mixtures thereof. Preferably, the coding sequence(s) are derived from monoclonal antibodies, advantageously mouse monoclonal antibodies. The coding sequences may be "native" sequences or may be "humanized" sequences wherein the complementarity determining regions (CDRs) from a monoclonal antibody from one species has been grafted onto the framework regions (FRs) from another species. The techniques which can be used for "humanizing" antibodies have been described by Reichmann et al. [5].

According to a second aspect of the invention, there is provided an Fv structure wherein one or both of the domains comprises a fusion protein having the variable domain at its N-terminal end and an enzyme, ligand or toxin at its C-terminal end.

The domain encoding sequences will be under the control of a promoter in the operon. Preferably the promoter is a strong promoter such as the promoter from the human cytomegalovirus (HCMV) major immediate early (MIE) gene.

The eukaryotic host cells to be transformed are preferably mammalian cells, and such cells as CHO cells may be used. However, it is most preferred that the host cells are mammalian myeloid cells, and in particular myeloma cells which, prior to transformation, do not secrete whole antibody or light chains. Such cell lines are well known and widely available.

The techniques by which expression vectors suitable for use in the present invention can be produced and transformed into host cells are well known in the art, and are described, for instance, by Maniatis [6].

The Fv fragment produced by the transformed host cell may be collected by any of the methods currently available. For instance, the antigen may be immobilised on a chromatography medium and the culture supernatant passed over the medium. This separates the Fv fragment from the remaining constituents of the supernatant.

The present invention also includes eukaryotic expression vectors for use in the method of the present invention, eukaryotic host cells transformed with the vectors and Fv fragments produced by the method of the present invention.

Given the failure to produce easily recoverable quantities of functional Fv structures using recombinant DNA technology in microorganisms (except using the particular expression system of Skerra and Pluckthun [3]), it is surprising that Fvs can be produced in eukaryotic cells in good yields as stable products which are secreted into the culture medium. Yields of Fvs of about 10 mg/l of culture have been obtained and improved yields are expected with further development of the eukaryotic expression systems. Thus, the present invention provides the unexpected benefit of being able to produce Fv structures in commercially useful quantities by recombinant DNA technology. Additionally, an advantage of the use of eukaryotic host cells is that the secreted Fv structures are typically produced in the absence of bacterial pyrogens.

A further surprising feature is that the Fv fragment can be assembled properly in the absence of a binding site in the Fv fragment for BIP (heavy chain binding protein). It has been conjectured that binding of BIP is essential to ensure correct assembly of antibody.

It is believed that the process of the present invention will be generally applicable to any Fv fragment and will thus enable the production of Fv fragments having desired specificities in an easy manner. Moreover, since the Fv fragments may be produced in mammalian cells, it will be possible readily to obtain regulatory approval for their use in vivo.

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Some embodiments of the present invention are now described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 shows the construction of a vector for use in producing a humanised anti-lysozyme Fv fragment;

Figure 2 shows the nucleotide sequence and corresponding amino acid sequence of the reshaped HuVLLYS gene used in the vector of Figure 1;

Figure 3 shows the construction of a vector for use in producing an anti-TAG72 Fv fragment; and

Figure 4 shows a fluorograph of an SDS polyacrylamide gel of B72.3 Fvs.

Example 1

The heavy chain variable domain of antibody D1.3 (a mouse monoclonal antibody specific for lysozyme (Verhoeyen et al.[7]) was "humanised" by grafting the DNA sequences encoding its CDRs onto the DNA sequences encoding the FRs of the monoclonal antibody produced by the human myeloma NEW (Saul et al. [8]). The light chain variable domain of D1.3 was also humanised by grafting the DNA sequences encoding its CDRs onto the DNA sequences encoding the FRs of a human kappa chain concensus sequence (Kabat et al. [9]) similar to the sequence of a Bence Jones protein REI (EPP et al. [10]). The grafting was carried out using site directed mutagenesis with long oligonucleotides according to the procedure of Reichmann et al. [4].

For the expression of an Fv structure, stop codons were introduced at the 3' end of the coding sequences for the humanised light and heavy chain variable domains.

In a first construct, the coding sequences were placed in a single vector, but independently under the control of an immunoglobulin heavy chain promoter/enhancer sequence (Verhoeyen et al. [7]). In a second construct, the coding sequences were each placed under the control of the HCMV-MIE gene promoter/enhancer sequence (Stenberg et al. [11] and Boshart et al. [12]). The HCMV-MIE gene promoter/enhancer sequences were fused to the 5' flanking sequences of the signal sequences at the 5' end of the variable domain coding sequences utilizing the RNA start site of the HCMV sequence. The second construct, pLR1, is shown in Figure 1.

A "reshaped" light chain variable domain HuVLLYS and a "reshaped" heavy chain variable domain HuVHLYS (Verhoeyen et al. [7]) were each cloned as HindIII-BamHI fragments in M13. The nucleotide sequence and corresponding amino acid sequence of the reshaped HuVLLYS gene are shown in Figure 2. At the 3' end of each coding sequence two stop codons followed by a SacI site were introduced by site directed mutagenesis.

Between the RNA start site and the translation start of the leader sequence in each gene, a HindIII site was introduced as shown in Figure 2. The resulting variable domain genes were cloned as HindIII-BamHI fragments into a pSVgpt vector (Mulligan and Berg [14]). The vector contained a EcoRI-HindIII fragment of an Ig-heavy chain enhancer (IgHenh) as a linker (Neuberger et al. [15]). The 3' SacI-BamHI fragment of each gene was then exchanged with a SacI-BamHI fragment of the human kappa constant region (3' end C_K) (Hieter et al. [13]) to provide a polyadenylation signal. Into the HindIII site of each vector a HindIII fragment of the HCMV-MIE gene (Stenberg et al. [11] and Boshart et al. [12]) containing its enhancer, promoter and the first nontranslated exon (HCMV enh-pro) was cloned. The complete VL-gene (containing Ig-enhancer, HCMV-promoter, VL-coding region and polyadenylation signal) was then subcloned as an EcoRI fragment into pBGS18 (Spratt et al. [16]) and the resulting vector pBGS-HvVLLYS was cloned into the pSVgpt-HuVHLYS vector as a BamHI-fragment as shown in Figure 1. The final plasmid PLR1 further contained the resistance genes for the drugs ampicillin (amp^R), kanamycin (Kan^R) and mycophenolic acid (Eco gpt), two col E1 origins of replication (col E1 ori) and the SV40 enhancer (SV_{40} enhpro). The BamHI (B), HindIII (H), EcoRI (E) and SacI (S) restriction sites used for the cloning steps are indicated.

The plasmid was transfected by electroporation (Potter et al. [17]) into the non-producer myeloma cell line (NSO Galfre et al. [18]). Transfectants were selected with mycophenolic acid (Mulligan and Berg [14]).

Pools of transfected cell clones were screened by ^{35}S-methionine incorporation, affinity purification of culture supernatants with lysozyme-Sepharose, and analysis on SDS-acrylamide gels.

The yield of secreted Fv fragments was about 100 to 1000 fold higher when the HCMV promoter was used as compared to constructs with the Ig promoter. The cloned cell line used for the preparation of Fv fragments secreted about 8 mg/l when grown in roller bottles. Thus it is possible to produce Fv fragments in myeloma cells with yields similar to recombinant versions of intact antibodies.

The Fv fragment contains two chains of about 12kD (calculated values: 12,749 for VH and 11,875 for VL) when analysed on SDS gels. It is secreted in a functional form, as it can readily be purified from the culture supernatant with lysozyme-Sepharose. When the purified Fv fragment was investigated on an HPLC sizing column (Biozorbax GB250) in phosphate buffered saline (PBS), a single peak was observed and its retention time did

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not change between concentrations of 70 and 0.3 mg/l.

The Fv fragment was also analysed on native acrylamide gels at pH 7.5. The Fv fragment ran as a single band, which contained both the VH and the VL domain when analysed on SDS gels. This band was shifted on the native gel in the presence of lysozyme. The shifted band contained lysozyme and the VH and VL domains. Further, the isolated VL domain ran with a mobility different from that of the Fv fragment. The isolated VH did not run into the gel. These results strongly suggest that the predominant form of the Fv fragment at pH 7.5 is an associated VH-VL heterodimer.

Also its apparent molecular weight in ultracentrifuge sedimentation analysis was about 23.05 ± 0.35kD (assuming a partial specific volume of 0.73).

The formation of VH-VL heterodimers was further established by crosslinking with 3.7% formaldehyde/PBS at a protein concentration of 0.5mg/l. Crosslinked VH-VL heterodimers of about 25 kD were formed. Such heterodimers still bound to lysozyme-Sepharose. Overloading of SDS gels with crosslinked material also made visible a small fraction (less than 5%) of slightly lower molecular weight material suggesting the formation of crosslinked VL homodimers. No higher molecular weight band for possible VH homodimers was observed.

Nevertheless the Fv fragment dissociated on native acrylamide gels at pH 4.5. The VH and the VL each formed a single band. Incubation of antibodies at low pH has been used historically to facilitate their proteolytic digestion, probably reflecting the same underlying structural change.

Also at neutral pH, when the Fv fragment is predominantly associated, it is in a dynamic equilibrium. The purified, biosynthetically labelled VH domain exchanges with the unlabelled VH domain when incubated with unlabelled VH-VL heterodimer. The labelled VH-VL heterodimers could be trapped by crosslinking with formaldehyde. The exchange is fast enough to reach an equilibrium during the overnight crosslinking procedure without a pre-incubation in the absence of reagent.

The type of protein produced by the method of the present invention is of potential value for both structural studies and clinical applications. Thus these Fv fragments should especially simplify the assignment of signals in NMR spectra, because the same β-sheet frameworks can be used for different antigen binding sites. For clinical applications the reuse of hypervariable regions (and thereby the specificity) in the context of new frameworks might help to overcome a primary anti-immunoglobulin response to the frameworks of the original Fv-fragment. The dissociation of Fv fragments should not cause problems in diagnostic or therapeutic applications, although crosslinking might be advantageous in some situations. The crosslinking of the VH and VL domain is possible either chemically as shown here or by the introduction of peptide linkers on the gene level, which could contain for example cysteines for the formation of interdomain disulphide bridges.

Example 2

The light and heavy chain genes for antibody B72.3, an antitumour monoclonal antibody (Colcher et. al. [19] and Whittle et al. [20]) were subjected to site-directed mutagenesis (Kramer et al. [21]) to introduce EcoRI restriction sites and translation stops at the 3' ends of the variable domains. In addition, to facilitate the manipulation of the variable gene sequences, EcoRV and HindIII sites were also incorporated into VL to match the naturally occurring PvuII and BglI sites in VH. These genes were cloned, either separately or in tandem, into an expression vector under the control of the HCMV promoter with an SV-40 polyA addition sequence. A construct with the genes arranged in tandem is shown in Figure 2. These constructs were tested by transient expression in COS 1 cells. Synthesis and secretion of variable domains was assayed by biosynthetically labelling the transfected cells with ^{35}S-methionine (100 μCi/10^6 cells/ml for 48 hours). The cell supernatants were subjected to immunoprecipitation with antisera reacting with either VL or VH framework epitopes.

The VH/VL construct was transfected into Chinese hamster ovary (CHO) cells by electroporation.

VH and VL domains were detected in COS cell supernatants as 14 kD (cf. calculated mol. wt. of 12.6 kD) and 12 kD (cf. calculated mol. wt. of 11.8 kD) polypetides on both reducing and non-reducing SDS polyacrylamide gels. Apparently higher levels of expression of both domains were obtained from the dual VH/VL plasmid compared to the single VH or VL plasmids. VH was co-precipitated with VL from both labelled COS cell media by the antiserum specific for VL implying some degree of association of the domains. However, insufficient amounts of material were produced by either the COS cells to enable the function of the putative Fv fragment to be tested in an antigen-binding competition assay. Higher levels of expression are required to investigate whether the B72.3 variable domains are functionally assembled in vivo. To this end, the VH/VL single and dual plasmids have been introduced into CHO cells, which with these plasmids appear to give greater yields of recombinant antibodies than the COS cells.

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Figure 4 shows a fluorograph of an SDS polyacrylamide gel of B72.3 Fvs. CHO cells transfected with the vector of Figure 1 were labelled with 35_S methionine (100 µCi/ml, 48h) and cell supernatants were subjected to immunoprecipitation using a rat antiserum specific for the B72.3 VL domain. Samples were analysed on 15% SDS polyacrylamide gels under reducing conditions. Tracks 1 to 5 show the results for five independent CHO cell lines. The numbers on the left refer to size markers.

The B72.3 Fv polypeptides were isolated from the culture supernatant of one of these cell lines (Fv3, Track 3, Figure 4) by affinity chromatography on a mucin-Sepharose matrix. Mucin has been shown to mimic the B72.3 antigen [22]. The mucin-Sepharose used was prepared by coupling bovine submaxillary mucin to CNBr-activated Sepharose by standard techniques at 5mg/ml of gel.

100 ml of supernatant from CHO cell line Fv3 was incubated on a roller mixer at 4°C overnight with 1 ml of a 50% suspension of mucin-Sepharose (in 50mM Tris, pH 8.0). The mucin-Sepharose was then recovered by pouring the incubation mix into a column. The retained mucin-Sepharose was washed with 50mM Tris pH 8.0 until no absorbance was seen at 280 nm in the eluent from the column. Fv was then eluted with 1 ml of 0.1 M citric acid. The pH of the acid eluent was then adjusted to 5.5 and assayed for antigen binding activity by ELISA.

Samples were serially diluted in microtitre plates coated with 5 µg ml bovine submaxillary gland mucin and incubated for 50h at 4°C. After washing with phosphate buffered saline, pH 7.2, containing 0.2% Tween 20, 100 µ of a rabbit polyclonal antiserum raised to B72.3 $(Fab')_2$ was added to each well at a dilution of 1 in 2,000. This antiserum contains antibodies that recognise both B72.3 VH and VL and chains. The plates were incubated for 1h at room temperature, washed again and 100 µl goat anti-rabbit IgG Fc-horse radish peroxidase conjugate added to each well at a dilution of 1 in 5,000.

After a further 1h incubation at room temperature, the plates were washed again and bound antibody visualised by adding TMB substrate and reading optical density of the samples at 605nm. The results showed that the CHO Fv3 supernatant sample was capable of binding the solid-phase mucin in this assay.

It is by no means certain that the hydrophobic interactions which are involved in the association of VH and VL will be strong enough to maintain the FV fragment in an assembled state. Further modifications of the domains may therefore be necessary to enhance or stabilise the Fv fragment. For example, this may be achieved by the introduction of covalent linkages, such as disulphides.

It will be appreciated by the skilled man that the embodiments set out above have been described by way of illustration only and that variations of detail can be made without departing from the scope of the present invention.

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Claims

1. A method for producing Fv fragments, wherein the chains of the Fv fragment are secreted and correctly assembled by the host cell such that fully functional Fv fragments are produced in the culture supernatant, comprising:

   transforming a eukaryotic host cell with a eukaryotic expression vector comprising an operon having a DNA sequence which encodes the variable domain only of an antibody light or heavy chain,

   culturing the transformed host cell under conditions which cause the protein encoded by the DNA sequence to be synthesised; and

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collecting the synthesised protein.

2. The method of claim 1, wherein the host cell is also transformed with a second eukaryotic expression vector comprising an operon having a DNA sequence encoding a complementary heavy or light chain variable domain respectively.

3. The method of claim 1, wherein the first expression vector has a second operon comprising a DNA sequence encoding a complementary heavy or light chain variable domain respectively.

4. The method of claim 2 or claim 3, wherein the or each variable domain is expressed as a fusion protein having an effector protein, such as an enzyme, ligand or toxin, fused to its C-terminal end.

5. The method of any one of claims 2 to 4, wherein the or each DNA coding sequence may be altered towards its 3' end so that one or more cysteine residues are produced towards the C-terminal end of each variable domain.

6. The method of any one of claims 2 to 4, wherein the Fv fragment is stabilised by use of a vector having in the operon a first DNA sequence encoding a first variable domain and a second DNA sequence encoding a second variable domain, the first and second sequences being linked by a third DNA sequence which encodes a joining peptide sequence.

7. The method of any one of claims 1 to 6, wherein the coding sequence(s) are derived from monoclonal antibodies.

Patentansprüche

1. Ein Verfahren zur Herstellung von Fv-Fragmenten, bei welchem die Ketten des Fv-Fragments von der Wirtszelle ausgeschieden und korrekt zusammengefügt werden, sodaß voll funktionsfähige Fv-Fragmente im Kulturüberstand produziert werden, welches Verfahren umfaßt:

Transformation einer eukaryotischen Wirtszelle mit einem eukaryotischen Expressionsvektor, der ein Operon mit einer DNA-Sequenz, die für den variablen Bereich nur einer leichten oder schweren Antikörperkette codiert, enthält,

Züchtung der transformierten Wirtszelle unter Bedingungen, die die Synthetisierung des von der DNA-Sequenz codierten Proteins bewirken; und

Sammlung des synthetisierten Proteins.

2. Das Verfahren nach Anspruch 1, bei welchem die Wirtszelle auch mit einem zweiten eukaryotischen Expressionsvektor transformiert wird, der ein Operon mit einer DNA-Sequenz, die für einen komplementären variablen Bereich einer schweren bzw. leichten Kette codiert, enthält.

3. Das Verfahren nach Anspruch 1, bei welchem der erste Expressionsvektor ein zweites Operon mit einer DNA-Sequenz aufweist, die für einen komplementären variablen Bereich einer schweren bzw. leichten Kette codiert.

4. Das Verfahren nach Anspruch 2 oder Anspruch 3, bei welchem der oder jeder variable Bereich als ein Fusionsprotein mit einem Effektorprotein, wie etwa einem Enzym, Ligand oder Toxin, das an sein C-terminales Ende fusioniert ist, exprimiert wird.

5. Das Verfahren nach einem der Ansprüche 2 bis 4, bei welchem die oder jede DNA-Codierungssequenz gegen ihr 3'-Ende hin verändert sein kann, sodaß ein oder mehrere Cystein-Reste in Richtung zum C-terminalen Ende jedes variablen Bereichs produziert werden.

6. Das Verfahren nach einem der Ansprüche 2 bis 4, bei welchem das Fv-Fragment durch Verwendung eines Vektors stabilisiert ist, der in dem Operon eine erste DNA-Sequenz, die für einen ersten variablen Bereich codiert, und eine zweite DNA-Sequenz, die für einen zweiten variablen Bereich codiert, aufweist, wobei die ersten und zweiten Sequenzen durch eine dritte DNA-Sequenz verknüpft sind, die für eine Verbindungspeptidsequenz codiert.

7. Das Verfahren nach einem der Anspürche 1 bis 6, bei welchem die Codierungssequenz-(en) von monoklonalen Antikörpern abgeleitet sind.

Revendications

1. Procédé de production de fragments Fv, dans lequel les chaînes du fragment Fv sont sécrétées et correctement assemblées par la cellule hôte de telle sorte que des fragments Fv complètement fonctionnels sont produits dans le surnageant de culture, ledit procédé comprenant:

la transformation d'une cellule hôte eucaryote avec un vecteur d'expression eucaryote

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comprenant un opéron ayant une séquence d'ADN qui code uniquement pour le domaine variable d'une chaîne légère ou lourde d'anticorps,

la culture de la cellule hôte transformée dans des conditions qui provoquent la synthèse de la protéine codée par la séquence d'ADN; et

la récupération de la protéine synthétisée.

2. Procédé selon la revendication 1, dans lequel la cellule hôte est également transformée avec un deuxième vecteur d'expression eucaryote comprenant un opéron ayant une séquence d'ADN codant respectivement pour un domaine variable de chaîne lourde ou légère complémentaire.

3. Procédé selon la revendication 1, dans lequel le premier vecteur d'expression possède un deuxième opéron comprenant une séquence d'ADN qui code respectivement pour un domaine variable de chaîne lourde ou légère complémentaire.

4. Procédé selon la revendication 2 ou la revendication 3, dans lequel le ou chaque domaine variable est exprimé sous forme d'une protéine de fusion ayant une protéine effectrice, telle qu'une enzyme, un ligand ou une toxine, fusionnée à son extrémité C-terminale.

5. Procédé selon l'une quelconque des revendications 2 à 4, dans lequel la ou chaque séquence codante d'ADN peut être modifiée du côté de son extrémité 3' de manière à ce que un ou plusieurs résidus de type cystéine soient produits du côté de l'extrémité C-terminale de chaque domaine variable.

6. Procédé selon l'une quelconque des revendications 2 à 4, dans lequel le fragment Fv est stabilisé à l'aide d'un vecteur ayant dans l'opéron une première séquence d'ADN codant pour un premier domaine variable et une deuxième séquence d'ADN codant pour un deuxième domaine variable, la première et la deuxième séquence étant reliées par une troisième séquence d'ADN qui code pour une séquence peptidique de jonction.

7. Procédé selon l'une quelconque des revendications 1 à 6, dans lequel la ou les séquences codantes sont dérivées d'anticorps monoclonaux.

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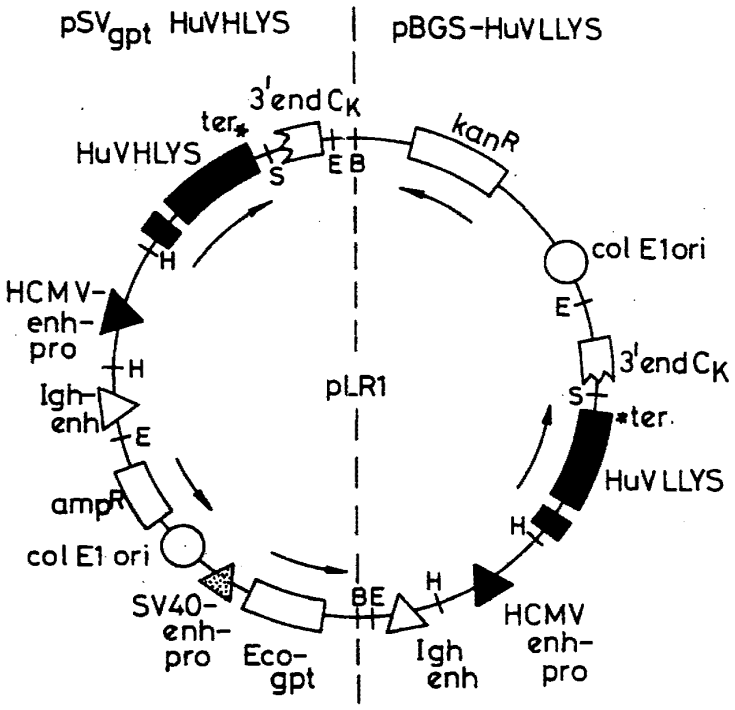

HuVHYS =reshaped heavy chain variable domain
HuVLLYS = reshaped light chain variable domain
AmpR = ampicillin resistance gene
KanR = Kanamycin resistance gene
Eco-gpt = mycophenolic acid resistance gene
col E1 ori = col E1 origin of replication
enh = enhancer
pro = promoter
Restriction sites: B = BamHI; H= Hind III; E= EcoRI;
S = Sac I

Fig. 1

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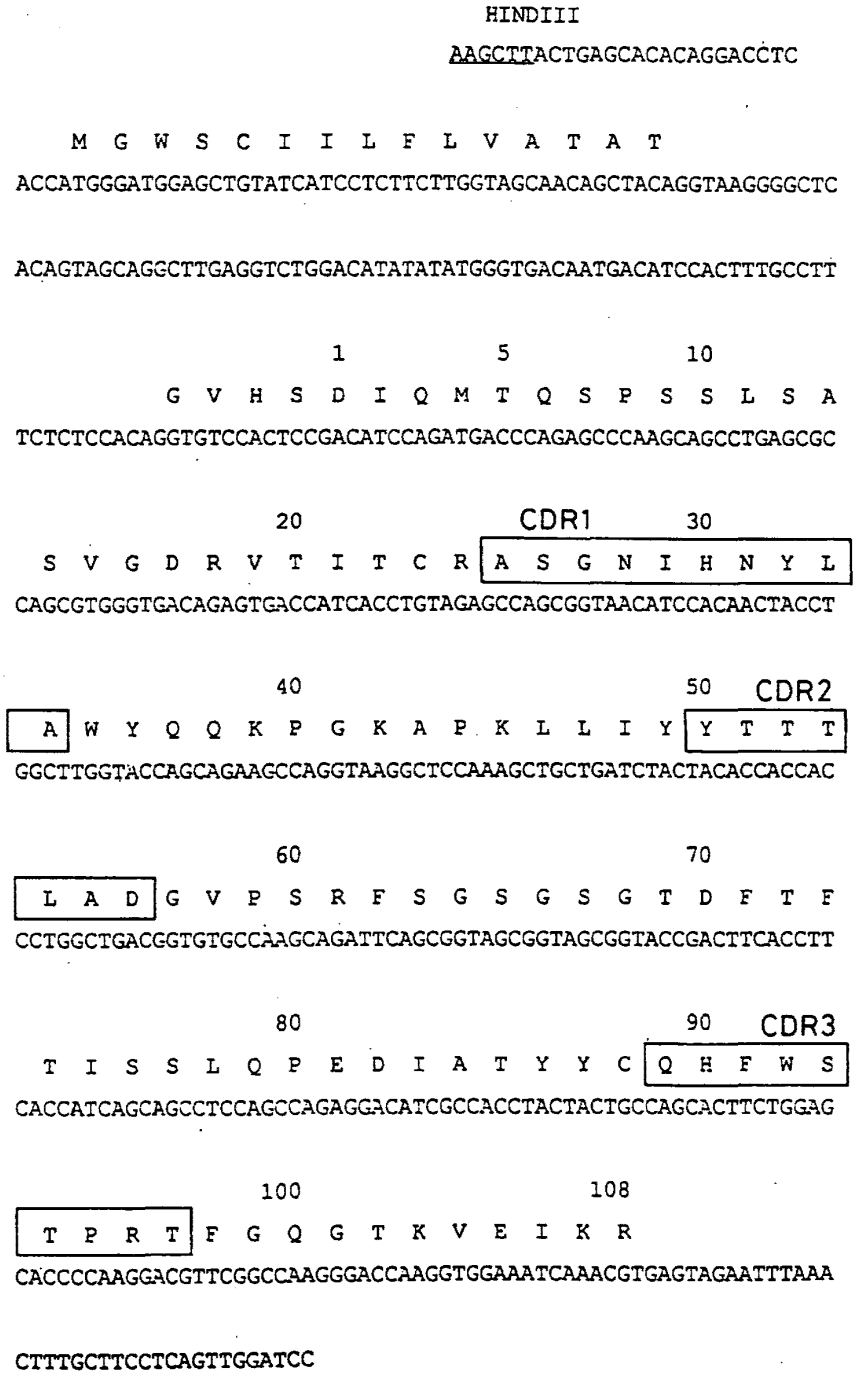
Fig. 2



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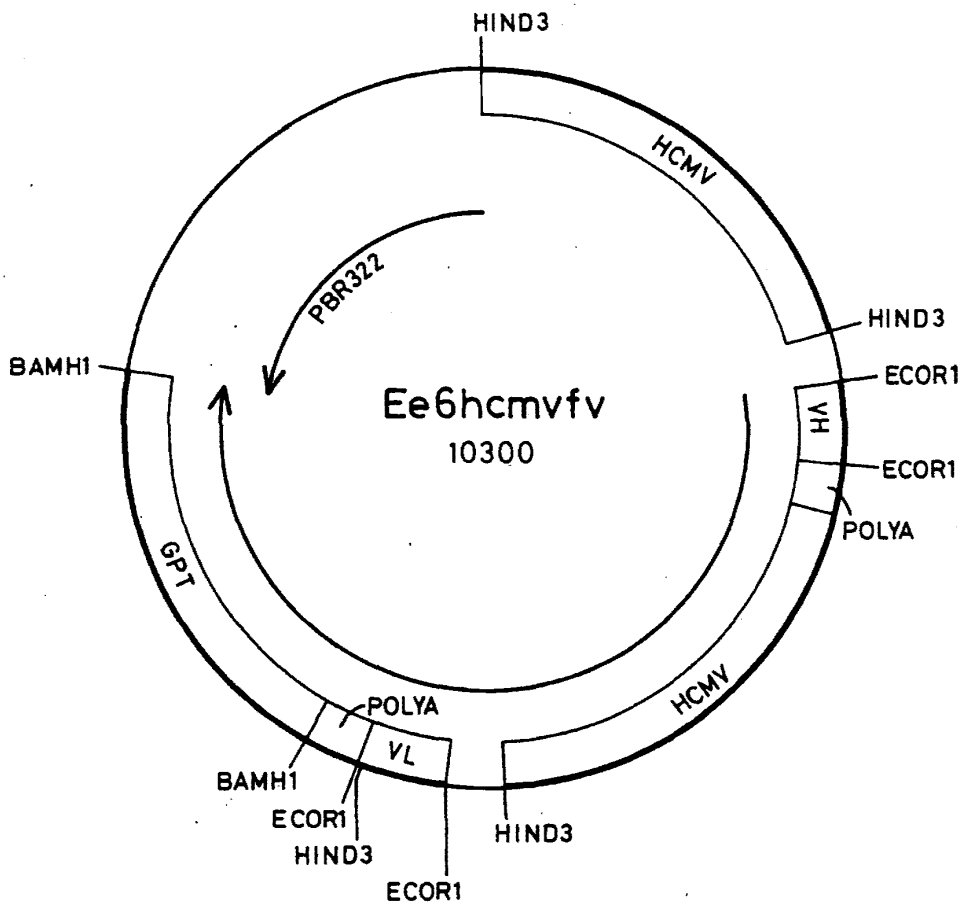

Fig. 3

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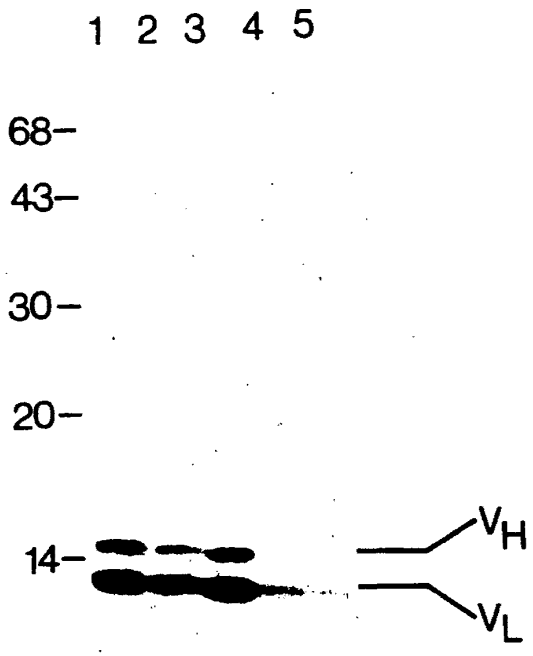

Fig. 4

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(51) INT CL⁴
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Microbiol. Immunol (1985) Vol 29 p. 959–972.
Lymphokines (N.Y.) (1984) vol. 9 p. 127–52.
J. of Immunology (U.S.) (1985) Vol. 134 p. 390 1–6
Molecular Immunology (1984) Vol. 21 No 12 p. 1229–36
Microbiology and Immunology (1986) Vol. 30 No. 4 p. 373–388.

(58) Field of search
C6F
C3H
Selected US specifications from IPC sub-classes A61K C12N C12P

(54) Monoclonal antibodies recognizing human interleukin-2-receptor

(57) Hybrid cell lines have been produced for the production of monoclonal antibody to an antigen found on activated human lymphocytes, the interleukin-2-receptor (iL-2-R) to the antibody so produced, and to therapeutic and diagnostic methods and compositions employing this antibody.

The antibody may be in the form of a chimeric animal-human antibody recognising iL-2-R wherein the F_C region is obtained from a human and the F_{AB} region is obtained from an animal.

GB 2 188 941 A

The drawings originally filed were informal and the print here reproduced is taken from a later filed formal copy.

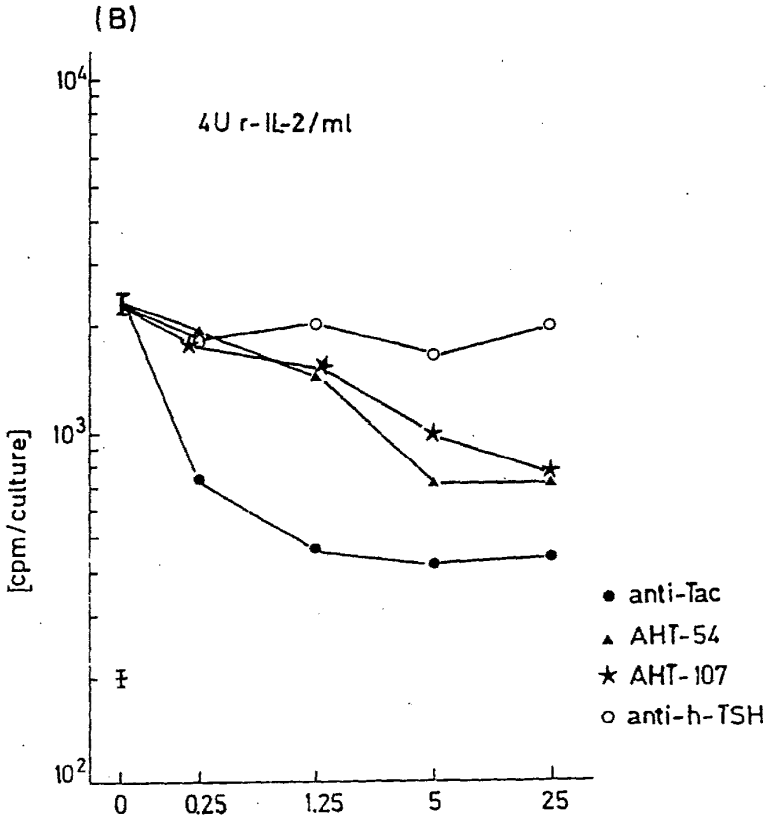

(B)
4U r-IL-2/ml
[cpm/culture]
10^4
10^3
10^2
0
0.25
1.25
5
25
anti-Tac
AHT-54
AHT-107
anti-h-TSH

FIG. 1a

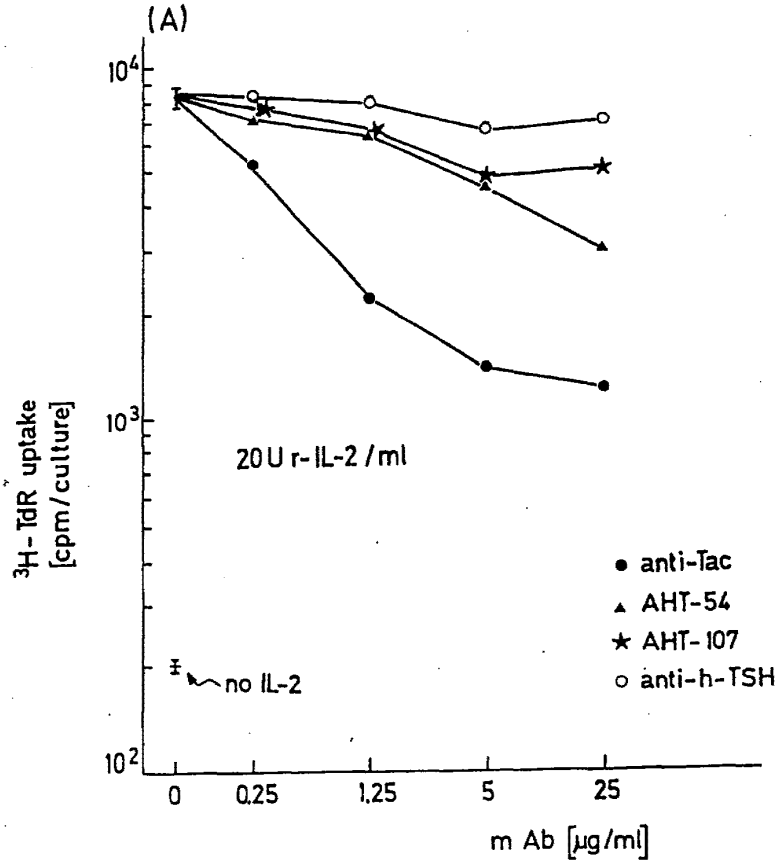
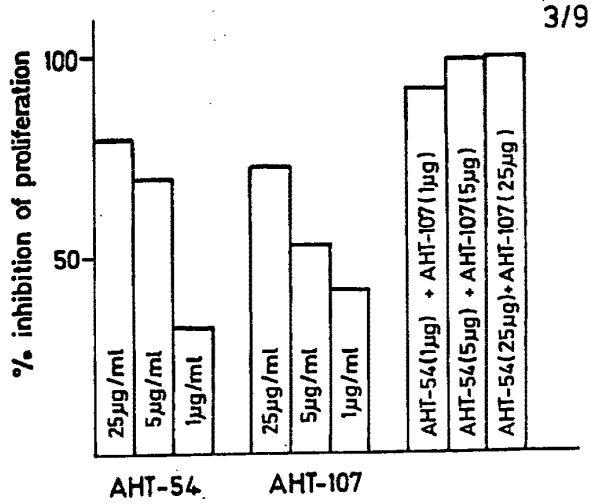

(A)
10^4
10^3
10^2
^{3}H-TdR uptake [cpm/culture]
20 U r-IL-2 / ml
no IL-2
anti-Tac
AHT-54
AHT-107
anti-h-TSH
0
0.25
1.25
5
25
m Ab [µg/ml]

FIG. 1b

FIG. 2



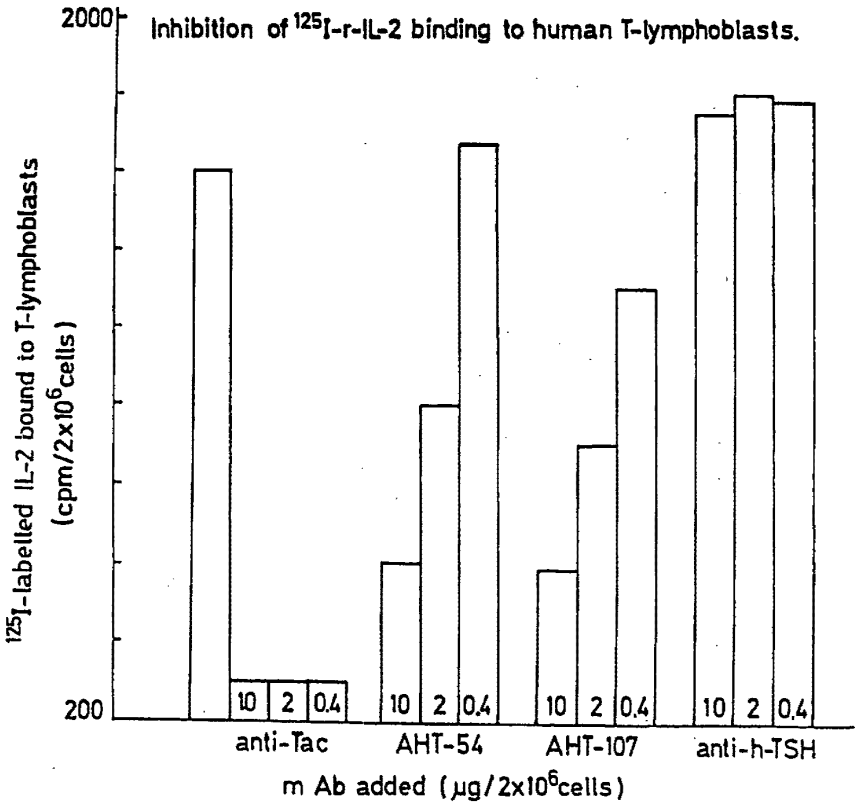


FIG. 4

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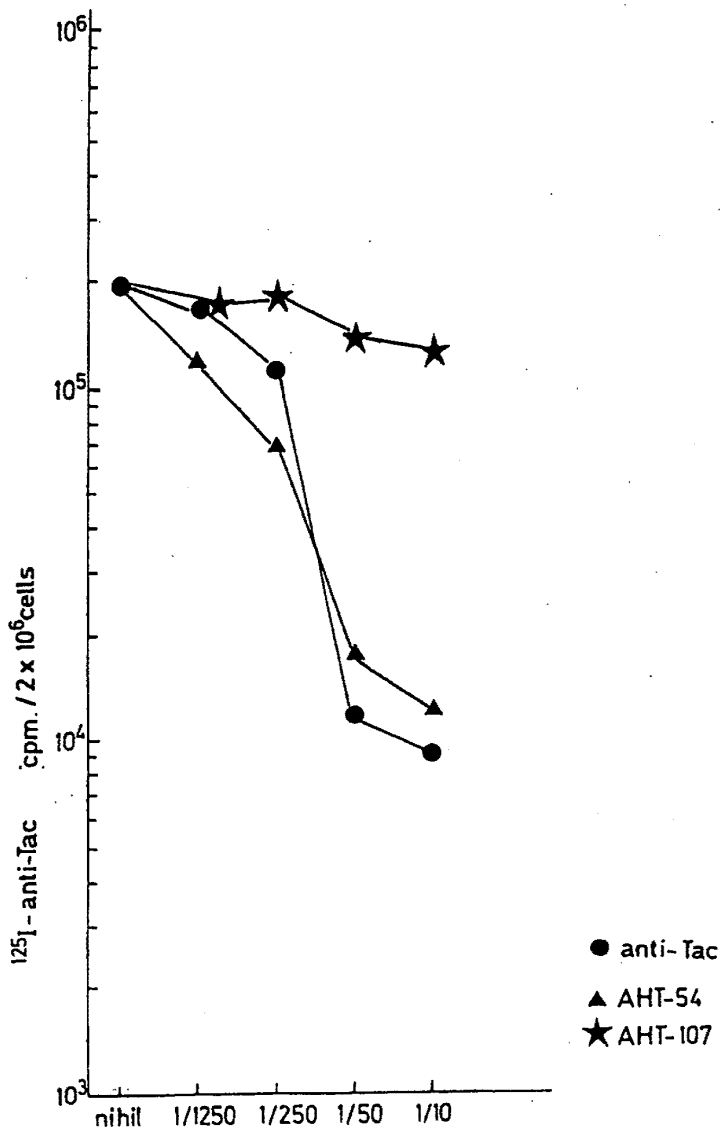
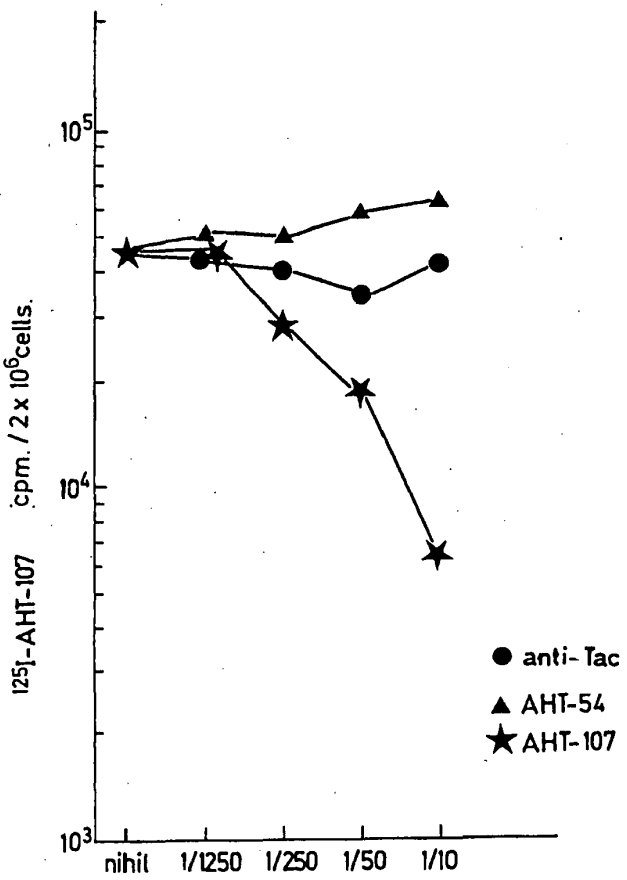

10^6
10^5
10^4
10^3
^{125}I-anti-Tac cpm. / 2 x 10^6cells
nihil
1/1250
1/250
1/50
1/10
anti-Tac
AHT-54
AHT-107

FIG. 3a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10^4
10^3
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nihil
1/1250
1/250
1/50
1/10
anti-Tac
AHT-54
AHT-107

FIG. 3b

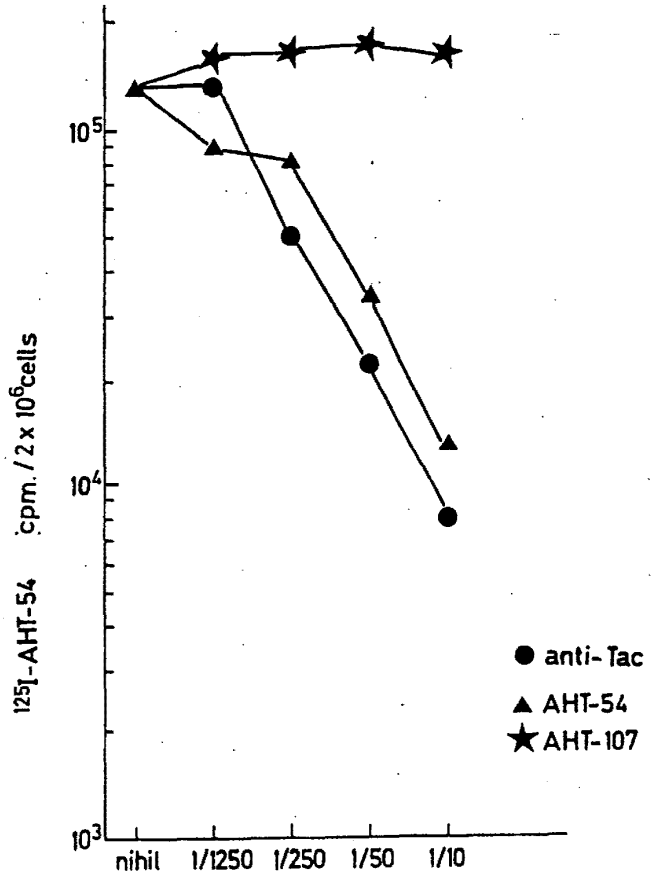

10^5
10^4
10^3
^{125}I-AHT-54 cpm. / 2 x 10^6cells
nihil
1/1250
1/250
1/50
1/10
anti-Tac
AHT-54
AHT-107

FIG. 3c

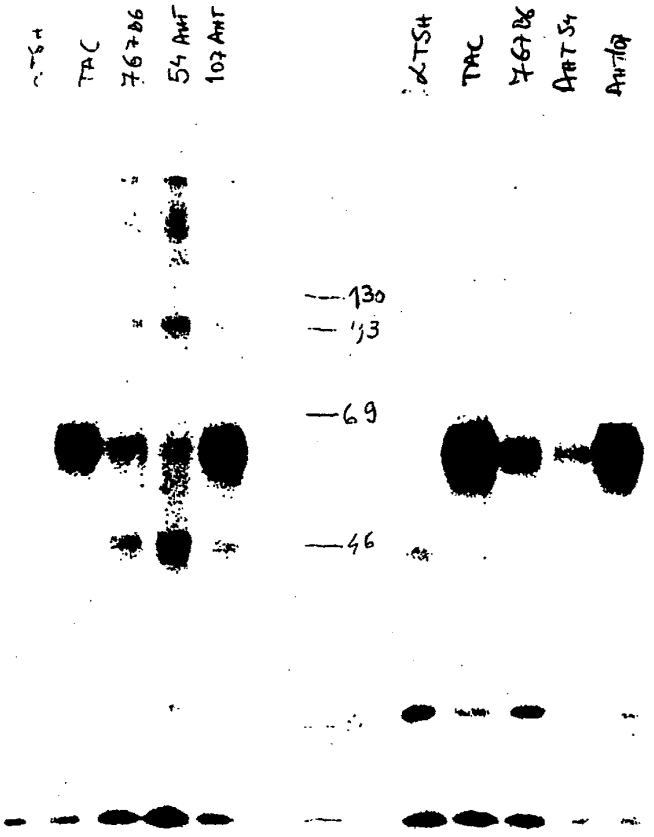


FIG. 5

FIG. 6

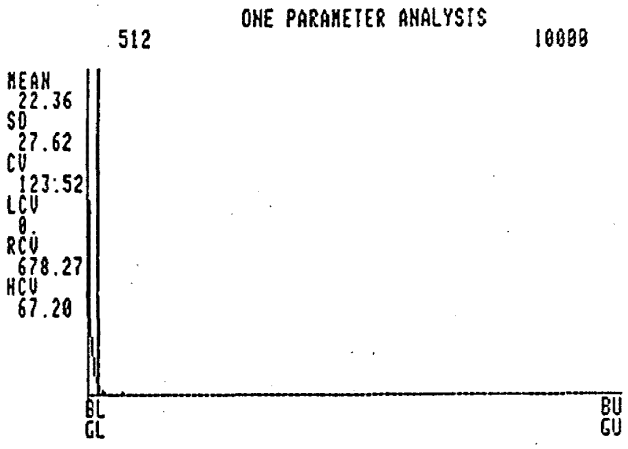


FIG. 7

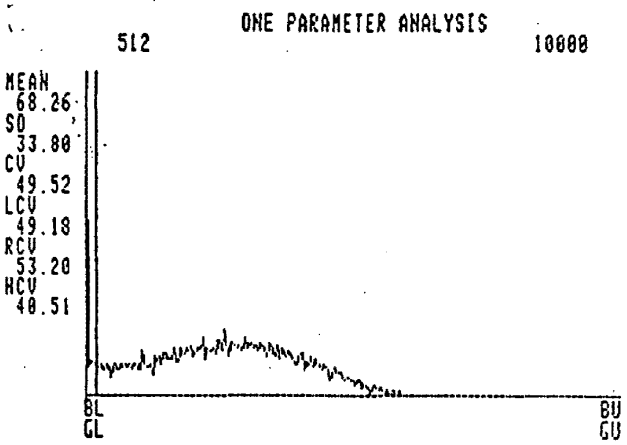


FIG. 8

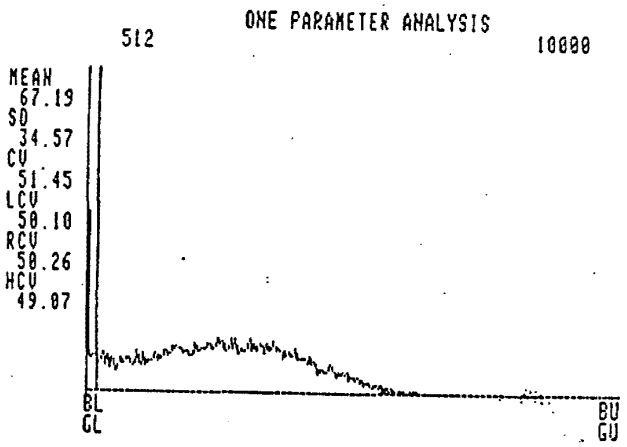


FIG. 9

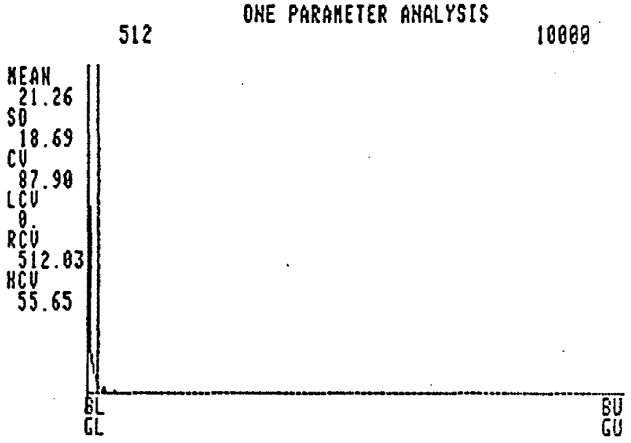


FIG. 10

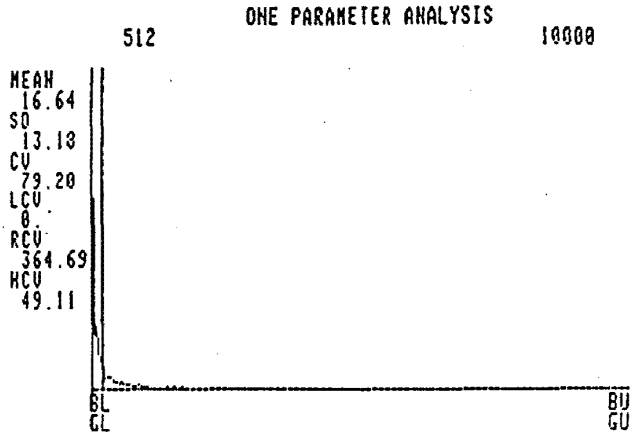
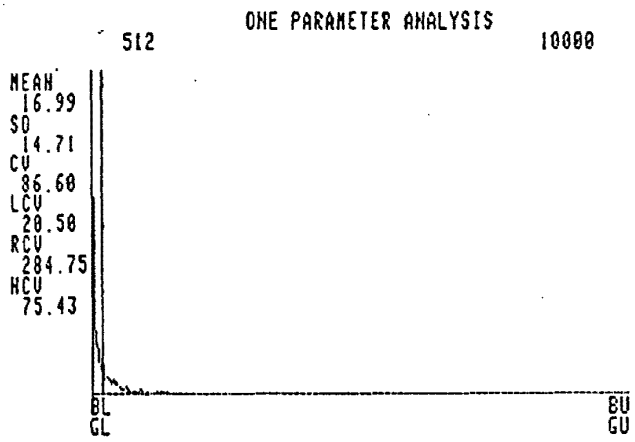


FIG. 11



SPECIFICATION

Monoclonal antibodies recognizing human interleukin-2-receptor

This invention relates generally to new hybrid cell lines and more specifically to hybrid cell lines for production of monoclonal antibody to an antigen found on activated human lymphocytes, the interleukin-2-receptor, to the antibody so produced, and to therapeutic and diagnostic methods and compositions employing this antibody.

INTRODUCTION

For many, if not all cells, the initial trigger for proliferation appears to be the interaction of growth factors with the cell surface growth factor receptor. Activation of the growth factor receptor leads in turn to yet undefined cytoplasmic signalling systems.

Resting T lymphocytes are long living cells in the G_0 phase of the cell cycle. They only enter proliferative cycles under antigenic stimulation in the presence of a T cell growth factor, interleukin 2 (IL-2). Receptors for IL-2 are not detectable on the surface of resting T cells. Expression of IL-2 receptors (IL-2R) is the consequence of interaction of antigen presenting cells with the antigen receptor. As shown recently, IL-2 receptor expression is a transient even and repeated restimulation by lectins (Cantrell, P.A., and K.A. Smith. (1984). Science (Wash. DC) 224:1312); (Osawa, H., and Diamantstein, T. (1984). J. Immunol. 132:2445) or the antigen (Reske-Kunz, A.B., D.v. Steldern, E. Rüde, H. Osawa and T. Diamantstein, (1984). J. Immunol. 133:1356) is required for continuous IL-2 receptor expression and consequently for long term cell growth.

Since, IL-2-R are expressed exclusively on activated lymphocytes monoclonal antibody (mAb) that reacts with the IL-2-R may be useful as specific and selective immunosuppressive agent. Furthermore, such antibodies may serve as diagnostic reagents in order to detect qualitatively and quantitatively activated lymphocytes as well as neoplastic cells expressing IL-2-R.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a composition of at least two monoclonal antibodies recognizing human interleukin-2-receptor capable to inhibit interleukin 2 induced lymphocyte proliferation. In an preferred embodiment one or more of the antibodies are of the IgG_1 class. In another preferred emodiment the antibody composition is capable to inhibit interleukin-2 binding to the receptor. The present invention provides monoclonal antibodies of the IgG_1 class recognizing human interleukin-2-receptor capable to inhibit interleukin-2 binding to the receptor and in particular antibodies capable to inhibit interleukin-2 dependent lymphozyte proliferation. The antibodies of the present invention can be used for preparation of chimeric animal-human antibodies recognizing human interleukin-2-receptor wherein the constant F_c region of the immunoglobulin is obtained from human and the variance Fab region is obtained from an animal. Preferably the Fab region is obtained from mice.

The present invention further provides hybridoma cell lines, characterized by the production of monoclonal antibodies of the IgG_1 class recognizing human interleukin-2-receptor. Particular preferred are the hybridoma cell lines having the NTCC designation number . . . and . . . . .

Human T-lymphoblasts expressing IL-2-R were prepared by known methods (Osawa, H., and Diamantstein, T. (1983) J. Immunol. 130:51.) were used to produce a mouse monoclonal antibody against IL-2-R by the technique of Köhler and Milstein (Köhler, G., and C. Milstein, (1975) Nature 256:495). The fusion resulted in two hybrid clones AHT-54 and AHT-107 producing anti-IL-2-R antibodies of IgG_1 subclass. The hybrid clones secreting anti-IL-2-R antibodies were selected as preferred embodiment of the present invention.

Both mAb i) inhibit binding of 125J labelled IL-2 to IL-2-R positive human lymphocytes, ii) inhibit IL-2 dependent proliferation in vitro and iii) precipitates the identical cell surface molecule of 55KD, the IL-2 binding protein. Competitive binding of AHT-54 and AHT-107 revealed that they recognize different epilops of the IL-2-R molecule.

AHT-107 is different from anti-Tac (Uchiyama, T. et al., (1981) J. Immunol. 126:1398) i) because competitive inhibition studies revealed that they recognize two different epitops of the IL-2-R molecule; AHT-107 is also different from 7G7 B6, a recently published antihuman IL-2-R mAb (A. Rubin, C. Kurman, E. Biddison, D. Goldman, and L. Nelson (1985) Hybridoma Vol. 4:91), because in contrast to 7G7 B6 AHT-107 inhibits binding of IL-2 to the IL-2-R as well as IL-2 dependent proliferation of lymphocytes.

Both mAb react specifically with activated lymphocyte (T and B) but not with resting lymphocytes or other non-lymphoid cells. This statement is based on FACS-analysis data (Fig. 6–11).

According to previous studies in animal models such Ab reacting with the rat (ART-18) and with the mouse IL-2-R (AMT-13 and M7/20) has been shown to inhibit selectively and specifically i) local GVH-reaction (Diamantstein, T. and H. Osawa, (1986), Immune Rev. 92 in press.) ii) cardiac allograft rejection (L. Kirkman, E. Kelley, A. Koltun, J. Schoen, A. Ythier and B. Strom, (1985), Transplantation 40:719), (L. Kirkman, L.V. Barrett, N. Gaulton, E. Kelley, A. Ythier and B. Strom,

(1985), J. Exp. Med. 162: 358.) and iii) T-cell-mediated autoimmune reaction such as acute autoimmune encephalomyelitis and adjuvant arthritis induced by T-cell transfer (Wekerle, H. and T. Diamantstein, (1986), Autoimmunity: Experimental and Clinical Aspects Eds: R.S. Schwarz, N.R. Rose. Ann. New York Acad, Sci., In press.)

The anti-IL-R monoclonal antibodies of the present invention are also useful as therapeutic agents in clinical syndromes which are associated with pathological proliferation of IL-2 dependent cells. Thus, for example, hyperimmune syndromes such as Host versus Graft(HvG) Graft versus Host (GvH) diseases and autoimmune diseases (e.g. multiples sclerosis, autoimmune diabetis, Crohn's disease) may be treated. In a preferred embodiment of the present invention, the anti-IL-2-R monoclonal antibodies are used as therapeutic agents directly without further modification thereof. Furthermore, the invention includes preparation of anti IL-2-R chimeric antibodies using human heavy chain of different classes and subclasses in combination with the variable region of the AHT-54 and AHT-107 mAb, in order to optimise for therapeutic use.

Alternatively, the antibodies may be coupled to drugs including cytotoxic agents. The monoclonal antibodies of the present invention are capable of recognizing specifically cells expressing IL-2 receptors, inhibiting their function and of eliminating them selectively.

The monoclonal antibodies of the present invention are also useful diagnostic reagents for cells which contain IL-2-R either on the cell surface or within the cells and in body fluids. Thus by means of the present invention, cells containing IL-2-R may be identified in samples having different kinds of cells. Localization of IL-2-containing cells is possible in cultured cell colonies or in tissue specimens. When used in this manner the monoclonal antibodies are preferably coupled to fluorescent, color-forming substances such as an enzyme or chromophor, or a radioactive substance (ELISA, RIA).

DETAILED DESCRIPTION OF THE INVENTION

The following description is intended to illustrate this invention without limiting the same in any manner especially with respect to substantially functional equivalents of hybridomas and monoclonal antibodies as described herein.

I. *Production of mAb*

Source of IL-2-R

IL-2-R expressing cells were prepared as described using human-T-lymphoblast. Mixed human peripheral blood lymphocytes were stimulated with 3 mg/ml of concanavalin A (Con A) for 3 days. The cells were converted, treated with α-methyl mannoside (20 mg per ml), washed and used as immunogens in culture medium. Cultures were performed in Click's RPMI medium (Seromed GmbH, München, F.R.G.) supplemented with 2×10^{-3} M L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 U ml^{-1} penicillin, 100μg ml^{-1} streptomycin, and 5 to 10% (v/v) fetal calf serum (FCS; batch No. 104; Seromed GmbH).

Immunization, cell fusion, cloning, and production of monoclonal antibodies (mAb)

Ten-week-old BALB/c mice were primed with 2×10^7 T lymphoblasts. The cells were injected in 0,1 ml portions (10^6 cells) subcutaneously into the footpads and into the necks of the mice as well as i.V. (10^7 cells in 0.5 ml). Four weeks later, the mice were challenged i.v. with 10^7 T-lymphoblasts. Three days later, spleen cells from the immunized mice were fused with X63-Ag8.653 mouse myeloma cells in the presence of polyethylene glycol (Köhler and Milstein, (1975), Nature 256:495, as modified by Lemke H., G.J. Hämmerling, C. Höhmann and K. Rajewsky, (1978), Nature 271:249). Fused cells suspended in HAT medium were distributed into each well of ten 24-well tissue culture plates (1 to 2×10^6 spleen cells/well). Supernatants of the wells in which vigorous growth was observed after 3 to 4 wk were screened for their capacity to bind a) human T lymphoblasts, b) mouse T lymphoblasts, and c) human thymocytes attached to the surface of the wells of microtiter plates. Cell-bound immunoglobulin was then detected by enzyme-linked immunosorbent assay (ELISA) as described (Kincade, P.W., G. Lee, L. Sun, and T. Watanabe, (1981), J. Immunol. Methods 42:17.) by using β-galactosidase-coupled sheep $F(ab')_2$ anti-mouse immunoglobulin (New England Nuclear, Dreieich, F.R.G.) as a second antibody. The hybridomas grown in HAT or RPMI medium that constantly produced antibodies binding specifically to human T lymphoblasts were selected. Supernatants of growing hybridomas were repeatedly tested and selected for hybridomas producing supernatants active in the functional assay (inhibition of the T-lymphoblast response to IL-2) as well as the absorption assay (inhibition of the capacity of T lymphoblasts to absorb IL 2 after preincubation). Positive hybridomas were cloned by limiting dilution with mouse thymocytes used as a feeder layer. The clones were retested and expanded. The supernatants of the relevant clones were used for isolation and purification of the mAb.

Purification of the mAb

As tested in the Ouchterlongy double immunodiffusion test with rabbit anti-mouse IgM, IgA, IgG1, IgG2a, IgG2b, and IgG_3 sera (Miles Laboratories, Ltd., Slough, England), the hybridoma clone AHT-54 and AHT-107 were found to produce IgG1 antibodies. Excepting the initial screening experiments, in which unpurified culture supernatants were used, the

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following experiments were performed with purified IgG1. Purification was achieved by successive binding/elution from protein A-Sepharose (Pharmacia Fine Chemicals) according to the method described by Ey et al, (Ey, P.L., S.J. Prowse and C.R. Jenkin, (1978), Immunochemistry 15:429). About 600 ml of the culture supernatants, brought to pH 8.0, were passed over a 5 ml protein A-Sepharose column equilibrated in 0.1 M sodium phosphate buffer (pH 8.0) IgG1 was eluted from the column with 0.1 M sodium citrate buffer (pH 6.0). The purified antibody was then dialyzed against a buffer containing 0.01 M HEPES (pH 7.4) and 0.9% NaCl. The purity of mAb was confirmed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis performed in reducing conditions as described (Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:429). The protein concentration of the purified IgG1 was determined by absorption of ultaviolet light at 280 nm, assuming an extinction coeficient (1% w/v:1cm) of 14, and by the method of Lowry et al, 1951 (Lowry, O.H., NJ. Rosebrogh, A.L. Farr, and R.J. Randall (1951). J. Biol. Chem. 193:265.) with bovine serum albumin (BSA) used as the standard.

Recombinant Interleukin-2 provided from Sanolez Mena was used. ^{125} I-labelled recombinant IL-2 was produced from NEN.

Labelling of the mAB with ^{125}J

MoAb were labelled with ^{125}J according to McConahey and Dixon (McConahey, P.J., and F.J. Dixon, (1980) Methods Enzymol, 70:210). Briefly, 20 μg of IgG1 dissolved in 60 μl of $Na^{125}J$(100 mCi ml $^{-1}$, carrier-free; Amersham Buchler). Ten microliters of chloramine-T (2.5 mg ml^{-1} in 0.05 M Na-P) were added to the mixture. After 45 sec of incubation at room temperature, 20 μl of $Na_2S_2O_5$ (3 mg ml^{-1} in 0.05 Na-P) were added to the tube. The mixture was immediately loaded onto a 15-ml Sephadex G-75 column (prewashed with 0.05 M Na-P containing 4% BSA and washed consecutively with 0.05 M Na-P until the eluate was protein-free), and the radiolabel in the excluded fraction was collected.

Detailed description of the drawings:

Figure 1

Inhibition of IL-2 dependent human T-lymphoblast proliferation by different mAbs.

2×10^6 human T-lymphoblasts were incubated for 3 days in 0.2 ml of medium containing the indicated amounts of recombinant IL-2 (Fig. 1a, 20U/ml; Fib. 1b,4U/ml) in the absence or presence of the different mAbs, anti-Tac (–O–), HT-54 (–Δ–), AHT-107 (–☆–) and of a control mAb anti-human-TSH (–O–). The cells were pulsed with ^{3}H-thymidine for the last 4 h of the incubated period. Incorporation of ^{3}H-thymidine was measured according to the standard procedure (Diamantstein et al, Mol. Immunol., (1984), 21:1229.

Figure 2

Synergistic action of AHT-54 and AHT-107 mAbs on IL-2 dependent proliferation

T-lymphoblasts were cultured with 20U/ml of r-IL-2 for 3 days (for detail see Fig. 1) in the presence of either AHT-54 or AHT-107 mAB or in combination of both mAbs.

Figure 3

Competition for the binding of ^{125} I-labelled antibodies

2×10^6 human T blasts were first suspended in 100 μl of a binding buffer (PBS=0.5% BSA/10 mM NaN_3) containing different dilutions of the mAbs anti-Tac (–O–), AHT-54 (–Δ–) and AHT-107 (–☆–). The suspensions were mixed with 100 μl of a 1:40 dilution of the ^{125}I-labelled mAb.

The mixture was incubated for 1h at 4°C. The relative amount of ^{125} I-labelled mAb (cpm) bound to the pelleted cells was measured by using a gamma-radiation counter after washing them twice with the binding buffer.

Figure 4

Inhibition of ^{125}I-IL-2 binding to human T blasts by different mAbs

2×10^6 human T blasts were first incubated for 30 min. at 37°C in 0.25 ml of a buffer (RPMI/Hepes/BSA/NaN_3) containing the indicated amounts of different mAbs. The incubation was further continued at 37°C in the presence of ^{125}I-IL-2. After 40 min. the incubation mixture was centrifuged to pellet the cells and the pelleted cells were taken up in 100 μl of the buffer and overlaid on the oil phase consisting of dibutylphthalate/olive oil (10+3). After centrifugation the tips of the tube containing the cell pellets were cut out and counted in a gamma-radiation counter.

Figure 5

SDS-PAGE analysis of immunoprecipitates with different mAbs

2×10^7 human T blasts were surface-iodinated with 0.5 mCi of Na [^{125}I] and lysed in 0.5 ml of the lysis buffer. The lysate was centrifuged and preabsorbed with 1/5 volume of protein A-sepharose beads (10 μl) through a bridge antibody rabbit anti-mouse IgG. After 1h at 4°C the beads were washed three times with a buffer containing 50mM Tris-HCL, pH 8.3, 450 mM NaCl, 5mM KI, 0.02% NaN_3 and 0.5 Nonidet P-40, and extracted with 100 μl of the sample buffer. 50 μl aliquots of the extracts were subjected to SDS-PAGE analysis either under non-reducing (lanes 1–6) or reducing (lanes 7–12) conditions. the mAbs used were control mouse UPC-10 ascites (lanes 1 & 7), anti Tac ascites (lanes 2 & 8),

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AHT-54 ascites lanes 4 & 10), AHT-107 ascites (lanes 5 & 11) and AHT-107 culture supernatants (lanes 6 & 12).

Figures 6 to 11
FACS-analysis

Human peripheral blood cells (HPBL) and activated T-lymphoblasts derived from HPL were incubated at 4°C in presence of 0.1 % NaN_3 for 30 min with AHT-54 or AHT-107 mAB (1:1000 ascites fluid and as negative control with a TSH, washed and stained using saturating amount of goat anti-mouse IgG labelled with FITC. Fluorescence activated cell sorter analysis performed with Epics V.

Fig. 6 shows the negative control. The α TSH antibody did not bind to the T-lymphoblasts. In Fig. 7 and 8 it is shown that AHT 54 (Fig. 7) and AHT 107 (Fig. 8) are capable to bind to the lymphoblasts. Fig. 9 to 11 are refering to the same experiment except the lymphoblasts are substituted by HPBL. With none of the three antibodies a reaction occurs.

CLAIMS

1. A composition of at least two monoclonal antibodies regognizing human interleukin-2-receptor capable to inhibit interleukin-2 dependent lymphocyte proliferation.

2. A composition according to claim 1, wherein at least one of the monoclonal antibodies is of the IgG_1 class.

3. A composition according to any of claims 1 and 2 inhibiting interleukin-2 binding to the receptor.

4. A monoclonal antibody of the IgG_1 class recognizing human interleukin-2-receptor capable to inhibit interleukin-2 binding to the receptor.

5. A monoclonal antibody according to claim 4 capable to inhibit interleukin-2 induced lymphozyte proliferation.

6. A chimeric animal-human antibody recognizing human interleukin-2-receptor.

7. A chimeric antibody according to claim 6, wherein the F_c region is obtained from human and the Fab region is obtained from an animal.

8. A chimeric antibody according to any of claims 6 and 7, wherein the Fab region is obtained from mice.

9. Hybridoma cell lines, characterized by the production of monoclonal antibodies of the IgG_1 class recognizing human interleukin-2-receptor.

10. The hybridoma cell lines of claim 9 having the NTCC designation number . . . and . . . . .

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(54) Resurfacing of rodent antibodies.

(57) A method for determining how to humanize a rodent antibody or fragment thereof by resurfacing, said method comprising :

(a) determining the conformational structure of the variable region of said rodent antibody or fragment thereof by constructing a three-dimensional model of said rodent antibody variable region ;

(b) generating sequence alignments from relative accessibility distributions from x-ray crystallographic structures of a sufficient number of rodent antibody variable region heavy and light chains to give a set of heavy and light chain framework positions wherein said set is identical in 98% of said sufficient number of rodent antibody heavy and light chains ;

(c) defining for said rodent antibody or fragment thereof to be humanized a set of heavy and light chain surface exposed amino acid residues using said set of framework positions generated in said step (b) ;

(d) identifying from human antibody amino acid sequences a set of heavy and light chain surface exposed amino acid residues that is most closely identical to said set of surface exposed amino acid residues defined in said step (c), wherein said heavy and light chain from said human antibody are or are not naturally paired ;

(e) substituting, in the amino acid sequence of said rodent antibody or fragment thereof to be humanized said set of heavy and light chain surface exposed amino acid residues defined in said step (c) with said set of heavy and light chain surface exposed amino acid residues identified in said step (d) ;

(f) constructing a three-dimensional model of said variable region of said rodent antibody or fragment thereof resulting from the substituting specified in said step (e) ;

(g) identifying, by comparing said three-dimensional models constructed in said steps (a) and (f), any amino acid residues from said set identified in said step (d), that are within 5 Angstroms of any atom of any residue of the complementarity determining regions of said rodent antibody or fragment thereof to be humanized ; and

(h) changing any residues identified in said step (g) from the human to the original rodent amino acid residue to thereby define a rodent antibody humanizing set of surface exposed amino acid residues ; with the proviso that said step (a) need not be conducted first, but must be conducted prior to said step (g).

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FIELD OF THE INVENTION

The present invention relates to the development of prediction rules that can be used to accurately model the variable regions (V-regions) of antibodies. The development of these rules and their application in the predictive molecular restructuring of the surfaces of variable domains of non-human monoclonal antibodies enables changing of the surface, resurfacing, of these monoclonal antibody V-regions to replicate the surface characteristics found on human antibody V-regions. This method of resurfacing non-human monoclonal antibody V-regions to resemble human antibody V-regions is expected to permit the production of functional altered antibodies, which retain the binding parameters of the original non-human monoclonal antibody, with improved therapeutic efficacy in patients due to the presentation of a human surface on the V-region.

BACKGROUND OF THE INVENTION

General Background of Antibodies

Murine monoclonal antibodies are widely used as diagnostic and therapeutic agents in the treatment of human disease. Mice can be readily immunized with foreign antigens to produce a broad spectrum of high affinity antibodies. Invariably, the introduction of murine or other rodent antibodies into humans results in the production of a human anti-mouse antibody (HAMA) response due to the presentation of a foreign protein in the body. The production of HAMA in patients can result from the introduction of foreign antibody in a single dose or from extended use in therapy, for example, for the treatment of cancer. Extended use of murine antibody is generally limited to a term of days or weeks in patients before concerns of anaphylaxis arise. Moreover, once HAMA has developed in a patient, future use of murine antibodies for diagnostic or therapeutic purposes is often precluded for the same reasons.

Beyond ethical considerations, attempts to produce human monoclonal antibodies have not been highly successful for a number of reasons. The production *in vitro* of human monoclonals rarely results in high affinity antibodies. *In vitro* cultures of human lymphocytes yield a restricted range of antibody responses relative to the broad spectrum of reactive antibodies produced *in vivo* through direct immunization of mice. Additionally, in humans, immune tolerance prevents the successful generation of antibodies to self-antigens. All of these factors have contributed to the search for ways to modify the structures of murine monoclonal antibodies to improve their use in patients. Many investigators have attempted to alter, reshape or humanize murine monoclonal antibodies in an effort to improve the therapeutic application of these molecules in patients.

Strategies of Antibody Humanization

The earliest reports of the controlled rearrangement of antibody domains to create novel proteins was demonstrated using rabbit and human antibodies as described by Bobrzecka, K. et al. (Bobrzecka, K., Konieczny, L., Laidler, P. and Rybarska, J. (1980), Immunology Letters **2**, pp. 151-155) and by Konieczny et al. (Konieczny, L., Bobrzecka, K., Laidler, P. and Rybarska, J. (1981), Haematologia **14** (I), pp. 95-99). In those reports, the protein subunits of antibodies, rabbit Fab fragments and human Fc fragments, were joined through protein disulfide bonds to form new, artificial protein molecules or chimeric antibodies.

Recombinant DNA technology was used to construct gene fusions between DNA sequences encoding mouse antibody variable light and heavy chain domains and human antibody light chain (LC) and heavy chain (HC) constant domains to permit expression of the first recombinant "near-human" antibody (chimeric antibody) product (Morrison, S.L., Johnson, M.J., Herzenberg, L.A. and Oi, V.T. (1984), Proc. Natl. Acad. Sci. U.S.A. **81**, pp. 6851-6855).

The kinetics and immune response in man to chimeric antibodies has been examined (LoBuglio, A.F., Wheeler, R.H., Trang, J., Haynes, A., Rogers, K., Harvey, E.B., Sun, L., Ghrayeb, J. and Khazaeli, M.B. (1989), Proc. Natl. Acad. Sci. **86**, pp. 4220-4224).

Chimeric antibodies contain a large number of non-human amino acid sequences and are immunogenic in man. The result is the production of human anti-chimera antibodies (HACA) in patients. HACA is directed against the murine V-region and can also be directed against the novel V-region/C-region (constant region) junctions present in recombinant chimeric antibodies.

To overcome some of the limitations presented by the immunogenicity of chimeric antibodies, the DNA sequences encoding the antigen binding portions or complementarity determining regions (CDR's) of murine monoclonal antibodies have been grafted by molecular means in the DNA sequences encoding the frameworks of human antibody heavy and light chains (Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S. and Winter, G. (1986), Nature **321**, pp. 522-525; Riechmann, L., Clark, M., Waldmann, H. and Winter, G. (1988), Nature **332**,

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pp. 323-327). The expressed recombinant products called reshaped or humanized antibodies are comprised of the framework of a human antibody light or heavy chain and the antigen recognition portions, CDR's, of a murine monoclonal antibody. Several patent applications have been filed in this area including, for example, European Patent Application, Publication No. 0239400; European Patent Application, Publication Nos. 0438310A1 and 0438310A2; International Patent Publication No. WO 91/09967; and International Patent Publication No. WO 90/07861.

However, it is questionable whether European Patent Application (EP), Publication No. 0239400 is truly enabling. It is not assured in this patent that the best fit is made to assure proper presentation of the CDR loops at the antibody combining site.

EP Publication Nos. 0438310A1 and 0438310A2 go a step beyond EP Publication No. 0239400 by protecting the importance of uniquely selected human frameworks for the human light chain (LC) and heavy chain (HC) V-regions. These V-region frameworks should show a high degree of sequence similarity with the frameworks of the murine monoclonal antibody and present the CDR's in the appropriate configuration. However, the criteria for sequence matching are no more sophisticated than simple homology searching of the antibody protein or DNA databases.

International Patent Publication No. WO 91/09967 attempts a further variation of the method disclosed in EP Publication No. 0239400. In International Patent Publication No. WO 91/09967, homology of the donor sequences and the acceptor framework is not important, rather it discloses that a selected set of residues in the LC and HC are critically important to humanization. The ability to make changes at these positions is the basis of International Patent Publication No. WO 91/09967.

International Patent Publication No. WO 90/07861 proposes four important criteria for designing humanized antibodies. 1) Homology between human acceptor and non-human donor sequences. 2) Use donor rather than acceptor amino acids where the acceptor amino acid is unusual at that position. 3) Use donor framework amino acids at positions adjacent to the CDR. 4) Use donor amino acids at framework positions where the sidechain atom is within 3 Angstroms of the CDR in a 3-D model. The first antibody humanized by this method retained less than 1/3 the affinity of the original monoclonal antibody.

None of the above methods for designing a humanized antibody are predictable due to the questions that surround CDR framework interactions. By replacement of murine framework with human framework, there is no guarantee of identical conformations for CDR's because i) the V_L-V_H interaction is not identical in all V-regions and ii) accurate prediction of the CDR-framework interactions are key to faithful reproduction of the antigen binding contacts.

The above methods do not offer a general solution to solving the issues surrounding antibody humanization, rather the methods as outlined in each reference above involve a substantial amount of trial and error searching to obtain the desired affinity in the final humanized product. More importantly, there is no guarantee that corrective changes in framework amino acids will leave the reshaped V-regions resembling the surface character of a truly human antibody. Therefore, it can be argued that antibodies humanized by the above methods may be immunogenic in man.

Antigenicity of Antibodies

The antigenicity/immunogenicity of an antibody, including recombinant reshaped antibody products, introduced into humans can be viewed as a surface phenomenon. In general one can view the immune system as scanning the surface of a protein introduced to the body. If the F_V portion of a humanized antibody 'opens-up' in the circulation then internal residues can be presented to the immune system. On the other hand, if the F_V portion is stable and tightly packed then only the surface residues presented by the V-regions and the interface between the V_L and V_H regions will be 'scanned'.

Surface Reshaping or Resurfacing of Antibobies

The notion of surface presentation of proteins to the immune system raises the prospect of redesigning murine monoclonal antibodies to resemble human antibodies by humanizing only those amino acids that are accessible at the surface of the V-regions of the recombinant F_V. The resurfacing of murine monoclonal antibodies to reduce their immunogenicity could be beneficial in maintaining the avidity of the original monoclonal antibody in the reshaped version, because the natural framework-CDR interactions are retained. The value of maintaining the integrity of the framework-CDR interactions has been illustrated as summarized below.

In a recent research report, two different reshaped versions of the rat monoclonal antibody, Campath-9 (anti-human CD4), were generated (Gorman, S.D., Clark, M.R., Routledge, E.G., Cobbold, S.P. and Waldmann, H. (1991), Proc. Natl. Acad. Sci. U.S.A. **88**, pp. 4181-4185). In one version, pV_HNEW/C_{G1}, the acceptor V_H fra-

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mework was from the human NEW-based heavy chain, which has 47% identical residues to the Campath-9 V_H. While in the second version, pV_HKOL/C_{G1}, the acceptor V_H framework was from the human KOL antibody, which has 72% identical residues to Campath-9 V_H. Each reshaped antibody contained the identical V_L domain from the human REI antibody sequence. However, the recombinant product of pV_HKOL/C_{G1} had an avidity for CD4 that was substantially greater than the product of pV_HNEW/C_{G1}. The authors proposed a reshaping strategy where human sequences, that are highly homologous to the rodent antibody of interest, are transferred, by in vitro mutagenesis, into the rodent V-region to create a "bestfit" reshaped antibody. This strategy uses the term "bestfit" to describe the modeling process, however, there is no quantitative formula employed to assess "bestfit", and so in effect, the process is subjective. Additionally, there is no resurfacing concept presented in that paper.

The concept of reducing rodent-derived antibody immunogenicity through the replacement of exposed residues in the antibody framework regions which differ from those of human origin is discussed in a recent paper (Padlan, E.A. (1991), Molecular Immunology **28**, pp. 489-498). In that paper, the variable domains of two antibody structures, KOL (human) and J539 (mouse), are examined. The crystal structures of the Fab fragments of these two antibodies have been elucidated to high resolution. The solvent accessibility of the exposed framework residues in the variable domains of these two antibodies were compared to a sequence database of human and murine antibody V-region subgroups. On the basis of his findings, Padlan proposed to reduce the antigenicity of allogeneic variable domains [murine V-regions], through replacement of the exposed residues in the framework regions with residues usually found in human antibodies. In murine sequences with the highest similarity to a given human sequence, the number of changes necessary to "humanize" a murine V-region surface would range from 6-15 amino acid changes per V-region. This reference suggests how to convert one antibody surface into another but no general method is developed. Application of the procedure is provided by two examples, a worst-case and a best-case.

Worst Case:

Among the representative murine kappa V_L sequences examined for which its autologous V_H has been sequenced, $S107V_L$ has the most residues that need to be replaced to humanize it. $S107V_L$ is most similar to the members of the human subgroup VKIV and JK2. The exposed or partially exposed residues that need to be replaced are those at positions 9, 10, 14, 15, 16, 17, 18, 22, 41, 63, 80, 83, 85, 100 and 106. Murine V-region $S107V_H$ is most similar in its framework to the members of the human subgroup VHIII and JH6. The exposed or partially exposed residues in $S107V_H$ that need to be replaced are those at positions 3, 40, 68, 73, 75, 76, 82b and 89. A total of 23 residues need to be replaced to humanize the variable domains of S107.

Best Case:

Among the murine V_H sequences examined for which the autologous V_L has also been sequenced, $MOPC21V_H$ has the least number of residues that need to be replaced to humanize it. $MOPC21V_H$ is most similar in its framework to the members of the human subgroup HIII and JH6. The exposed or partially exposed residues that need to be replaced are those at positions 1, 42, 74, 82a, 84, 89 and 108. $MOPC21V_L$ is most similar in its framework to human subgroup VKIV and JK4. The exposed or partially exposed residues that need to be replaced are those at positions 1, 9, 12, 15, 22, 41, 63, 68, 83 and 85. A total of 17 amino acids need to be replaced to humanize the variable domains of MOPC21.

Of the light chains in the Best- and Worst-Case examples cited above, $S107V_L$ required changes at 15 positions and $MOPC21V_L$ required changes at 10 positions. Only seven of the changes are common to both of these light chain sequences (see underlined residues). Moreover, of the heavy chain residues that need to be replaced to humanize the respective V-regions, $S107V_H$ required changes at 8 positions and $MOPC21V_H$ required changes at 7 positions. In this instance, only one position is common to both of these heavy chain sequences (see residues in boldface).

An analysis of S107 V-regions alone would not have led to the prediction of which residues to change in MOPC21. The reason for this is that the surface residues in Padlan's analysis are only determined by reference to the crystal structure analysis of one antibody. In addition, the basis for defining the surface exposure of an amino acid at a particular position on that crystal structure is a continuous gradient of change, e.g., the fractional solvent accessibility values (Padlan, E.A. (1990), Molecular Immunology **28**, pp. 489-498) were computed, where: 0 to 0.2 = completely buried, 0.2 to 0.4 = mostly buried, 0.4 to 0.6 = partly buried/partly exposed, 0.6 to 0.8 = mostly exposed, and 0.8 or above = completely exposed. By limiting the analysis of exposed surface residues to a single crystal structure and by superimposing a broad range of solvent accessibility ratios on exposed residues, such a modeling strategy could be expected to have a wide margin of error in its calculations.

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This model fails to take into account the great majority of structural information available in the database for other antibody crystal structures.

SUMMARY OF THE INVENTION

Accordingly, it is an object of this invention to provide humanized rodent antibodies or fragments thereof, and in particular, humanized rodent monoclonal antibodies that have improved therapeutic efficacy in patients due to the presentation of a human surface on the V-region. This and other objects have been attained by providing a method for determining how to humanize a rodent antibody or fragment thereof by resurfacing the method comprising:

(a) determining the conformational structure of the variable region of the rodent antibody or fragment thereof by constructing a three-dimensional model of the rodent antibody variable region;

(b) generating sequence alignments from relative accessibility distributions from x-ray crystallographic structures of a sufficient number of rodent antibody variable region heavy and light chains to give a set of heavy and light chain framework positions wherein the set is identical in 98% of the sufficient number of rodent antibody heavy and light chains;

(c) defining for the rodent antibody or fragment thereof to be humanized a set of heavy and light chain surface exposed amino acid residues using the set of framework positions generated in step (b);

(d) identifying from human antibody amino acid sequences a set of heavy and light chain surface exposed amino acid residues that is most closely identical to the set of surface exposed amino acid residues defined in step (c), wherein the heavy and light chain from the human antibody are or are not naturally paired;

(e) substituting, in the amino acid sequence of the rodent antibody or fragment thereof to be humanized the set of heavy and light chain surface exposed amino acid residues defined in step (c) with the set of heavy and light chain surface exposed amino acid residues identified in step (d);

(f) constructing a three-dimensional model of the variable region of the rodent antibody or fragment thereof resulting from the substituting specified in step (e);

(g) identifying, by comparing the three-dimensional models constructed in steps (a) and (f), any amino acid residues from the set identified in step (d), that are within 5 Angstroms of any atom of any residue of the complementarity determining regions of the rodent antibody or fragment thereof to be humanized; and

(h) changing any residues identified in step (g) from the human to the original rodent amino acid residue to thereby define a rodent antibody humanizing set of surface exposed amino acid residues; with the proviso that step (a) need not be conducted first, but must be conducted prior to step (g).

Also provided is a method for producing a humanized rodent antibody or fragment thereof from a rodent antibody or fragment thereof, the method comprising:

(I) carrying out the above-described method for determining how to humanize a rodent antibody or fragment thereof by resurfacing; and

(II) modifying the rodent antibody or fragment thereof by replacing the set of rodent antibody surface exposed amino acid residues with the rodent antibody humanizing set of surface exposed amino acid residues defined in step (h) of the above-described method.

In a preferred embodiment, the rodent antibody or fragment thereof is a murine antibody, and most preferably murine antibody N901.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows an algorithm that can be used for constructing a three-dimensional model of the rodent antibody variable region.

Figure 2 is a diagram showing the approach to determine how to humanize a rodent antibody or fragment thereof according to the present invention.

Figures 3A and 3B are plots of relative accessibility of amino acid residues for twelve antibody F_V structures, mapped onto the sequence alignment of these structures. Structures Glb2 (Jeffrey, P.D., Doctor of Philosophy Thesis, University of Oxford, United Kingdom, 1991), D1.3 (Amit, A.G., Mariuzza, R.A., Phillips, S.E.V. and Poljak, R.J. (1986), Science **233**, pp. 747-753), 3D6 (Grunow, R., Jahn, S., Porstman, T., Kiessig, T., Steinkeller, H., Steindl, F., Mattanovich, D., Gurtler, L., Deinhardt, F., Katinger, H. and von R., B. (1988), J. Immunol. Meth. **106**, pp. 257-265) and 36-71 (5fab) (Rose, D.R., Strong, R.K., Margolis, M.N., Gefter, M.L. and Petsko, G.A. (1990), Proc. Natl. Acad. Sci. U.S.A. **87**, pp. 338-342) are not yet present in the Brookhaven database. The other structures used were: 2hfl (Sheriff, S., Silverton, E.W., Padlan, E.A., Cohen, G.H., Smith-Gill, S.J., Finzel, B.C. and Davies, D.R. (1987), Proc. Natl. Acad. Sci. U.S.A. **84**, pp. 8075-8079), 3hfm (Padlan, E., Silverton, E., Sheriff, S., Cohen, G., Smith-Gill, S. and Davies, D. (1989), Proc. Natl. Acad. Sci. U.S.A. **86**, pp.

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5938-5942), 2fbj (Mainhart, C.R., Potter, M. and Feldmann, R.J. (1984), Mol. Immunol. **21**, pp. 469-478), 3fab (Saul, F.A., Amzel, L.M. and Poljak, R.J. (1978), J. Biol. Chem. **253**, pp. 585-597), 4fab (Herron, J., He, X., Mason, M., Voss, E. and Edmunson, A. (1989), Proteins: Struct., Funct., Genet. **5**, pp. 271-280), 2mcp (Segal, D., Padlan, E., Cohen, G., Rudikoff, S., Potter, M. and Davies, D. (1974), Proc. Natl. Acad. Sci. U.S.A. **71**, pp. 4298-[??]), 2fb4 (Marquart, M. Deisenhofer, J. and Huber, R. (1980), J. Mol. Biol. **141**, pp. 369-391), and 1f19 (Lascombe, M. Alzari, P., Boulot, G., Salujian, P., Tougard, P., Berek, C., Haba, S., Rosen, E., Nisonof, A. and Poljak, R. (1989), Proc. Natl. Acad. Sci. U.S.A. **86**, p. 607). These structures are designated by their Brookhaven entry code. The sequence numbering used here is described in Figures 4A and 4B. Figure 3A graphically shows the relative accessibility for the heavy chain and Figure 3B graphically shows the relative accessibility for the light chain.

Figures 4A and 4B show alignments of sequences generated using the three methods of humanization. Sequences are: 1) Original rodent N901. 2+3) KOL (Marquart, M. Deisenhofer, J. and Huber, R. (1980), J. Mol. Biol. **141**, pp. 369-391) and reshaped N901 using KOL surface. 4+5) Most homologous sequences, L(KV2F) (Klobeck, H., Meindl, A., Combriato, G., Solomon, A. and Zachau, H. (1985), Nucleic Acids Res. pp. 6499-6513) and H(G36005) (Schroeder, H. and Wang, J. (1990), Proc. Natl. Acad. Sci. U.S.A. **87**), and reshaped N901 using these sequences. 6+7) Most homologous with respect to surface residues, L(KV4B) (Klobeck, H., Bronkamp, G., Combriato, G., Mocikat, R., Pohelnz, H. and Zachau, H. (1985), Nucleic Acids Res. 3, pp. 6515-6529) and H(PLO123) (Bird, J., Galili, N., Link, M., Sites, D. and Sklar, J. (1988), J. Exp. Med. **168**, pp. 229-245), and reshaped N901 using these sequences. The numbering is the same as used in the antibody modelling program ABM (trademark for commercial software, Oxford Molecular Ltd., Oxford, U.K.), which is based on structural conservation and not sequence homology as used by Padlan et al. (Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M. and Gottesman, K.S. (1987), Sequences of Proteins of Immunological Interest. U.S. Department of Health and Human Services, Fourth Edition). The sequence changes which have to be introduced in order to resurface N901 with a given sequence are marked with bars, back-mutations as determined from F_V models are marked with stars. The sequence homology of given sequences to N901 are shown in brackets after each sequence.

Figure 5 is a stereo plot of mean antibody β-barrel, coordinates determined by iterative multiple fitting of eight antibody structures. Strands 7 and 8 comprise the 'take off' positions for CDR H3 and are not included in the fitting of V_L and V_H regions.

Figure 6 is a plot of RMS deviation from the mean of the eight β-sheet strands comprising the framework. The RMS was calculated from structures F19.9, 4-4-20, NEW, FBJ, KOL, HyHEL-5, HyHEL-10 and McPC603. N,Cα,C atoms are included in the plot. The residues used are shown in the alignment (Table 2). The most disordered residues are all the residues of strand HFR4, the last residue of LFR1, and the first and last residue of HFR2. The nomenclature of the strands is explained in the alignment in Table 2. LFR1 - #1, LFR2 - #2, LFR3 - #3, LFR4 - #4, HFR1 - #5, HFR2 - #6, HFR3 - #7, HFRS4 - #8.

Figure 7 is a flowchart of the overall modelling protocol known as CAMAL.

Figure 8 is a plot of superimposed loop backbones for models and x-ray structures discussed in Example 2. The loops are positioned after global framework fit. This does not represent the best local least squares fit, but shows how the loops are positioned globally onto the framework.

Figures 9A to 9D are stereo (N,C-α,C,O) representations of crystal structures and models of D1.3, 3671 and Gloop-2 variable domain and β-barrel strands described in Example 2. Crystal structures are shown with open bonds, model with solid bonds. The difference between the 3D6-H3 in the model and the crystal structure is due to a 5-7° twist in the extended β-sheet conformation of this loop, Figure 9A: D1.3, Figure 9B: 36-71, Figure 9C: Gloop-2, Figure 9D: 3D6.

Figure 10 is a histogram showing the distribution of loop length for CDR H3 loops, data from Kabat et al. (Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M. and Gottesman, K.S. (1987), Sequences of Proteins of Immunological Interest. U.S. Department of Health and Human Services, Fourth Edition).

DETAILED DESCRIPTION OF THE INVENTION

The existence of specific, yet different, surface patches in murine and human antibodies may be the origin of the inherited immunogenicity of murine antibodies in humans. Statistical analysis of a database of unique human and murine antibody F_V fragments has revealed that certain combinations of residues in exposed surface positions are specific for human and murine sequences. The combinations are not the same in human and murine F_V domains. However, it is possible to define families of surface residues for the two species of antibodies. These families reveal a novel method for the "humanization" or reshaping of murine antibodies. Humanization is the modification of the solvent accessible surface of a non-human antibody or fragment thereof to resemble the surface of a chosen human antibody or fragment thereof such that the modified non-human antibody or fragment thereof exhibits lower immunogenicity when administered to humans. Such a process

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applies in the present application to antibody variable regions but could equally well apply to any other antibody fragment. The method is considered to be generally applicable to humanization of rodent antibodies.

According to the present invention, a statistical analysis is presented which is based on accessibility calculated for a range of antibody crystal structures. When this information is applied to an antibody sequence database, it is possible to discriminate between human and murine antibodies at the sequence level purely on the basis of their surface residue profiles.

Rational Resurfacing Approach

There are several key features of the resurfacing approach of the present invention.

1) This method uses as a starting point, construction of a three-dimensional model of a rodent variable region by known methods;

2) A large number (e.g., twelve) of antibody F_V or Fab fragment x-ray crystallographic structures are analyzed to produce an unambiguous set of surface exposed amino acid residues that will be positionally identical for a majority (98%) of antibodies. The set is produced by identifying all those residues whose solvent accessibility is above a given cut-off (typically 30%), calculated using a modification of the method of Kabsch and Sander (Kabsch, W. and Sander, C. (1983), Biopolymers **22**, pp. 2257-2637) in which explicit atomic radii are used for each atom type to predict sidechain positions as is described below in more detail;

3) Using a complete human antibody database, the best set of human heavy and light chain surface exposed amino acid residues is selected on the basis of their closest identity to the set of surface amino acid residues of the murine antibody;

4) In order to retain the conformational structure- of the CDRs of the rodent antibody, replacement of any human surface exposed amino acid with the original rodent surface exposed amino acid residue is carried out whenever a surface residue is calculated from the three-dimensional model to be within 5 Angstroms of a CDR residues.

The general resurfacing approach of the present invention is illustrated in Figure 2. The approach can be divided into two stages. In the first, the rodent framework (white) is retained and only the surface residues changed from rodent (dark grey circles) to the closest human pattern (light grey circles). This should remove the antigenicity of the rodent antibody. In the second stage, surface residues within 5 Angstroms of the CDRs are replaced with the rodent equivalents in an attempt to retain antigen binding and CDR conformation.

The method of the present invention is applicable to whole antibodies as well as antibody fragments. Suitable antibody fragments that can be used can readily be determined by the skilled artisan. Examples of some suitable fragments include a single chain antibody (SCA), an antibody F_V fragment, Fab fragment, Fab_2 fragment, Fab' fragment, or other portion of an antibody comprising the binding site thereof.

According to the present invention, an important step in the method for determining how to modify a rodent antibody or fragment thereof by resurfacing is to determine the conformational structure of the variable region of the rodent antibody or fragment thereof to be humanized by constructing a three-dimensional model of the rodent antibody variable region. This can be done by known methods such as those described, for example, in Martin et al. (Martin, A.C.R., Cheetham, J.C. and Rees, A.R. (1989), Proc. Natl. Acad. Sci. U.S.A. **86**, pp. 9268-9272; Methods in Enzymology (1991), **203**, pp. 121-152) and as described in detail in Example 2.

Martin et al. describe an algorithm which is depicted in Figure 1. The algorithm applies to murine and human antibodies equally well. The present inventors therefore expect that, based on sequence similarity between antibodies of different species (Kabat, E.A. Segments of Proteins of Immunological Interest, National Institutes of Health, U.S.A. 1991), the algorithm will work equally well for rat and other rodent antibodies.

Briefly, the algorithm depicted in Figure 1 can be summarized as follows. The framework region of an antibody to be modelled is selected on the basis of sequence homology and constructed by a least squares fit onto the six conserved strands of the variable region β-barrel. Light and heavy chain complementarity determining regions are constructed using a combination of canonical structures (Chothia, C. and Lesk, A.M. (1987), J. Molec. Bio. **196**, pp. 901-917), database searching and conformational searching. Detailed descriptions of these methods are described in Example 2 herein and in the above two references (Martin et al. 1989 and 1991).

According to the present invention, another three-dimensional model is also constructed. The other three-dimensional model is of the rodent antibody variable region having human antibody surface amino acid residues substituted therein at particular rodent antibody surface residue positions.

This other three-dimensional model is constructed by carrying out the series of steps described next.

The first of the steps is to generate sequence alignments from relative accessibility distributions from x-ray crystallographic structures of a sufficient number of rodent antibody variable region heavy and light chains to give a set of framework positions of surface exposed amino acid residues which is identical in a majority

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(98%) of the variable regions.

As used herein, the term "framework" means the antibody variable region from which the complementarity determining regions have been excluded.

"Complementarity determining regions" means those amino acid sequences corresponding to the following numbering system as defined by Kabat, E.A. (In Sequences of Immunological Interest, N.I.H., U.S.A., 1991).

Light Chain	L1	residues	24-34
Light Chain	L2	residues	50-56
Light Chain	L3	residues	89-97
Heavy Chain	H1	residues	31-358
Heavy Chain	H2	residues	50-58
Heavy Chain	H3	residues	95-102

A sufficient number of rodent antibody fragments that need to be analyzed in order to produce the set of framework positions of surface exposed amino acid residues can readily be determined by the skilled artisan through routine experimentation using a database of antibody sequences. Thus, this step can be conducted using suitable databases now in existence or later compiled.

The x-ray crystallographic structures are used to determine relative accessibility distributions of surface exposed amino acid residues. The relative accessibility distributions identify all those residues whose solvent accessibility is above a given cut-off (typically 30%), calculated using a modification of the method of Kabsch and Sander (Kabsch, W. and Sander C. (1983), Biopolymers **22**, pp. 2257-2637) in which explicit atomic radii are used for each atom type.

The relative accessibility distributions determined from the x-ray crystallographic structures can then be used to generate sequence alignments which give a set of framework positions of surface exposed amino acid residues which is identical in a majority (98%) of the variable regions.

The set of framework positions of surface exposed amino acid residues for the variable regions of murine antibodies is shown in Table 1, set forth in Example 1, and was produced using the sequence alignments and accessibility distributions shown in Figures 3A and 3B.

Once a set of framework positions of surface exposed amino acid residues for the variable regions of the rodent antibodies have been generated, the surface exposed residues of the heavy and light chain pair of the rodent antibody, or fragment thereof, to be humanized can be identified using an alignment procedure such as that described in Example 1 and shown in Figures 3A and 3B. This defines a set of surface exposed amino acid residues of a heavy and light chain pair of a rodent antibody or antibody fragment to be humanized.

Next, a complete human antibody sequence database is used to identify a set of surface exposed amino acid residues from a human antibody variable region that have the closest positional identity to the set of surface exposed amino acid residues of the variable region of the rodent antibody that is to be humanized. The set of surface exposed amino acid residues from the human antibodies can be separately identified for a heavy chain and for a light chain that are not naturally paired and/or a set can be identified from a natural human heavy and light chain pair, that is, a pair originating from a single B cell or hybridoma clone. Preferably, the set is one from a natural human heavy and light chain pair.

A humanized rodent antibody that gives the appearance of a human antibody is then predicted by substituting the set of surface exposed amino acid residues from the rodent antibody or fragment thereof to be humanized with the set of surface exposed amino acid residues from the human antibody.

A three-dimensional model can then be constructed from the resulting, fully substituted variable region of the rodent antibody or fragment thereof. The three-dimensional model is constructed using the same known methods mentioned above for constructing a 3-D model of the original rodent antibody or fragment thereof.

While the antigenicity of this fully "resurfaced" or humanized antibody should be removed, an additional factor to be addressed is the binding affinity or the binding strength of the resurfaced antibody. Changes in the framework of the variable domain introduced through resurfacing can influence the conformation of the CDR loops and therefore antigen binding of the antibody. According to the present invention, this problem is removed by the next step which is to identify, by means of a comparison of both of the above-described three-dimensional models of the rodent antibody variable region, any residues from the set of surface exposed amino acid residues of the variable region heavy and light chain pair of the human antibody identified that are within 5 Angstroms of any atom of any residue of the rodent antibody or antibody fragment complementarity deter-

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mining regions (CDRs).

Any residue(s) so identified is then changed back from the human to the original rodent amino acid residue(s).

The results of this method can then be applied to a particular rodent antibody by well known methods. Briefly, genes for the humanized variable heavy and light chain regions are constructed using standard recombinant DNA methods (Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989), Molecular Cloning, Second Edition). For example, a PCR method can be used (Daugherty et al. (1991), Nucleic Acids Research **19**, pp. 2471-2476).

Variable heavy chain or variable light chain gene constructs are subcloned into appropriate expression vectors. Suitable expression vectors contain either a human gamma or human kappa constant region gene, a suitable promoter, a sequence coding for a human immunoglobulin leader peptide (for example: met-gly-trp-ser-cys-ile-ile-leu-phe-leu-val-ala-thr-ala-thr (SEQ ID NO:39), Olandi et al. (1989), PNAS **86**, pp. 3833-3837), and a drug selectable marker.

Heavy and light chain expression plasmids can be co-transfected, for example, by electroporation into suitable cells, for example, SP2/0 cells, and selected with an appropriate drug, G418, for example. Screening for intact antibody can be accomplished by ELISA assay. 96-well plates are coated with, for example, goat anti-human kappa chain antibody, and light chains are detected with, for example, goat anti-human antibody conjugated to alkaline phosphatase.

As another approach, light chain constructs are transfected, for example, by electroporation into suitable cells, for example, SP2/0 cells and selected, for example, in hygromycin. Screening for light chain expression can be accomplished by ELISA assay. 96-well plates are coated with, for example, goat anti-human kappa chain antibody, and light chains are detected with, for example, goat anti-human antibody conjugated to alkaline phosphatase.

A light chain producing line is then used as a host to electroporate in the heavy chain construct. The heavy chain plasmid is co-transfected with a plasmid containing the gene coding for another drug marker, for example, neomycin resistance and selected in the presence of the drug G418. Screening for intact antibody is accomplished by ELISA assay. 96-well plates are coated with, for example, goat anti-human Fc and detected with, for example, goat anti-human light chain conjugated to alkaline phosphatase.

EXAMPLE 1 AND COMPARATIVE EXAMPLES

The superiority of the presently claimed method for determining how to modify a rodent antibody or fragment thereof by resurfacing in order to produce a humanized rodent antibody will now be described by reference to the following example and comparative examples which are illustrative and are not meant to limit the present invention.

A) Analysis for Murine Antibodies

In order to determine the positions which are usually accessible on the surface of the F_V domain of murine antibodies, the accessibility was calculated for twelve Fab x-ray crystallographic structures obtained from the Brookhaven database (Bernstein, F., Koetzle, T., Williams, G., Meyer, E., Brice, M., Rodgers, J., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977), J. Mol. Biol. **112**, pp. 535-542). The relative accessibility was calculated using the program MC (Pedersen, J. (1991)), which implements a modified version of the DSSP (Kabsch, W. and Sander, C. (1983), Biopolymers **22**, pp. 2257-2637) accessibility calculation routine in which explicit atomic radii are specified for every atom. A residue was defined as being surface accessible when the relative accessibility was greater than 30%. The alignment positions of these residues were conserved in all twelve structures (98% identity). Surface accessible framework positions constitute 40% of the F_V surface area. The remaining surface accessible residues are in the CDRs and in the interdomain C-terminal region. Figures 3A and 3B show a sequence alignment of the twelve crystal structures, the average relative accessibility, and the 30% accessibility cutoff. Figure 3A shows the alignments relative accessibility for the twelve murine antibody light chains and Figure 3B shows the alignments and relative accessibility for the murine antibody heavy chains.

The surface accessible framework positions were mapped onto a database of unique human and mouse F_V sequences (see lists at the end of this Example). The frequency of particular residues in each of these positions is shown in Table 1. Only residue frequencies higher than 5% are listed.

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Light chain		
Position	Human	Mouse
1	D 51 E 34 A 5 S 5	D 76 Q 9 E 6
3	V 38 Q 24 S 24 Y 6	V 63 Q 22 L 5
5	T 61 L 37	T 87
9	P 26 S 26 G 17 A 14 L 7	S 36 A 29 L 17 P 5
15	P 62 V 25 L 12	L 47 P 30 V 8 A 7
18	R 57 S 18 T 13 P 6	R 38 K 22 S 13 Q 12 T 9
46	P 94	P 82 S 9
47	G 89	G 71 D 18
51	K 43 R 31	K 70 Q 13 R 8 T 5
63	G 91	G 98
66	D 43 S 25 A 9	D 38 A 26 S 26
73	S 96	S 90 I 5
76	D 43 T 18 S 16 E 15	D 67 S 15 A 5 K 5
86	P 44 A 27 S 17 T 8	A 50 P 11 T 8 E 7 Q 6
87	E 71 D 11 G 7	E 91 D 6
111	K 74 R 12 N 6	K 93
115	K 54 L 40	K 87 L 5
116	R 60 G 33 S 5	R 89 G 9
117	Q 50 T 37 E 6 P 6	A 74 Q 14 P 5 R 5
Heavy chain		
Position	Human	Mouse
118	E 47 Q 46	E 59 Q 29 D 10
120	Q 83 T 7	Q 68 K 26
122	V 59 L 15 Q 13	Q 57 V 27 L 5 K 5
126	G 54 A 23 P 18	G 36 P 30 A 29
127	G 53 E 22 A 14 D 7	E 45 G 43 S 6
128	L 61 V 31 F 7	L 96
130	K 46 Q 41 E 5	K 52 Q 27 R 17
131	P 95	P 91 A 5
132	G 74 S 16 T 7	G 82 S 17
136	R 53 K 23 S 17 T 7	K 66 S 17 R 13
143	G 96	G 98
145	T 46 S 32 N 9 I 7	T 63 S 19 N 7 A 5 D 5
160	P 84 S 10	P 89 H 7
161	G 93	G 71 E 24
162	K 76 Q 10 R 8	K 50 Q 30 N 10 H 5
163	D 26 P 25 A 17 Q 10 T 7	E 31 P 22 D 17 A 12 Q 11
184	S 70 K 9 P 8	K 42 S 37 T 6
186	K 53 Q 22 R 7 N 5	K 83 Q 7
187	G 66 S 21 T 5	G 62 S 18 D 10
195	T 30 D 26 N 19 K 7	T 36 K 30 N 26 D 6
196	S 91	S 76 A 16
197	K 65 I 8 T 8 R 5	S 46 K 34 Q 11
208	R 46 T 18 K 17 D 6	T 55 R 26 K 8
209	A 50 P 21 S 13 T 8	S 67 A 14 T 11
210	E 46 A 18 D 13 S 9 Z 8 V 5	E 88 D 7
212	T 91	T 53 S 43
222	G 17 D 11 P 10 Y 9 V N 8	D 67 A 18

Table 1: Distribution of accessible residues in human V_H and V_L chain sequences. All of the positions appear to be conserved, which leads to the hypothesis that immunogenecity arises from a specific combination of these surface residues. The sequence numbering is explaned in Figures 3A and 3B.

None of the entire combinations of surface residues in the human sequences are found in the murine sequences and *vice versa* (see lists at the end of this Example). However, the residues in individual positions appear to be conserved (see Table 1). There are few residues which differ significantly between the species;

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these are at positions 54 and 91 of the L chain and 168 and 216 of the H chain. Of these positions only position 216 is a non conservative (V to Y) mutation. Differences between human and murine antigenicities are therefore believed to arise from the combinations of residues in these positions.

In order to determine whether the mouse sequences are more distantly related to human F_V sequences than to other mouse F_V sequences, the homology was calculated using a Dayhoff mutation matrix (Dayhoff, M., Barker, W. and Hunt, L. (1983), Meth. Enz. **91**, pp. 524-545). The homology was calculated between all the sequences in a pool of both human and mouse sequence patches made up of the surface accessible residues. The data was then represented as a density map (not shown) in which the sequences are plotted against each other. The density map can be used to discriminate "murine surfaces" from "human surfaces".

B) Reshaping of Antibody N901

In order to test the resurfacing approach suggested by the above analysis, three humanization experiments were set up. 1) Traditional loop grafting (Verhoeyen, M.E., Saunders, J.A., Broderick, E.L., Eida, S.J. and Badley, R.A. (1991), Disease markers **9**, pp. 3-4) onto a human F_V framework of known structure (KOL). 2) Resurfacing approach using most similar chain. 3) Resurfacing approach using human sequences with most similar surface residues.

The antibody used was the murine anti-N901 antibody (Griffin et al. (1983), J. Imm. **130**, pp. 2947-2951). The anti-N901 antibody (also referred to herein as the "N901 antibody") is available commercially from Coulter Corporation under the name NKH-1.

The alignment of the light chain sequences and heavy chain sequences in Figures 4A and 4B, respectively, show the original N901 antibody and the sequences used in each of the three approaches outlined here.

Figures 4A and 4B show alignments of sequences generated using the three methods of humanization. Sequences are: 1) Original rodent N901. 2+3) KOL (Marquart, M. Deisenhofer, J. and Huber, R. (1980), J. Mol. Biol. **141**, pp. 369-391) and reshaped N901 using KOL surface. 4+5) Most homologous sequences, L(KV2F) (Klobeck, H., Meindl, A., Combriato, G., Solomon, A. and Zachau, H. (1985), Nucleic Acids Res., pp. 6499-6513) and H(G36005) (Schroeder, H. and Wang, J. (1990), Proc. Natl. Acad. Sci. U.S.A. **87**) and reshaped N901 using these sequences. 6+7) Most homologous with respect to surface residues, L(KV4B) (Klobeck, H., Bronkamp, G., Combriato, G., Mocikat, R., Pohelnz, H. and Zachau, H. (1985), Nucleic Acids Res. 3, pp. 6515-6529) and H(PLO123) (Bird, J., Galili, N., Link, M., Sites, D. and Sklar, J. (1988), J. Exp. Med. **168**, pp. 229-245), and reshaped N901 using these sequences. The numbering is the same as used in the antibody modelling program ABM (ABM is a trademark for commercial software, Oxford Molecular Ltd., Oxford, U.K.), which is based on structural conservation and not sequence homology as used by Padlan et al. (Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M. and Gottesman, K.S. (1987), Sequences of Proteins of Immunological Interest. U.S. Department of Health and Human Services, Fourth Edition). The sequence changes which have to be introduced in order to reshape N901 with a given sequence are marked with bars, and back-mutations as determined from F_V models are marked with stars. The sequence homology of a given sequence to N901 is shown in brackets after each sequence.

(1) Classical Humanization

In classical humanization the rationale is to graft the rodent CDR's onto a framework of known structure, such that CDR-framework interactions can be accurately monitored by homology modelling. The model of the humanized antibody is compared to that of the original rodent antibody, and possible CDR interacting framework residues are back mutated (marked with '*' in alignment) in order to retain the three-dimensional shape of the CDR's. In this example the antibody KOL was used, giving a low homology score of only 77 and 46 in the heavy and light chains respectively.

(2) Most Similar Chain Resurfacing

A database of nonredundant human antibody sequences was compiled from available protein and nucleotide sequences. A total of 164 H and 129 L chains were sampled.

Each of the rodent chains, L and H, were then matched and the most similar human sequence found independently (G36005/KV2F) (Schroeder, H. and Wang, J. (1990), Proc. Natl. Acad. Sci. U.S.A. **87**); Klobeck, H., Meindl, A., Combriato, G., Solomon, A. and Zachau, H. (1985), Nucleic Acids Res., pp. 6499-6513). Surface residues, as outlined in Table 1, were then changed in the rodent sequences to match those of the human sequences. Subsequently a model was built of the resurfaced antibody and compared to the model of the original rodent antibody and back mutation of any CDR interacting residues was performed.

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(3) Most Similar Surface Replacement According to the Present Invention

This method is identical to the above method, except that the similarity is calculated only over the surface residues outlined in Table 1 above.

The same procedure of surface mutation and subsequent back mutation was performed as in the previous methods. In this case the chosen sequences were PLO123/KV4B (Bird, J., Galili, N., Link, M., Sites, D. and Sklar, J. (1988), J. Exp. Med. **168**, pp. 229-245); Klobeck, H., Bronkamp, G., Combriato, G., Mocikat, R., Pohelnz, H. and Zachau, H. (1985), Nucleic Acids Res. **3**, pp. 6515-6529).

The following lists show the surface residue patterns in mouse and human light and heavy chain antibody variable regions. The sequences are ordered on similarity to one another. There are no pattern matches between mouse and human sequences although there are matches within a species.

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MOUSE LIGHT CHAIN SURFACE PATCHES

1	KV5E$MOUSE	:KTSLRPGKGSSDYEKK*	(SEQ ID NO: 40)
2	PL0101	:KTSLRPGKGSSEYEKK*	(SEQ ID NO: 41)
3	N$1F19L	:QTSLRPDKGSSDHEKK*	(SEQ ID NO: 42)
4	KV5U$MOUSE	:QTSLRPDKGSSDQEKK*	(SEQ ID NO: 43)
5	MUSIGLDD	:QSSLRPDKGSSDQEKK*	(SEQ ID NO: 44)
6	PL0220	:QTSLRPDKGSSDPEKK*	(SEQ ID NO: 45)
7	KV5J$MOUSE	:QTSLRPDKGSSDPZKK*	(SEQ ID NO: 46)
8	MUSIGKABB	:QTSLRPDKGSSDPEKT*	(SEQ ID NO: 47)
9	MUSIGKCLG	:QTSLRADKGSSDQEKK*	(SEQ ID NO: 48)
10	MUSIGGVJ2	:QTSLRPDKGKSDSEKK*	(SEQ ID NO: 49)
11	MUSIGKCRN	:QTSLRPARGSSDQEKK*	(SEQ ID NO: 50)
12	MUSIGKCLP	:QTSLKPGRGSSDPEKK*	(SEQ ID NO: 51)
13	MUSIGKACM	:QTSLRPGRGSSDTEKK*	(SEQ ID NO: 52)
14	MUSIGKABE	:QISLRPGKGSSDSEKK*	(SEQ ID NO: 53)
15	KV5P$MOUSE	:QTSLRPGKGDSDEDKK*	(SEQ ID NO: 54)
16	MUSIGKCMK	:ETALRPGKGASDADKK*	(SEQ ID NO: 55)
17	KV3D$MOUSE	:VTALRPGKGASDEDKK*	(SEQ ID NO: 56)
18	MUSIGKAAW	:VTALRPGKGASDEEKK*	(SEQ ID NO: 57)
19	KV3G$MOUSE	:VTALRPGKGASBABKK*	(SEQ ID NO: 58)
20	KV3E$MOUSE	:VTALRPGKGASDEDDE*	(SEQ ID NO: 59)
21	MUSIGKAAZ	:QTSLRPDKGSSDQETT*	(SEQ ID NO: 60)
22	MUSIGKCNE	:QNSLTPGKGSSSPEKK*	(SEQ ID NO: 61)
23	MUSIGKBA	:VTKVRPGKGDSDSDKK*	(SEQ ID NO: 62)
24	KV5A$MOUSE	:VTKVRPGKGDSDAEKK*	(SEQ ID NO: 63)
25	MUSIGKV	:VTRVRPGKGDSDAEKK*	(SEQ ID NO: 64)
26	MUSIGKCNM	:LTKVRPGKGDSDSEKK*	(SEQ ID NO: 65)
27	MUSIGKCL1	:VTKVRPGKGDSDSEQK*	(SEQ ID NO: 66)
28	KV5B$MOUSE	:VTKVRPEKGDSDAEKK*	(SEQ ID NO: 67)
29	MUSIGKCSA	:VTKVRPEKGDSDSEKK*	(SEQ ID NO: 68)
30	MUSIGKCSR	:VTKVSPGKGDSDAEKK*	(SEQ ID NO: 69)
31	MUSIGKCST	:VTKVRSGKGESDAEKK*	(SEQ ID NO: 70)
32	MUSIGKAB	:VTSVKPGKGDSDAEKK*	(SEQ ID NO: 71)
33	PL0014	:VSSVKPGKGDSDAEKK*	(SEQ ID NO: 72)
34	MUSIGKACU	:VTSAKPGKGDSDAEKK*	(SEQ ID NO: 73)
35	PS0023	:VSSAKPGKGDSDAEKK*	(SEQ ID NO: 74)
36	N$2MCPL	:VTSARPGKGDSDAEKK*	(SEQ ID NO: 75)
37	MUSIGKADV	:VSPAKPGKGDSDAEKK*	(SEQ ID NO: 76)
38	MUSIGKCPP	:VTKARPGKGDSDVEKN*	(SEQ ID NO: 77)
39	MUSIGLDB	:VTLIPPGKGDSDAEKK*	(SEQ ID NO: 78)
40	MUSIGKCNS	:VTLLQPGKGDSDAEKK*	(SEQ ID NO: 79)
41	B27887	:VTLLQPGKGDSDADKK*	(SEQ ID NO: 80)
42	H28840	:VTLLQPGKGDSDAERK*	(SEQ ID NO: 81)
43	KV2G$MOUSE	:VTLLQAGKGDSDAEKK*	(SEQ ID NO: 82)
44	C27887	:VTLLQPGEGDSDAEKK*	(SEQ ID NO: 83)
45	JL0029	:LTLLQPGNGDSDAEKK*	(SEQ ID NO: 84)
46	MUSIGKAEH	:VTLLQPGKGDSDAEKI*	(SEQ ID NO: 85)
47	PS0074	:VTLPQPGQGDSDPEKK*	(SEQ ID NO: 86)
48	MUSIGKCNY	:VTLPQPGKGDSDAEKK*	(SEQ ID NO: 87)
49	MUSIGKCNX	:VTLPQPGKGDWDAEKK*	(SEQ ID NO: 88)
50	KV2D$MOUSE	:VTFLSPGQGDSDAEKK*	(SEQ ID NO: 89)

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51	MUSIGKADW	:ESSARPGKGDSDAEKK*	(SEQ ID NO: 90)
52	KV2A$MOUSE	:VTLSSPGQGDSDAEKK*	(SEQ ID NO: 91)
53	KV1A$MOUSE	:VTTAKPEKGDSDVEKK*	(SEQ ID NO: 92)
54	F30534	:VTTPKPDKGDSDVEKK*	(SEQ ID NO: 93)
55	MUSIGKCLO	:VTAPRPGKGASSAEKK*	(SEQ ID NO: 94)
56	G27887	:VTAPKPGKGTSSAEKK*	(SEQ ID NO: 95)
57	MUSIGVKV3	:VTTPKPGKGASSAEKK*	(SEQ ID NO: 96)
58	MUSIGKCNA	:VSAPKPGKGASSAEKK*	(SEQ ID NO: 97)
59	S03410	:VTAPRSGKGASSAEKK*	(SEQ ID NO: 98)
60	B32456	:VTAPKSGKGASSAEKK*	(SEQ ID NO: 99)
61	PL0013	:VTAPKPDKGVSSAEKK*	(SEQ ID NO: 100)
62	MUSIGLAET	:VTAPKSEKGVSSAEKK*	(SEQ ID NO: 101)
63	MUSIGVKV1	:FTAPKPGKGASSAEKK*	(SEQ ID NO: 102)
64	KV6K$MOUSE	:LTAPKPGRGVSSAEKK*	(SEQ ID NO: 103)
65	G30560	:VTAPKSGKGASSAEKR*	(SEQ ID NO: 104)
66	MUSIGKBO	:VSAPKPGKEGSSAEKK*	(SEQ ID NO: 105)
67	MUSIGKCNB	:VTAPKPRKGASSAEKK*	(SEQ ID NO: 106)
68	H33730	:VTFLSPGQGNSDAELP*	(SEQ ID NO: 107)
69	MUSIGKCPC	:VTFLSPGQGNSDEDLP*	(SEQ ID NO: 108)
70	KV2C$MOUSE	:VTLSSPQRGDSDAEKK*	(SEQ ID NO: 109)
71	MUSIGLAV	:VTAPKSSKGGSSAEKK*	(SEQ ID NO: 110)
72	MUSIGKCNH	:QTSPTPGKGSSDPEKK*	(SEQ ID NO: 111)
73	KV5R$MOUSE	:QISLIPGKGSYDDEKK*	(SEQ ID NO: 112)
74	KV6E$MOUSE	:VTALKSGKGASSAEKK*	(SEQ ID NO: 113)
75	MUSIGKCNI	:VTALKSDKGASSGEKK*	(SEQ ID NO: 114)
76	MUSIGLDA	:VTPPSPGQGDSAAEKK*	(SEQ ID NO: 115)
77	C26317	:VTPPSPGQGDSAREKK*	(SEQ ID NO: 116)
78	PS0073	:VTVRKPGKGDSSDEKK*	(SEQ ID NO: 117)
79	A23986	:QTSVRLGQGSSDPEKK*	(SEQ ID NO: 118)
80	MUSIGKABW	:KTSLRPWKGSSDSDKK*	(SEQ ID NO: 119)
81	KV5D$MOUSE	:QTDVTQGQGSSQPEKK*	(SEQ ID NO: 120)
82	MUSIGE6L	:QTAVSQGQGSSQSEKK*	(SEQ ID NO: 121)
83	MUSIGKCOE	:LTAPRTNRGSSDSEKK*	(SEQ ID NO: 122)
84	MUSIGKCKN	:VTAPSSHRGSSDTEKK*	(SEQ ID NO: 123)
85	MUSIGLVD	:LLSLSPLKGDSDPEKV*	(SEQ ID NO: 124)
86	S06822	:VTAPTPDTGAIKTEKL*	(SEQ ID NO: 125)
87	S06821	:VTIPTPDTGAIKTEKL*	(SEQ ID NO: 126)
88	MUSIGLAS	:AVSPTPDTGAIKTEKL*	(SEQ ID NO: 127)
89	MUSIGLAR	:AVSPTPDTGAIKTEKL*	(SEQ ID NO: 128)
90	LV2B$MOUSE	:AVSPTPDTGVIKTEKL*	(SEQ ID NO: 129)
91	MUSIGLAN	:AVSPTPDTGAIKTEPS*	(SEQ ID NO: 130)

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HUMAN LIGHT CHAIN SURFACE PATCHES

1	LV4A$HUMAN	:YLPPTPGVIRSTAMKL*	(SEQ ID NO: 131)
2	LV4B$HUMAN	:YLPPTPGVIRSTAMRL*	(SEQ ID NO: 132)
3	LV4E$HUMAN	:YLPPTPGLIRSTSMKL*	(SEQ ID NO: 133)
4	LV4D$HUMAN	:YLPPTPGLIRSTSVKL*	(SEQ ID NO: 134)
5	LV4C$HUMAN	:YLPPTPGVIRSTAEKL*	(SEQ ID NO: 135)
6	LV5A$HUMAN	:YLPPTPGVIRSTAGKL*	(SEQ ID NO: 136)
7	LV7A$HUMAN	:YLPATPGVVRSSAGML*	(SEQ ID NO: 137)
8	LV2G$HUMAN	:SLPPSPGKVRSTAEKL*	(SEQ ID NO: 138)
9	LV2I$HUMAN	:SLPPSPGKVRSTANKL*	(SEQ ID NO: 139)
10	N$2RHE	:SLPPRPGKVRSSSEKL*	(SEQ ID NO: 140)
11	HUMIGLAN	:SLPPRPGKVRSSSDKL*	(SEQ ID NO: 141)
12	LV1A$HUMAN	:SLPPRPGRVRSSSEKL*	(SEQ ID NO: 142)
13	LV1B$HUMAN	:SLPPRPGKVRSSSEQL*	(SEQ ID NO: 143)
14	LV1F$HUMAN	:SLPPRPGKVRSSSETL*	(SEQ ID NO: 144)
15	LV1C$HUMAN	:SLPPKPGKIRSSTGKL*	(SEQ ID NO: 145)
16	A29700	:SLPPKPGRIRSSTGKL*	(SEQ ID NO: 146)
17	HUMIGLAM4	:SLPPKPGKIRSSTGQL*	(SEQ ID NO: 147)
18	LV1D$HUMAN	:SLPPEPGKIRSSTGRL*	(SEQ ID NO: 148)
19	LV2K$HUMAN	:SLAPSPGKIRSTAEKL*	(SEQ ID NO: 149)
20	LV1I$HUMAN	:SLPPRPGKIRSSTGNV*	(SEQ ID NO: 150)
21	LV2E$HUMAN	:SLRPSPGKVRSTAEKL*	(SEQ ID NO: 151)
22	LV2D$HUMAN	:SLRPSPGKVRSTADKL*	(SEQ ID NO: 152)
23	LV2C$HUMAN	:SLRPSPGKVRSTAENL*	(SEQ ID NO: 153)
24	LV2J$HUMAN	:SLRPSPGKVRSAVEKL*	(SEQ ID NO: 154)
25	LV1E$HUMAN	:SLPPRPGK-RSSAEKL*	(SEQ ID NO: 155)
26	LV2B$HUMAN	:SLAPSPGKVRSTVERL*	(SEQ ID NO: 156)
27	N$1MCMW	:SLAPSPDKIRSTPDKL*	(SEQ ID NO: 157)
28	LV2H$HUMAN	:SLALSPGKVRSTAEKL*	(SEQ ID NO: 158)
29	N$3MCG2	:SLPLSAGKVRSTAEKL*	(SEQ ID NO: 159)
30	LV2A$HUMAN	:SLAPSPGKVRSTAEYL*	(SEQ ID NO: 160)
31	S02083	:SLPLTPGLIRSTAEKL*	(SEQ ID NO: 161)
32	HUMIGLAM2	:SLPLTPRVIRSTAEKL*	(SEQ ID NO: 162)
33	LV6C$HUMAN	:FLHPTPGTDSSSTEKL*	(SEQ ID NO: 163)
34	LV6D$HUMAN	:FLLPTPGTDSSSTERL*	(SEQ ID NO: 164)
35	LV6E$HUMAN	:FLHPTRVTDSSSTEKL*	(SEQ ID NO: 165)
36	LV6B$HUMAN	:LLPPTPGTNSSSNDKL*	(SEQ ID NO: 166)
37	HUMIGLK5G	:VLPLSPHRIRSESENL*	(SEQ ID NO: 167)
38	HUMIGLVC	:SLAPSPAKVRSTAERD*	(SEQ ID NO: 168)
39	HUMIGVLLS	:VTAPRPGRIRSDPEKK*	(SEQ ID NO: 169)
40	HUMIGKAX	:VTAPRPGRVRSDPEKK*	(SEQ ID NO: 170)
41	E30609	:VTGPRPGRIRSDPEKK*	(SEQ ID NO: 171)
42	KV3B$HUMAN	:VTGPRPGRIRSDPDKK*	(SEQ ID NO: 172)
43	G30607	:VTGPRPGRVRSDPEKK*	(SEQ ID NO: 173)
44	KV3M$HUMAN	:VTGPRPGRIRSDPXKK*	(SEQ ID NO: 174)
45	KV3H$HUMAN	:VTAPRPGRIRSESERK*	(SEQ ID NO: 175)
46	KV3K$HUMAN	:VTGPSRGRIRSDPEKK*	(SEQ ID NO: 176)
47	KV3F$HUMAN	:VTVPRPSRIRSESERK*	(SEQ ID NO: 177)
48	B26555	:VTAPGPGRIRSESERK*	(SEQ ID NO: 178)
49	KV1Q$HUMAN	:QTSVRPGRVRSDPERK*	(SEQ ID NO: 179)
50	KV1W$HUMAN	:QTSVRPGKVRSDPERK*	(SEQ ID NO: 180)

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51	KV1M$HUMAN	:QTSVRPGKVRSDPEKK*	(SEQ ID NO: 181)
52	KV1R$HUMAN	:QTSVRPGKVRSEPEKK*	(SEQ ID NO: 182)
53	KV1F$HUMAN	:QTSVRPGKVRSEPDKK*	(SEQ ID NO: 183)
54	KV1G$HUMAN	:QTSVRPGKVRAEPEKK*	(SEQ ID NO: 184)
55	KV1K$HUMAN	:QTSVRPGKVRSBPZKK*	(SEQ ID NO: 185)
56	KV1D$HUMAN	:QTSVRPGKVRSDPBKK*	(SEQ ID NO: 186)
57	KV1H$HUMAN	:QTSVRPGQVRSDPERK*	(SEQ ID NO: 187)
58	KV1B$HUMAN	:QTSVRPGKVRSHPEKK*	(SEQ ID NO: 188)
59	B27585	:QTSVRPGNVRSDPDKK*	(SEQ ID NO: 189)
60	N$1REIA	:QTSVRPGKVRSDPEKT*	(SEQ ID NO: 190)
61	KV1X$HUMAN	:QTSVRPGTVRSEPEKK*	(SEQ ID NO: 191)
62	KV1L$HUMAN	:QTSVRPEKVRSEPDKK*	(SEQ ID NO: 192)
63	IMGL38	:QTSVRPGKVRSESDKK*	(SEQ ID NO: 193)
64	A27585	:QTSVRPGEVRSEPDKK*	(SEQ ID NO: 194)
65	KV1N$HUMAN	:QTSVRPGBVRSBPZRK*	(SEQ ID NO: 195)
66	KV1C$HUMAN	:QTSVSPGKVRSDPEKK*	(SEQ ID NO: 196)
67	KV1V$HUMAN	:QTSVRPGKVNSDPEKK*	(SEQ ID NO: 197)
68	KV1T$HUMAN	:QTSVRPGKVRSDPDTK*	(SEQ ID NO: 198)
69	KV1U$HUMAN	:QTSVRPKKVRSDPZKK*	(SEQ ID NO: 199)
70	KV1A$HUMAN	:QTSVRPKKVRFDPEKK*	(SEQ ID NO: 200)
71	KV1S$HUMAN	:QTSVRSGKVRSEPETK*	(SEQ ID NO: 201)
72	KV4A$HUMAN	:VTNLRPGKVRSDAEKK*	(SEQ ID NO: 202)
73	KV4C$HUMAN	:VTDLRPGKVRSDAEKK*	(SEQ ID NO: 203)
74	HUMIGK2A1	:QTSVSPGNIRSESDKK*	(SEQ ID NO: 204)
75	HUMIGKBA	:KTSVTPGKFRSEPEKK*	(SEQ ID NO: 205)
76	HUMIGKBC	:VTLLPPGRVRSDAEKK*	(SEQ ID NO: 206)
77	KV2B$HUMAN	:VTLLPPGEVRSDAEKK*	(SEQ ID NO: 207)
78	KV2D$HUMAN	:VTLPPPGZVRSDAERK*	(SEQ ID NO: 208)
79	KV2C$HUMAN	:VTLPPPGZVRSBAZNK*	(SEQ ID NO: 209)
80	KV2E$HUMAN	:VTLPPPQQVRSDAEKK*	(SEQ ID NO: 210)
81	S03876	:VTLPPPGQVTSDAEKK*	(SEQ ID NO: 211)
82	KV2A$HUMAN	:VTLPPAGQVRSDAEKR*	(SEQ ID NO: 212)
83	HUMIGLAM5	:ALSPSSGQSSSASERL*	(SEQ ID NO: 213)

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MOUSE HEAVY CHAIN SURFACE PATCHES

1	MUSIGHIT	:EKVGGLQPGRGTPGKASRGDSQRPES*	(SEQ ID NO: 214)
2	MUSIGHIU	:EKVGGLQPGRGTPGKVSRGDSQRPES*	(SEQ ID NO: 215)
3	MUSIGHIV	:EKVGGLQPGTGAPGKASRGDSQRPES*	(SEQ ID NO: 216)
4	MUSIGHYM	:EKVGGLQPGRGTPGKASKGNSQRAES*	(SEQ ID NO: 217)
5	PU0003	:EKMGGLQPGRGTPGKASKGNSQRAES*	(SEQ ID NO: 218)
6	MUSIGHFO	:EKVGGLQPGRGTPGKASKGTSQRAES*	(SEQ ID NO: 219)
7	A30515	:EKVGGLQPGRGTPGKASKGTSQRAET*	(SEQ ID NO: 220)
8	PL0018	:EKVGGLKPGRGTPGKASKGTSQRAET*	(SEQ ID NO: 221)
9	MUSIGHFK	:ENVGGLQPGRGTPGKASKGTSQRAET*	(SEQ ID NO: 222)
10	MUSIGHFQ	:EKVGGLQSGRGTPGKASKGTSQRAET*	(SEQ ID NO: 223)
11	PU0001	:EKVGGLQSGRGTPGKASKGTSQRAES*	(SEQ ID NO: 224)
12	E30540	:EKVGGLQPGRGTPGKASKGISQRAER*	(SEQ ID NO: 225)
13	HV17$MOUSE	:EKVGGLQPGRGTPGKSAKGBSZRAQS*	(SEQ ID NO: 226)
14	MUSIGHLN	:EKVGGLQPGSGTPGKASKGNSQRAES*	(SEQ ID NO: 227)
15	MUSIGHKG	:EKVGGLQPGSGTPGKASKGSSQRAES*	(SEQ ID NO: 228)
16	PU0004	:EKVGGLQPGRGTPRKASKGNSQRAES*	(SEQ ID NO: 229)
17	MUSIGHKJ	:EKMGNLQPGSGTPGKASKGNSQRPDS*	(SEQ ID NO: 230)
18	HV56$MOUSE	:EKVGGLKPGKGTPEKDSKGNARRSET*	(SEQ ID NO: 231)
19	C27888	:EKVGGLKPGKGAPEKDSKGNARRSET*	(SEQ ID NO: 232)
20	MUSIGHAAF	:EKVGGLKPGKGTPERDSKGNARRSET*	(SEQ ID NO: 233)
21	PH0097	:DKVGGLKPGKGTPEKDSKGNAKRSET*	(SEQ ID NO: 234)
22	E27888	:DKVGGLKPGKGTPEKDSKGNAKKSET*	(SEQ ID NO: 235)
23	MUSIGHJB	:DKVGGLKPGKGTPDKDNKGNAKKSET*	(SEQ ID NO: 236)
24	MUSIGHADL	:EKVGGLTPGKGTPEKDSKGNGRRSET*	(SEQ ID NO: 237)
25	A27888	:EMVGGLKPGKGTPEKDSKGNDRRSET*	(SEQ ID NO: 238)
26	H27887	:EMVGGLKPGKGTPEKDSKGNDKRSET*	(SEQ ID NO: 239)
27	B27888	:EMVGGLKPGKGTPEKDSKGNAKRSET*	(SEQ ID NO: 240)
28	B27889	:EQVGGLKPGKGTPEKDSKGNAKKSET*	(SEQ ID NO: 241)
29	D27889	:EQVGGLKPGKGTPEKDTKGNAKKSET*	(SEQ ID NO: 242)
30	HV55$MOUSE	:EQVGGLKPGKGAPEKDTKGNAKKSET*	(SEQ ID NO: 243)
31	MUSIGHAGT	:EKVGGLQPGKGTPEKDSKGNAKKSET*	(SEQ ID NO: 244)
32	MUSIGVH50	:EKVGGLQPGKGTPEKDTKGNAKKSET*	(SEQ ID NO: 245)
33	MUSIGHIW	:EKVGGLQPGRGTPEKDTKGNAKKSET*	(SEQ ID NO: 246)
34	MUSIGHAGZ	:EKVGGLQPGKGSPEKDSKGNAKKSET*	(SEQ ID NO: 247)
35	PH0098	:DKMGGLKPGKGTPEKDSKGNAKQSET*	(SEQ ID NO: 248)
36	MUSIGHID	:EQVGGLQPGKGTPDKDSKGNAKKSET*	(SEQ ID NO: 249)
37	MUSIGHAGZ	:EKVGGLQPGKGTPEKDSKGNAKKSET*	(SEQ ID NO: 250)
38	MUSIGHMF	:EQVGDLKPGKGTPEKDTKGNARRSET*	(SEQ ID NO: 251)
39	D27888	:EMVGDLKPGKGAPEKDSKGNARRSET*	(SEQ ID NO: 252)
40	MUSIGHIP	:EQVGGLQPGKGTSDKDSKGNAKKSET*	(SEQ ID NO: 253)
41	MUSIGHAGS	:EQVGGLQPGKGTPEKDSKGNAKKSGT*	(SEQ ID NO: 254)
42	HV16$MOUSE	:DQVGGLQPGKGTPEKDTKGNPKRSET*	(SEQ ID NO: 255)
43	B34871	:DQVGGLQPGQGTPEKDTKGNPKRSDT*	(SEQ ID NO: 256)
44	PH0094	:EKVGGLQPGKGTSEKDIKGNAKKSET*	(SEQ ID NO: 257)
45	PH0096	:DKVGGLKPGKRTPEKDNKGNAKKSET*	(SEQ ID NO: 258)
46	MUSIGVH62	:DKVGGLKLGKGTPEKDTKGNAKKSET*	(SEQ ID NO: 259)
47	MUSIGHAGR	:EKVGGLQPGKGTPEKDSKGNAMTSET*	(SEQ ID NO: 260)
48	HV58$MOUSE	:EMVGGLKPGKGTPEKDSKGNAGRSET*	(SEQ ID NO: 261)
49	H27888	:EQVGGLQPGNGTPEKDTTGNAKRSET*	(SEQ ID NO: 262)
50	HV34$MOUSE	:EKEGGLQPGKGTPEKESKGDSKRAET*	(SEQ ID NO: 263)

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51	HV33$MOUSE	:EKEGGLQPGKGTPEKESKGDSKRPET*	(SEQ ID NO: 264)
52	MUSIGHZAB	:EKEGGLQPGKGSPEKESKGDSKRAET*	(SEQ ID NO: 265)
53	N$4FABH	:EKDGGLQPGKGTPEKDSKGDSKRVEM*	(SEQ ID NO: 266)
54	I27888	:EQVGGLKPGRGTPEKDTTGDAQRSET*	(SEQ ID NO: 267)
55	G27888	:EQVGGLKPGRGTPEKDTTGNAKGSET*	(SEQ ID NO: 268)
56	HV59$MOUSE	:EKVGGSKPGKGTPEKDSKGNAKTSET*	(SEQ ID NO: 269)
57	MUSIGHOE	:SDQGGLKPGKGTPEKDTKGNARRSES*	(SEQ ID NO: 270)
58	N$2FVWH	:EKIGGLQPGKGDPGKPSKDNAKRSET*	(SEQ ID NO: 271)
59	MUSIGHJT	:EKLGGLQPGKGDPGKPSKDNAKRSET*	(SEQ ID NO: 272)
60	MUSIGHLY	:EKLGGLQPGKGDPGKPFKDNAKRSET*	(SEQ ID NO: 273)
61	S06816	:EKLGGLQPGKGDPGKLMKENAKRSET*	(SEQ ID NO: 274)
62	S06817	:ENLGGLQPGKGDPGKLKXENAKRPET*	(SEQ ID NO: 275)
63	MUSIGHAAI	:EKLGGLQPGNGDLGKPSKDNAKRSET*	(SEQ ID NO: 276)
64	HV42$MOUSE	:EKLGPLQLGKGDPGKPSKDDAKRSET*	(SEQ ID NO: 277)
65	MUSIGHAAL	:EQLGGLQPGGGTPGKPSKDNDKRSET*	(SEQ ID NO: 278)
66	MUSIGHABO	:EQLGGLQPGGGTPGKASKDNDKRSET*	(SEQ ID NO: 279)
67	MUSIGHEG	:EQVGGLKARKGTPEKDTTGNAKRSET*	(SEQ ID NO: 280)
68	MUSIGHWN	:EMVGVLEPGKGTPEKRQEGNAKRSET*	(SEQ ID NO: 281)
69	MUSIGKCLT	:EQVGGLQPKKGSPGKDSKDDSQKTET*	(SEQ ID NO: 282)
70	MUSIGHZAE	:EQVGGLQPKKGSPGKDSKDDSQKTER*	(SEQ ID NO: 283)
71	MUSIGHAAD	:QQVPELKPGRGTPGKEDKGTSARNDT*	(SEQ ID NO: 284)
72	MUSIGHAAW	:QQVPELKPGKGTPGKDDKGTSAKNET*	(SEQ ID NO: 285)
73	MUSIGHAMA	:QQVPELKPGKGTPGKDDKGTSAKMEM*	(SEQ ID NO: 286)
74	MUSIGHXZ	:QQKPELKPGKGSPGQEKKGTSSTSET*	(SEQ ID NO: 287)
75	A30502	:EQQPELKPGKGTPGQEKKGKSSTSES*	(SEQ ID NO: 288)
76	MUSIGHAAG	:EQQPELRPGKGTPGQEKKGKSSTSES*	(SEQ ID NO: 289)
77	B30502	:EQQPELKPGKGTPGQEKKGKSSASES*	(SEQ ID NO: 290)
78	MUSIGHADG	:EQQPELKPGKGTPGKQKKGKSSTSES*	(SEQ ID NO: 291)
79	MUSIGHFV	:EQQPELKPGKGTHGKQKKGKSSTSES*	(SEQ ID NO: 292)
80	MUSIGHAAMA	:EQQPELKPGKGSHGKQKKGKSSTSES*	(SEQ ID NO: 293)
81	MUSIGHZR	:EQQPELKPGKGSHGKQKKGKSSASES*	(SEQ ID NO: 294)
82	MUSIGHAI	:EQQPELKPGKGTHGKQKKGKSSTTES*	(SEQ ID NO: 295)
83	MUSIGHALA	:EQQPELKPGKGTHGKQKQGKSSTTES*	(SEQ ID NO: 296)
84	PL0011	:EQQPELKPGKGTHGKEKKDKSSTSES*	(SEQ ID NO: 297)
85	MUSIGKCLS	:EQQAELKPGKGSHGKQKKGKSSTSES*	(SEQ ID NO: 298)
86	MUSIGHADY	:EQQPELKPGKGTHGKQKKSNSSTSES*	(SEQ ID NO: 299)
87	MUSIGHWX	:QQQAELRPGKGAPGQEKKGKSSTSES*	(SEQ ID NO: 300)
88	MUSIGHADO	:QQQAELRPGKGAPGQEKKGKSSTSDS*	(SEQ ID NO: 301)
89	MUSIGHVBM	:QQQAELRPGKGVPGQEKKGKSSTSDS*	(SEQ ID NO: 302)
90	A24672	:QQQPELKPGKGAPGKEKKGKSSTSES*	(SEQ ID NO: 303)
91	MUSIGHJG	:QQQPELKPGKGAPGKEKKDKSSTSES*	(SEQ ID NO: 304)
92	JL0044	:EQQPEAKPGKGTHGKQKKGKSSTSDS*	(SEQ ID NO: 305)
93	MUSIGHBA	:QQQAELKPGKGTHGKEKKDKSSTSDS*	(SEQ ID NO: 306)
94	MUSIGHAGP	:QQQAELRPGKGAPGQGKKGKSSTSES*	(SEQ ID NO: 307)
95	MUSIGHVBK	:QQQAELKPGRGTPGQEKKGKSSTSES*	(SEQ ID NO: 308)
96	A36194	:EQQAELRAGKGTPGQEKKGKSSTSES*	(SEQ ID NO: 309)
97	MUSIGHVBJ	:EQQAELRPGKGTPGQEKKGTSSTSES*	(SEQ ID NO: 310)
98	MUSIGHADV	:QQQAELRPGKGTPGHEKKGTSSTSES*	(SEQ ID NO: 311)
99	MUSIGHAAT	:QQQAELKPGKGTPGHEKKGTSSTSES*	(SEQ ID NO: 312)
100	MUSIGHJL	:QQQAELRPGKGTPGHENKGTSSTSES*	(SEQ ID NO: 313)

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101	MUSIGHABM	:QQQAEVRPGKGTPGHEKKGTSSTSES*	(SEQ ID NO: 314)
102	MUSIGHFU	:QQQAELKPGKGTPGHENKGTSSTSES*	(SEQ ID NO: 315)
103	MUSIGHZZB	:QQQAELRPGKGTPGQQKKGKSSASES*	(SEQ ID NO: 316)
104	HV06$MOUSE	:HQQAELKPGKGTPGQQKKGKSSTSES*	(SEQ ID NO: 317)
105	MUSIGHRD	:EQQVELRAGKGTPGQEKKGKSSTSES*	(SEQ ID NO: 318)
106	MUSIGHVBH	:EQQAELRPGKGTPGQEKQGTSSTSES*	(SEQ ID NO: 319)
107	HV01$MOUSE	:EQQAELRPGKGTPGHDNKGTSSTSES*	(SEQ ID NO: 320)
108	MUSIGHADN	:QQQAEVRPGKGTPGHEKKGRSSTSES*	(SEQ ID NO: 321)
109	HV05$MOUSE	:QQQAELRPGKGTPGQQKKDKSSTSES*	(SEQ ID NO: 322)
110	MUSIGHAEF	:QQQAELKPGKGTPGQQKKDKSSTSES*	(SEQ ID NO: 323)
111	MUSIGHAAN	:QQQAELKPGKGTPGQQKKDKSSTSDS*	(SEQ ID NO: 324)
112	MUSIGHAAB	:QQQAELKPGKGSPGQQKKDKSSTSES*	(SEQ ID NO: 325)
113	C30560	:QHQAELKPGKGTPGQQKKNKSSTSES*	(SEQ ID NO: 326)
114	PS0024	:QQQAELKPGKGTPGQQNKDKSSTSES*	(SEQ ID NO: 327)
115	MUSIGHRG	:EQQAELRAGKGIPGQEKKGKSSTSES*	(SEQ ID NO: 328)
116	MUSIGHAAE	:QQQAELKPGKGTPGQEKKSKSSTSES*	(SEQ ID NO: 329)
117	MUSIGHLX	:QQQSELKPGKGTPGQEKKSKSSTSES*	(SEQ ID NO: 330)
118	HV04$MOUSE	:QQQTELKPGKGTPGQEKKSKSSTSES*	(SEQ ID NO: 331)
119	MUSIGHVBG	:EQQAELRTGKGTPGQERKGKSSTSES*	(SEQ ID NO: 332)
120	MUSIGHMX	:QQQAELKPGKGTPGQQKKDKSSTFES*	(SEQ ID NO: 333)
121	MUSIGHAAR	:EQQAELRPGTGAPGQEKKGKSSTSES*	(SEQ ID NO: 334)
122	HV15$MOUSE	:QQQPEVRPGKGTHAKQKKGKSSTSES*	(SEQ ID NO: 335)
123	MUSIGHAAU	:QQQPEVRPGKDTHAKQKKGKSSTSES*	(SEQ ID NO: 336)
124	MUSIGHVBO	:QQQAELKPGKGTPEQEKKGKSSTSES*	(SEQ ID NO: 337)
125	A26405	:EQQTELRAGKGTPGQEKKGRSSTSEA*	(SEQ ID NO: 338)
126	HV10$MOUSE	:QQQAELKPGKGTPGREKKSKPSTSES*	(SEQ ID NO: 339)
127	MUSIGJB44	:QQQSELKPGKGTPGREKKSKPSTSES*	(SEQ ID NO: 340)
128	MUSIGJB62	:QQRAELKPGKDTPGREKKNKPSTSES*	(SEQ ID NO: 341)
129	HV09$MOUSE	:QQQAELKPGKGTPGREKKSTSSTSES*	(SEQ ID NO: 342)
130	MUSIGKCLP	:QQQAELKPGKGTPGQEKKSTSSTSDS*	(SEQ ID NO: 343)
131	MUSIGBH	:QQQAELRPGKGTPIQQKKDKSSTSES*	(SEQ ID NO: 344)
132	HV11$MOUSE	:QQQAEPKPGKGTPGREHRSKPSTSES*	(SEQ ID NO: 345)
133	MUSIGHMC	:QQQAELRPGKGALGQEKKGKSSTSDS*	(SEQ ID NO: 346)
134	MUSIGHAGW	:QQQPEVKPGKGAPGKGNTDKSSTSES*	(SEQ ID NO: 347)
135	MUSIGHRF	:EQQAEVRAGKGSPGQEKKGKSSTSES*	(SEQ ID NO: 348)
136	MUSIGHVAD	:QQLAELKPGKGTPGHEKKGISSTSES*	(SEQ ID NO: 349)
137	MUSIGHVAF	:QQQAELKPGKGKPEQEKKGTSSTSES*	(SEQ ID NO: 350)
138	PL0012	:QQQPELKPGKGRNGKENKGKSSTSES*	(SEQ ID NO: 351)
139	MUSIGGVD2	:QQQTELRPGRGTTGQERKGKSSTSES*	(SEQ ID NO: 352)
140	S06824	:QHQAELKPGKGTPGHENKVTSSTSES*	(SEQ ID NO: 353)
141	MUSIGHRB	:EQQAELRAGKGTPGQEQKAKSSTSES*	(SEQ ID NO: 354)
142	MUSIGHAAE	:QQQAELKPGKGTPGQQRTGTSSTTES*	(SEQ ID NO: 355)
143	MUSIGHHS	:QQQAELKPGKGNPGQEKKSTSSASES*	(SEQ ID NO: 356)
144	MUSIGHAXA	:EQQTVLRPGKGTPGQQKKGTSATNES*	(SEQ ID NO: 357)
145	HV50$MOUSE	:QQLTELKPGNGTPGQEKKSKSSTSES*	(SEQ ID NO: 358)
146	MUSIGHVBP	:QQQSVLRPGKGTPGQEKKGTSSTSKS*	(SEQ ID NO: 359)
147	PH0100	:LQQPVLKPGKGSHGKQKKDKSSTSES*	(SEQ ID NO: 360)
148	MUSIGHAYA	:EQQPETKPGKGTLGKQKKSKSSTSES*	(SEQ ID NO: 361)
149	MUSIGHCP2	:QQQAELKPGQGTPGQEKKNKSSTPEF*	(SEQ ID NO: 362)
150	MUSIGHDZ	:EQQAELRPGKGMPEQPKQGTSSTSET*	(SEQ ID NO: 363)

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151	MUSIGHNPI	:EQQAELRPGKGNPEQPKQGTSTTSET*	(SEQ ID NO: 364)
152	S06823	:EQQAELKPGKGNPEQPKQGTSSTSET*	(SEQ ID NO: 365)
153	MUSIGHASA	:EQQAELKPGKGNPEQPKQDTSSTSET*	(SEQ ID NO: 366)
154	S03484	:EQQAELKPGKGNPEQPKQGTSSTSGT*	(SEQ ID NO: 367)
155	MUSIGHVAA	:EQQAEVKPGKGNPEQPKQGTSSTSET*	(SEQ ID NO: 368)
156	MUSIGHNPD	:EQQAELRPGKGNPEQPKQVTSSTSET*	(SEQ ID NO: 369)
157	MUSIGHNPB	:EQQAELRPGKGNPEQPKQITSSTSET*	(SEQ ID NO: 370)
158	MUSIGHEC	:EQQAELRPGRGNPEQPKQVTSSTSET*	(SEQ ID NO: 371)
159	MUSIGHNPC	:EQQAELRPGRGNPEQPKHVTSSTSET*	(SEQ ID NO: 372)
160	MUSIGHNPF	:EQQAELRPGKGNTEQPKQVTSSTSET*	(SEQ ID NO: 373)
161	MUSIGHNPE	:EQQAELKPGKGNTEQPKLITSSTSET*	(SEQ ID NO: 374)
162	A27635	:TGQAELRPGKGAPEQGKKGKSSTSDR*	(SEQ ID NO: 375)
163	MUSIGHXW	:QYQAELRPGKGTPRQQKKGKSSTSES*	(SEQ ID NO: 376)
164	MUSIGHIZA	:QQQAVLRHGKGTHGQEKKGKSSTSES*	(SEQ ID NO: 377)
165	MUSIGHEH	:QQQTKLGPGRGTPGQGRKGKSSTSGS*	(SEQ ID NO: 378)
166	MUSIGHRH	:EQQAELRAGKGTPGQEKKGKSSVYPA*	(SEQ ID NO: 379)
167	HV00$MOUSE	:EQQAELKAGKGTPGQQKQGESTRSET*	(SEQ ID NO: 380)
168	N$1F19H	:QQKAELAASKGTPGQEKKGRSSTSES*	(SEQ ID NO: 381)
169	MUSIGHZAD	:QQQTELRPGKGTPGQEKRGKSSNLRL*	(SEQ ID NO: 382)
170	B30515	:EKVGGLQGSSFDPGKASKGTSQRAET*	(SEQ ID NO: 383)
171	MUSIGHEB	:EQQADLKLGKGNPEQPKLATPSTSET*	(SEQ ID NO: 384)
172	E27889	:EQVGGLKPGKGTPDKSDVKDNAKSET*	(SEQ ID NO: 385)
173	MUSIGHAAC	:DQQPDLKPSSGSPGHPSKSTSKTTET*	(SEQ ID NO: 386)
174	HV61$MOUSE	:DQQPDLKPSSGSPGNPSKSTSKTTET*	(SEQ ID NO: 387)
175	MUSIGVHR2	:DQQPDLKPSSGSPGNPSKSTSKTAET*	(SEQ ID NO: 388)
176	PL0100	:DQQPGLKPSSGSPGNPSKSTSKTTET*	(SEQ ID NO: 389)
177	MUSIGHAAO	:DQQPGLKPSSGSPGNPSKNTSKTTET*	(SEQ ID NO: 390)
178	MUSIGHGA6	:DQQPGLKPSSGSPGDPSKTTSKTTET*	(SEQ ID NO: 391)
179	MUSIGHJY	:DQQPGLKPSSGSPGNPSKTTSKTTET*	(SEQ ID NO: 392)
180	MUSIGHGA1	:DHQPGLKPSSGSPGNPSKNTSKTTET*	(SEQ ID NO: 393)
181	MUSIGHXX	:DQQPGLKPSSGSPGNPSRSTSKTTET*	(SEQ ID NO: 394)
182	HV62$MOUSE	:DQQPGLKPSAGSPGNPSKSTSKTAET*	(SEQ ID NO: 395)
183	MUSIGHAAGA	:EQQPGLKPSSGSPGNPSKSTSKTSET*	(SEQ ID NO: 396)
184	MUSIGHGA5	:DQQPGLKPSSGSPGNPSKNTSKTIET*	(SEQ ID NO: 397)
185	MUSIGHGA4	:DQQPGLKPSSGSPGDPSKNTSKTPET*	(SEQ ID NO: 398)
186	MUSIGHAGI	:EQQPSLKPSSGSPGNPSKSTSKTTET*	(SEQ ID NO: 399)
187	PL0102	:DQQPGLKPSSGSPGNPSKNTSETTET*	(SEQ ID NO: 400)
188	HV46$MOUSE	:DQQPGLKPSSGSPGNPSKNTSETTZT*	(SEQ ID NO: 401)
189	MUSIGHIT	:EQQPSLKPSSGSPGNPSKSTSKTSET*	(SEQ ID NO: 402)
190	MUSIGHAGD	:EQQPSLKPSSGSPGNPSKSTSKTTET*	(SEQ ID NO: 403)
191	MUSIGHAGQ	:EQQPSLKPSSGSPGNPSKSTSKTAET*	(SEQ ID NO: 404)
192	MUSIGAMJ2	:DQQPDLKPSSGFPGNPSKSTSKTTET*	(SEQ ID NO: 405)
193	MUSIGHAFX	:EQQPSLKPSSGSPGKPSKSTSKTNET*	(SEQ ID NO: 406)
194	MUSIGHAGE	:EQQPSLKPSSGSPGNPSKSTFKTSET*	(SEQ ID NO: 407)
195	MUSIGHAGB	:EQQPSLKPSSGSPGNPSKSTSTTSET*	(SEQ ID NO: 408)
196	MUSIGHAGC	:EQQLSLKPSSGSPGNPSKSTSKTTET*	(SEQ ID NO: 409)
197	MUSIGHAAM	:QQQPGLKPSFGPPGKPSQSTSKTTET*	(SEQ ID NO: 410)
198	HV43$MOUSE	:QQKPGLAPSSGSPGKSTKSNSKQTDT*	(SEQ ID NO: 411)
199	MUSIGMUV1	:QQKPGLAPSSGSPGKSARSNSKQTDT*	(SEQ ID NO: 412)
200	MUSIGHAEI	:QQKPGLAPSSGSPGKSAMSNSKQTDT*	(SEQ ID NO: 413)
201	MUSIGHBP	:QQKPGLAPSSGSPGKSAISNSKQTDT*	(SEQ ID NO: 414)
202	MUSIGHZZA	:QQKPGLQPSSGSPGKAAISNSKQSMT*	(SEQ ID NO: 415)
203	MUSIGMUV2	:QQKPGLQPSSGSPGKAAISNSKQAMT*	(SEQ ID NO: 416)
204	A32456	:QQKPVLAPSSGSPGKSAMSNSKQIDT*	(SEQ ID NO: 417)
205	MUSIGHMB	:QQKPSLQPSSDSPGKAAMSNSKQADT*	(SEQ ID NO: 418)

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HUMAN HEAVY CHAIN SURFACE PATCHES

1	HUMIGHVS	:ERVGDLEPGRGIPGKAPKGDSKKIET*	(SEQ ID NO: 419)
2	HUMIGHVR	:ERVGDLEPERGIPGKAPKGDSKKIET*	(SEQ ID NO: 420)
3	H36005	:EQVGGLKPGRGTPGKAPKGDSKKTET*	(SEQ ID NO: 421)
4	PL0122	:EQVGGLQPGKGTSGKASKGDSKKTET*	(SEQ ID NO: 422)
5	HV3D$HUMAN	:EQLGGLQPGRGTPGKBSKGDSKRAET*	(SEQ ID NO: 423)
6	HUMIGHAT	:EQLGGLQPGRGTPGKDSKGNSKRAET*	(SEQ ID NO: 424)
7	B34964	:EQLGGLQPGRGTPGKDSRGNSKRAET*	(SEQ ID NO: 425)
8	A34964	:EQVGGLQPGRGTPGKDSKGNSKRAET*	(SEQ ID NO: 426)
9	PL0123	:EQVGGLQPGRGTPGKDSKGNAKRAET*	(SEQ ID NO: 427)
10	HV3F$HUMAN	:EQVGGLQPGRGTPGKDSKGDSRRAET*	(SEQ ID NO: 428)
11	JL0048	:EQVGGLQPGRGTPGKDSKGNSRRAET*	(SEQ ID NO: 429)
12	HV3B$HUMAN	:QQVGGLEPGRGTPGKDSKGBSKRAET*	(SEQ ID NO: 430)
13	HUMIGHBV	:EQLGDLQPGRGTPGKASKGNSKRAET*	(SEQ ID NO: 431)
14	HV3E$HUMAN	:EQVGGLQPGRGTTGKDSKGDSKRAET*	(SEQ ID NO: 432)
15	PL0116	:QQVGGVQPGRGTPGKDSKGNSKRAET*	(SEQ ID NO: 433)
16	HV3K$HUMAN	:QQVGGVQPGRGIPGKDSKGNSKRPET*	(SEQ ID NO: 434)
17	N$2FB4H	:EQVGGVQPGRGIPGKDSKGDSKRPET*	(SEQ ID NO: 435)
18	HV3I$HUMAN	:QQVGGVQPGRGTPGKDSNGDSKRPET*	(SEQ ID NO: 436)
19	HV3J$HUMAN	:QKVGGVQPGRGTPGKDSKGNSKRTET*	(SEQ ID NO: 437)
20	HV3G$HUMAN	:QEVGGVZPGRGTPGKBSKGBSKRAET*	(SEQ ID NO: 438)
21	HV3M$HUMAN	:EQLGGLQPGRGTPGKDSNGDSKQAZT*	(SEQ ID NO: 439)
22	HV3O$HUMAN	:EQLGGLQPGRGSPGKDTNGDSKEAZT*	(SEQ ID NO: 440)
23	HV3N$HUMAN	:AQLGGLQPGRGTPGKDSNGDSKQAZS*	(SEQ ID NO: 441)
24	HV3R$HUMAN	:EQLGGLQPGRGTPGKVSQGDSKQAZT*	(SEQ ID NO: 442)
25	HV3P$HUMAN	:EQVGGLQPGRGTPGKVSQGDSKEPZT*	(SEQ ID NO: 443)
26	HUMIGHCV	:EQLGGLQPERGTPGKESKGNSKRAET*	(SEQ ID NO: 444)
27	HV3T$HUMAN	:EQVGDLQPGRGBPGKDSKGNAKRVET*	(SEQ ID NO: 445)
28	HV3U$HUMAN	:EQVGDLQPGRGNPGKDSKGNAQRPET*	(SEQ ID NO: 446)
29	PL0098	:QQVGGVQPGRGTLGKDSKGNSKRAET*	(SEQ ID NO: 447)
30	HV3H$HUMAN	:QZVGGAZPGRGSPGKASKGBSKRAET*	(SEQ ID NO: 448)
31	HV3A$HUMAN	:QQVGGLKPGRGSPGKDSKGNAQRTZT*	(SEQ ID NO: 449)
32	HV3S$HUMAN	:DQVGGLKPGRGTPGKNSNGDSKTPZT*	(SEQ ID NO: 450)
33	HUMIGHAW	:EQLGGLQPGRGTSREDSKGNSKRAET*	(SEQ ID NO: 451)
34	HV3Q$HUMAN	:EQVGALQPGRGTPGKDSQADSKEAZT*	(SEQ ID NO: 452)
35	A36040	:EQLGGLQPGRGTPGK----VEGSVET*	(SEQ ID NO: 453)
36	HUMIGHAB	:EQVGAFQPGRGNSGKASKGDSKRPDT*	(SEQ ID NO: 454)
37	HUMIGHAO	:EQVGAFQPGKGNSGKASKGDSKRPDT*	(SEQ ID NO: 455)
38	HUMIGHAR	:EQVGAFQPGKGNSGKASKGDSNRPDT*	(SEQ ID NO: 456)
39	HV3L$HUMAN	:QQVGGVQAGRANPGKDSRGISKRTET*	(SEQ ID NO: 457)
40	HV1A$HUMAN	:QQVAEVKPGKGTPGQQKQGESTRSET*	(SEQ ID NO: 458)
41	A32483	:QQVAEVKPGKGTPGQQKQGTSTRSET*	(SEQ ID NO: 459)
42	HUMIGHAY	:QQVAEVKPGKGTPGQQKQGTSARSET*	(SEQ ID NO: 460)
43	HUMIGHCU	:QQVAEVKPGKGTPGQQKQGTSIRSDT*	(SEQ ID NO: 461)
44	HUMIGHBS	:QQVAEVKPGKGTPGQEKQGTSIRSDT*	(SEQ ID NO: 462)
45	HUMIGVHLS	:QQVAEVKPGKGTPGQQMQGTSTRSDT*	(SEQ ID NO: 463)
46	HUMIGHBX	:QQVGEVKPGRGTPGQQKQDTSTRSDT*	(SEQ ID NO: 464)
47	HV1C$HUMAN	:QQVAEVKPGRGTPGHPRQGASTRSDS*	(SEQ ID NO: 465)
48	H34964	:QQVSELKPGKGTPGQQGTGTSVKAET*	(SEQ ID NO: 466)
49	HUMIGHCY	:EQVAEVKPGKGSPGKPSQGKSIKAST*	(SEQ ID NO: 467)
50	PL0119	:EQVAEVKPGRGSPGKPSQGKSIKAST*	(SEQ ID NO: 468)

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51	HV1F$HUMAN	:QQVAEVKPGRGDPGRPRQASSTISAT*	(SEQ ID NO: 469)
52	D34964	:EQVAEVPQGKGRPGKSLQGKSLKAST*	(SEQ ID NO: 470)
53	HV1D$HUMAN	:QQMAEVKPGRGTPGKPGVVPSFFSET*	(SEQ ID NO: 471)
54	HV1E$HUMAN	:QQVAEVKPGRGTPGRYIWEPSFFNEG*	(SEQ ID NO: 472)
55	JL0047	:QQQAGLKPSSGSPGKPSKSTSKTAAT*	(SEQ ID NO: 473)
56	HUMIGHBW	:QQQPGLKPSSGSPGKPSKSTSKTAAT*	(SEQ ID NO: 474)
57	E34964	:QQQPGLKPSSGSPGKPSKSTSNTAAT*	(SEQ ID NO: 475)
58	HUMIGHCW	:QQQPGLKPSSGSAGKPSKSTSKTAAT*	(SEQ ID NO: 476)
59	HV2F$HUMAN	:RQQPGLKPSSGPPGKPSRGTSRSAAT*	(SEQ ID NO: 477)
60	HV2I$HUMAN	:QQQAGLKPSSGSPGRTSKSTSKTAAT*	(SEQ ID NO: 478)
61	HV2G$HUMAN	:QQEPGLRPSSGTPGRTPRSTSKTAAT*	(SEQ ID NO: 479)
62	N$3FABH	:XQEPGLRPSSGSPGRTPRSTSKTAAT*	(SEQ ID NO: 480)
63	PS0091	:QQQPGLKPSSGSPSRVSKSTSKTPET*	(SEQ ID NO: 481)
64	HUMIGHDA	:QHQAGLKRSSGPPGKPSTSTSKTAAT*	(SEQ ID NO: 482)
65	A26555	:ZQESGLKPTSGSPGKPSKSRSKAADA*	(SEQ ID NO: 483)
66	HV2E$HUMAN	:QTKPTLKPTTGSPGRPSKSTSKDPVT*	(SEQ ID NO: 484)
67	HV2D$HUMAN	:QTKPTLKPTTGSPGKPSRSTSRDPVS*	(SEQ ID NO: 485)
68	A36005	:ETRPALKPTTGSPGKTSKTTSKDPVT*	(SEQ ID NO: 486)
69	HV2H$HUMAN	:QNRPALKATTGSPGKTSETTSKDPAT*	(SEQ ID NO: 487)
70	HV2A$HUMAN	:QTTPALKPKTGSPGKTSRTDSKNPVT*	(SEQ ID NO: 488)
71	HV2C$HUMAN	:QTRPALRPTTGSPGEASETTSKGPGT*	(SEQ ID NO: 489)
72	HV2B$HUMAN	:QTRPALKPTTGSPGKTSETTSRDTAY*	(SEQ ID NO: 490)
73	JL0049	:LEGVQLWGGRGISRKYAKGNGKRDES*	(SEQ ID NO: 491)

EXAMPLE 2

DETAILED DESCRIPTION OF METHOD FOR CONSTRUCTING THREE-DIMENSIONAL MODEL OF ANTIBODY VARIABLE REGION

The references cited in the text below are listed at the end of this Example.

The first antibody Fab structure was determined in 1972. Since then, no more than about twelve Fab structures have been published, a number that represents a very small fraction of the total antibody repertoire (>10^8 antibodies). To understand the molecular basis of this antibody diversity will require knowledge of either a large number of x-ray structures, or the rules by which combining site topography is governed. The development of such prediction rules has now reached the point where variable regions of antibodies can be modelled to an accuracy approaching that of the medium resolution x-ray structure.

The interaction of an antibody with its cognate antigen is one of the most widely accepted paradigms of molecular recognition. To understand the antibody-antigen interaction in atomic detail requires knowledge of the three-dimensional structure of antibodies and of their antigen complexes. Traditionally such information has come from x-ray crystallographic studies (see Davies et al. for review (Davies et al., 1988)).

The modelling of antibody combining sites was first attempted by Padlan & Davies (Padlan et al., 1976) at a time when very few antibody structures were known. Nonetheless, Padlan and colleagues recognized that the key lay in high structural homology that existed within the β-sheet framework regions of different antibody variable domains. The antigen combining site is formed by the juxtaposition of six interstrand loops, or CDRs (Complementarity Determining Regions) (Kabat et al., 1987), on this framework. If the framework could be modelled by homology then it might be possible to model the CDRs in the same way. Padlan and Davies (Padlan et al., 1976) reasoned that CDR length was the important determinant of backbone conformation though the number of antibody structures was insufficient to thoroughly test this maximum overlap procedure (MOP). This notion was not picked up again until the early 1980's when Pedersen and Rees proposed a similar approach to modelling antibody combining sites based on a more extensive analysis of antibody structures (de la Pas et al., 1986).

Those essentially knowledge-based procedures are best exemplified for antibodies by the work of Chothia & Lesk (Chothia et al., 1986) who, in 1986, extended and modified the MOP procedure by introducing the concept of "key" residues. These residues allow the further subdivision of CDRs of the same length into "canonical" structures which differ in having residues at specified positions that, through packing, hydrogen bonding or the ability to assume unusual values of the torsion angels ϕ, ψ and ω, determine the precise CDR conformation

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(Chothia et al., 1989). Similar knowledge-based methods have been proposed for predicting loop conformations in general (Thornton et al., 1988; Tramontano et al., 1989). These methods rely on the crystallographic database of protein structures. However, none of the above knowledge-based methods has been totally successful. In particular, the MOP or canonical structure approaches have succeeded in modelling only five of the six CDRs. This stems from the fact that the third CDR of the heavy chain, H3, is more variable in sequence, length and structure than any of the other CDRs.

To deal with this problem several groups have attempted to use *ab initio* methods to model the combining site (Bruccoleri and Karplus, 1987). The requirement with such methods is that the total allowable conformational space accessible to a particular CDR is sampled. Typical of purely geometric approaches is that of Go & Sheraga (Go and Sheraga, 1970) and more recently Palmer & Sheraga (Palmer and Sheraga, 1991), where the problem is reduced to one in which the central region of the polypeptide backbone, having characteristic bond length and bond angles, is constructed between the end points of the loop (CDR if an antibody loop) by a "chain closure" algorithm. In a modification of this algorithm, Bruccoleri & Karplus (Bruccoleri and Karplus, 1987) introduced an energy minimization procedure which greatly expanded the domain of conformational space searched during the chain closure procedure. This modification is incorporated into the conformational search program CONGEN (Bruccoleri and Karplus, 1987), which also allows the user to choose any set of standard bond length and bond angels such as the CHARMM (Brooks et al., 1983) standard geometry parameter sets. Other approaches such as minimization (Moult and James, 1986), or molecular dynamics (Fine et al., 1986) either fail to saturate conformational space or are unable to deal with the problem of long CDRs. Whichever of the *ab initio* methods is employed however, the problem is one of defining the selection criteria in such a way as to allow the unambiguous identification of the *correct* structure (in this context *correct* is defined by reference to an appropriate X-ray structure) within the ensemble of candidates, for every CDR. To date this has not been possible.

Recently a more holistic approach has been taken to the modelling of CDRs which combines the advantages of knowledge-based and *ab initio* methods in a single algorithm known as CAMAL (Combined Algorithm for Modelling Antibody Loops) (Martin et al., 1989; Martin et al., 1991). Previously this algorithm has been used to model individual CDRs in the presence of the crystal structure conformations of the other five. As is demonstrated below, CAMAL is able to predict the backbone conformations of all six CDRs of the antibody combining site to an accuracy approaching that of medium resolution x-ray structures. In addition the algorithm includes a procedure for selecting and fitting together the light and heavy chain framework regions prior to generation of CDR conformations, thus making possible the prediction of the entire variable region. Furthermore a new Monte Carlo (MC) simulated annealing method has been developed for the determination of sidechain conformations.

The Framework Region

Antibody framework regions consist of conserved β-strands that form the β-barrel structure characteristic of immunoglobulin V-type regions. In the procedure described here each V-region is built from a database of known antibody structures, using sequence homology for selection of the light (L) and heavy (H) chain V-domains. The two domains are then paired by least squares fitting on the most conserved strands of the antibody β-barrel (Table 2 and Figures 5 & 6. The strand orientations were determined by analyzing the barrels of known antibody crystal structures. Eight antibodies were analyzed using a multiple structure fitting program as follows. Seven structures were fitted onto one of the set selected at random and mean coordinates were calculated. All eight structures were then fitted onto these mean coordinates and new mean coordinates determined. This procedure was iterated until the mean coordinate set converged (5-10 cycles). The variance for the mean coordinates at each barrel point (N,Cα,C) was calculated. In Figure 5 this variance is plotted against the projected positions of these points onto the conjugate axis of the barrel.

Strand 8 and all but two residues of strand 7 in both light and heavy chains were eliminated as they showed deviations greater than 3σ (standard deviation units) from the mean coordinates. These two strands comprised the takeoff points of CDR H3, and suggests that any knowledge-based prediction of CDR H3 would have to account not only for sequence and length variation in the CDR itself, but also for the position of the participating strands. The remaining mean coordinates were used as a scaffold onto which the L and H chains were fitted. Strands 7 and 8 in the final framework were obtained from the database structure used in the construction. The framework strands are marked + in the multialignment in Table 2.

The sidechains were then replaced using a 'maximum overlap' method, in which sidechain templates were fitted on backbone atoms with the sidechain torsion angles being adjusted to match those of equivalent torsions in the parent sidechain.

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The Combining Site

The procedure for predicting the structure of combining sites combines a database search with a conformational search procedure. The architecture of the program suite to perform this task is outlined in Figure 7.

The database search utilizes distance constraints for each of the six CDR loops determined from known antibody structures. These constraints were determined by calculating Cα-Cα distances within known loops and using a search range of $\bar{x} + 3.5\sigma$ (the mean ± 3.5 standard deviation units). A database containing all the proteins in the Brookhaven Protein Databank (Bernstein et al., 1977) is then searched for fragments which satisfy the constraints for a loop of the required length. The middle section of the loop is then deleted and reconstructed using the conformational search program CONGEN (Bruccoleri and Karplus, 1987). For loops of six or seven residues, the structure database appears to saturate the conformational space available to the backbone adequately and only sidechains are built by conformational search. Loops shorter than six residues are built by conformational search alone since this is computationally feasible and the number of loops selected from the database becomes unacceptably large as loop length decreases.

When modelling a complete combining site, loops of 6 or more residues are modelled individually with the other loops absent. If the loops are built consecutively, small errors can accumulate leading to a poor result (Martin, 1990). All the loop conformations are then evaluated using a solvent modified potential, which excludes the attractive van der Waals and electrostatic terms of the non-bonded energy function contained within the GROMOS (Åqvist et al., 1985) potential. The lowest five energy conformations are selected and filtered using a "structurally determining residue" algorithm (FILTER), based on backbone torsion angles observed in the original database loops. Since the database search is not used for the shortest loops of 5 residues or fewer, the FILTER algorithm cannot be used. Energy is thus the only available selection criterion and the short loops are built last, in the presence of the longer loops.

Side Chains

The determination of sidechain positions was previously done using the iterative sidechain determination algorithm described by Bruccoleri et al. (Bruccoleri and Karplus, 1987). Unfortunately the CHARMM (Brooks et al., 1983) force field fails to select the correct conformations of exposed hydrophobic sidechains. There is no penalty for having an exposed uncharged atom, without solvent present. CONGEN is also unable to saturate the conformational space for a large number of sidechains (more than 6 residues).

Recently Lee et al. (Lee and Levitt, 1991; Lee and Subbiah, 1991) has proposed a method for searching conformational space for a large number of sidechains using MC simulated annealing. A simple energy function is used for the evaluation of conformations generated by a biased random walk:

$$E = \sum_{i=1}^{n} \epsilon_o \left(\left(\frac{r_o}{r}\right)^6 - 2\left(\frac{r_o}{r}\right)^{12} \right) + \kappa_o \cdot COS(3\omega)$$

Where the first term is a simple *Lennard-Jones* potential which evaluates the non-bonded contacts between the atoms in a given molecule, the second term is a simple torsional term which only applies to C-C bonds. The torsional term biases the function towards 60° rotamers. ε_o and κ_o are constants. The metropolis function:

$$P = C^{\frac{-\delta E}{T}}$$

is used to evaluate the energy function. Any move which results in a decrease in energy is accepted, and any move which results in a positive δE is only accepted with the probability P. This simple method can be used to search the large conformational space defined by a set of torsion angles in amino-acid sidechains, and find or define the global minimum which exist for a set of sidechains. T is the simulation temperature.

When searching sidechain conformations using this method the simulation system usually gets trapped in an energetic minima well before the global minimum is encountered, at a high temperature, without the solution space having been searched sufficiently. This problem can be solved by truncating the *Lennard-Jones* potential, thus allowing atoms to pass through each other. In reality this function would converge towards infinity when the distance r between the atoms approaches zero.

The evaluation of sidechain conformations generated is done solely on the basis of energy, for internal (core) residues, since good van der Waal's interactions are considered to be equal to a good packing of the sidechains. The situation becomes more complicated when trying to predict the conformation of surface residues. The lowest van der Waal's interaction is obtained by a combination of sidechain conformations which minimize the overlap of atoms, this means that the lowest energy is obtained with extended conformations of

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sidechains, without considering good packing of sidechains.

Using the fact that hydrophobic, bulky residues will be shielded by the hydrophilic sidechains, and will be buried in the surface, it is possible to generate a simple function which will evaluate these macroscopic observations. These functions can either be implemented in the objective evaluation function of the Monte Carlo simulation, or as is done here, added as a post processing step. Including an accessibility/hydrophobicity term in the evaluation function would slow down the calculation considerably, hence the term has been added as a post processing function. The function used is a sum of the product of relative exposed surface area multiplied by the residual hydrophobicities. The hydrophobicities used are taken from Cornette et al. (Cornette et al., 1987).

$$f_{conformation} = \sum_{i=1}^{n} -A_{irel} \cdot H_{irel}$$

n is the number of sidechains reconstructed. The surface area is calculated using the tesselated icosahedron approach (Chau and Dean, 1987), which is not very precise (0.1 percent), but is able to evaluate a large number of conformations. The function is evaluated for the final 2,000 conformations and the lowest value conformation selected as the best.

Using this simple approach it is possible to integrate over a large phase space with many degrees of freedom, and get a complete sampling of the space.

Predicted Structures of an Anti-hapten, Anti-peptide and Two Anti-protein Antibodies

In the following section the predicted structures of four different antibody F_V regions are presented and analyzed. The antibodies are:

- Gloop-2 (Darsley and Rees, 1985), an anti-lysozyme antibody whose Fab structure was determined by Jeffrey et al., (Jeffrey et al., 1991) and which was used as a learning exercise during the development of CAMAL.
- D1.3 (Amit et al., 1986), an anti-lysozyme antibody whose uncomplexed F_V coordinates were supplied by R. Poljak et al. after the model coordinates had been deposited.
- 36-71 (Rose et al., 1990), an anti-phenylarsonate antibody whose Fab structure was carried out by D. R. Rose, et al., and whose coordinates were obtained after the model coordinates had been deposited.
- 3D6 (Grunow et al., 1988), an anti-protein (GP41 of HIV) antibody whose Fab structure was carried out by D. Carter et al. (Carter, 1991) and whose coordinates were obtained after the model coordinates had been deposited. For this antibody, the model was generated using the canonical loop method of Chothia & Lesk (Chothia et al., 1989; Chothia et al., 1986) for CDRs L1, L2, H1 and H2, while L3 and H3, which cannot be modelled using canonical structures, were constructed using CAMAL.

All four models were subjected to both restrained and unrestrained energy minimization using the DISCOVER (TM Biosym Technology) potential with 300 cycles of steepest descents, followed by conjugate gradient minimization until convergence to within 0.01 Kcal occurred.

The resolution and R-factors of the x-ray structures are given in Table 3 together with the parent frameworks selected in building the models. The structures and models were compared by global fits of the loops. The β-barrel strands 1 to 6, as described above, were least squares fitted and the RMS deviation was then calculated over the loops. The backbone (N,Cα,C) RMS values for fitting model and crystal structure frameworks were between 0.4 and 0.9 Å, illustrating the conservation of the core β-barrel. Using all eight strands RMS deviations between 0.6 and 1.2 Å were observed.

Global fits (Table 4) give a more realistic measure of the accuracy of the model than a local least-squares fit over the loops since they account for the overall positioning of the loops in the context of the F_V structure. Local fits, which give lower RMS deviations, are also shown in Table 4. Differences between local and global RMS deviations arise from differences in V_H/V_L domain packing and differences in loop 'take off' angles and positions.

Table 5 shows the canonical loops selected from modelling 3D6. Backbone structures of the modelled CDRs, superimposed on the x-ray structures after global fitting are shown in Figure 8. General features and points of interest for each of the six CDRs are discussed below.

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Analysis of the CDR Regions

During the comparison of CDR conformations in the V-region models and the x-ray Fab structures it was observed that at certain positions in a CDR, the peptide backbone may adopt either of two conformations by undergoing a "peptide flip" (1,4 shift). This phenomenon is also seen in type 2 β-turns (Paul et al., 1990). Dynamics simulations of β-turns show that the transformation energy between $\phi 1 = -00$, $\psi 1 = -30$, $\phi 2 = -90$, $\psi 2 = 0$ and $\phi 1 = -00$, $\psi 1 = 120$, $\phi 2 = 90$, $\psi 2 = 0$ has a maximum value of 5 kcal (Paul et al., 1990). This is low enough to allow selection of either conformation. The peptide flip is observed within several canonical classes (as described by Chothia et al. (Chothia et al., 1989)) and the hydrogen bonding pattern used to determine the conformation of a canonical class does not disallow the peptide flip. Any modelling procedure should therefore take these, or any other multiple conformations, into consideration where the transformation energies are sufficiently low to permit population of the different conformational forms. Table 6 shows an example of the "peptide-flip" phenomenon from the crystallographic database of antibody structures. It should be noted that a single crystal structure will not show multiple conformations since the crystallization will 'freeze out' one of the conformations. During the modelling procedure the two populations of conformers are easily extracted from a set of *ab initio* generated loops, by using a torsional clustering algorithm.

CDR-L1

In Gloop-2 and D1.3, all five low energy conformations were very similar with RMS deviations differing by less than 0.25 Å (backbone) and 0.35 Å (all atoms). The FILTER algorithm was unable to distinguish between the conformations and the lowest energy structure was selected.

Although CDR-L1 of 3D6 was originally built using the canonical loop from HyHEL-10, the mid-section was rebuilt by conformational search, for the following reason. HyHEL-10 and REI CDR-L1 loops are placed in the same canonical ensemble (Chothia et al., 1989) although they contain a 1-4 shift (peptide flip) relative to one another between the fifth and eighth residues of the loop (residues 28-31) (see Table 6).

36-71 shows the same 1-4 shift between the model and crystal structure CDRs. Both crystal structure and model were compared with other loops of the same canonical class as defined by Chothia et al. (Chothia et al., 1989). It was found that the hydrogen bonding pattern which determines the conformation was conserved.

CDR-L2

CDR-L2 of D1.3 has two adjacent threonines (49, 50) which in the x-ray structure are packed against the tyrosine at the fourth position of CDR-H3, thus minimizing the exposed hydrophobic sidechains. In the unminimized model the threonine sidechains are exposed to the solvent, but after energy minimization, this packing is observed.

CDR-L3

In Gloop-2, D1.3 and 36-71 the proline at the seventh position in the loop is correctly predicted in the *cis* conformation. It has previously been suggested that the conformation of CDR-L3 is dictated by the presence of a proline in position 8 or 9 (Chothia et al., 1989) within the loop. 3D6 does not have a proline in either position. Only 7 out of 290 CDR-L3 sequences (Kabat et al., 1987) lack a proline at both positions and in all of the published x-ray structures this proline is present. This is an example of a situation where either a new canonical class may need to be defined or where the canonical rule breaks down altogether, and an alternative method must be employed.

The 3D6 L3 loop is 7 residues in length and was built using database loops alone where conformational space is saturated by means of fragments selected from the crystallographic database (Global RMS: 2.01 Å, N,Cα,C), and by using CAMAL (Construction: Q[Q(YNS)Y]S, Global RMS: 1.97 Å, N,Cα,C). The similarity of the structures generated by the two procedures illustrates the utility of the database search and suggests that, for shorter loops it is capable of saturating the available conformational space.

CDR-H1

Using the Kabat and Wu definition of CDR-H1 places this loop as an extension of the β-sheet. The extended nature of this stretch of peptide limits its conformational flexibility and CDR-H1 is generally modelled accurately (Martin et al., 1989; Chothia et al., 1989).

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In Gloop-2 and D1.3, the Phe or Tyr sidechain at the second position in the loop is poorly placed and packs against Leu at the penultimate position in HFR1 (see Table 2). 36-71 has a well-placed Asn at this position, rather than the more common bulky hydrophobic sidechain.

CDR-H2

CDR-H2 of 36-71 is similar in sequence to F19.9 (Strong et al., 1991), (36-71: YNNPGNGYIA (SEQ ID NO:492); F19.9: YINPGKGYLS (SEQ ID NO:493)). While the structurally determining residues specified by Chothia and Lesk (Chothia et al., 1989) are conserved, the backbone conformations are different: F19.9 has a bulge at the -PGN- Gly, compared with 36-71, giving the loop a 'kink' in the middle. The model of 36-71 shows a 1-4 shift, though the sidechains are still well placed.

In Gloop-2, the all atom RMS deviation is poor (3.00 Å) (Jeffrey et al., 1991) when compared with the $P2_1$ crystal structure, owing to rotations of the Phe at position 3 in the loop and Tyr at position 10 by approximately 120° about the χ_2 torsion angle. Gloop-2 has been solved in two different crystal forms, $P2_1$ and P1 (Jeffrey et al., 1991; Jeffrey, 1989). When compared with the P1 structure, the sidechains are placed almost perfectly and the all atom RMS (global fit) drops to 2.23 Å.

This concerted sidechain motion between crystal forms illustrates the effects of crystallization conditions on surface sidechain placement. Even though surface sidechains may show low temperature factors indicating low mobility in the crystal, their mobility in solution may be high. In the Gloop-2 P1 structure, the mean side-chain temperature factor for the F_V domain is 13.46 (σ = 8.20) while the sidechains of these two residues of H2 show mean temperature factors of 5.56 (σ = 0.68) for the Phe at position 3 and 7.10 (σ = 1.73) for the Tyr at position 10.

CDR-H3

CDR-H3 is the most variable of the six CDR's with all lengths up to 21 residues being represented in Kabat et al., (Kabat et al., 1987). This extreme variability results from V-D-J splicing (Schilling et al., 1980) and has always been a problem when attempting to model antibodies. Such loops may be divided into short (up to 7 residues), medium (up to 14 residues) and long (15 or more residues). Using the CAMAL procedure, short and medium CDR-H3's can be modelled as accurately as other CDR's of similar lengths. Although long CDR-H3's are more difficult and cannot, at present, be built to the same accuracy, the chain trace is still correct.

It is unlikely that the longer loops consist of 'pure' loops (i.e., all random coil or turn). In crystal structures of antibodies with medium to long CDR-H3 loops (McPC603 (Rudikoff et al., 1981): 11 amino acids (aa); KOL (Marquart et al., 1980): 17 aa; F19.9 (Lascombe et al., 1989): 15 aa) the loops consist of a disordered β-sheet extension from the β-barrel core and a 5-8 residue random coil/turn connecting these two strands.

To determine the nature of medium to long loops (>8 residues) which satisfy the CDR-H3 constraints, a complete search of the Protein Databank for loops of length 8-20 residues, was performed using the inter-Cα distance constraints determined from known antibody crystal structures for CDR-H3. The resulting loops were then analyzed using the DSSP (Kabsch and Sander, 1983) program, which is able to assign secondary structure to polypeptide structures. The amount of secondary structure for each length of loop was calculated, and it was observed that for loops longer than 12 residues the amount of secondary structure within each of the classes described in DSSP was constant. The number of loops selected is also constant (approximately 150 loops) for loops longer than 12 residues. A closer inspection of each of the length ensembles shows indeed that the loops are the same between the groups.

This analysis shows that, like the long CDR-H3 crystal structures, the selected fragments consist of β-strands connected by 5-8 residue loops. For loops above 12-13 residues in length, the same loops are selected, but with extensions to the β-strands. This is called the "sliding-ladder" effect. In addition, the maximum size of a random coil or turn fragment in any of the structures contained in the Protein Databank tends not to exceed 8 residues, as determined by DSSP. This implies that the conformational space of longer loops is not saturated by the database and, although it is unlikely that long loops in antibodies will differ significantly from long loops in other structures, confidence in the prediction must be correspondingly reduced.

By how much is the usefulness of the CAMAL algorithm reduced by this observation ?

The frequency of occurrence of different CDR-H3 lengths in antibody sequences described by Kabat et al. (Kabat et al., 1987) was analyzed. Figure 10 shows that more than 85% of H3 loops have lengths between 4 and 14 residues which can be modelled accurately by the CAMAL algorithm.

CDR-H3 of D1.3 is of average length (8 residues), though no loops of this length are seen in the available antibody structures. The crystal structure coordinate set showed an RMS of 1.9 Å compared with the model.

The 36-71 loop is 12 residues long. The conformation is correctly predicted as a short loop connecting an

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extension of the β-sheet.

The 3D6 H3 loop is 17 residues long. While KOL (Marquart et al., 1980) has the same length it has only one residue in common with 3D6 and only one conservative mutation. There is thus no reason to believe that the conformations would be similar. The final predicted conformation of 3D6 is an extended β-sheet, as in the crystal structure. The difference between the predicted and the crystal structure of 3D6-H3 is due to a twist of 5-7° in the extended β-sheet loop (see Figures 9A-9D). Such a twist has also been observed for complexed and uncomplexed antibodies by Wilson et al. (Wilson and others). This suggests that long CDR-H3 loops may be flexible and actively involved in antigen binding.

The Complete Variable Region

Prediction of the strand positions and V_L-V_H orientation in the framework β-barrel was exact for all of the four antibodies. The backbone (N,Cα,C) RMS deviations from the crystal structures were between 0.56 and 0.86 Å, despite the fact that, in all cases the V_L and V_H regions of a particular model were derived from different antibody structures. This suggests that this method will do well in procedures such as humanization (Gorman et al., 1991), where correct framework positioning is important. The backbones of all six CDRs in all four antibodies are essentially correctly predicted, as shown in Figure 8. There are two important points to make about these predictions. First, the position of each CDR on its framework barrel is correct. Thus, CDR-framework interactions can be confidently monitored. The only deviation from the x-ray structure is CDR-H3 of antibody 3D6 which has been previously discussed. Second, the all atom RMS deviation between models and x-ray structures is dominated by sidechain positions. In most instances this deviation is due to a small number of incorrectly positioned, exposed sidechains (for example, in D1.3 the only sidechains which are incorrectly predicted are Tyr 9 of L1, Trp 4 of L3, Tyr 2 of H1 and Tyr 4 of H3). Since each CDR is constructed in the absence of other CDRs, the force field may choose a rotamer which is 120° away from that found in the crystal structure. This effect has also been observed by Lee et al. (Lee and Levitt, 1991).

Conclusion

For antibodies having CDR H3 regions of 14 residues or less the complete variable domain can be modelled to an accuracy approaching that of medium resolution x-ray structures. For antibodies with longer H3 loops the CAMAL algorithm is likely to need an additional procedure in which molecular dynamics simulations are also incorporated.

The canonical approach of Chothia et al. appears to work well (at least in modelling backbones) where it may be applied and may be used successfully in combination with the CAMAL procedure.

One important observation that has emerged from these studies is that a given loop can exist in several conformations. In particular, this seems likely for CDR-L1 and, to a lesser extent, CDR-L3 and longer CDR-H3's. A simple combinatorial calculation shows that, if each of these three loops can exist in three separate conformations, a given combining site can have $3^3 = 27$ different topographies. Clearly, this would explain the origins of cross reactivity and would allow for induced fit of antigens.

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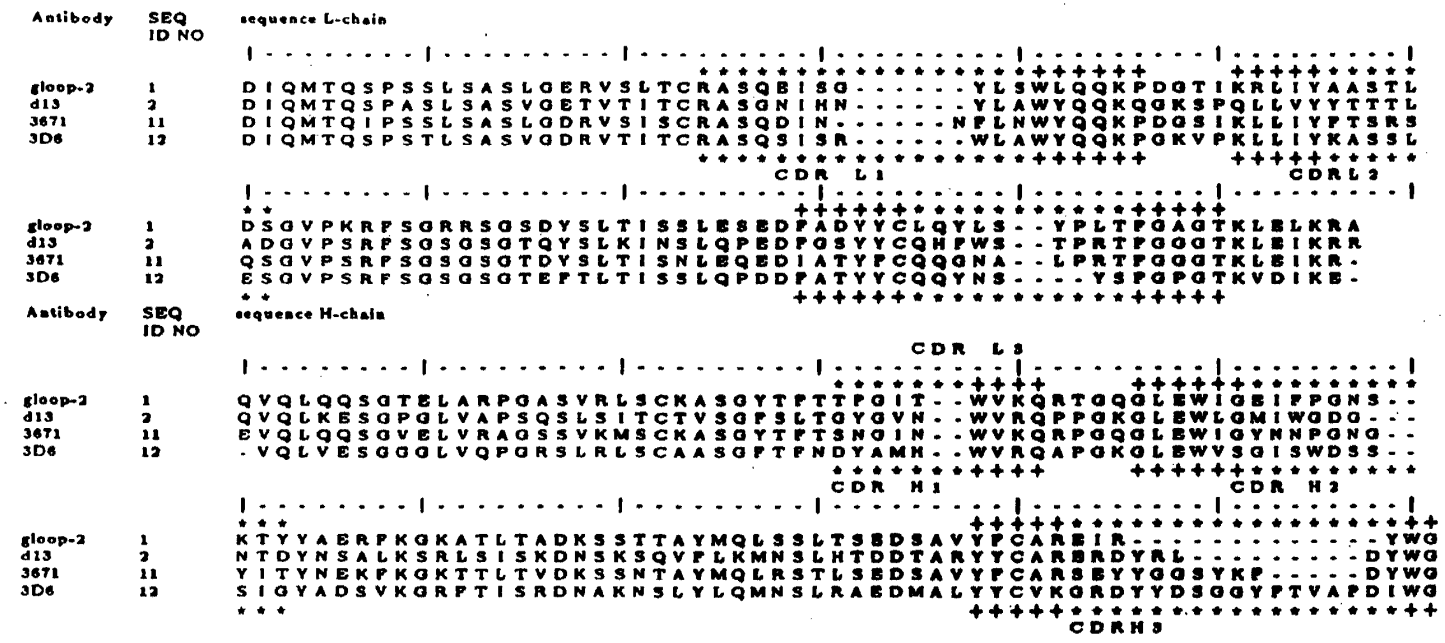

Table 2: Alignment of antibody sequences used in the modelling. '*' indicates CDR regions; '+' indicates β-strand regions used in the fitting for modelling frameworks. Nomenclature for β-barrel strands is (H or L - Chain) - FR(Framework region)-(Strand number), thus for example strand one of the heavy chain becomes HFR1.

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Antibody	Resolution	R-factor	Framework Model	
			Light	Heavy
Gloop-2	2.80	21.2	REI	HyHEL-5
D1.3	-	-	REI	NEW
36–71	1.90	20.9	Gloop2	NEW
3D6	2.70	17.7	REI	KOL

Table 3: Details of the antibody crystal structures against which the models were compared and the parent frameworks used to build the models. Resolution data for D1.3 has not yet been published.

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Antibody	CDR	sequence	SEQ ID NO	RMS local (Å)				RMS global (Å)			
				Cα	N,Cα,C	All CO	All MC	Cα	N,Cα,C	All CO	All MC
Gloop-2	L1	RAS[Q(EIS)G]YLS	494	0.73	0.71	2.05	1.96	0.86	0.87	2.09	2.12
D1.3		RAS[G(NIH)N]YLA	495	2.29	1.93	4.34	3.94	2.72	2.43	4.59	4.32
36-71		RAS[Q(DIN)N]FLN	496	2.71	2.43	4.69	4.59	3.51	3.31	5.19	5.07
3D6		RAS[Q(SIG)N]NLH	497	0.51	0.54	2.48	1.62	0.61	0.76	2.68	1.98
Gloop-2	L2	AASTLDS	498	0.25	0.23	0.89	1.00	0.86	0.86	1.10	1.10
D1.3		Y[T(TTL)A]D	499	0.67	0.73	1.89	1.40	0.99	1.02	2.01	1.98
36-71		F[T(SRS)Q]S	500	0.64	0.66	2.34	2.23	0.73	0.73	2.48	2.40
3D6		KASSLES	501	0.41	0.42	1.37	1.20	0.63	0.86	1.73	1.60
Gloop-2	L3	LQ[Y(LSY)P]LT	502	0.58	0.62	1.73	1.60	0.75	0.74	2.00	1.98
D1.3		QH[F(WST)P]RT	503	1,41	1.36	2.69	2.98	1.76	1.79	3.46	3.20
36-71		QQ[G(NAL)P]RT	504	1.09	1.06	2.26	2.10	1.48	1.36	2.87	2.28
3D6		Q[Q(YNS)Y]S	505	1.48	1.66	3.84	3.90	2.31	1.97	3.96	3.90
Gloop-2	H1	[T(FGI)T]	506	0.60	0.70	2.00	1.69	1.03	1.01	2.04	2.00
D1.3		[G(YGV)N]	507	0.46	0.62	2.33	2.00	0.88	0.90	3.24	2.98
36-71		[S(NGI)N]	508	0.90	0.83	2.22	1.96	1.04	0.97	2.81	2.23
3D6		DYAMH	509	0.67	0.77	1.82	1.11	0.81	0.73	1.59	1.20
Gloop-2	H2	EI[F(PGN)S]KTY	510	0.63	0.64	1.69	1.70	1.20	0.94	2.23	2.10
D1.3		MI[W(GDG)N]TD	511	0.42	0.42	1.55	1.49	0.87	0.85	1.86	1.89
36-71		YNN[P(GNG)Y]IA	512	0.84	0.78	2.01	2.20	1.47	1.41	1.79	1.96
3D6		ISWDSSSIG	513	0.45	0.52	2.35	2.03	0.95	0.89	2.85	2.10
Gloop-2	H3	[R(EIR)Y]	514	0.66	0.89	3.44	3.90	0.87	1.07	3.68	4.15
D1.3		ER[D(YRL)D]V	515	0.38	0.53	1.68	1.20	1.25	0.81	1.96	1.35
36-71		SEYY[G(GSY)K]FDY	516	1.95	1.75	4.40	4.00	2.65	2.53	4.60	4.09
3D6		GRDYY[D(SGG)YF]TVAFDI	517	3.66	3.42	5.93	4.01	4.39	3.95	6.30	6.20

Table 4: Sequence and conformational search construction scheme for each of the 24 CDRs, [ ]=construction area, ( )= Chain closure, all sidechains are constructed. RMS(Root Mean Square) difference between model and crystal structure loop coordinates. The RMS values are a global fit calculated by least-squares fitting the conserved core of the two structures upon each other and calculating the RMS over the loops. The total RMS of the frameworks (N,Cα,C) is 0.81, 0.60, 0.86 and 0.56 respectivly

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Loop	Canonical	Sequence	SEQ ID NO
L1	HyHEL-10	R A S Q S I S R W L A	518
	(3D6)	R A S Q S I G N N L H	497
L2	REI	E A S N D L A	519
	(3D6)	K A S S L E S	501
H1	McPC603	D F Y M E	520
	(3D6)	D Y A M H	509
H2	KOL	I I W D D G S D Q	521
	(3D6)	I S W D S S S I G	513

Table 5: Canonical loops selected for the model of 3D6(taken from Chothia *et al* (1989)).

	Residue Number	24	25	26	27	28*	29*	
REI	Sequence	Q	A	S	Q	S	I	
	ϕ/ψ	-/138	-103/157	-96/7	-158/142	-40/108	-112/9	
HyHEL-10	Sequence	R	A	S	Q	S	I	
	ϕ/ψ	-/108	-85/135	-88/64	172/160	-64/-38	9/63	
	Residue Number	30*	31*	32	33	32		
REI	Sequence	I	K	Y	L	N		SEQ ID NO: 522
	ϕ/ψ	79/-77	-146/21	-104/89	-143/133	-144/-		
HyHEL-10	Sequence	G	N	N	L	H		SEQ ID NO: 518
	ϕ/ψ	-63/107	85/-15	-105/72	-129/118	-126/-		

Table 6: Backbone ϕ and ψ angles of residues in CDR-L1 from HyHEL-10 and REI classified in the same canonical group by Chothia *et al* (1989). The residues exhibiting a peptide flip are indicated by a *.

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(1) INFORMATION FOR SEQ ID NO:1

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

(ii) MOLECULE TYPE: peptide

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

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

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Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln Glu Ile Ser Gly Tyr
20 25 30

Leu Ser Trp Leu Gln Gln Lys Pro Asp Gly Thr Ile Lys Arg Leu Ile
35 40 45

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

Arg Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Ser
65 70 75 80

Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln Tyr Leu Ser Tyr Pro Leu
85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala
100 105

(2) INFORMATION FOR SEQ ID NO:2

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

(ii) MOLECULE TYPE: peptide

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

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

Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Gly Asn Ile His Asn Tyr
20 25 30

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

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

Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Ser Thr Pro Arg
85 90 95

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

(3) INFORMATION FOR SEQ ID NO:3

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

(ii) MOLECULE TYPE: peptide

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

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

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Asn Tyr Met
20 25 30

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

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

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Thr Glu
65 70 75 80

Asp Ala Ala Glu Tyr Tyr Cys Gln Gln Trp Gly Arg Asn Pro Thr Phe
85 90 95

Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
100 105

(4) INFORMATION FOR SEQ ID NO:4

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

(ii) MOLECULE TYPE: peptide

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

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

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```
Asn Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Gly Asn Asn
            20                  25                  30

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
        35                  40                  45

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

Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Thr
65                  70                  75                  80

Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser Asn Ser Trp Pro Tyr
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
            100                 105
```

(5) INFORMATION FOR SEQ ID NO:5

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

Gln Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Ser Leu
            20                  25                  30

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

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

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Asn Thr Met Glu Ala Glu
65                  70                  75                  80

Asp Ala Ala Ile Tyr Tyr Cys Gln Gln Trp Thr Tyr Pro Leu Ile Thr
                85                  90                  95
```


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Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala
100 105

(6) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

Arg Val Thr Ile Ser Cys Thr Gly Thr Ser Ser Asn Ile Gly Ser Ile
20 25 30

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

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

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Glu
65 70 75 80

Ala Glu Asp Glu Ser Asp Tyr Tyr Cys Ala Ser Trp Asn Ser Ser Asp
85 90 95

Asn Ser Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly Gln
100 105 110

(7) INFORMATION FOR SEQ ID NO:7

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

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Glu Arg Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

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

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

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
85 90 95

Asp His Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile
100 105 110

Lys Arg Ala
115

(8) INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 103 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Asn
20 25 30

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

Ile Phe His Asn Asn Ala Arg Phe Ser Val Ser Lys Ser Gly Ser Ser
50 55 60

Ala Thr Leu Ala Ile Thr Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr
65 70 75 80

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

Lys Leu Thr Val Leu Arg Gln
100

(9) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 114 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Gln Gly Asn Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Val Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95

Thr His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg Ala

(10) INFORMATION FOR SEQ ID NO:10

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

(ii) MOLECULE TYPE: peptide

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

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

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

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Val
        35                  40                  45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu His
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Ser Thr Thr Pro Arg
                85                  90                  95

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

(11) INFORMATION FOR SEQ ID NO:11

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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

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

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

Tyr Phe Thr Ser Arg Ser Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
```


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

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Ala Leu Pro Arg
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
100 105

(12) INFORMATION FOR SEQ ID NO:12

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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

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

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

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

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

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

Gly Pro Gly Thr Lys Val Asp Ile Lys Arg Thr
100 105

(13) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 104 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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

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

Gly Ile Thr Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

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

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65                  70                  75                  80

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

Ala Arg Glu Ile Arg Tyr Trp Gly
            100
```

(14) INFORMATION FOR SEQ ID NO:14

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

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

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Gly Tyr
            20                  25                  30

Gly Val Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45

Gly Met Ile Trp Gly Asp Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys
    50                  55                  60
```


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

Lys Met Asn Ser Leu His Thr Asp Asp Thr Ala Arg Tyr Tyr Cys Ala
85 90 95

Arg Glu Arg Asp Tyr Arg Leu Asp Tyr Trp Gly
100 105

(15) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 106 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Asp Tyr Trp
20 25 30

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

Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr His Glu Arg Phe Lys
50 55 60

Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Ser Thr Ala Tyr Met
65 70 75 80

Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Gly Val Tyr Tyr Cys Leu
85 90 95

His Gly Asn Tyr Asp Phe Asp Gly Trp Gly
100 105

(16) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 104 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

Thr Leu Ser Leu Thr Cys Ser Val Thr Gly Asp Ser Ile Thr Ser Asp
            20                  25                  30

Tyr Trp Ser Trp Ile Arg Lys Phe Pro Gly Asn Arg Leu Glu Tyr Met
        35                  40                  45

Gly Tyr Val Ser Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys
    50                  55                  60

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

Asp Leu Asn Ser Val Thr Thr Glu Asp Thr Ala Thr Tyr Tyr Cys Ala
                85                  90                  95

Asn Trp Asp Gly Asp Tyr Trp Gly
            100
```

(17) INFORMATION FOR SEQ ID NO:17

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Lys Tyr
            20                  25                  30

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

Gly Glu Ile His Pro Asp Ser Gly Thr Ile Asn Tyr Thr Pro Ser Leu
    50                  55                  60
```


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

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

Ala Arg Leu His Tyr Tyr Gly Tyr Asn Ala Tyr Trp Gly
100 105

(18) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Glu 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 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 Asn Asp 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
115

(19) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid

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(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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

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

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

Ala Ala Ser Arg Asn Lys Gly Asn Lys Tyr Thr Thr Glu Tyr Ser Ala
    50                  55                  60

Ser Val Lys Gly Arg Phe Ile Val Ser Arg Asp Thr Ser Gln Ser Ile
65                  70                  75                  80

Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Thr Ala Ile Tyr
                85                  90                  95

Tyr Cys Ala Arg Asn Tyr Tyr Gly Ser Thr Trp Tyr Phe Asp Val Trp
            100                 105                 110

Gly
```

(20) INFORMATION FOR SEQ ID NO:20

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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

Leu Ser Leu Thr Cys Thr Val Ser Gly Thr Ser Phe Asp Asp Tyr Tyr
            20                  25                  30
```


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Ser Thr Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly
35 40 45

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

Arg Val Thr Met Leu Val Asn Thr Ser Lys Asn Gln Phe Ser Leu Arg
65 70 75 80

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
85 90 95

Asn Leu Ile Ala Gly Cys Ile Asp Val Trp Gly
100 105

(21) INFORMATION FOR SEQ ID NO:21

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Glu Val Lys Leu Asp Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Pro Met Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
35 40 45

Ala Gln Ile Arg Asn Lys Pro Tyr Asn Tyr Glu Thr Tyr Tyr Ser Asp
50 55 60

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

Val Tyr Leu Gln Met Asn Asn Leu Arg Val Glu Asp Met Gly Ile Tyr
85 90 95

Tyr Cys Thr Gly Ser Tyr Tyr Gly Met Asp Tyr Trp Gly
100 105

(22) INFORMATION FOR SEQ ID NO:22

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

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

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Gly Val Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Gly Lys Gly Tyr Leu Ser Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Gly Lys Thr Thr Leu Thr Val Asp Arg Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

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

Ala Arg Ser Phe Tyr Gly Gly Ser Asp Leu Ala Val Tyr Tyr Phe Asp
            100                 105                 110

Ser Trp Gly
        115
```

(23) INFORMATION FOR SEQ ID NO:23

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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


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```
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Asn
                20                  25                  30

Gly Ile Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
            35                  40                  45

Gly Tyr Asn Asn Pro Gly Asn Gly Tyr Ile Ala Tyr Asn Glu Lys Phe
        50                  55                  60

Lys Gly Lys Thr Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
    65                  70                  75                  80

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

Ala Arg Ser Glu Tyr Tyr Gly Gly Ser Tyr Lys Phe Asp Tyr Trp Gly
            100                 105                 110
```

(24) INFORMATION FOR SEQ ID NO:24

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Leu Tyr Tyr Cys
                85                  90                  95
```


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Val Lys Gly Arg Asp Tyr Tyr Asp Ser Gly Gly Tyr Phe Thr Val Ala
100 105 110

Phe Asp Ile Trp Gly
115

(25) INFORMATION FOR SEQ ID NO:25

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile Ile His Ser
20 25 30

Asp Gly Asn Thr Tyr Leu Glu Trp Phe Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro His Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
100 105 110

(26) INFORMATION FOR SEQ ID NO:26

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

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

Arg Val Thr Ile Ser Cys Ser Gly Thr Ser Ser Asn Ile Gly Ser Ser
            20                  25                  30

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

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

Gly Ser Lys Ser Gly Ala Ser Ala Ser Leu Ala Ile Gly Gly Leu Gln
65                  70                  75                  80

Ser Glu Asp Glu Thr Asp Tyr Tyr Cys Ala Ala Trp Asp Val Ser Leu
                85                  90                  95

Asn Ala Tyr Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu
            100                 105                 110
```

(27) INFORMATION FOR SEQ ID NO:27

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

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

Gln Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile Ile His Ser
            20                  25                  30

Asp Gly Asn Thr Tyr Leu Glu Trp Phe Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

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


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Ser Arg Val Glu Ala Glu Asp Glu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro His Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
100 105 110

(28) INFORMATION FOR SEQ ID NO:28

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val Tyr Ser
20 25 30

Asp Gly Asn Thr Tyr Leu Asn Trp Phe Gln Gln Arg Pro Gly Gln Ser
35 40 45

Pro Arg Arg Leu Ile Tyr Lys Val Ser Asn Arg Asp Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

Thr His Trp Ser Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

(29) INFORMATION FOR SEQ ID NO:29

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

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

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile Ile His Ser
            20                  25                  30

Asp Gly Asn Thr Tyr Leu Glu Trp Phe Gln Gln Arg Pro Gly Gln Ser
        35                  40                  45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95

Ser His Val Pro His Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
            100                 105                 110
```

(30) INFORMATION FOR SEQ ID NO:30

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

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

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
            20                  25                  30

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

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80
```


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Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Asp Thr Ile Pro Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

(31) INFORMATION FOR SEQ ID NO:31

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

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

Asp Arg Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile Ile His Ser
20 25 30

Asp Gly Asn Thr Tyr Leu Glu Trp Phe Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro His Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
100 105 110

(32) INFORMATION FOR SEQ ID NO:32

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

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

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

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

Ala Tyr Ile Ser Ser Gly Ser Phe Thr Ile Tyr His Ala Asp Thr Val
    50                  55                  60

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

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

Ala Arg Met Arg Lys Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser
        115
```

(33) INFORMATION FOR SEQ ID NO:33

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Glu 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 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
```


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Lys Gly Arg Phe Thr Ile Ser Arg Asn Asp 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
115 120 125

(34) INFORMATION FOR SEQ ID NO:34

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Glu 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 Ala Ala Ser Gly Phe Ile Phe Ser Ser Phe
20 25 30

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

Ala Tyr Ile Ser Ser Asp Gly Phe Thr Ile Tyr His Ala Asp Ser Val
50 55 60

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

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

Ala Arg Met Arg Lys Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser
115

(35) INFORMATION FOR SEQ ID NO:35

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
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 Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

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

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

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

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

Ala Arg Asp Arg Lys Asp Trp Gly Trp Ala Leu Phe Asp Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser
        115                 120
```

(36) INFORMATION FOR SEQ ID NO:36

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

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


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

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

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

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

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

Ala Arg Met Arg Lys Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser
115

(37) INFORMATION FOR SEQ ID NO:37

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

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

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

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

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60

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

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

Ala Arg

(38) INFORMATION FOR SEQ ID NO:38

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

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

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

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

Ala Tyr Ile Ser Ser Gly Ser Phe Thr Ile Tyr His Ala Asp Ser Val
50 55 60

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

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

Ala Arg Met Arg Lys Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser
115

(39) INFORMATION FOR SEQ ID NO:39

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

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

(40) INFORMATION FOR SEQ ID NO:40

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Lys Thr Ser Leu Arg Pro Gly Lys Gly Ser Ser Asp Tyr Glu Lys Lys
1 5 10 15

(41) INFORMATION FOR SEQ ID NO:41

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Lys Thr Ser Leu Arg Pro Gly Lys Gly Ser Ser Glu Tyr Glu Lys Lys
1 5 10 15

(42) INFORMATION FOR SEQ ID NO:42

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Gln Thr Ser Leu Arg Pro Asp Lys Gly Ser Ser Asp His Glu Lys Lys
1 5 10 15

(43) INFORMATION FOR SEQ ID NO:43

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gln Thr Ser Leu Arg Pro Asp Lys Gly Ser Ser Asp Gln Glu Lys Lys
1 5 10 15

(44) INFORMATION FOR SEQ ID NO:44

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gln Ser Ser Leu Arg Pro Asp Lys Gly Ser Ser Asp Gln Glu Lys Lys
1 5 10 15

(45) INFORMATION FOR SEQ ID NO:45

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Gln Thr Ser Leu Arg Pro Asp Lys Gly Ser Ser Asp Pro Glu Lys Lys
1 5 10 15

(46) INFORMATION FOR SEQ ID NO:46

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Gln Thr Ser Leu Arg Pro Asp Lys Gly Ser Ser Asp Pro Glx Lys Lys
1 5 10 15

(47) INFORMATION FOR SEQ ID NO:47

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Gln Thr Ser Leu Arg Pro Asp Lys Gly Ser Ser Asp Pro Glu Lys Thr
1 5 10 15

(48) INFORMATION FOR SEQ ID NO:48

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Gln Thr Ser Leu Arg Ala Asp Lys Gly Ser Ser Asp Gln Glu Lys Lys
1 5 10 15

(49) INFORMATION FOR SEQ ID NO:49

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Gln Thr Ser Leu Arg Pro Asp Lys Gly Lys Ser Asp Ser Glu Lys Lys
1 5 10 15

(50) INFORMATION FOR SEQ ID NO:50

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Gln Thr Ser Leu Arg Pro Ala Arg Gly Ser Ser Asp Gln Glu Lys Lys
1 5 10 15

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(51) INFORMATION FOR SEQ ID NO:51

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gln Thr Ser Leu Lys Pro Gly Arg Gly Ser Ser Asp Pro Glu Lys Lys
1 5 10 15

(52) INFORMATION FOR SEQ ID NO:52

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gln Thr Ser Leu Arg Pro Gly Arg Gly Ser Ser Asp Thr Glu Lys Lys
1 5 10 15

(53) INFORMATION FOR SEQ ID NO:53

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Gln Ile Ser Leu Arg Pro Gly Lys Gly Ser Ser Asp Ser Glu Lys Lys
1 5 10 15

(54) INFORMATION FOR SEQ ID NO:54

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

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Gln Thr Ser Leu Arg Pro Gly Lys Gly Asp Ser Asp Glu Asp Lys Lys
1 5 10 15

(55) INFORMATION FOR SEQ ID NO:55

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Glu Thr Ala Leu Arg Pro Gly Lys Gly Ala Ser Asp Ala Asp Lys Lys
1 5 10 15

(56) INFORMATION FOR SEQ ID NO:56

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Val Thr Ala Leu Arg Pro Gly Lys Gly Ala Ser Asp Glu Asp Lys Lys
1 5 10 15

(57) INFORMATION FOR SEQ ID NO:57

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Val Thr Ala Leu Arg Pro Gly Lys Gly Ala Ser Asp Glu Glu Lys Lys
1 5 10 15

(58) INFORMATION FOR SEQ ID NO:58

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Val Thr Ala Leu Arg Pro Gly Lys Gly Ala Ser Asx Ala Asx Lys Lys
1 5 10 15

(59) INFORMATION FOR SEQ ID NO:59

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Val Thr Ala Leu Arg Pro Gly Lys Gly Ala Ser Asp Glu Asp Asp Glu
1 5 10 15

(60) INFORMATION FOR SEQ ID NO:60

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Gln Thr Ser Leu Arg Pro Asp Lys Gly Ser Ser Asp Gln Glu Thr Thr
1 5 10 15

(61) INFORMATION FOR SEQ ID NO:61

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Gln Asn Ser Leu Thr Pro Gly Lys Gly Ser Ser Ser Pro Glu Lys Lys
1 5 10 15

(62) INFORMATION FOR SEQ ID NO:62

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Val Thr Lys Val Arg Pro Gly Lys Gly Asp Ser Asp Ser Asp Lys Lys
1 5 10 15

(63) INFORMATION FOR SEQ ID NO:63

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Val Thr Lys Val Arg Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(64) INFORMATION FOR SEQ ID NO:64

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Val Thr Arg Val Arg Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(65) INFORMATION FOR SEQ ID NO:65

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Leu Thr Lys Val Arg Pro Gly Lys Gly Asp Ser Asp Ser Glu Lys Lys
1 5 10 15

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(66) INFORMATION FOR SEQ ID NO:66

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Val Thr Lys Val Arg Pro Gly Lys Gly Asp Ser Asp Ser Glu Gln Lys
1 5 10 15

(67) INFORMATION FOR SEQ ID NO:67

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Val Thr Lys Val Arg Pro Glu Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(68) INFORMATION FOR SEQ ID NO:68

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Val Thr Lys Val Arg Pro Glu Lys Gly Asp Ser Asp Ser Glu Lys Lys
1 5 10 15

(69) INFORMATION FOR SEQ ID NO:69

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

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Val Thr Lys Val Ser Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(70) INFORMATION FOR SEQ ID NO:70

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Val Thr Lys Val Arg Ser Gly Lys Gly Glu Ser Asp Ala Glu Lys Lys
1 5 10 15

(71) INFORMATION FOR SEQ ID NO:71

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Val Thr Ser Val Lys Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(72) INFORMATION FOR SEQ ID NO:72

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Val Ser Ser Val Lys Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(73) INFORMATION FOR SEQ ID NO:73

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Val Thr Ser Ala Lys Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(74) INFORMATION FOR SEQ ID NO:74

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Val Ser Ser Ala Lys Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(75) INFORMATION FOR SEQ ID NO:75

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Val Thr Ser Ala Arg Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(76) INFORMATION FOR SEQ ID NO:76

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Val Ser Pro Ala Lys Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(77) INFORMATION FOR SEQ ID NO:77

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Val Thr Lys Ala Arg Pro Gly Lys Gly Asp Ser Asp Val Glu Lys Asn
1 5 10 15

(78) INFORMATION FOR SEQ ID NO:78

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Val Thr Leu Ile Pro Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(79) INFORMATION FOR SEQ ID NO:79

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

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

(80) INFORMATION FOR SEQ ID NO:80

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

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

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(81) INFORMATION FOR SEQ ID NO:81

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Val Thr Leu Leu Gln Pro Gly Lys Gly Asp Ser Asp Ala Glu Arg Lys
1 5 10 15

(82) INFORMATION FOR SEQ ID NO:82

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Val Thr Leu Leu Gln Ala Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(83) INFORMATION FOR SEQ ID NO:83

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

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

(84) INFORMATION FOR SEQ ID NO:84

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

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Leu Thr Leu Leu Gln Pro Gly Asn Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(85) INFORMATION FOR SEQ ID NO:85

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

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

(86) INFORMATION FOR SEQ ID NO:86

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

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

(87) INFORMATION FOR SEQ ID NO:87

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

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

(88) INFORMATION FOR SEQ ID NO:88

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Val Thr Leu Pro Gln Pro Gly Lys Gly Asp Trp Asp Ala Glu Lys Lys
1 5 10 15

(89) INFORMATION FOR SEQ ID NO:89

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Val Thr Phe Leu Ser Pro Gly Gln Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(90) INFORMATION FOR SEQ ID NO:90

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Glu Ser Ser Ala Arg Pro Gly Lys Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(91) INFORMATION FOR SEQ ID NO:91

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

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

(92) INFORMATION FOR SEQ ID NO:92

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Val Thr Thr Ala Lys Pro Glu Lys Gly Asp Ser Asp Val Glu Lys Lys
1 5 10 15

(93) INFORMATION FOR SEQ ID NO:93

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Val Thr Thr Pro Lys Pro Asp Lys Gly Asp Ser Asp Val Glu Lys Lys
1 5 10 15

(94) INFORMATION FOR SEQ ID NO:94

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Val Thr Ala Pro Arg Pro Gly Lys Gly Ala Ser Ser Ala Glu Lys Lys
1 5 10 15

(95) INFORMATION FOR SEQ ID NO:95

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Val Thr Ala Pro Lys Pro Gly Lys Gly Thr Ser Ser Ala Glu Lys Lys
1 5 10 15

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(96) INFORMATION FOR SEQ ID NO:96

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

Val Thr Thr Pro Lys Pro Gly Lys Gly Ala Ser Ser Ala Glu Lys Lys
1 5 10 15

(97) INFORMATION FOR SEQ ID NO:97

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

Val Ser Ala Pro Lys Pro Gly Lys Gly Ala Ser Ser Ala Glu Lys Lys
1 5 10 15

(98) INFORMATION FOR SEQ ID NO:98

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

Val Thr Ala Pro Arg Ser Gly Lys Gly Ala Ser Ser Ala Glu Lys Lys
1 5 10 15

(99) INFORMATION FOR SEQ ID NO:99

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

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Val Thr Ala Pro Lys Ser Gly Lys Gly Ala Ser Ser Ala Glu Lys Lys
1 5 10 15

(100) INFORMATION FOR SEQ ID NO:100

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

Val Thr Ala Pro Lys Pro Asp Lys Gly Val Ser Ser Ala Glu Lys Lys
1 5 10 15

(101) INFORMATION FOR SEQ ID NO:101

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

Val Thr Ala Pro Lys Ser Glu Lys Gly Val Ser Ser Ala Glu Lys Lys
1 5 10 15

(102) INFORMATION FOR SEQ ID NO:102

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

Phe Thr Ala Pro Lys Pro Gly Lys Gly Ala Ser Ser Ala Glu Lys Lys
1 5 10 15

(103) INFORMATION FOR SEQ ID NO:103

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

Leu Thr Ala Pro Lys Pro Gly Arg Gly Val Ser Ser Ala Glu Lys Lys
1 5 10 15

(104) INFORMATION FOR SEQ ID NO:104

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

Val Thr Ala Pro Lys Ser Gly Lys Gly Ala Ser Ser Ala Glu Lys Arg
1 5 10 15

(105) INFORMATION FOR SEQ ID NO:105

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

Val Ser Ala Pro Lys Pro Gly Lys Glu Gly Ser Ser Ala Glu Lys Lys
1 5 10 15

(106) INFORMATION FOR SEQ ID NO:106

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

Val Thr Ala Pro Lys Pro Arg Lys Gly Ala Ser Ser Ala Glu Lys Lys
1 5 10 15

(107) INFORMATION FOR SEQ ID NO:107

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

Val Thr Phe Leu Ser Pro Gly Gln Gly Asn Ser Asp Ala Glu Leu Pro
1 5 10 15

(108) INFORMATION FOR SEQ ID NO:108

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

Val Thr Phe Leu Ser Pro Gly Gln Gly Asn Ser Asp Glu Asp Leu Pro
1 5 10 15

(109) INFORMATION FOR SEQ ID NO:109

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

Val Thr Leu Ser Ser Pro Gln Arg Gly Asp Ser Asp Ala Glu Lys Lys
1 5 10 15

(110) INFORMATION FOR SEQ ID NO:110

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

Val Thr Ala Pro Lys Ser Ser Lys Gly Gly Ser Ser Ala Glu Lys Lys
1 5 10 15

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(111) INFORMATION FOR SEQ ID NO:111

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

Gln Thr Ser Pro Thr Pro Gly Lys Gly Ser Ser Asp Pro Glu Lys Lys
1 5 10 15

(112) INFORMATION FOR SEQ ID NO:112

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

Gln Ile Ser Leu Ile Pro Gly Lys Gly Ser Tyr Asp Asp Glu Lys Lys
1 5 10 15

(113) INFORMATION FOR SEQ ID NO:113

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

Val Thr Ala Leu Lys Ser Gly Lys Gly Ala Ser Ser Ala Glu Lys Lys
1 5 10 15

(114) INFORMATION FOR SEQ ID NO:114

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

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Val Thr Ala Leu Lys Ser Asp Lys Gly Ala Ser Ser Gly Glu Lys Lys
1 5 10 15

(115) INFORMATION FOR SEQ ID NO:115

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

Val Thr Pro Pro Ser Pro Gly Gln Gly Asp Ser Ala Ala Glu Lys Lys
1 5 10 15

(116) INFORMATION FOR SEQ ID NO:116

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

Val Thr Pro Pro Ser Pro Gly Gln Gly Asp Ser Ala Arg Glu Lys Lys
1 5 10 15

(117) INFORMATION FOR SEQ ID NO:117

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

Val Thr Val Arg Lys Pro Gly Lys Gly Asp Ser Ser Asp Glu Lys Lys
1 5 10 15

(118) INFORMATION FOR SEQ ID NO:118

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

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

(119) INFORMATION FOR SEQ ID NO:119

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

Lys Thr Ser Leu Arg Pro Trp Lys Gly Ser Ser Asp Ser Asp Lys Lys
1 5 10 15

(120) INFORMATION FOR SEQ ID NO:120

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

Gln Thr Asp Val Thr Gln Gly Gln Gly Ser Ser Gln Pro Glu Lys Lys
1 5 10 15

(121) INFORMATION FOR SEQ ID NO:121

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

Gln Thr Ala Val Ser Gln Gly Gln Gly Ser Ser Gln Ser Glu Lys Lys
1 5 10 15

(122) INFORMATION FOR SEQ ID NO:122

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

Leu Thr Ala Pro Arg Thr Asn Arg Gly Ser Ser Asp Ser Glu Lys Lys
1 5 10 15

(123) INFORMATION FOR SEQ ID NO:123

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

Val Thr Ala Pro Ser Ser His Arg Gly Ser Ser Asp Thr Glu Lys Lys
1 5 10 15

(124) INFORMATION FOR SEQ ID NO:124

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

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

(125) INFORMATION FOR SEQ ID NO:125

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

Val Thr Ala Pro Thr Pro Asp Thr Gly Ala Ile Lys Thr Glu Lys Leu
1 5 10 15

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(126) INFORMATION FOR SEQ ID NO:126

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

Val Thr Ile Pro Thr Pro Asp Thr Gly Ala Ile Lys Thr Glu Lys Leu
1 5 10 15

(127) INFORMATION FOR SEQ ID NO:127

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

Ala Val Ser Pro Thr Pro Asp Thr Gly Ala Ile Lys Thr Glu Lys Leu
1 5 10 15

(128) INFORMATION FOR SEQ ID NO:128

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

Ala Val Ser Pro Thr Pro Asp Thr Gly Ala Ile Lys Thr Glu Lys Leu
1 5 10 15

(129) INFORMATION FOR SEQ ID NO:129

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

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Ala Val Ser Pro Thr Pro Asp Thr Gly Val Ile Lys Thr Glu Lys Leu
1 5 10 15

(130) INFORMATION FOR SEQ ID NO:130

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

Ala Val Ser Pro Thr Pro Asp Thr Gly Ala Ile Lys Thr Glu Pro Ser
1 5 10 15

(131) INFORMATION FOR SEQ ID NO:131

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

Tyr Leu Pro Pro Thr Pro Gly Val Ile Arg Ser Thr Ala Met Lys Leu
1 5 10 15

(132) INFORMATION FOR SEQ ID NO:132

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

Tyr Leu Pro Pro Thr Pro Gly Val Ile Arg Ser Thr Ala Met Arg Leu
1 5 10 15

(133) INFORMATION FOR SEQ ID NO:133

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

Tyr Leu Pro Pro Thr Pro Gly Leu Ile Arg Ser Thr Ser Met Lys Leu
1 5 10 15

(134) INFORMATION FOR SEQ ID NO:134

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

Tyr Leu Pro Pro Thr Pro Gly Leu Ile Arg Ser Thr Ser Val Lys Leu
1 5 10 15

(135) INFORMATION FOR SEQ ID NO:135

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

Tyr Leu Pro Pro Thr Pro Gly Val Ile Arg Ser Thr Ala Glu Lys Leu
1 5 10 15

(136) INFORMATION FOR SEQ ID NO:136

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

Tyr Leu Pro Pro Thr Pro Gly Val Ile Arg Ser Thr Ala Gly Lys Leu
1 5 10 15

(137) INFORMATION FOR SEQ ID NO:137

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

Tyr Leu Pro Ala Thr Pro Gly Val Val Arg Ser Ser Ala Gly Met Leu
1 5 10 15

(138) INFORMATION FOR SEQ ID NO:138

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

Ser Leu Pro Pro Ser Pro Gly Lys Val Arg Ser Thr Ala Glu Lys Leu
1 5 10 15

(139) INFORMATION FOR SEQ ID NO:139

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

Ser Leu Pro Pro Ser Pro Gly Lys Val Arg Ser Thr Ala Asn Lys Leu
1 5 10 15

(140) INFORMATION FOR SEQ ID NO:140

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

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

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(141) INFORMATION FOR SEQ ID NO:141

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

Ser Leu Pro Pro Arg Pro Gly Lys Val Arg Ser Ser Ser Asp Lys Leu
1 5 10 15

(142) INFORMATION FOR SEQ ID NO:142

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

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

(143) INFORMATION FOR SEQ ID NO:143

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

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

(144) INFORMATION FOR SEQ ID NO:144

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

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Ser Leu Pro Pro Arg Pro Gly Lys Val Arg Ser Ser Ser Glu Thr Leu
1 5 10 15

(145) INFORMATION FOR SEQ ID NO:145

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

Ser Leu Pro Pro Lys Pro Gly Lys Ile Arg Ser Ser Thr Gly Lys Leu
1 5 10 15

(146) INFORMATION FOR SEQ ID NO:146

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

Ser Leu Pro Pro Lys Pro Gly Arg Ile Arg Ser Ser Thr Gly Lys Leu
1 5 10 15

(147) INFORMATION FOR SEQ ID NO:147

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

Ser Leu Pro Pro Lys Pro Gly Lys Ile Arg Ser Ser Thr Gly Gln Leu
1 5 10 15

(148) INFORMATION FOR SEQ ID NO:148

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

Ser Leu Pro Pro Glu Pro Gly Lys Ile Arg Ser Ser Thr Gly Arg Leu
1 5 10 15

(149) INFORMATION FOR SEQ ID NO:149

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

Ser Leu Ala Pro Ser Pro Gly Lys Ile Arg Ser Thr Ala Glu Lys Leu
1 5 10 15

(150) INFORMATION FOR SEQ ID NO:150

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

Ser Leu Pro Pro Arg Pro Gly Lys Ile Arg Ser Ser Thr Gly Asn Val
1 5 10 15

(151) INFORMATION FOR SEQ ID NO:151

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

Ser Leu Arg Pro Ser Pro Gly Lys Val Arg Ser Thr Ala Glu Lys Leu
1 5 10 15

(152) INFORMATION FOR SEQ ID NO:152

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

Ser Leu Arg Pro Ser Pro Gly Lys Val Arg Ser Thr Ala Asp Lys Leu
1 5 10 15

(153) INFORMATION FOR SEQ ID NO:153

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

Ser Leu Arg Pro Ser Pro Gly Lys Val Arg Ser Thr Ala Glu Asn Leu
1 5 10 15

(154) INFORMATION FOR SEQ ID NO:154

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

Ser Leu Arg Pro Ser Pro Gly Lys Val Arg Ser Ala Val Glu Lys Leu
1 5 10 15

(155) INFORMATION FOR SEQ ID NO:155

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

Ser Leu Pro Pro Arg Pro Gly Lys Arg Ser Ser Ala Glu Lys Leu
1 5 10 15

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(156) INFORMATION FOR SEQ ID NO:156

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

Ser Leu Ala Pro Ser Pro Gly Lys Val Arg Ser Thr Val Glu Arg Leu
1 5 10 15

(157) INFORMATION FOR SEQ ID NO:157

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

Ser Leu Ala Pro Ser Pro Asp Lys Ile Arg Ser Thr Pro Asp Lys Leu
1 5 10 15

(158) INFORMATION FOR SEQ ID NO:158

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

Ser Leu Ala Leu Ser Pro Gly Lys Val Arg Ser Thr Ala Glu Lys Leu
1 5 10 15

(159) INFORMATION FOR SEQ ID NO:159

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

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Ser Leu Pro Leu Ser Ala Gly Lys Val Arg Ser Thr Ala Glu Lys Leu
1 5 10 15

(160) INFORMATION FOR SEQ ID NO:160

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

Ser Leu Ala Pro Ser Pro Gly Lys Val Arg Ser Thr Ala Glu Tyr Leu
1 5 10 15

(161) INFORMATION FOR SEQ ID NO:161

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

Ser Leu Pro Leu Thr Pro Gly Leu Ile Arg Ser Thr Ala Glu Lys Leu
1 5 10 15

(162) INFORMATION FOR SEQ ID NO:162

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

Ser Leu Pro Leu Thr Pro Arg Val Ile Arg Ser Thr Ala Glu Lys Leu
1 5 10 15

(163) INFORMATION FOR SEQ ID NO:163

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

Phe Leu His Pro Thr Pro Gly Thr Asp Ser Ser Ser Thr Glu Lys Leu
1 5 10 15

(164) INFORMATION FOR SEQ ID NO:164

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

Phe Leu Leu Pro Thr Pro Gly Thr Asp Ser Ser Ser Thr Glu Arg Leu
1 5 10 15

(165) INFORMATION FOR SEQ ID NO:165

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

Phe Leu His Pro Thr Arg Val Thr Asp Ser Ser Ser Thr Glu Lys Leu
1 5 10 15

(166) INFORMATION FOR SEQ ID NO:166

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:

Leu Leu Pro Pro Thr Pro Gly Thr Asn Ser Ser Ser Asn Asp Lys Leu
1 5 10 15

(167) INFORMATION FOR SEQ ID NO:167

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:

Val Leu Pro Leu Ser Pro His Arg Ile Arg Ser Glu Ser Glu Asn Leu
1 5 10 15

(168) INFORMATION FOR SEQ ID NO:168

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:

Ser Leu Ala Pro Ser Pro Ala Lys Phe Arg Ser Thr Ala Glu Arg Asp
1 5 10 15

(169) INFORMATION FOR SEQ ID NO:169

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:

Val Thr Ala Pro Arg Pro Gly Arg Ile Arg Ser Asp Pro Glu Lys Lys
1 5 10 15

(170) INFORMATION FOR SEQ ID NO:170

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:

Val Thr Ala Pro Arg Pro Gly Arg Val Arg Ser Asp Pro Glu Lys Lys
1 5 10 15

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(171) INFORMATION FOR SEQ ID NO:171

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:

Val Thr Gly Pro Arg Pro Gly Arg Ile Arg Ser Asp Pro Glu Lys Lys
1 5 10 15

(172) INFORMATION FOR SEQ ID NO:172

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:

Val Thr Gly Pro Arg Pro Gly Arg Ile Arg Ser Asp Pro Asp Lys Lys
1 5 10 15

(173) INFORMATION FOR SEQ ID NO:173

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:

Val Thr Gly Pro Arg Pro Gly Arg Val Arg Ser Asp Pro Glu Lys Lys
1 5 10 15

(174) INFORMATION FOR SEQ ID NO:174

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:174:

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Val Thr Gly Pro Arg Pro Gly Arg Ile Arg Ser Asp Pro Xaa Lys Lys
1 5 10 15

(175) INFORMATION FOR SEQ ID NO:175

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:175:

Val Thr Ala Pro Arg Pro Gly Arg Ile Arg Ser Glu Ser Glu Arg Lys
1 5 10 15

(176) INFORMATION FOR SEQ ID NO:176

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:176:

Val Thr Gly Pro Ser Arg Gly Arg Ile Arg Ser Asp Pro Glu Lys Lys
1 5 10 15

(177) INFORMATION FOR SEQ ID NO:177

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:177:

Val Thr Val Pro Arg Pro Ser Arg Ile Arg Ser Glu Ser Glu Arg Lys
1 5 10 15

(178) INFORMATION FOR SEQ ID NO:178

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:178:

Val Thr Ala Pro Gly Pro Gly Arg Ile Arg Ser Glu Ser Glu Arg Lys
1 5 10 15

(179) INFORMATION FOR SEQ ID NO:179

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:179:

Gln Thr Ser Val Arg Pro Gly Arg Val Arg Ser Asp Pro Glu Arg Lys
1 5 10 15

(180) INFORMATION FOR SEQ ID NO:180

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:180:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser Asp Pro Glu Arg Lys
1 5 10 15

(181) INFORMATION FOR SEQ ID NO:181

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:181:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser Asp Pro Glu Lys Lys
1 5 10 15

(182) INFORMATION FOR SEQ ID NO:182

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:182:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser Glu Pro Glu Lys Lys
1 5 10 15

(183) INFORMATION FOR SEQ ID NO:183

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:183:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser Glu Pro Asp Lys Lys
1 5 10 15

(184) INFORMATION FOR SEQ ID NO:184

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:184:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ala Glu Pro Glu Lys Lys
1 5 10 15

(185) INFORMATION FOR SEQ ID NO:185

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:185:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser Asx Pro Glx Lys Lys
1 5 10 15

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(186) INFORMATION FOR SEQ ID NO:186

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:186:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser Asp Pro Asx Lys Lys
1 5 10 15

(187) INFORMATION FOR SEQ ID NO:187

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:187:

Gln Thr Ser Val Arg Pro Gly Gln Val Arg Ser Asp Pro Glu Arg Lys
1 5 10 15

(188) INFORMATION FOR SEQ ID NO:188

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:188:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser His Pro Glu Lys Lys
1 5 10 15

(189) INFORMATION FOR SEQ ID NO:189

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:189:

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Gln Thr Ser Val Arg Pro Gly Asn Val Arg Ser Asp Pro Asp Lys Lys
1 5 10 15

(190) INFORMATION FOR SEQ ID NO:190

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:190:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser Asp Pro Glu Lys Thr
1 5 10 15

(191) INFORMATION FOR SEQ ID NO:191

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:191:

Gln Thr Ser Val Arg Pro Gly Thr Val Arg Ser Glu Pro Glu Lys Lys
1 5 10 15

(192) INFORMATION FOR SEQ ID NO:192

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:192:

Gln Thr Ser Val Arg Pro Glu Lys Val Arg Ser Glu Pro Asp Lys Lys
1 5 10 15

(193) INFORMATION FOR SEQ ID NO:193

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser Glu Ser Asp Lys Lys
1 5 10 15

(194) INFORMATION FOR SEQ ID NO:194

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:

Gln Thr Ser Val Arg Pro Gly Glu Val Arg Ser Glu Pro Asp Lys Lys
1 5 10 15

(195) INFORMATION FOR SEQ ID NO:195

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:195:

Gln Thr Ser Val Arg Pro Gly Asx Val Arg Ser Asx Pro Glx Arg Lys
1 5 10 15

(196) INFORMATION FOR SEQ ID NO:196

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:196:

Gln Thr Ser Val Ser Pro Gly Lys Val Arg Ser Asp Pro Glu Lys Lys
1 5 10 15

(197) INFORMATION FOR SEQ ID NO:197

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:197:

Gln Thr Ser Val Arg Pro Gly Lys Val Asn Ser Asp Pro Glu Lys Lys
1 5 10 15

(198) INFORMATION FOR SEQ ID NO:198

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:198:

Gln Thr Ser Val Arg Pro Gly Lys Val Arg Ser Asp Pro Asp Thr Lys
1 5 10 15

(199) INFORMATION FOR SEQ ID NO:199

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:199:

Gln Thr Ser Val Arg Pro Lys Lys Val Arg Ser Asp Pro Glx Lys Lys
1 5 10 15

(200) INFORMATION FOR SEQ ID NO:200

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:200:

Gln Thr Ser Val Arg Pro Lys Lys Val Arg Phe Asp Pro Glu Lys Lys
1 5 10 15

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(201) INFORMATION FOR SEQ ID NO:201

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:201:

Gln Thr Ser Val Arg Ser Gly Lys Val Arg Ser Glu Pro Glu Thr Lys
1 5 10 15

(202) INFORMATION FOR SEQ ID NO:202

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:202:

Val Thr Asn Leu Arg Pro Gly Lys Val Arg Ser Asp Ala Glu Lys Lys
1 5 10 15

(203) INFORMATION FOR SEQ ID NO:203

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:203:

Val Thr Asp Leu Arg Pro Gly Lys Val Arg Ser Asp Ala Glu Lys Lys
1 5 10 15

(204) INFORMATION FOR SEQ ID NO:204

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:204:

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Gln Thr Ser Val Ser Pro Gly Asn Ile Arg Ser Glu Ser Asp Lys Lys
1 5 10 15

(205) INFORMATION FOR SEQ ID NO:205

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:205:

Lys Thr Ser Val Thr Pro Gly Lys Phe Arg Ser Glu Pro Glu Lys Lys
1 5 10 15

(206) INFORMATION FOR SEQ ID NO:206

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:206:

Val Thr Leu Leu Pro Pro Gly Arg Val Arg Ser Asp Ala Glu Lys Lys
1 5 10 15

(207) INFORMATION FOR SEQ ID NO:207

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:207:

Val Thr Leu Leu Pro Pro Gly Glu Val Arg Ser Asp Ala Glu Lys Lys
1 5 10 15

(208) INFORMATION FOR SEQ ID NO:208

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:208:

Val Thr Leu Pro Pro Pro Gly Glx Val Arg Ser Asp Ala Glu Arg Lys
1 5 10 15

(209) INFORMATION FOR SEQ ID NO:209

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:209:

Val Thr Leu Pro Pro Pro Gly Glx Val Arg Ser Asx Ala Glx Asn Lys
1 5 10 15

(210) INFORMATION FOR SEQ ID NO:210

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:210:

Val Thr Leu Pro Pro Pro Gln Gln Val Arg Ser Asp Ala Glu Lys Lys
1 5 10 15

(211) INFORMATION FOR SEQ ID NO:211

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:211:

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

(212) INFORMATION FOR SEQ ID NO:212

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212:

Val Thr Leu Pro Pro Ala Gly Gln Val Arg Ser Asp Ala Glu Lys Arg
1 5 10 15

(213) INFORMATION FOR SEQ ID NO:213

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:213:

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

(214) INFORMATION FOR SEQ ID NO:214

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214:

Glu Lys Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Arg Gly Asp Ser Gln Arg Pro Glu Ser
20 25

(215) INFORMATION FOR SEQ ID NO:215

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:215:

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Glu Lys Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Val
1 5 10 15

Ser Arg Gly Asp Ser Gln Arg Pro Glu Ser
20 25

(216) INFORMATION FOR SEQ ID NO:216

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:216:

Glu Lys Val Gly Gly Leu Gln Pro Gly Thr Gly Ala Pro Gly Lys Ala
1 5 10 15

Ser Arg Gly Asp Ser Gln Arg Pro Glu Ser
20 25

(217) INFORMATION FOR SEQ ID NO:217

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:217:

Glu Lys Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Asn Ser Gln Arg Ala Glu Ser
20 25

(218) INFORMATION FOR SEQ ID NO:218

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:218:

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Glu Lys Met Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Asn Ser Gln Arg Ala Glu Ser
20 25

(219) INFORMATION FOR SEQ ID NO:219

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:219:

Glu Lys Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Thr Ser Gln Arg Ala Glu Ser
20 25

(220) INFORMATION FOR SEQ ID NO:220

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:220:

Glu Lys Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Thr Ser Gln Arg Ala Glu Thr
20 25

(221) INFORMATION FOR SEQ ID NO:221

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:221:

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Glu Lys Val Gly Gly Leu Lys Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Thr Ser Gln Arg Ala Glu Thr
20 25

(222) INFORMATION FOR SEQ ID NO:222

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:222:

Glu Asn Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Thr Ser Gln Arg Ala Glu Thr
20 25

(223) INFORMATION FOR SEQ ID NO:223

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223:

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

Ser Lys Gly Thr Ser Gln Arg Ala Glu Thr
20 25

(224) INFORMATION FOR SEQ ID NO:224

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:224:

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

Ser Lys Gly Thr Ser Gln Arg Ala Glu Ser
20 25

(225) INFORMATION FOR SEQ ID NO:225

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:225:

Glu Lys Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Ile Ser Gln Arg Ala Glu Arg
20 25

(226) INFORMATION FOR SEQ ID NO:226

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:226:

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

Ala Lys Gly Asx Ser Glx Arg Ala Gln Ser
20 25

(227) INFORMATION FOR SEQ ID NO:227

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:227:

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

Ser Lys Gly Asn Ser Gln Arg Ala Glu Ser
20 25

(228) INFORMATION FOR SEQ ID NO:228

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:228:

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

Ser Lys Gly Ser Ser Gln Arg Ala Glu Ser
20 25

(229) INFORMATION FOR SEQ ID NO:229

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:229:

Glu Lys Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Arg Lys Ala
1 5 10 15

Ser Lys Gly Asn Ser Gln Arg Ala Glu Ser
20 25

(230) INFORMATION FOR SEQ ID NO:230

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:230:

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Glu Lys Met Gly Asn Leu Gln Pro Gly Ser Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Asn Ser Gln Arg Pro Asp Ser
20 25

(231) INFORMATION FOR SEQ ID NO:231

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:231:

Glu Lys Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Arg Arg Ser Glu Thr
20 25

(232) INFORMATION FOR SEQ ID NO:232

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:232:

Glu Lys Val Gly Gly Leu Lys Pro Gly Lys Gly Ala Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Arg Arg Ser Glu Thr
20 25

(233) INFORMATION FOR SEQ ID NO:233

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:233:

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Glu Lys Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Arg Asp
1 5 10 15

Ser Lys Gly Asn Ala Arg Arg Ser Glu Thr
20 25

(234) INFORMATION FOR SEQ ID NO:234

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:234:

Asp Lys Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Arg Ser Glu Thr
20 25

(235) INFORMATION FOR SEQ ID NO:235

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:235:

Asp Lys Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(236) INFORMATION FOR SEQ ID NO:236

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:236:

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Asp Lys Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Asp Lys Asp
1 5 10 15

Asn Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(237) INFORMATION FOR SEQ ID NO:237

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:237:

Glu Lys Val Gly Gly Leu Thr Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Gly Arg Arg Ser Glu Thr
20 25

(238) INFORMATION FOR SEQ ID NO:238

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:238:

Glu Met Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Asp Arg Arg Ser Glu Thr
20 25

(239) INFORMATION FOR SEQ ID NO:239

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:239:

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Glu Met Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Asp Lys Arg Ser Glu Thr
20 25

(240) INFORMATION FOR SEQ ID NO:240

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:240:

Glu Met Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Arg Ser Glu Thr
20 25

(241) INFORMATION FOR SEQ ID NO:241

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:241:

Glu Gln Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(242) INFORMATION FOR SEQ ID NO:242

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:242:

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Glu Gln Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(243) INFORMATION FOR SEQ ID NO:243

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:243:

Glu Gln Val Gly Gly Leu Lys Pro Gly Lys Gly Ala Pro Glu Lys Asp
1 5 10 15

Thr Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(244) INFORMATION FOR SEQ ID NO:244

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:244:

Glu Lys Val Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(245) INFORMATION FOR SEQ ID NO:245

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:245:

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Glu Lys Val Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(246) INFORMATION FOR SEQ ID NO:246

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:246:

Glu Lys Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(247) INFORMATION FOR SEQ ID NO:247

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:247:

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

Ser Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(248) INFORMATION FOR SEQ ID NO:248

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:248:

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Asp Lys Met Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Gln Ser Glu Thr
20 25

(249) INFORMATION FOR SEQ ID NO:249

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:249:

Glu Gln Val Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Asp Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(250) INFORMATION FOR SEQ ID NO:250

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:250:

Glu Lys Val Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Glu Lys Ser Glu Thr
20 25

(251) INFORMATION FOR SEQ ID NO:251

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:251:

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Glu Gln Val Gly Asp Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Lys Gly Asn Ala Arg Arg Ser Glu Thr
20 25

(252) INFORMATION FOR SEQ ID NO:252

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:252:

Glu Asn Val Gly Asp Leu Lys Pro Gly Lys Gly Ala Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Arg Arg Ser Glu Thr
20 25

(253) INFORMATION FOR SEQ ID NO:253

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:253:

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

Ser Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(254) INFORMATION FOR SEQ ID NO:254

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:254:

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Glu Gln Val Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Lys Ser Gly Thr
20 25

(255) INFORMATION FOR SEQ ID NO:255

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:255:

Asp Gln Val Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Lys Gly Asn Pro Lys Arg Ser Glu Thr
20 25

(256) INFORMATION FOR SEQ ID NO:256

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:256:

Asp Gln Val Gly Gly Leu Gln Pro Gly Gln Gly Thr Pro Glu Lys Asn
1 5 10 15

Thr Lys Gly Asn Pro Lys Arg Ser Asp Thr
20 25

(257) INFORMATION FOR SEQ ID NO:257

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:257:

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

Ile Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(258) INFORMATION FOR SEQ ID NO:258

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:258:

Asp Lys Val Gly Gly Leu Lys Pro Gly Lys Arg Thr Pro Glu Lys Asp
1 5 10 15

Asn Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(259) INFORMATION FOR SEQ ID NO:259

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:259:

Asp Lys Val Gly Gly Leu Lys Leu Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Lys Gly Asn Ala Lys Lys Ser Glu Thr
20 25

(260) INFORMATION FOR SEQ ID NO:260

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:260:

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Glu Lys Val Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Asn Thr Ser Glu Thr
20 25

(261) INFORMATION FOR SEQ ID NO:261

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:261:

Glu His Val Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Gly Arg Ser Glu Thr
20 25

(262) INFORMATION FOR SEQ ID NO:262

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:262:

Glu Gln Val Gly Gly Leu Gln Pro Gly Asn Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Thr Gly Asn Ala Lys Arg Ser Glu Thr
20 25

(263) INFORMATION FOR SEQ ID NO:263

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:263:

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Glu Lys Glu Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Glu Lys Glu
1 5 10 15

Ser Lys Gly Asp Ser Lys Arg Ala Glu Thr
20 25

(264) INFORMATION FOR SEQ ID NO:264

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:264:

Glu Lys Glu Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Glu Lys Glu
1 5 10 15

Ser Lys Gly Asp Ser Lys Arg Pro Glu Thr
20 25

(265) INFORMATION FOR SEQ ID NO:265

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:265:

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

Ser Lys Gly Asp Ser Lys Arg Ala Glu Thr
20 25

(266) INFORMATION FOR SEQ ID NO:266

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:266:

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Glu Lys Asp Gly Gly Leu Gln Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asp Ser Lys Arg Val Glu Met
20 25

(267) INFORMATION FOR SEQ ID NO:267

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:267:

Glu Gln Val Gly Gly Leu Lys Pro Gly Arg Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Thr Gly Asp Ala Gln Arg Ser Glu Thr
20 25

(268) INFORMATION FOR SEQ ID NO:268

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:268:

Glu Gln Val Gly Gly Leu Lys Pro Gly Arg Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Thr Gly Asn Ala Lys Gly Ser Glu Thr
20 25

(269) INFORMATION FOR SEQ ID NO:269

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:269:

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Glu Lys Val Gly Gly Ser Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Thr Ser Glu Thr
20 25

(270) INFORMATION FOR SEQ ID NO:270

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:270:

Ser Asp Gln Gly Gly Leu Lys Pro Gly Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Lys Gly Asn Ala Arg Arg Ser Glu Ser
20 25

(271) INFORMATION FOR SEQ ID NO:271

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:271:

Glu Lys Ile Gly Gly Leu Gln Pro Gly Lys Gly Asp Pro Gly Lys Pro
1 5 10 15

Ser Lys Asp Asn Ala Lys Arg Ser Glu Thr
20 25

(272) INFORMATION FOR SEQ ID NO:272

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:272:

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Glu Lys Leu Gly Gly Leu Gln Pro Gly Lys Gly Asp Pro Gly Lys Pro
1 5 10 15

Ser Lys Asp Asn Ala Lys Arg Ser Glu Thr
20 25

(273) INFORMATION FOR SEQ ID NO:273

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:273:

Glu Lys Leu Gly Gly Leu Gln Pro Gly Lys Gly Asp Pro Gly Lys Pro
1 5 10 15

Phe Lys Asp Asn Ala Lys Arg Ser Glu Thr
20 25

(274) INFORMATION FOR SEQ ID NO:274

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:274:

Glu Lys Leu Gly Gly Leu Gln Pro Gly Lys Gly Asp Pro Gly Lys Leu
1 5 10 15

Met Lys Glu Asn Ala Lys Arg Ser Glu Thr
20 25

(275) INFORMATION FOR SEQ ID NO:275

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:275:

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Glu Asn Leu Gly Gly Leu Gln Pro Gly Lys Gly Asp Pro Gly Lys Leu
1 5 10 15

Lys Xaa Glu Asn Ala Lys Arg Pro Glu Thr
20 25

(276) INFORMATION FOR SEQ ID NO:276

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:276:

Glu Lys Leu Gly Gly Leu Gln Pro Gly Asn Gly Asp Leu Gly Lys Pro
1 5 10 15

Ser Lys Asp Asn Ala Lys Arg Ser Glu Thr
20 25

(277) INFORMATION FOR SEQ ID NO:277

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:277:

Glu Lys Leu Gly Pro Leu Gln Leu Gly Lys Gly Asp Pro Gly Lys Pro
1 5 10 15

Ser Lys Asp Asp Ala Lys Arg Ser Glu Thr
20 25

(278) INFORMATION FOR SEQ ID NO:278

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:278:

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Glu Gln Leu Gly Gly Leu Gln Pro Gly Gly Gly Thr Pro Gly Lys Pro
1 5 10 15

Ser Lys Asp Asn Asp Lys Arg Ser Glu Thr
20 25

(279) INFORMATION FOR SEQ ID NO:279

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:279:

Glu Gln Leu Gly Gly Leu Gln Pro Gly Gly Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Asp Asn Asp Lys Arg Ser Glu Thr
20 25

(280) INFORMATION FOR SEQ ID NO:280

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:280:

Glu Gln Val Gly Gly Leu Lys Ala Arg Lys Gly Thr Pro Glu Lys Asp
1 5 10 15

Thr Thr Gly Asn Ala Lys Arg Ser Glu Thr
20 25

(281) INFORMATION FOR SEQ ID NO:281

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:281:

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Glu Met Val Gly Val Leu Glu Pro Gly Lys Gly Thr Pro Glu Lys Arg
1 5 10 15

Gln Glu Gly Asn Ala Lys Arg Ser Glu Thr
20 25

(282) INFORMATION FOR SEQ ID NO:282

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:282:

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

Ser Lys Asp Asp Ser Gln Lys Thr Glu Thr
20 25

(283) INFORMATION FOR SEQ ID NO:283

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:283:

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

Ser Lys Asp Asp Ser Gln Lys Thr Glu Arg
20 25

(284) INFORMATION FOR SEQ ID NO:284

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:284:

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Gln Gln Val Pro Glu Leu Lys Pro Gly Arg Gly Thr Pro Gly Lys Glu
1 5 10 15

Asp Lys Gly Thr Ser Ala Arg Asn Asp Thr
20 25

(285) INFORMATION FOR SEQ ID NO:285

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:285:

Gln Gln Val Pro Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Lys Asp
1 5 10 15

Asp Lys Gly Thr Ser Ala Lys Asn Glu Thr
20 25

(286) INFORMATION FOR SEQ ID NO:286

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:286:

Gln Gln Val Pro Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Lys Asp
1 5 10 15

Asp Lys Gly Thr Ser Ala Lys Asn Glu Met
20 25

(287) INFORMATION FOR SEQ ID NO:287

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:287:

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

Lys Lys Gly Thr Ser Ser Thr Ser Glu Thr
20 25

(288) INFORMATION FOR SEQ ID NO:288

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:288:

Glu Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(289) INFORMATION FOR SEQ ID NO:289

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:289:

Glu Gln Gln Pro Glu Leu Arg Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(290) INFORMATION FOR SEQ ID NO:290

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:290:

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Glu Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Ala Ser Glu Ser
20 25

(291) INFORMATION FOR SEQ ID NO:291

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:291:

Glu Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Lys Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(292) INFORMATION FOR SEQ ID NO:292

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:292:

Glu Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Thr His Gly Lys Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(293) INFORMATION FOR SEQ ID NO:293

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:293:

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

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(294) INFORMATION FOR SEQ ID NO:294

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:294:

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

Lys Lys Gly Lys Ser Ser Ala Ser Glu Ser
20 25

(295) INFORMATION FOR SEQ ID NO:295

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:295:

Glu Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Thr His Gly Lys Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Phe Glu Ser
20 25

(296) INFORMATION FOR SEQ ID NO:296

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:296:

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Glu Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Thr His Gly Lys Gln
1 5 10 15

Lys Gln Gly Lys Ser Ser Thr Phe Glu Ser
20 25

(297) INFORMATION FOR SEQ ID NO:297

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:297:

Glu Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Thr His Gly Lys Glu
1 5 10 15

Lys Lys Asp Lys Ser Ser Thr Ser Glu Ser
20 25

(298) INFORMATION FOR SEQ ID NO:298

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:298:

Glu Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Ser His Gly Lys Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(299) INFORMATION FOR SEQ ID NO:299

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:299:

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Glu Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Thr His Gly Lys Gln
1 5 10 15

Lys Lys Ser Asn Ser Ser Thr Ser Glu Ser
20 25

(300) INFORMATION FOR SEQ ID NO:300

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:300:

Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Ala Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(301) INFORMATION FOR SEQ ID NO:301

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:301:

Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Ala Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Asp Ser
20 25

(302) INFORMATION FOR SEQ ID NO:302

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:302:

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Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Val Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Asp Ser
20 25

(303) INFORMATION FOR SEQ ID NO:303

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:303:

Gln Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Ala Pro Gly Lys Gly
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(304) INFORMATION FOR SEQ ID NO:304

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:304:

Gln Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Ala Pro Gly Lys Gly
1 5 10 15

Lys Lys Asp Lys Ser Ser Thr Ser Glu Ser
20 25

(305) INFORMATION FOR SEQ ID NO:305

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:305:

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Glu Gln Gln Pro Glu Ala Lys Pro Gly Lys Gly Thr His Gly Lys Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Asp Ser
20 25

(306) INFORMATION FOR SEQ ID NO:306

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:306:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr His Gly Lys Glu
1 5 10 15

Lys Lys Asp Lys Ser Ser Thr Ser Asp Ser
20 25

(307) INFORMATION FOR SEQ ID NO:307

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:307:

Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Ala Pro Gly Gln Gly
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(308) INFORMATION FOR SEQ ID NO:308

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:308:

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Gln Gln Gln Ala Glu Leu Lys Pro Gly Arg Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(309) INFORMATION FOR SEQ ID NO:309

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:309:

Glu Gln Gln Ala Glu Leu Arg Ala Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(310) INFORMATION FOR SEQ ID NO:310

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:310:

Glu Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Thr Ser Ser Thr Ser Glu Ser
20 25

(311) INFORMATION FOR SEQ ID NO:311

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:311:

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Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Thr Pro Gly His Glu
1 5 10 15

Lys Lys Gly Thr Ser Ser Thr Ser Glu Ser
20 25

(312) INFORMATION FOR SEQ ID NO:312

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:312:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly His Glu
1 5 10 15

Lys Lys Gly Thr Ser Ser Thr Ser Glu Ser
20 25

(313) INFORMATION FOR SEQ ID NO:313

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:313:

Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Thr Pro Gly His Glu
1 5 10 15

Asn Lys Gly Thr Ser Ser Thr Ser Glu Ser
20 25

(314) INFORMATION FOR SEQ ID NO:314

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:314:

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Gln Gln Gln Ala Glu Val Arg Pro Gly Lys Gly Thr Pro Gly His Glu
1 5 10 15

Lys Lys Gly Thr Ser Ser Thr Ser Glu Ser
20 25

(315) INFORMATION FOR SEQ ID NO:315

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:315:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly His Glu
1 5 10 15

Asn Lys Gly Thr Ser Ser Thr Ser Glu Ser
20 25

(316) INFORMATION FOR SEQ ID NO:316

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:316:

Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Ala Ser Glu Ser
20 25

(317) INFORMATION FOR SEQ ID NO:317

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:317:

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His Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(318) INFORMATION FOR SEQ ID NO:318

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:318:

Glu Gln Gln Val Glu Leu Arg Ala Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(319) INFORMATION FOR SEQ ID NO:319

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:319:

Glu Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Gln Gly Thr Ser Ser Thr Ser Glu Ser
20 25

(320) INFORMATION FOR SEQ ID NO:320

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:320:

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Glu Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Thr Pro Gly His Asp
1 5 10 15

Asn Lys Gly Thr Ser Ser Thr Ser Glu Ser
20 25

(321) INFORMATION FOR SEQ ID NO:321

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:321:

Gln Gln Gln Ala Glu Val Arg Pro Gly Lys Gly Thr Pro Gly His Glu
1 5 10 15

Lys Lys Gly Arg Ser Ser Thr Ser Glu Ser
20 25

(322) INFORMATION FOR SEQ ID NO:322

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:322:

Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Lys Asp Lys Ser Ser Thr Ser Glu Ser
20 25

(323) INFORMATION FOR SEQ ID NO:323

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:323:

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Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Lys Asp Lys Ser Ser Thr Ser Glu Ser
20 25

(324) INFORMATION FOR SEQ ID NO:324

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:324:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Lys Asp Lys Ser Ser Thr Ser Asp Ser
20 25

(325) INFORMATION FOR SEQ ID NO:325

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:325:

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

Lys Lys Asp Lys Ser Ser Thr Ser Glu Ser
20 25

(326) INFORMATION FOR SEQ ID NO:326

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:326:

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Gln His Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Lys Asn Lys Ser Ser Thr Ser Glu Ser
20 25

(327) INFORMATION FOR SEQ ID NO:327

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:327:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Asn Lys Asp Lys Ser Ser Thr Ser Glu Ser
20 25

(328) INFORMATION FOR SEQ ID NO:328

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:328:

Glu Gln Gln Ala Glu Leu Arg Ala Gly Lys Gly Ile Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(329) INFORMATION FOR SEQ ID NO:329

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:329:

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Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Ser Lys Ser Ser Thr Ser Glu Ser
20 25

(330) INFORMATION FOR SEQ ID NO:330

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:330:

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

Lys Lys Ser Lys Ser Ser Thr Ser Glu Ser
20 25

(331) INFORMATION FOR SEQ ID NO:331

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:331:

Gln Gln Gln Thr Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Ser Lys Ser Ser Thr Ser Glu Ser
20 25

(332) INFORMATION FOR SEQ ID NO:332

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:332:

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Glu Gln Gln Ala Glu Leu Arg Thr Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Arg Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(333) INFORMATION FOR SEQ ID NO:333

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:333:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Lys Asp Lys Ser Ser Thr Phe Glu Ser
20 25

(334) INFORMATION FOR SEQ ID NO:334

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:334:

Glu Gln Gln Ala Glu Leu Arg Pro Gly Thr Gly Ala Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(335) INFORMATION FOR SEQ ID NO:335

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:335:

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Gln Gln Gln Pro Glu Val Arg Pro Gly Lys Gly Thr His Ala Lys Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(336) INFORMATION FOR SEQ ID NO:336

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:336:

Gln Gln Gln Pro Glu Val Arg Pro Gly Lys Asp Thr His Ala Lys Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(337) INFORMATION FOR SEQ ID NO:337

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:337:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Glu Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(338) INFORMATION FOR SEQ ID NO:338

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:338:

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Glu Gln Gln Thr Glu Leu Arg Ala Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Arg Ser Ser Thr Ser Glu Ala
20 25

(339) INFORMATION FOR SEQ ID NO:339

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:339:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Arg Glu
1 5 10 15

Lys Lys Ser Lys Pro Ser Thr Ser Glu Ser
20 25

(340) INFORMATION FOR SEQ ID NO:340

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:340:

Gln Gln Gln Ser Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Arg Glu
1 5 10 15

Lys Lys Ser Lys Pro Ser Thr Ser Glu Ser
20 25

(341) INFORMATION FOR SEQ ID NO:341

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:341:

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Gln Gln Arg Ala Glu Leu Lys Pro Gly Lys Asp Thr Pro Gly Arg Glu
1 5 10 15

Lys Lys Asn Lys Pro Ser Thr Ser Glu Ser
20 25

(342) INFORMATION FOR SEQ ID NO:342

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:342:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Arg Glu
1 5 10 15

Lys Lys Ser Thr Ser Ser Thr Ser Glu Ser
20 25

(343) INFORMATION FOR SEQ ID NO:343

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:343:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Ser Thr Ser Ser Thr Ser Asp Ser
20 25

(344) INFORMATION FOR SEQ ID NO:344

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:344:

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Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Thr Pro Ile Gln Gln
1 5 10 15

Lys Lys Asp Lys Ser Ser Thr Ser Glu Ser
20 25

(345) INFORMATION FOR SEQ ID NO:345

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:345:

Gln Gln Gln Ala Glu Phe Lys Pro Gly Lys Gly Thr Pro Gly Arg Glu
1 5 10 15

His Arg Ser Lys Pro Ser Thr Ser Glu Ser
20 25

(346) INFORMATION FOR SEQ ID NO:346

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:346:

Gln Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Ala Leu Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Asp Ser
20 25

(347) INFORMATION FOR SEQ ID NO:347

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:347:

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Gln Gln Gln Pro Glu Val Lys Pro Gly Lys Gly Ala Pro Gly Lys Gly
1 5 10 15

Asn Thr Asp Lys Ser Ser Thr Ser Glu Ser
20 25

(348) INFORMATION FOR SEQ ID NO:348

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:348:

Glu Gln Gln Ala Glu Val Arg Ala Gly Lys Gly Ser Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(349) INFORMATION FOR SEQ ID NO:349

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:349:

Gln Gln Leu Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly His Glu
1 5 10 15

Lys Lys Gly Ile Ser Ser Thr Ser Glu Ser
20 25

(350) INFORMATION FOR SEQ ID NO:350

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:350:

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Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Lys Pro Glu Gln Glu
1 5 10 15

Lys Lys Gly Thr Ser Ser Thr Ser Glu Ser
20 25

(351) INFORMATION FOR SEQ ID NO:351

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:351:

Gln Gln Gln Pro Glu Leu Lys Pro Gly Lys Gly Arg Asn Gly Lys Glu
1 5 10 15

Asn Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(352) INFORMATION FOR SEQ ID NO:352

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:352:

Gln Gln Gln Thr Glu Leu Arg Pro Gly Arg Gly Thr Thr Gly Gln Glu
1 5 10 15

Arg Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(353) INFORMATION FOR SEQ ID NO:353

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:353:

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Gln His Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly His Glu
1 5 10 15

Asn Lys Val Thr Ser Ser Thr Ser Glu Ser
20 25

(354) INFORMATION FOR SEQ ID NO:354

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:354:

Glu Gln Gln Ala Glu Leu Arg Ala Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Gln Lys Ala Lys Ser Ser Thr Ser Glu Ser
20 25

(355) INFORMATION FOR SEQ ID NO:355

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:355:

Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Thr Gly Thr Ser Ser Thr Thr Glu Ser
20 25

(356) INFORMATION FOR SEQ ID NO:356

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:356:

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Gln Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Asn Pro Gly Gln Glu
1 5 10 15

Lys Lys Ser Thr Ser Ser Ala Ser Glu Ser
20 25

(357) INFORMATION FOR SEQ ID NO:357

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:357:

Glu Gln Gln Thr Val Leu Arg Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Lys Gly Thr Ser Ala Thr Asn Glu Ser
20 25

(358) INFORMATION FOR SEQ ID NO:358

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:358:

Gln Gln Leu Thr Glu Leu Lys Pro Gly Asn Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Ser Lys Ser Ser Thr Ser Glu Ser
20 25

(359) INFORMATION FOR SEQ ID NO:359

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:359:

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

Lys Lys Gly Thr Ser Ser Thr Ser Lys Ser
20 25

(360) INFORMATION FOR SEQ ID NO:360

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:360:

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

Lys Lys Asp Lys Ser Ser Thr Ser Glu Ser
20 25

(361) INFORMATION FOR SEQ ID NO:361

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:361:

Glu Gln Gln Pro Glu Thr Lys Pro Gly Lys Gly Thr Leu Gly Lys Gln
1 5 10 15

Lys Lys Ser Lys Ser Ser Thr Ser Glu Ser
20 25

(362) INFORMATION FOR SEQ ID NO:362

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:362:

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Gln Gln Gln Ala Glu Leu Lys Pro Gly Gln Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Asn Lys Ser Ser Thr Pro Glu Phe
20 25

(363) INFORMATION FOR SEQ ID NO:363

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:363:

Glu Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Gln Gly Thr Ser Ser Thr Ser Glu Thr
20 25

(364) INFORMATION FOR SEQ ID NO:364

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:364:

Glu Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Gln Gly Thr Ser Thr Thr Ser Glu Thr
20 25

(365) INFORMATION FOR SEQ ID NO:365

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:365:

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Glu Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Gln Gly Thr Ser Ser Thr Ser Glu Thr
20 25

(366) INFORMATION FOR SEQ ID NO:366

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:366:

Glu Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Gln Asp Thr Ser Ser Thr Ser Glu Thr
20 25

(367) INFORMATION FOR SEQ ID NO:367

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:367:

Glu Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Gln Gly Thr Ser Ser Thr Ser Gly Thr
20 25

(368) INFORMATION FOR SEQ ID NO:368

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:368:

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Glu Gln Gln Ala Glu Val Lys Pro Gly Lys Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Gln Gly Thr Ser Ser Thr Ser Glu Thr
20 25

(369) INFORMATION FOR SEQ ID NO:369

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:369:

Glu Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Gln Val Thr Ser Ser Thr Ser Glu Thr
20 25

(370) INFORMATION FOR SEQ ID NO:370

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:370:

Glu Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Gln Ile Thr Ser Ser Thr Ser Glu Thr
20 25

(371) INFORMATION FOR SEQ ID NO:371

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:371:

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Glu Gln Gln Ala Glu Leu Arg Pro Gly Arg Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Gln Val Thr Ser Ser Thr Ser Glu Thr
20 25

(372) INFORMATION FOR SEQ ID NO:372

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:372:

Glu Gln Gln Ala Glu Leu Arg Pro Gly Arg Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys His Val Thr Ser Ser Thr Ser Glu Thr
20 25

(373) INFORMATION FOR SEQ ID NO:373

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:373:

Glu Gln Gln Ala Glu Leu Arg Pro Gly Lys Gly Asn Thr Glu Gln Pro
1 5 10 15

Lys Gln Val Thr Ser Ser Thr Ser Glu Thr
20 25

(374) INFORMATION FOR SEQ ID NO:374

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:374:

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Glu Gln Gln Ala Glu Leu Lys Pro Gly Lys Gly Asn Thr Glu Gln Pro
1 5 10 15

Lys Leu Ile Thr Ser Ser Thr Ser Glu Thr
20 25

(375) INFORMATION FOR SEQ ID NO:375

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:375:

Thr Gly Gln Ala Glu Leu Arg Pro Gly Lys Gly Ala Pro Glu Gln Gly
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Asp Arg
20 25

(376) INFORMATION FOR SEQ ID NO:376

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:376:

Gln Tyr Gln Ala Glu Leu Arg Pro Gly Lys Gly Thr Pro Arg Gln Gln
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(377) INFORMATION FOR SEQ ID NO:377

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:377:

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Gln Gln Gln Ala Val Leu Arg His Gly Lys Gly Thr His Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Thr Ser Glu Ser
20 25

(378) INFORMATION FOR SEQ ID NO:378

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:378:

Gln Gln Gln Thr Lys Leu Gly Pro Gly Arg Gly Thr Pro Gly Gln Gly
1 5 10 15

Arg Lys Gly Lys Ser Ser Thr Ser Gly Ser
20 25

(379) INFORMATION FOR SEQ ID NO:379

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:379:

Glu Gln Gln Ala Glu Leu Arg Ala Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Lys Ser Ser Val Tyr Phe Ala
20 25

(380) INFORMATION FOR SEQ ID NO:380

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:380:

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Glu Gln Gln Ala Glu Leu Lys Ala Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Gln Gly Glu Ser Thr Arg Ser Glu Thr
20 25

(381) INFORMATION FOR SEQ ID NO:381

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:381:

Gln Gln Lys Ala Glu Leu Ala Ala Ser Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Lys Gly Arg Ser Ser Thr Ser Glu Ser
20 25

(382) INFORMATION FOR SEQ ID NO:382

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:382:

Gln Gln Gln Thr Glu Leu Arg Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Arg Gly Lys Ser Ser Asn Leu Arg Leu
20 25

(383) INFORMATION FOR SEQ ID NO:383

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:383:

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

Ser Lys Gly Thr Ser Gln Arg Ala Glu Thr
20 25

(384) INFORMATION FOR SEQ ID NO:384

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:384:

Glu Gln Gln Ala Asp Leu Lys Leu Gly Lys Gly Asn Pro Glu Gln Pro
1 5 10 15

Lys Leu Ala Thr Pro Ser Thr Ser Glu Thr
20 25

(385) INFORMATION FOR SEQ ID NO:385

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:385:

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

Asp Val Lys Asp Asn Ala Lys Ser Glu Thr
20 25

(386) INFORMATION FOR SEQ ID NO:386

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:386:

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Asp Gln Gln Pro Asp Leu Lys Pro Ser Ser Gly Ser Pro Gly His Pro
1 5 10 15

Ser Lys Ser Thr Ser Lys Thr Thr Glu Thr
20 25

(387) INFORMATION FOR SEQ ID NO:387

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:387:

Asp Gln Gln Pro Asp Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Ser Thr Ser Lys Thr Thr Glu Thr
20 25

(388) INFORMATION FOR SEQ ID NO:388

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:388:

Asp Gln Gln Pro Asp Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Ser Thr Ser Lys Thr Ala Glu Thr
20 25

(389) INFORMATION FOR SEQ ID NO:389

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:389:

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Asp Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Ser Thr Ser Lys Thr Thr Glu Thr
20 25

(390) INFORMATION FOR SEQ ID NO:390

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:390:

Asp Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Asn Thr Ser Lys Thr Thr Glu Thr
20 25

(391) INFORMATION FOR SEQ ID NO:391

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:391:

Asp Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asp Pro
1 5 10 15

Ser Lys Thr Thr Ser Lys Thr Thr Glu Thr
20 25

(392) INFORMATION FOR SEQ ID NO:392

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:392:

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Asp Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Thr Thr Ser Lys Thr Thr Glu Thr
20 25

(393) INFORMATION FOR SEQ ID NO:393

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:393:

Asp His Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Asn Thr Ser Lys Thr Thr Glu Thr
20 25

(394) INFORMATION FOR SEQ ID NO:394

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:394:

Asp Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Arg Ser Thr Ser Lys Thr Thr Glu Thr
20 25

(395) INFORMATION FOR SEQ ID NO:395

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:395:

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Asp Gln Gln Pro Gly Leu Lys Pro Ser Ala Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Ser Thr Ser Lys Thr Ala Glu Thr
20 25

(396) INFORMATION FOR SEQ ID NO:396

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:396:

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

Ser Lys Ser Thr Ser Lys Thr Ser Glu Thr
20 25

(397) INFORMATION FOR SEQ ID NO:397

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:397:

Asp Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Asn Thr Ser Lys Thr Ile Glu Thr
20 25

(398) INFORMATION FOR SEQ ID NO:398

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:398:

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Asp Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asp Pro
1 5 10 15

Ser Lys Asn Thr Ser Lys Thr Pro Glu Thr
20 25

(399) INFORMATION FOR SEQ ID NO:399

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:399:

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

Ser Lys Ser Thr Ser Lys Thr Thr Glu Thr
20 25

(400) INFORMATION FOR SEQ ID NO:400

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:400:

Asp Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Asn Thr Ser Glu Thr Thr Glu Thr
20 25

(401) INFORMATION FOR SEQ ID NO:401

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:401:

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Asp Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Asn Pro
1 5 10 15

Ser Lys Asn Thr Ser Glu Thr Thr Glx Thr
20 25

(402) INFORMATION FOR SEQ ID NO:402

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:402:

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

Ser Lys Ser Thr Ser Lys Thr Ser Glu Thr
20 25

(403) INFORMATION FOR SEQ ID NO:403

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:403:

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

Ser Lys Ser Thr Ser Arg Thr Thr Glu Thr
20 25

(404) INFORMATION FOR SEQ ID NO:404

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:404:

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

Ser Lys Ser Thr Ser Lys Thr Ala Glu Thr
20 25

(405) INFORMATION FOR SEQ ID NO:405

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:405:

Asp Gln Gln Pro Asp Leu Lys Pro Ser Ser Gly Phe Pro Gly Asn Pro
1 5 10 15

Ser Lys Ser Thr Ser Lys Thr Thr Glu Thr
20 25

(406) INFORMATION FOR SEQ ID NO:406

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:406:

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

Ser Lys Ser Thr Ser Lys Thr Asn Glu Thr
20 25

(407) INFORMATION FOR SEQ ID NO:407

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:407:

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

Ser Lys Ser Thr Phe Lys Thr Ser Glu Thr
20 25

(408) INFORMATION FOR SEQ ID NO:408

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:408:

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

Ser Lys Ser Thr Ser Thr Thr Ser Glu Thr
20 25

(409) INFORMATION FOR SEQ ID NO:409

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:409:

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

Ser Lys Ser Thr Ser Lys Thr Thr Glu Thr
20 25

(410) INFORMATION FOR SEQ ID NO:410

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:410:

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Gln Gln Gln Pro Gly Leu Lys Pro Ser Phe Gly Pro Pro Gly Lys Pro
1 5 10 15

Ser Gln Ser Thr Ser Lys Thr Thr Glu Thr
20 25

(411) INFORMATION FOR SEQ ID NO:411

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:411:

Gln Gln Lys Pro Gly Leu Ala Pro Ser Ser Gly Ser Pro Gly Lys Ser
1 5 10 15

Thr Lys Ser Asn Ser Lys Gln Thr Asp Thr
20 25

(412) INFORMATION FOR SEQ ID NO:412

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:412:

Gln Gln Lys Pro Gly Leu Ala Pro Ser Ser Gly Ser Pro Gly Lys Ser
1 5 10 15

Ala Lys Ser Asn Ser Lys Gln Thr Asp Thr
20 25

(413) INFORMATION FOR SEQ ID NO:413

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:413:

Gln Gln Lys Pro Gly Leu Ala Pro Ser Ser Gly Ser Pro Gly Lys Ser
1 5 10 15

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Ala Met Ser Asn Ser Lys Gln Thr Asp Thr
20 25

(414) INFORMATION FOR SEQ ID NO:414

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:414:

Gln Gln Lys Pro Gly Leu Ala Pro Ser Ser Gly Ser Pro Gly Lys Ser
1 5 10 15

Ala Ile Ser Asn Ser Lys Gln Thr Asp Thr
20 25

(415) INFORMATION FOR SEQ ID NO:415

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:415:

Gln Gln Lys Pro Gly Leu Gln Pro Ser Ser Gly Ser Pro Gly Lys Ala
1 5 10 15

Ala Ile Ser Asn Ser Lys Gln Ser Asn Thr
20 25

(416) INFORMATION FOR SEQ ID NO:416

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:416:

Gln Gln Lys Pro Gly Leu Gln Pro Ser Ser Gly Ser Pro Gly Lys Ala
1 5 10 15

Ala Ile Ser Asn Ser Lys Gln Ala Asn Thr
20 25

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(417) INFORMATION FOR SEQ ID NO:417

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:417:

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

Ala Met Ser Asn Ser Lys Gln Ile Asp Thr
20 25

(418) INFORMATION FOR SEQ ID NO:418

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:418:

Gln Gln Lys Pro Ser Leu Gln Pro Ser Ser Asp Ser Pro Gly Lys Ala
1 5 10 15

Ala Met Ser Asn Ser Lys Gln Ala Asp Thr
20 25

(419) INFORMATION FOR SEQ ID NO:419

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:419:

Glu Arg Val Gly Asp Leu Glu Pro Gly Arg Gly Ile Pro Gly Lys Ala
1 5 10 15

Pro Lys Gly Asp Ser Lys Lys Ile Glu Thr
20 25

(420) INFORMATION FOR SEQ ID NO:420

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:420:

Glu Arg Val Gly Asp Leu Glu Pro Glu Arg Gly Ile Pro Gly Lys Ala
1 5 10 15

Pro Lys Gly Asp Ser Lys Lys Ile Glu Thr
20 25

(421) INFORMATION FOR SEQ ID NO:421

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:421:

Glu Gln Val Gly Gly Leu Lys Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Pro Lys Gly Asp Ser Lys Lys Thr Glu Thr
20 25

(422) INFORMATION FOR SEQ ID NO:422

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:422:

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

Ser Lys Gly Asp Ser Lys Lys Thr Glu Thr
20 25

(423) INFORMATION FOR SEQ ID NO:423

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:423:

Glu Gln Leu Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asx
1 5 10 15

Ser Lys Gly Asp Ser Lys Arg Ala Glu Thr
20 25

(424) INFORMATION FOR SEQ ID NO:424

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:424:

Glu Gln Leu Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ser Lys Arg Ala Glu Thr
20 25

(425) INFORMATION FOR SEQ ID NO:425

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:425:

Glu Gln Leu Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Arg Gly Asn Ser Lys Arg Ala Glu Thr
20 25

(426) INFORMATION FOR SEQ ID NO:426

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:426:

Glu Gln Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ser Lys Arg Ala Glu Thr
20 25

(427) INFORMATION FOR SEQ ID NO:427

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:427:

Glu Gln Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Arg Ala Glu Thr
20 25

(428) INFORMATION FOR SEQ ID NO:428

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:428:

Glu Gln Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asp Ser Arg Arg Ala Glu Thr
20 25

(429) INFORMATION FOR SEQ ID NO:429

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:429:

Glu Gln Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ser Arg Arg Ala Glu Thr
20 25

(430) INFORMATION FOR SEQ ID NO:430

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:430:

Gln Gln Val Gly Gly Leu Glu Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asx Ser Lys Arg Ala Glu Thr
20 25

(431) INFORMATION FOR SEQ ID NO:431

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:431:

Glu Gln Leu Gly Asp Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Asn Ser Lys Arg Ala Glu Thr
20 25

(432) INFORMATION FOR SEQ ID NO:432

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:432:

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Glu Gln Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Thr Gly Lys Asp
1 5 10 15

Ser Lys Gly Asp Ser Lys Arg Ala Glu Thr
20 25

(433) INFORMATION FOR SEQ ID NO:433

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:433:

Gln Gln Val Gly Gly Val Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ser Lys Arg Ala Glu Thr
20 25

(434) INFORMATION FOR SEQ ID NO:434

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:434:

Gln Gln Val Gly Gly Val Gln Pro Gly Arg Gly Ile Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ser Lys Arg Pro Glu Thr
20 25

(435) INFORMATION FOR SEQ ID NO:435

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:435:

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Glu Gln Val Gly Gly Val Gln Pro Gly Arg Gly Ile Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asp Ser Lys Arg Pro Glu Thr
20 25

(436) INFORMATION FOR SEQ ID NO:436

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:436:

Gln Gln Val Gly Gly Val Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Asn Gly Asp Ser Lys Arg Pro Glu Thr
20 25

(437) INFORMATION FOR SEQ ID NO:437

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:437:

Gln Lys Val Gly Gly Val Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ser Lys Arg Thr Glu Thr
20 25

(438) INFORMATION FOR SEQ ID NO:438

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:438:

Gln Glu Val Gly Gly Val Glx Pro Gly Arg Gly Thr Pro Gly Lys Asx
1 5 10 15

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Ser Lys Gly Asx Ser Lys Arg Ala Glu Thr
20 25

(439) INFORMATION FOR SEQ ID NO:439

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:439:

Glu Gln Leu Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Asn Gly Asp Ser Lys Gln Ala Glx Thr
20 25

(440) INFORMATION FOR SEQ ID NO:440

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:440:

Glu Gln Leu Gly Gly Leu Gln Pro Gly Arg Gly Ser Pro Gly Lys Asp
1 5 10 15

Thr Asn Gly Asp Ser Lys Glu Ala Glx Thr
20 25

(441) INFORMATION FOR SEQ ID NO:441

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:441:

Ala Gln Leu Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Asn Gly Asp Ser Lys Gln Ala Glx Ser
20 25

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(442) INFORMATION FOR SEQ ID NO:442

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:442:

Glu Gln Leu Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Val
1 5 10 15

Ser Gln Gly Asp Ser Lys Gln Ala Glx Thr
20 25

(443) INFORMATION FOR SEQ ID NO:443

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:443:

Glu Gln Val Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Val
1 5 10 15

Ser Gln Gly Asp Ser Lys Glu Pro Glx Thr
20 25

(444) INFORMATION FOR SEQ ID NO:444

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:444:

Glu Gln Leu Gly Gly Leu Gln Pro Glu Arg Gly Thr Pro Gly Lys Glu
1 5 10 15

Ser Lys Gly Asn Ser Met Arg Ala Glu Thr
20 25

(445) INFORMATION FOR SEQ ID NO:445

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:445:

Glu Gln Val Gly Asp Leu Gln Pro Gly Arg Gly Asx Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Lys Arg Val Glu Thr
20 25

(446) INFORMATION FOR SEQ ID NO:446

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:446:

Glu Gln Val Gly Asp Leu Gln Pro Gly Arg Gly Asn Pro Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ala Gln Arg Pro Glu Thr
20 25

(447) INFORMATION FOR SEQ ID NO:447

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:447:

Gln Gln Val Gly Gly Val Gln Pro Gly Arg Gly Thr Leu Gly Lys Asp
1 5 10 15

Ser Lys Gly Asn Ser Lys Arg Ala Glu Thr
20 25

(448) INFORMATION FOR SEQ ID NO:448

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:448:

Gln Glx Val Gly Gly Ala Glx Pro Gly Arg Gly Ser Pro Gly Lys Ala
1 5 10 15

Ser Lys Gly Asx Ser Lys Arg Ala Glu Thr
20 25

(449) INFORMATION FOR SEQ ID NO:449

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:449:

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

Ser Lys Gly Asn Ala Gln Arg Thr Glx Thr
20 25

(450) INFORMATION FOR SEQ ID NO:450

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:450:

Asp Gln Val Gly Gly Leu Lys Pro Gly Arg Gly Thr Pro Gly Lys Asn
1 5 10 15

Ser Asn Gly Asp Ser Lys Thr Pro Glx Thr
20 25

(451) INFORMATION FOR SEQ ID NO:451

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:451:

Glu Gln Leu Gly Gly Leu Gln Pro Gly Arg Gly Thr Ser Arg Glu Asp
1 5 10 15

Ser Lys Gly Asn Ser Lys Arg Ala Glu Thr
20 25

(452) INFORMATION FOR SEQ ID NO:452

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:452:

Glu Gln Val Gly Ala Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Asp
1 5 10 15

Ser Gln Ala Asp Ser Lys Glu Ala Glx Thr
20 25

(453) INFORMATION FOR SEQ ID NO:453

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:453:

Glu Gln Leu Gly Gly Leu Gln Pro Gly Arg Gly Thr Pro Gly Lys Val
1 5 10 15

Glu Gly Ser Val Glu Thr
20

(454) INFORMATION FOR SEQ ID NO:454

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:454:

Glu Gln Val Gly Ala Phe Gln Pro Gly Arg Gly Asn Ser Gly Lys Ala
1 5 10 15

Ser Lys Gly Asp Ser Lys Arg Pro Asp Thr
20 25

(455) INFORMATION FOR SEQ ID NO:455

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:455:

Glu Gln Val Gly Ala Phe Gln Pro Gly Lys Gly Asn Ser Gly Lys Ala
1 5 10 15

Ser Lys Gly Asp Ser Lys Arg Pro Asp Thr
20 25

(456) INFORMATION FOR SEQ ID NO:456

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:456:

Glu Gln Val Gly Ala Phe Gln Pro Gly Lys Gly Asn Ser Gly Lys Ala
1 5 10 15

Ser Lys Gly Asp Ser Asn Arg Pro Asp Thr
20 25

(457) INFORMATION FOR SEQ ID NO:457

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:457:

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Gln Gln Val Gly Gly Val Gln Ala Gly Arg Ala Asn Pro Gly Lys Asp
1 5 10 15

Ser Arg Gly Ile Ser Lys Arg Thr Glu Thr
20 25

(458) INFORMATION FOR SEQ ID NO:458

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:458:

Gln Gln Val Ala Glu Val Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Gln Gly Glu Ser Thr Arg Ser Glu Thr
20 25

(459) INFORMATION FOR SEQ ID NO:459

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:459:

Gln Gln Val Ala Glu Val Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Gln Gly Thr Ser Thr Arg Ser Glu Thr
20 25

(460) INFORMATION FOR SEQ ID NO:460

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:460:

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Gln Gln Val Ala Glu Val Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Gln Gly Thr Ser Ala Arg Ser Glu Thr
20 25

(461) INFORMATION FOR SEQ ID NO:461

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:461:

Gln Gln Val Ala Glu Val Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Gln Gly Thr Ser Ile Arg Ser Asp Thr
20 25

(462) INFORMATION FOR SEQ ID NO:462

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:462:

Gln Gln Val Ala Glu Val Lys Pro Gly Lys Gly Thr Pro Gly Gln Glu
1 5 10 15

Lys Gln Gly Thr Ser Ile Arg Ser Asp Thr
20 25

(463) INFORMATION FOR SEQ ID NO:463

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:463:

Gln Gln Val Ala Glu Val Lys Pro Gly Lys Gly Thr Pro Gly Gln Gln
1 5 10 15

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Asn Gln Gly Thr Ser Thr Arg Ser Asp Thr
20 25

(464) INFORMATION FOR SEQ ID NO:464

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:464:

Gln Gln Val Gly Glu Val Lys Pro Gly Arg Gly Thr Pro Gly Gln Gln
1 5 10 15

Lys Gln Asp Thr Ser Thr Arg Ser Asp Thr
20 25

(465) INFORMATION FOR SEQ ID NO:465

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:465:

Gln Gln Val Ala Glu Val Lys Pro Gly Arg Gly Thr Pro Gly His Pro
1 5 10 15

Arg Gln Gly Ala Ser Phe Arg Ser Asp Ser
20 25

(466) INFORMATION FOR SEQ ID NO:466

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:466:

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

Gly Thr Gly Thr Ser Val Lys Ala Glu Thr
20 25

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(467) INFORMATION FOR SEQ ID NO:467

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:467:

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

Ser Gln Gly Lys Ser Ile Lys Ala Ser Thr
20 25

(468) INFORMATION FOR SEQ ID NO:468

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:468:

Glu Gln Val Ala Glu Val Lys Pro Gly Arg Gly Ser Pro Gly Lys Pro
1 5 10 15

Ser Gln Gly Lys Ser Ile Lys Ala Ser Thr
20 25

(469) INFORMATION FOR SEQ ID NO:469

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:469:

Gln Gln Val Ala Glu Val Lys Pro Gly Arg Gly Asp Pro Gly Arg Pro
1 5 10 15

Arg Gln Ala Ser Ser Thr Ile Ser Ala Thr
20 25

(470) INFORMATION FOR SEQ ID NO:470

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:470:

Glu Gln Val Ala Glu Val Pro Gln Gly Lys Gly Arg Pro Gly Lys Ser
1 5 10 15

Leu Gln Gly Lys Ser Leu Lys Ala Ser Thr
20 25

(471) INFORMATION FOR SEQ ID NO:471

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:471:

Gln Gln Met Ala Glu Val Lys Pro Gly Arg Gly Thr Pro Gly Lys Pro
1 5 10 15

Gly Val Val Pro Ser Phe Phe Ser Glu Thr
20 25

(472) INFORMATION FOR SEQ ID NO:472

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:472:

Gln Gln Val Ala Glu Val Lys Pro Gly Arg Gly Thr Pro Gly Arg Tyr
1 5 10 15

Ile Trp Glu Pro Ser Phe Phe Asn Glu Gly
20 25

(473) INFORMATION FOR SEQ ID NO:473

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:473:

Gln Gln Gln Ala Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Lys Pro
1 5 10 15

Ser Lys Ser Thr Ser Lys Thr Ala Ala Thr
20 25

(474) INFORMATION FOR SEQ ID NO:474

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:474:

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

Ser Lys Ser Thr Ser Lys Thr Ala Ala Thr
20 25

(475) INFORMATION FOR SEQ ID NO:475

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:475:

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

Ser Lys Ser Thr Ser Asn Thr Ala Ala Thr
20 25

(476) INFORMATION FOR SEQ ID NO:476

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:476:

Gln Gln Gln Pro Gly Leu Lys Pro Ser Ser Gly Ser Ala Gly Lys Pro
1 5 10 15

Ser Lys Ser Thr Ser Lys Thr Ala Ala Thr
20 25

(477) INFORMATION FOR SEQ ID NO:477

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:477:

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

Ser Arg Gly Thr Ser Arg Ser Ala Ala Thr
20 25

(478) INFORMATION FOR SEQ ID NO:478

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:478:

Gln Gln Gln Ala Gly Leu Lys Pro Ser Ser Gly Ser Pro Gly Arg Thr
1 5 10 15

Ser Lys Ser Thr Ser Lys Thr Ala Ala Thr
20 25

(479) INFORMATION FOR SEQ ID NO:479

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:479:

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

Pro Arg Ser Thr Ser Lys Thr Ala Ala Thr
20 25

(480) INFORMATION FOR SEQ ID NO:480

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:480:

Xaa Gln Glu Pro Gly Leu Arg Pro Ser Ser Gly Ser Pro Gly Arg Thr
1 5 10 15

Pro Arg Ser Thr Ser Lys Thr Ala Ala Thr
20 25

(481) INFORMATION FOR SEQ ID NO:481

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:481:

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

Ser Lys Ser Thr Ser Lys Thr Pro Glu Thr
20 25

(482) INFORMATION FOR SEQ ID NO:482

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:482:

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Gln His Gln Ala Gly Leu Lys Arg Ser Ser Gly Pro Pro Gly Lys Pro
1 5 10 15

Ser Thr Ser Thr Ser Lys Thr Ala Ala Thr
20 25

(483) INFORMATION FOR SEQ ID NO:483

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:483:

Glx Gln Glu Ser Gly Leu Lys Pro Thr Ser Gly Ser Pro Gly Lys Pro
1 5 10 15

Ser Lys Ser Arg Ser Lys Ala Ala Asp Ala
20 25

(484) INFORMATION FOR SEQ ID NO:484

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:484:

Gln Thr Lys Pro Thr Leu Lys Pro Thr Thr Gly Ser Pro Gly Arg Pro
1 5 10 15

Ser Lys Ser Thr Ser Lys Asp Pro Val Thr
20 25

(485) INFORMATION FOR SEQ ID NO:485

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:485:

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Gln Thr Lys Pro Thr Leu Lys Pro Thr Thr Gly Ser Pro Gly Lys Pro
1 5 10 15

Ser Arg Ser Thr Ser Arg Asp Pro Val Ser
20 25

(486) INFORMATION FOR SEQ ID NO:486

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:486:

Glu Thr Arg Pro Ala Leu Lys Pro Thr Thr Gly Ser Pro Gly Lys Thr
1 5 10 15

Ser Lys Thr Thr Ser Lys Asp Pro Val Thr
20 25

(487) INFORMATION FOR SEQ ID NO:487

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:487:

Gln Asn Arg Pro Ala Leu Lys Ala Thr Thr Gly Ser Pro Gly Lys Thr
1 5 10 15

Ser Glu Thr Thr Ser Lys Asp Pro Ala Thr
20 25

(488) INFORMATION FOR SEQ ID NO:488

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:488:

Gln Thr Thr Pro Ala Leu Lys Pro Lys Thr Gly Ser Pro Gly Lys Thr
1 5 10 15

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Ser Arg Thr Asp Ser Lys Asn Pro Val Thr
20 25

(489) INFORMATION FOR SEQ ID NO:489

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:489:

Gln Thr Arg Pro Ala Leu Arg Pro Thr Thr Gly Ser Pro Gly Glu Ala
1 5 10 15

Ser Glu Thr Thr Ser Lys Gly Pro Gly Thr
20 25

(490) INFORMATION FOR SEQ ID NO:490

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:490:

Gln Thr Arg Pro Ala Leu Lys Pro Thr Thr Gly Ser Pro Gly Lys Thr
1 5 10 15

Ser Glu Thr Thr Ser Arg Asp Thr Ala Tyr
20 25

(491) INFORMATION FOR SEQ ID NO:491

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:491:

Leu Glu Gly Val Gln Leu Trp Gly Gly Arg Gly Ile Ser Arg Lys Tyr
1 5 10 15

Ala Lys Gly Asn Gly Lys Arg Glu Asp Ser
20 25

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(492) INFORMATION FOR SEQ ID NO:492

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:492:

Tyr Asn Asn Pro Gly Asn Gly Tyr Ile Ala
1 5 10

(493) INFORMATION FOR SEQ ID NO:493

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:493:

Tyr Ile Asn Pro Gly Lys Gly Tyr Leu Ser
1 5 10

(494) INFORMATION FOR SEQ ID NO:494

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:494:

Arg Ala Ser Gln Glu Ile Ser Gly Tyr Leu Ser
1 5 10

(495) INFORMATION FOR SEQ ID NO:495

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:495:

Arg Ala Ser Gly Asn Ile His Asn Tyr Leu Ala
1 5 10

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(496) INFORMATION FOR SEQ ID NO:496

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:496:

Arg Ala Ser Gln Asp Ile Asn Asn Phe Leu Asn
1 5 10

(497) INFORMATION FOR SEQ ID NO:497

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:497:

Arg Ala Ser Gln Ser Ile Gly Asn Asn Leu His
1 5 10

(498) INFORMATION FOR SEQ ID NO:498

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:498:

Ala Ala Ser Thr Leu Asp Ser
1 5

(499) INFORMATION FOR SEQ ID NO:499

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:499:

Tyr Thr Thr Thr Leu Ala Asp
1 5

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(500) INFORMATION FOR SEQ ID NO:500

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:500:

Phe Thr Ser Arg Ser Gln Ser
1 5

(501) INFORMATION FOR SEQ ID NO:501

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:501:

Lys Ala Ser Ser Leu Glu Ser
1 5

(502) INFORMATION FOR SEQ ID NO:502

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:502:

Leu Gln Tyr Leu Ser Tyr Pro Leu Thr
1 5

(503) INFORMATION FOR SEQ ID NO:503

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:503:

Gln His Phe Trp Ser Thr Pro Arg Thr
1 5

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(504) INFORMATION FOR SEQ ID NO:504

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:504:

Gln Gln Gly Asn Ala Leu Pro Arg Thr
1 5

(505) INFORMATION FOR SEQ ID NO:505

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:505:

Gln Gln Tyr Asn Ser Tyr Ser
1 5

(506) INFORMATION FOR SEQ ID NO:506

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:506:

Thr Phe Gly Ile Thr
1 5

(507) INFORMATION FOR SEQ ID NO:507

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:507:

Gly Tyr Gly Val Asn
1 5

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(508) INFORMATION FOR SEQ ID NO:508

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:508:

Ser Asn Gly Ile Asn
1 5

(509) INFORMATION FOR SEQ ID NO:509

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:509:

Asp Tyr Ala Met His
1 5

(510) INFORMATION FOR SEQ ID NO:510

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:510:

Glu Ile Phe Pro Gly Asn Ser Lys Thr Tyr
1 5 10

(511) INFORMATION FOR SEQ ID NO:511

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:511:

Met Ile Trp Gly Asp Gly Asn Thr Asp
1 5

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(512) INFORMATION FOR SEQ ID NO:512

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:512:

Tyr Asn Asn Pro Gly Asn Gly Tyr Ile Ala
1 5 10

(513) INFORMATION FOR SEQ ID NO:513

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:513:

Ile Ser Trp Asp Ser Ser Ser Ile Gly
1 5

(514) INFORMATION FOR SEQ ID NO:514

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:514:

Arg Glu Ile Arg Tyr
1 5

(515) INFORMATION FOR SEQ ID NO:515

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:515:

Glu Arg Asp Tyr Arg Leu Asp Tyr
1 5

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(516) INFORMATION FOR SEQ ID NO:516

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:516:

Ser Glu Tyr Tyr Gly Gly Ser Tyr Lys Phe Asp Tyr
1 5 10

(517) INFORMATION FOR SEQ ID NO:517

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:517:

Gly Arg Asp Tyr Tyr Asp Ser Gly Gly Tyr Phe Thr Val Ala Phe Asp
1 5 10 15

Ile

(518) INFORMATION FOR SEQ ID NO:518

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:518:

Arg Ala Ser Gln Ser Ile Ser Arg Trp Leu Ala
1 5 10

(519) INFORMATION FOR SEQ ID NO:519

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:519:

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Glu Ala Ser Asn Asp Leu Ala
1 5

(520) INFORMATION FOR SEQ ID NO:520

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:520:

Asp Phe Tyr Met Glu
1 5

(521) INFORMATION FOR SEQ ID NO:521

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

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:521:

Ile Ile Trp Asp Asp Gly Ser Asp Gln
1 5

(522) INFORMATION FOR SEQ ID NO:522

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:522:

Gln Ala Ser Gln Ser Ile Ile Lys Tyr Leu Asn
1 5 10

Claims

1. A method for determining how to humanize a rodent antibody or fragment thereof by resurfacing, said method comprising:
(a) determining the conformational structure of the variable region of said rodent antibody or fragment thereof by constructing a three-dimensional model of said rodent antibody variable region;
(b) generating sequence alignments from relative accessibility distributions from x-ray crystallographic structures of a sufficient number of rodent antibody variable region heavy and light chains to give a set of heavy and light chain framework positions wherein said set is identical in 98% of said sufficient number of rodent antibody heavy and light chains;

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(c) defining for said rodent antibody or fragment thereof to be humanized a set of heavy and light chain surface exposed amino acid residues using said set of framework positions generated in said step (b);
(d) identifying from human antibody amino acid sequences a set of heavy and light chain surface exposed amino acid residues that is most closely identical to said set of surface exposed amino acid residues defined in said step (c), wherein said heavy and light chain from said human antibody are or are not naturally paired;
(e) substituting, in the amino acid sequence of said rodent antibody or fragment thereof to be humanized said set of heavy and light chain surface exposed amino acid residues defined in said step (c) with said set of heavy and light chain surface exposed amino acid residues identified in said step (d);
(f) constructing a three-dimensional model of said variable region of said rodent antibody or fragment thereof resulting from the substituting specified in said step (e);
(g) identifying, by comparing said three-dimensional models constructed in said steps (a) and (f), any amino acid residues from said set identified in said step (d), that are within 5 Angstroms of any atom of any residue of the complementarity determining regions of said rodent antibody or fragment thereof to be humanized; and
(h) changing any residues identified in said step (g) from the human to the original rodent amino acid residue to thereby define a rodent antibody humanizing set of surface exposed amino acid residues;
with the proviso that said step (a) need not be conducted first, but must be conducted prior to said step (g).

2. The method of claim 1, wherein said rodent antibody is an antibody fragment.

3. The method of claim 2, wherein said rodent antibody fragment is a single chain antibody, a F_V fragment, a Fab fragment, a Fab_2 fragment or a Fab' fragment.

4. The method of claim 1 or 2, wherein said step (d) identifies a set of naturally paired heavy and light chain surface exposed amino acid residues that is most closely identical to said set of surface exposed amino acid residues defined in said step (c).

5. The method of claim 1 or 2, wherein said surface exposed amino acid residues are those residues whose solvent accessibility is above 30%.

6. The method of claim 1 or 2, wherein the rodent antibody or fragment thereof to be humanized is a murine antibody.

7. The method of claim 6, wherein said set of framework positions of surface exposed amino acid residues is defined by the set shown in Table 1 and the alignments set forth in Figures 3A and 3B.

Light Chain		
Position	Human	Mouse
1	D 51 E 34 A 5 S 5	D 76 Q 9 E 6
3	V 38 Q 24 S 24 Y 6	V 63 Q 22 L 5
5	T 61 L 37	T 87
9	P 26 S 26 G 17 A 14 L 7	S 36 A 29 L 17 P 5
15	P 62 V 25 L 12	L 47 P 30 V 8 A 7
18	R 57 S 18 T 13 P 6	R 38 K 22 S 13 Q 12 T 9
46	P 94	P 82 S 9
47	G 89	G 71 D 18
51	K 43 R 31	K 70 Q 13 R 8 T 5
63	G 91	G 98

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66	D 43 S 25 A 9	D 38 A 26 S 26
73	S 96	S 90 I 5
76	D 43 T 18 S 16 E 15	D 67 S 15 A 5 K 5
86	P 44 A 27 S 17 T 8	A 50 P 11 T 8 E 7 Q 6
87	E 71 D 11 G 7	E 91 D 6
111	K 74 R 12 N 6	K 93
115	K 54 L 40	K 87 L 5
116	R 60 G 33 S 5	R 89 G 9
117	Q 50 T 37 E 6 P 6	A 74 Q 14 P 5 R 5

Heavy Chain

Position	Human	Mouse
118	E 47 Q 46	E 59 Q 29 D 10
120	Q 83 T 7	Q 68 K 26
122	V 59 L 15 Q 13	Q 57 V 27 L 5 K 5
126	G 54 A 23 P 18	G 36 P 30 A 29
127	G 53 E 22 A 14 D 7	E 45 G 43 S 6
128	L 61 V 31 F 7	L 96
130	K 46 Q 41 E 5	K 52 Q 27 R 17
131	P 95	P 91 A 5
132	G 74 S 16 T 7	G 82 S 17
136	R 53 K 23 S 17 T 7	K 66 S 17 R 13
143	G 96	G 98
145	T 46 S 32 N 9 I 7	T 63 S 19 N 7 A 5 D 5
160	P 84 S 10	P 89 H 7
161	G 93	G 71 E 24
162	K 76 Q 10 R 8	K 50 Q 30 N 10 H 5
183	D 26 P 25 A 17 Q 10 T 7	E 31 P 22 D 17 A 12 Q 11
184	S 70 K 9 P 8	K 42 S 37 T 6
186	K 53 Q 22 R 7 N 5	K 83 Q 7
187	G 66 S 21 T 5	G 62 S 18 D 10
195	T 30 D 26 N 19 K 7	T 36 K 30 N 26 D 6
196	S 91	S 76 A 16
197	K 65 I 8 T 8 R 5	S 46 K 34 Q 11

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208	R 46 T 18 K 17 D 6	S 67 A 14 T 11
209	A 50 P 21 S 13 T 8	E 88 D 7
210	E 46 A 18 D 13 S 9 Z 8 V 5	T 53 S 43
212	T 91	

Table 1

8. The method of claim 1 or 2, wherein the rodent antibody or fragment thereof to be humanized is murine antibody anti-N901.

9. The method of claim 8, wherein said set of framework positions of surface exposed amino acid residues is defined by the set shown in Table 1 and the alignments set forth in Figures 3A and 3B.

Light Chain		
Position	Human	Mouse
1	D 51 E 34 A 5 S 5	D 76 Q 9 E 6
3	V 38 Q 24 S 24 Y 6	V 63 Q 22 L 5
5	T 61 L 37	T 87
9	P 26 S 26 G 17 A 14 L 7	S 36 A 29 L 17 P 5
15	P 62 V 25 L 12	L 47 P 30 V 8 A 7
18	R 57 S 18 T 13 P 6	R 38 K 22 S 13 Q 12 T 9
46	P 94	P 82 S 9
47	G 89	G 71 D 18
51	K 43 R 31	K 70 Q 13 R 8 T 5
63	G 91	G 98
66	D 43 S 25 A 9	D 38 A 26 S 26
73	S 96	S 90 I 5
76	D 43 T 18 S 16 E 15	D 67 S 15 A 5 K 5
86	P 44 A 27 S 17 T 8	A 50 P 11 T 8 E 7 Q 6
87	E 71 D 11 G 7	E 91 D 6
111	K 74 R 12 N 6	K 93
115	K 54 L 40	K 87 L 5
116	R 60 G 33 S 5	R 89 G 9
117	Q 50 T 37 E 6 P 6	A 74 Q 14 P 5 R 5
Heavy Chain		
Position	Human	Mouse
118	E 47 Q 46	E 59 Q 29 D 10

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120	Q 83 T 7	Q 68 K 26
122	V 59 L 15 Q 13	Q 57 V 27 L 5 K 5
126	G 54 A 23 P 18	G 36 P 30 A 29
127	G 53 E 22 A 14 D 7	E 45 G 43 S 6
128	L 61 V 31 F 7	L 96
130	K 46 Q 41 E 5	K 52 Q 27 R 17
131	P 95	P 91 A 5
132	G 74 S 16 T 7	G 82 S 17
136	R 53 K 23 S 17 T 7	K 66 S 17 R 13
143	G 96	G 98
145	T 46 S 32 N 9 I 7	T 63 S 19 N 7 A 5 D 5
160	P 84 S 10	P 89 H 7
161	G 93	G 71 E 24
162	K 76 Q 10 R 8	K 50 Q 30 N 10 H 5
183	D 26 P 25 A 17 Q 10 T 7	E 31 P 22 D 17 A 12 Q 11
184	S 70 K 9 P 8	K 42 S 37 T 6
186	K 53 Q 22 R 7 N 5	K 83 Q 7
187	G 66 S 21 T 5	G 62 S 18 D 10
195	T 30 D 26 N 19 K 7	T 36 K 30 N 26 D 6
196	S 91	S 76 A 16
197	K 65 I 8 T 8 R 5	S 46 K 34 Q 11
208	R 46 T 18 K 17 D 6	S 67 A 14 T 11
209	A 50 P 21 S 13 T 8	E 88 D 7
210	E 46 A 18 D 13 S 9 Z 8 V 5	T 53 S 43
212	T 91	

Table 1

10. A method for producing a humanized rodent antibody or fragment thereof from a rodent antibody or fragment thereof by resurfacing, said method comprising.

(I) carrying out the method of claim 1; and

(II) modifying the rodent antibody or fragment thereof by replacing the set of rodent antibody surface exposed amino acid residues with the rodent antibody humanizing set of surface exposed amino acid residues defined in said step (h).

11. The method of claim 10, wherein said rodent antibody is an antibody fragment.

12. The method of claim 11, wherein said rodent antibody fragment is a single chain antibody, a F_V fragment,

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a Fab fragment, a Fab_2 fragment or a Fab' fragment.

13. The method of claim 10 or 11, wherein said step (d) identifies a set of naturally paired heavy and light chain surface exposed amino acid residues that is most closely identical to said set of surface exposed amino acid residues defined in said step (c).

14. The method of claim 10 or 11, wherein said surface exposed amino acid residues are those residues whose solvent accessibility is above 30%.

15. The method of claim 10 or 11, wherein the rodent antibody or fragment thereof to be humanized is a murine antibody.

16. The method of claim 15, wherein said set of framework positions of surface exposed amino acid residues is defined by the set shown in Table 1 and the alignments set forth in Figures 3A and 3B.

Light Chain		
Position	Human	Mouse
1	D 51 E 34 A 5 S 5	D 76 Q 9 E 6
3	V 38 Q 24 S 24 Y 6	V 63 Q 22 L 5
5	T 61 L 37	T 87
9	P 26 S 26 G 17 A 14 L 7	S 36 A 29 L 17 P 5
15	P 62 V 25 L 12	L 47 P 30 V 8 A 7
18	R 57 S 18 T 13 P 6	R 38 K 22 S 13 Q 12 T 9
46	P 94	P 82 S 9
47	G 89	G 71 D 18
51	K 43 R 31	K 70 Q 13 R 8 T 5
63	G 91	G 98
66	D 43 S 25 A 9	D 38 A 26 S 26
73	S 96	S 90 I 5
76	D 43 T 18 S 16 E 15	D 67 S 15 A 5 K 5
86	P 44 A 27 S 17 T 8	A 50 P 11 T 8 E 7 Q 6
87	E 71 D 11 G 7	E 91 D 6
111	K 74 R 12 N 6	K 93
115	K 54 L 40	K 87 L 5
116	R 60 G 33 S 5	R 89 G 9
117	Q 50 T 37 E 6 P 6	A 74 Q 14 P 5 R 5
Heavy Chain		
Position	Human	Mouse
118	E 47 Q 46	E 59 Q 29 D 10
120	Q 83 T 7	Q 68 K 26
122	V 59 L 15 Q 13	Q 57 V 27 L 5 K 5

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126	G 54 A 23 P 18	G 36 P 30 A 29
127	G 53 E 22 A 14 D 7	E 45 G 43 S 6
128	L 61 V 31 F 7	L 96
130	K 46 Q 41 E 5	K 52 Q 27 R 17
131	P 95	P 91 A 5
132	G 74 S 16 T 7	G 82 S 17
136	R 53 K 23 S 17 T 7	K 66 S 17 R 13
143	G 96	G 98
145	T 46 S 32 N 9 I 7	T 63 S 19 N 7 A 5 D 5
160	P 84 S 10	P 89 H 7
161	G 93	G 71 E 24
162	K 76 Q 10 R 8	K 50 Q 30 N 10 H 5
183	D 26 P 25 A 17 Q 10 T 7	E 31 P 22 D 17 A 12 Q 11
184	S 70 K 9 P 8	K 42 S 37 T 6
186	K 53 Q 22 R 7 N 5	K 83 Q 7
187	G 66 S 21 T 5	G 62 S 18 D 10
195	T 30 D 26 N 19 K 7	T 36 K 30 N 26 D 6
196	S 91	S 76 A 16
197	K 65 I 8 T 8 R 5	S 46 K 34 Q 11
208	R 46 T 18 K 17 D 6	S 67 A 14 T 11
209	A 50 P 21 S 13 T 8	E 88 D 7
210	E 46 A 18 D 13 S 9 Z 8 V 5	T 53 S 43
212	T 91	

Table 1

17. The method of claim 10 or 11, wherein the rodent antibody or fragment thereof to be humanized is murine antibody anti-N901.

18. The method of claim 17, wherein said set of framework positions of surface exposed amino acid residues is defined by the set shown in Table 1 and the alignments set forth in Figures 3A and 3B.

Light Chain		
Position	Human	Mouse
1	D 51 E 34 A 5 S 5	D 76 Q 9 E 6
3	V 38 Q 24 S 24 Y 6	V 63 Q 22 L 5

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5	T 61 L 37	T 87
9	P 26 S 26 G 17 A 14 L 7	S 36 A 29 L 17 P 5
15	P 62 V 25 L 12	L 47 P 30 V 8 A 7
18	R 57 S 18 T 13 P 6	R 38 K 22 S 13 Q 12 T 9
46	P 94	P 82 S 9
47	G 89	G 71 D 18
51	K 43 R 31	K 70 Q 13 R 8 T 5
63	G 91	G 98
66	D 43 S 25 A 9	D 38 A 26 S 26
73	S 96	S 90 I 5
76	D 43 T 18 S 16 E 15	D 67 S 15 A 5 K 5
86	P 44 A 27 S 17 T 8	A 50 P 11 T 8 E 7 Q 6
87	E 71 D 11 G 7	E 91 D 6
111	K 74 R 12 N 6	K 93
115	K 54 L 40	K 87 L 5
116	R 60 G 33 S 5	R 89 G 9
117	Q 50 T 37 E 6 P 6	A 74 Q 14 P 5 R 5
	Heavy Chain	
Position	Human	Mouse
118	E 47 Q 46	E 59 Q 29 D 10
120	Q 83 T 7	Q 68 K 26
122	V 59 L 15 Q 13	Q 57 V 27 L 5 K 5
126	G 54 A 23 P 18	G 36 P 30 A 29
127	G 53 E 22 A 14 D 7	E 45 G 43 S 6
128	L 61 V 31 F 7	L 96
130	K 46 Q 41 E 5	K 52 Q 27 R 17
131	P 95	P 91 A 5
132	G 74 S 16 T 7	G 82 S 17
136	R 53 K 23 S 17 T 7	K 66 S 17 R 13
143	G 96	G 98
145	T 46 S 32 N 9 I 7	T 63 S 19 N 7 A 5 D 5
160	P 84 S 10	P 89 H 7
161	G 93	G 71 E 24

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162	K 76 Q 10 R 8	K 50 Q 30 N 10 H 5
183	D 26 P 25 A 17 Q 10 T 7	E 31 P 22 D 17 A 12 Q 11
184	S 70 K 9 P 8	K 42 S 37 T 6
186	K 53 Q 22 R 7 N 5	K 83 Q 7
187	G 66 S 21 T 5	G 62 S 18 D 10
195	T 30 D 26 N 19 K 7	T 36 K 30 N 26 D 6
196	S 91	S 76 A 16
197	K 65 I 8 T 8 R 5	S 46 K 34 Q 11
208	R 46 T 18 K 17 D 6	S 67 A 14 T 11
209	A 50 P 21 S 13 T 8	E 88 D 7
210	E 46 A 18 D 13 S 9 Z 8 V 5	T 53 S 43
212	T 91	

Table 1

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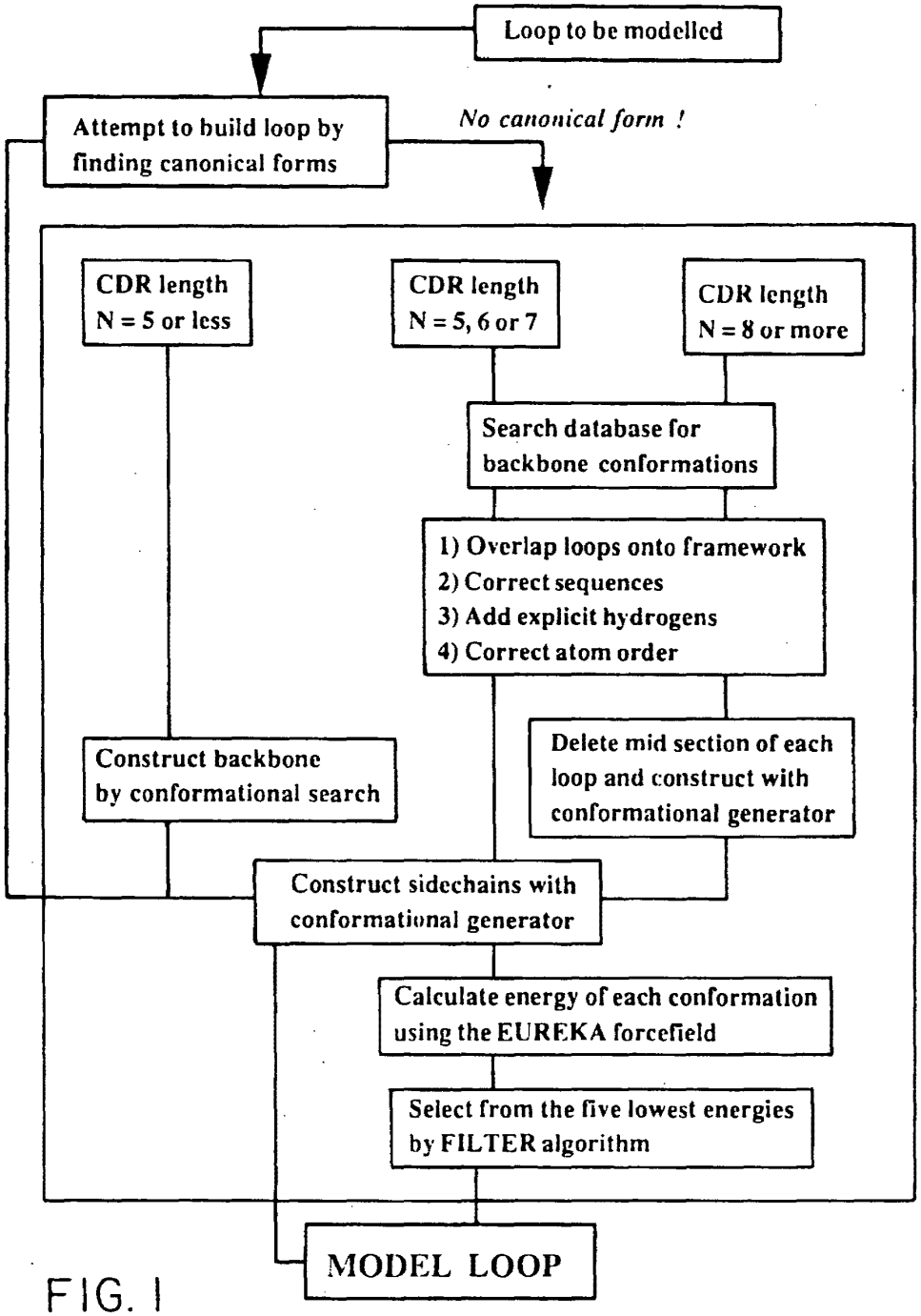

FIG. 1

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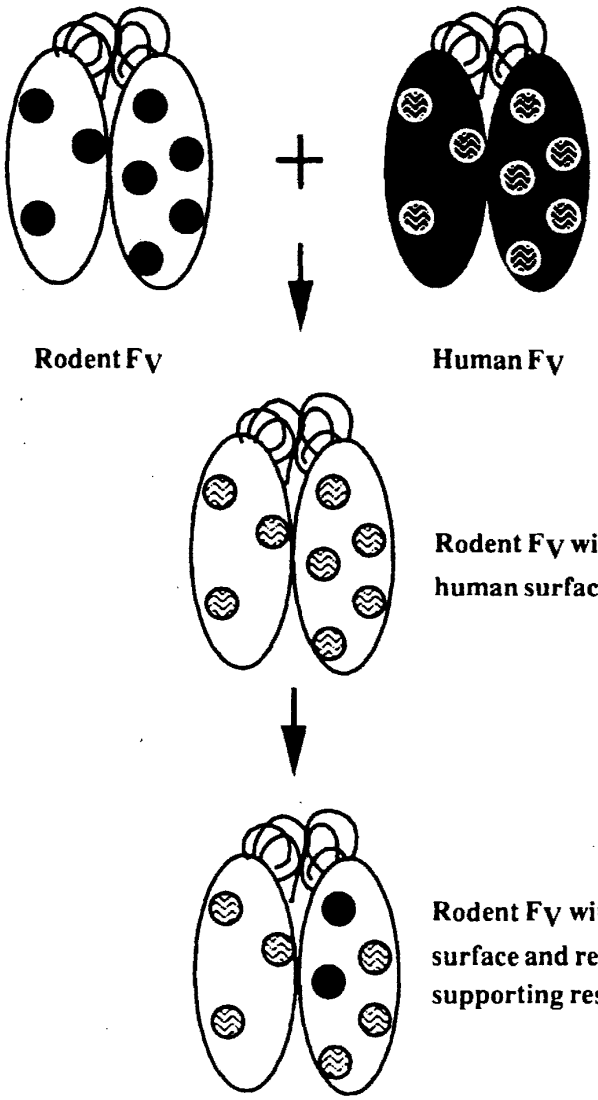

FIG. 2

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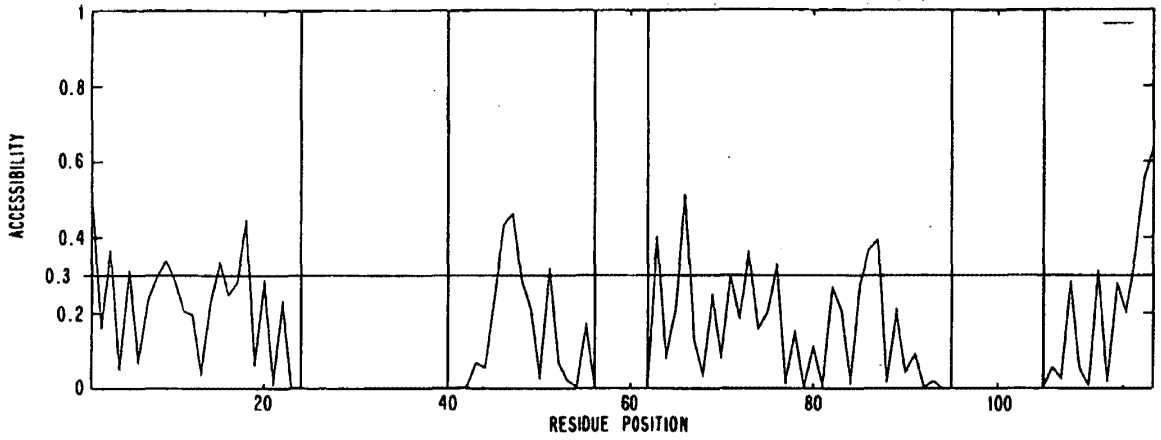
FIG. 3A

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glb2 DIQMTQSPSSLSASLGERVSLTCRASQEISG------YLSWLQQKPDGTIKRLIYAASTLDSGVPKRFSGRRSGSDYSLTISSLESEDFADYYCLQYLS--YPLTFGAGTKLELKRA  (SEQ ID NO: 1)
1fdl DIQMTQSPASLSASVGETVTITCRASGNIHN------YLAWYQQKQGKSPQLLVYYTTTLADGVPSRFSGSGSGTQYSLKINSLQPEDFGSYYCQHFWS--TPRTFGGGTKLEIKRR  (SEQ ID NO: 2)
2hfl DIVLTQSPAIMSASPGEKVTMTCSASSSVN-------YMYWYQQKSGTSPKRWIYDTSKLASGVPVRFSGSGSGTSYSLTISSMETEDAAEYYCQQWGR--NP-TFGGGTKLEIKRA  (SEQ ID NO: 3)
3hfm DIVLTQSPATLSVTPGNSVSLSCRASQSIGN------NLHWYQQKSHESPRLLIKYASQSISGIPSRFSGSGSGTDFTLSINSVETEDFGMYFCQQSNS--WPYTFGGGTKLEIKRA  (SEQ ID NO: 4)
2fbj EIVLTQSPAITAASLGQKVTITCSASSSVSS-------LHWYQQKSGTSPKPWIYEISKLASGVPARFSGSGSGTSYSLTINTMEAEDAAIYYCQQWTY--PLITFGAGTKLELKRA  (SEQ ID NO: 5)
2fb4 ESVLTQPPSASG-TPGQRVTISCTGTSSNIG----SITVNWYQQLPGMAPKLLIYRDAMRPSGVPTRFSGSKSGTSASLAISGLEAEDESDYYCASWNSSDNSYVFGTGTKVTVLGQ  (SEQ ID NO: 6)
2mcp DIVMTQSPSSLSVSAGERVTMSCKSSQSLLNSGNQKNFLAWYQQKPGQPPKLLIYGASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDHS--YPLTFGAGTKLEIKRA  (SEQ ID NO: 7)
7fab -SVLTQPPSVSG-APGQRVTISCTGSSSNIG---AGNEVKWYQQLPGTAPKLLIFHNNA-------RFSVSKSGSSATLAITGLQAEDEADYYCQSYDR--SLRVFGGGTKLTVLRQ  (SEQ ID NO: 8)
4fab DVVMTQTPLSLPVSLGDQASISCRSSQSLVHS-QGNTYLRWYLQKPGQSPKVLIYKVSNRFSGVPNRFSGSGSGTDFTLKISRVEAEDLGVYYFCSQSTH--VPWTFGGGTKLEIKRA  (SEQ ID NO: 9)
1f19 DIQMTQTTSSLSASLGDRVTISCRASQDISN------YLNWYQQKPDGTVKLLVYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEHEDIATYFCQQGST--TPRTFGGGTKLEIKRR  (SEQ ID NO: 10)
6fab DIQMTQIPSSLSASLGDRVSISCRASQDINN------FLNWYQQKPDGTIKLLIYFTSRSQSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNA--LPRTFGGGTKLEIKRA  (SEQ ID NO: 11)
1dfb DIQMTQSPSTLSASVGDRVTITCRASQSISR------WLAWYQQKPGKVPKLLIYKASSLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNS----YSFGPGTKVDIKRT  (SEQ ID NO: 12)
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