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 See application file for complete search history.

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(57) ABSTRACT

An altered antibody chain is produced in which the CDR's of the variable domain of the chain are derived from a first mammalian species. The framework-encoding regions of DNA encoding the variable domain of the first species are mutated so that the mutated framework-encoding regions encode a framework derived from a second different mammalian species. The or each constant domain of the antibody chain, if present, are also derived from the second mammalian species.

7 Claims, 33 Drawing Sheets

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Fig. 1

HindIII

1 AAGCTTATGAATATGCCAATCCCTCGAATCTACATGGTAAATATAGGTTTGTCTATACC 59

60 ACAACAGAAAACATGAGATCAGTTCTCTACAGTTACTGAGCACACAGGACCTCA 119

-19 M G W S C I I L F L V A T A T -5

120 CCATGGATGGAGCTGTATCATCCTTCTTGGTAGCAACAGCTACAGGTAAGGGTGCA 179

180 CAGTAGCAGGCTTGAGGCTCGACATATATATGGGTGACAATGACATCCACTTTCCTTT 239

-4 G V H S D I Q L T Q S P V S L S A 13

240 CTCGCCAGGTGTCCACTCCGACATCCAGCTGACCAGTCTCCAGTTTCCCTGCTGCA 299

CDR1

14 S L G E T V N I E C [L A S E D I Y S D L 33

300 TCTCTGGAGAAACTGTCAACATCGAATGTCTAGCAAGTGAGGACATTTACAGTGATTA 359

Fig. 1A

34 A W Y Q Q K P G K S P Q L L I Y N T D T 53
 360 GCATGGTATCAGCAGAACCCAGGAAATCTCCTCAACTCCTGATCTATAATACAGATACC 419

 54 L Q N G V P S R F S G S G T Q Y S L 73
 420 TTGCAAAATGGGTCCTTCCCTTACCGTTTAGTGGCAGTGGATCTGGCACACAGTATTCTTA 479

 74 K I N S L Q S E D V A T Y F C Q Q Y N N 93
 480 AAAATAAACAGCCCTGCAATCTGAAGATGTCGGACTTATTTCTGTCACAACAATAACAAT 539

 94 Y P W T F G G G T K L E I K R 108
 540 TATCCGTTGGACGTTCCGGTGGAGGACCAAGCTGGAGATCAAACCGTGTAGATTTAAAC 599

 600 TTGCTTCCCTCAGTTGGATCC 620

 BamHI

Fig. 2

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-19  HindIII           M G W S C I   -14
      1  AAGCTTGGCTCTACAGTTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTAIC  58

-13  I L F L V A T A T G V H S D I Q M T Q S   7
      59 ATCCTTCTTGGTAGCAACAGCTACAGGTGTCCTCCACTCCGACATCCAGATGCCAGACC  118
           CDR 1
      8  P S S L S A S V G D R V T I T C K A S Q   27
      119 CCAAGCAGCCTGAGCCGCCAGCGTGGTGACAGAGTGCACCTCATCCTGTAAAGCAAGTCAG  178

      28 N I D K Y L N W Y Q Q K P G K A P K L L   47
      179 AATATGACAAATACTTAAACTGGTACCAGCAGAGCCAGGTAAGCCTCCAAAGCTGCTG  238
           CDR 2
      48  I Y N T N N L Q T G V P S R F S G S G S   67
      239 ATCTACAATACAACAATTTGCCAAACGGGTGTGCCAAGCAGATTTCAGCGGTAGCGGTAGC  298

      68  G T D F T F T I S S L Q P E D I A T Y Y   87
      299 GGTACCGACTTCACCTTCACCATCAGCAGCCTCCAGCCAGAGGACATGCCACCTACTAC  358
    
```

Fig. 2A

CDR 3

88	C L Q H I S R P R T	F G Q G T K V E I K	107
359	TGCTTGCAGCATATAAGTAGGCCCGCCAGGTTCCGGCCAAGGACCAAGGTGGAAATCAAA		418
108	R T V A A P S V F I F P S D E Q L K S		127
419	CGAACTGTGGCTGCACCAATCTGTCTTCATCTTCCGCCATCTGATGAGCAGTTGAAATCT		478
128	G T A S V V C L L N N F Y P R E A K V Q		147
479	GGAACTGCCCTCTGTTGTGTCCTGCTGAATAACTTCTATCCAGAGAGGCCAAAGTACAG		538
148	W K V D N A L Q S G N S Q E S V T E Q D		167
539	TGGAAGGTGGATAACGCCCTCCAATCGGGTAACCTCCAGGAGAGTGTCCACAGAGCAGGAC		598
168	S K D S T Y S L S S T L T L S K A D Y E		187
599	AGCAGGACAGCACCTACAGCCTCAGCAGCACCTGACCGCTGAGCAAGCAGACTACGAG		658
188	K H K V Y A C E V T H Q G L S S P V T K		207
659	AAACACAAGTCTACGCCCTGGGAAGTCAACCCATCAGGGCCCTGAGCTCGCCCGTCACAAAG		
208	S F N R G E C Tm <i>HindIII</i>		214
719	AGCTTCAACAGGGAGAGTGTTAGAAGCTT		748

Fig. 3

-19 HindIII M G W S C I -14
 1 AAGCTTGGCTCTACAGTTACTGAGCACACAGGACCTCACCATGGCATGGAGCTGTATC 58

-13 I L F L V A T A T G V H S D I Q M T Q S 7
 59 ATCCCTTCTTGGTAGCACACAGCTACAGGTGTCCACTCCGACATCCAGATGACCCAGAGC 118
 CDR 1

8 P S S L S A S V G D R V T I T C L A S E 27
 119 CCAAGCAGCCTGAGCCCGGCTGGTGACAGAGTGACCATCACCCTGTCTAGCAAGTGAG 178

28 D I Y S D L A W Y Q Q K P G K A P K L L 47
 179 GACATTTACAGTGATTTAGCATGGTACCAGCAGAAGCCAGGTAAGCTCCAAGCTGCTG 238
 CDR 2

48 I Y N T D T L Q N G V P S R F S G S G S 67
 239 ATCTACAATACAGATACCTTTCGCAAAATCGTGTGCCAACACAGATTTCAGCGGTAGCGGTAGC 298

68 G T D F T F T I S S L Q P E D I A T Y Y 87
 299 GGTACCGACTTCACCTTACCATCAGCAGCCCTCCAGAGGACATCGCCACCTACTAC 358
 CDR 3

88 C Q Q Y N N Y P W T F G Q G T K V E I K 107
 359 TGCCAACAGTATAACAATTATCCGTGGACGTTCCGCCAAGGACCAAGGTGGAAATCAAA 418

Fig. 3A

108 R T V A A P S V F I F P P S D E Q L K S 127
419 CGAACTGTGCCCTGCACCATCTGCTTCATCTTCCCGCATCTCATGAGCAGTTGAAATCT 478

128 G T A S V V C L L N N F Y P R E A K V Q 147
479 GGAAC TGCCTCTGTGTGCTGCTGAATAA CTTCTATCCAGAGAGGCCAAAGTACAG 538

148 W K V D N A L Q S G N S Q E S V T E Q D 167
539 TGAAGGTGGATAACCCCTCCAATCGGTAAC TCCAGGAGAGTGTACAGAGCAGGAC 598

168 S K D S T Y S L S S T L T L S K A D Y E 187
599 AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAG 658

188 K H K V Y A C E V T H Q G L S S P V T K 207
659 AAACACAAGTCTACGCCCTGGGAAGTCA CCCCATCAGGGCCTGAGCTCGCCCGTCACAAAG 718

208 S F N R G E C Tm *Hind*III 214
719 ACCTTCAACAGGGGAGAGTGTAGAA GCTT 748

Fig. 4

HindIII

1 AAGCTTATGAATATGCCAAATCCCTGAACTACATCGTAAATAATAGGTTTGCTATACC 59
60 ACAACAGAAAACATGAGATCAGATCAGTTCCTCTACAGTTACTCAGCACACAGGACCITCA 119
-19 M G W S C I I L F L V A T A T -5
120 CCATGGCATGCAGCTGTATCATCTCTTCCTTCTGGTAGCAACACAGCTACAGGTAAGGGCTCA 179
180 CAGTAGCAGGCTTGAGGTCGGACATATAATATCGGTGACAAATGACATCCACTTGCCTTT 239
-4 G V H S Q V Q L Q E S G G L V Q 13
240 CTCCTCACAGGTGCCACTCCAGGTCCAACTGCAGGAGTCTGGGAGGCTTAGTGCAG 299
14 P G R S L K L S C A A S G L T F S N Y G 33
300 CCTGGAAGTCCCTGAAACTCTCTGTGCAGCCCTCTGGACTCACTTTCAGTAACTATGCC 359
34 M A W V R Q A P T K G L E W V A T I S H 53
360 ATGGCCCTGGTCCGCCAGGCTCCAACGAAGGGCTGGAGTGGGTCGCAACCATTAGTCAT 419
CDR 1
CDR 2

Fig. 4A

D G S D T Y F R D S V K G R F T I S R D 73
 54
GATGGTAGGACACTTACTTTCGAGACTCCGTGAAGGCCGATTCACTATCTCCAGAGAT 479
 420
 N G K S T L Y L Q M D S L R S E D T A T 93
 74
 AATGGAAAAGCACCCCTATACCTGCAATGGACAGTCTGAGGTCGAGGACACGGCCACT 539
 480
 Y Y C A R Q G T I A C I R H W G Q G T T 113
 94
 TATTACTGTCAAGACAAGGACTATAGCAGGTATACGTCACTGGGCCAAGGACCACG 599
 540
 V T V S S 118
 114
 GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTTCTTCTATTAGCTTAAATAGATT 659
 600
 TTACTGCATTGTGGGGGAAATGTGTATCTGAATTCAGGTCATGAAGGACTAGG 719
 660
 GACACCTTGGAGTCAGAAAGGGTCATTTGGGAGCCCGGCTGATGCAGACACATCCTC 779
 720
 AGCTCCAGACTTCATGGCCAGAGATTATAGGGATCC 817
 780

BanHI

Fig. 5

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-19 HindIII           M G W S C I I L           -12
1  AAGCTTACAGTACTGAGCACACAGGACCTCACCATGGATGGAGCTGTATCATCCTC 59

-11 F L V A T A T G V H S Q V Q L Q E S G P           9
60 TTCTTGGTAGCACAGCTACAGGTGTCCACTCCAGGTCCAACTGCAGGAGCGGTCCA 119

10 G L V R P S Q T L S L T C T V S G F T F           29
120 GGTCTTGAGACCTAGCCAGACCCTGAGCCCTGACCTGCACCCTGCTGGCTTACCTTC 179

30 T [D F Y M N] W V R Q P P G R G L E W I G           49
180 ACCGATTCTACATGAACCTGGTGCAGACACCCTGCAGGAGGCTTGTGAGTGGATTGGA 239

50 [F I R D K A K G Y T T E Y N P S V K G] R           69
240 TTTATTAGACAAAGCTAAAGGTTACACAACAGAGTACAATCCATCTGTCAAGCGGAGA 299

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CDR 1

CDR 2

Fig. 5A

70 V T M L V D T S K N Q F S L R L S S V T 89
 300 GTGACAATGCTGGTAGACACCAAGAACCACTTCAGCCTGAGACTCAGCAGCGTGACA 359

 CDR 3
 90 A A D T A V Y Y C A R [E G H T A A P F D] 109
 360 GCCGCCACACCGGCTATTATTGTGCAAGAGAGGGCCACACTGCTGCTCCTTTTGAT 419

 110 [Y] W G Q G S L V T V S S A S T K G P S V 129
 420 TACTGGGTCAGGCAGCCCTCGTCACAGTCTCCTCAGCCTCCACCAAGGCCCATCGGTC 479

 130 F P L A P S S K S T S G G T A A L G C L 149
 480 TTCCCCCTGGCACCCCTCCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCCTG 539

 150 V K D Y F P E P V T V S W N S G A L T S 169
 540 GTCAGGACTACTTCCCGAACCGGTGACGGTCTCGTGGAACTCAGGCGCCCTGACCAGC 599

Fig. 5B

170 G V H T F P A V L Q S S G L Y S L S S V 189
600 GCGTGCACACCTTCCGGCTGTCCCTACAGTCCCTCAGGACTTACTCCCTCAGCAGCGTG 659

190 V T V P S S L C T Q T Y I C N V N H K 209
660 GTGACCGTGCCCTCCAGCAGCTTGGGCACCAGACCTACATCTGCAACGTGATCACAAG 719

210 P S N T K V D K K V E P K S C D K T H T 229
720 CCCAGCAACACCAAGGTGGACAAGAAGTTGAGCCCAAATCTTGTGACAAACTCACACA 779

230 C P P C P A P E L L G G P S V F L F P P 249
780 TGCCACCGTGCCAGCACCTGAACCTCTGGGGGACCGTCTTCTTCTTCCCCCA 839

250 K P K D T L M I S R T P E V T C V V D 269
840 AAACCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGAC 899

Fig. 5C

270 V S H E D P E V K F N W Y V D G V E V H 289
900 GTGAGCCAGAGACCCTGAGGTCAAGTTCAACTGGTAGCGGCGGTGGAGGTCAT 959

290 N A K T K P R E E Q Y N S T Y R V V S V 309
960 AATGCCAAGACAAGCCCGGAGGAGCAGTACAACAGCAGTACCCTGCTCAGCGTC 1019

310 L T V L H Q D W L N G K E Y K C K V S N 329
1020 CTCACCGTCCCGCAGGACTGGCTGAATGGCAGGAGTACAAGTGAAGGTCCTCCAAC 1079

330 K A L P A P I E K T I S K A K G Q P R E 349
1080 AAAGCCCTCCAGCCCATCGAGAAACCAATCTCCAAGCCAAAGGCCAGCCCGAGAA 1139

350 P Q V Y T L P P S R D E L T K N Q V S L 369
1140 CCACAGGTGTACACCCTGCCCCATCCCGGATGAGCTGACCAAGAACCCAGGTCAGCCTG 1199

Fig. 5D

370 T C L V K G F Y P S D I A V E W E S N G 389
1200 ACCTGCCCTGCTCAAAGGCTTCTATCCAGGACATGCCCGTGGAGTGGAGAGCAATGGG 1259

390 Q P E N N Y K T T P P V L D S D G S F F 409
1260 CAGCCGGAGAACACTACAAGACCACGCCCTCCCGTGGACTCCGACGGCTCCTTCTTC 1319

410 L Y S K L T V D K S R W Q Q G N V F S C 429
1320 CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGGGAACGTTCTCTCATGC 1379

430 S V M H E A L H N H Y T Q K S L S L S F 448
1380 TCCGTGATGCATCAGGCTCTGCACAACCACCTACACCCAGAGCCTCTCCCTGTCCTCCG 1439

449 G K Tm HindIII 450
1440 GGTAATGAGTGGACGGCCCCAAGCTT 1467

Fig. 6

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-19 HindIII           M G W S C I I L           -12
   1 AAGCTTACAGTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTC   59

-11 F L V A T A T G V H S Q V Q L Q E S G P           9
   60 TTCTGGTAGCAACAGCTACAGGTGTCCACTCCAGTCCAACTGCAGGAGCGGTCCA   119

   10 G L V R P S Q T L S L T C T V S G F T F           29
   120 GGTCTTGAGACCTAGCCACACCCCTGAGCCCTGACCTCCACCGTCTCTGGCTTCACCTTC   179

                                CDR 1
   30 T N Y G M A W V R Q P P C R G L E W I G           49
   180 ACCAACTATGGCATGGCCCTGGGTGAGACAGCCACCTGGACGAGGCTCTTGAGTGGATTGGA   239

                                CDR 2
   50 T I S H D C S D T Y F R D S V K G R V T           69
   240 ACCATTAGTCATGATGATGACACTTACTTTCGAGACTCTGTGAAGGGAGAGTGACA   299

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Fig. 6A

70 M L V D T S K N Q F S L R L S S V T A A 89
300 ATGCTGGTAGACACCAGCAAGAACCAAGTTCAGCCTGAGACTCAGCAGCGTGACAGCCGCC 359
CDR 3
90 D T A V Y Y C A R Q G T I A G I R H W G 109
360 GACACCGGCTATTATTGTGCAAGACAAGGCACTATAGCTGGTATACGTCACCTGGGGT 419
110 Q G S L V T V S S A S T K G P S V F P L 129
420 CAAGCAGCCTCGTCACAGTCTCCTCAGCCTCCACCAAGGCCCAATCGGTCCTCCCCCTG 479
130 A P S S K S T S G G T A A L G C L V K D 149
480 GCACCTCCTCAAGAGACCTCTGGGGGCACAGCGCCCTGGGCTGGTCAAGGAC 539
150 Y F P E P V T V S W N S G A L T S G V H 169
540 TACTTCCCGAACCGGTGACGGTCTCGTGAACCTCAGGCGCCCTGACCAGCGCGGTGCAC 599

Fig. 6B

170 T F P A V L Q S S G L Y S L S S V V T V 189
600 ACCTCCGGCTCCTACAGTCCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG 659

190 P S S L G T Q T Y I C N V N H K P S N 209
660 CCTCCAGCAGCTTGGCCACCCAGACCTACATCTGCAACGTGATCACAAGCCCAAC 719

210 T K V D K K V E P K S C D K T H T C P P 229
720 ACCAAGTGGACAAGAAGTTGAGCCCAATCTTGTGACAAACTCACATGCCACCG 779

230 C P A P E L L G G P S V F L F P P K P K 249
780 TGCCAGCACCTGACTCCTGGGGGACCGTCAGTCTTCTTCCCCCAAACCCAAG 839

250 D T L M I S R T P E V T C V V D V S H 269
840 GACACCTCATGATCTCCCGACCCCTGAGCTCATGCTGGTGGACGTGAGCCAC 899

Fig. 6C

270 E D P E V K F N W Y V D G V E V H N A K 289
900 GAAGACCCTCAGGTCAGTTCAACTGGTACGTGGACGGCGGTGCATGATGCCAAG 959

290 T K P R E E Q Y N S T Y R V V S V L T V 309
960 ACAAGCCGGGAGGAGCAGTACAACAGCAGTACCCTGTGGTCAGCGTCCCTCACCGTC 1019

310 L H Q D W L N G K E Y K C K V S N K A L 329
1020 CTGCACCAGCACTGGCTGAATGGCAAGGAGTACAAGTCCCAAGTCTCCAACAAGCCCTC 1079

330 P A P I E K T I S K A K G Q P R E P Q V 349
1080 CCAGCCCCATCGAGAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCCACAGGTG 1139

350 Y T L P P S R D E L T K N Q V S L T C L 369
1140 TACACCCTGCCCCATCCGGGATGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCCTG 1199

Fig. 6D

370 V K G F Y P S D I A V E W E S N G Q P E 389
1200 GTC AAGGCTTCTATCCAGCCGACATCCCGTGGAGTGGCAGCAATGGCAGCCGGAG 1259

390 N N Y K T T P P V L D S D G S F F L Y S 409
1260 AACAACTACAAGACCAGCCCTCCCGTGGCTGGACTCCGACGGCTCTTCTTCCCTCTACAGC 1319

410 K L T V D K S R W Q Q G N V F S C S V M 429
1320 AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTTCTCATGCTCCGCTGATG 1379

430 H E A L H N H Y T Q K S L S L S P G K T M 448
1380 CATGAGGCTCTGCACAACCACTACACGCAGAGCCCTCTCCCTGCTCCGGGTAATGA 1439

HindIII

1440 GTCCGACGGCCCCAAGCTT 1458

Fig. 7

-19 *HindIII* M G W S C I I L -12

1 AAGCTTACAGTTACTGACACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTC 59

-11 F L V A T A T G V H S Q V Q L Q E S G P 9

60 TTCTTGGTAGCAACAGCTACAGGTGTCCTCCAGGTCCAACTGCAGGAGAGCGGTCCA 119

10 G L V R P S Q T L S L T C T V S G F T F 29

120 GGTCCTGTGAGACCTAGCCAGACCCCTGAGCCCTGACCTGCCACCGTCTGGCTTCACCTTC 179

CDR 1

30 S N Y G M A W V R Q P P G R G L E W I G 49

180 AGCAACTATGGCATGGCCCTGGGTGAGACAGCCACCTGGACGAGTCTTGAGTGGATTGGA 239

CDR 2

50 T I S H D G S D T Y F R D S V K G R V T 69

240 ACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGACTCTGTGAGGGGAGAGTGACA 299

Fig. 7A

70 M L V D T S K N Q F S L R L S S V T A A 89
300 ATGCTGGTAGACACCAGCAAGAACAGTTCAGCCTGAGACTCAGCAGCGTGACAGCCGCC 359
CDR 3
90 D T A V Y Y C A R [Q G T I A G I R H] W G 109
360 GACACCGGCTCTATTATGTCGAAGACAAGCCACTATAGCTGCTATACGTCACCTGGGGT 419
110 Q G S L V T V S S A S T K G P S V F P L 129
420 CAAGCAGCCTCGTACAGTCTCCTCAGCCTCCACCAAGGCCCATCGGCTCTCCCCCTG 479
130 A P S S K S T S G G T A A L G C L V K D 149
480 GCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCCGCCCTGGCTGCTCAAGGAC 539
150 Y F P E P V T V S W N S G A L T S G V H 169
540 TACTTCCCGAACCGGTGACGGTGTGGAACCTCAGGCCCTGACCAGCGCGCTGCAC 599

Fig. 7B

170 T F P A V L Q S S G L Y S L S V V T V 189
600 ACCTCCGGCTGCTACAGTCCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG 659

190 P S S L G T Q T Y I C N V N H K P S N 209
660 CCTCCAGCAGCTTGGCACCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC 719

210 T K V D K K V E P K S C D K T H T C P P 229
720 ACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCG 779

230 C P A P E L L G G P S V F L F P P K P K 249
780 TGCCAGCACCTGAACCTCCCTGGGGGACCGTCAGTCTTCTTCCCCCAAACCCAAG 839

250 D T L M I S R T P E V T C V V D V S H 269
840 GACACCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGACCGTGAGCCAC 899

Fig. 7C

270 E D P E V K F N W Y V D G V E V H N A K 289
900 GAAGACCTGAGGTCAGTCACTGCTAGCTGACGGCGTGGAGGTGCATAATGCCAAG 959

290 T K P R E E Q Y N S T Y R V V S V L T V 309
960 ACAAGCCGGGAGGAGCAGTACAACAGCAGCAGTACCGTGTGGTCCAGGTCCTCACCGTC 1019

310 L H Q D W L N G K E Y K C K V S N K A L 329
1020 CTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTCCAGGTCCTCCAAACAAGCCCTC 1079

330 P A P I E K T I S K A K G Q P R E P Q V 349
1080 CCAGCCCCCATCGAGAAACCATCTCCAAGCCAAAGGCCAGCCCGAGAACCCACAGGTG 1139

350 Y T L P P S R D E L T K N Q V S L T C L 369
1140 TACACCTGCCCCCATCCCGGATGACCTGACCACAAGAACCCAGGTCAGCCTGACCTGCCCTG 1199

Fig. 7D

370 V K G F Y P S D I A V E W E S N G Q P E 389
1200 GTC AAGGCTTCTATCCAGGACATCCCGTGGAGTGGAGAGCAATGGCAGCCGGAG 1259

390 N N Y K T T P P V L D S D G S F F L Y S 409
1260 AACACTACAAGACCACCGCTCCCGTGGACTCCGACGCCCTTCTTCTCTACAGC 1319

410 K L T V D K S R W Q Q G N V F S C S V M 429
1320 AAGTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTTCTCATGCTCCGTGATG 1379

430 H E A L H N H Y T Q K S L S L S P G K T M 448
1380 CATGAGGCTCTGCACAACCACTACACGAGAGCCCTCTCCCTCGGTAAATGA 1439

HindIII
1440 GTGGACGGCCCCAAGCTT 1458

Fig. 8

1 Q V Q L V E S G G G V V Q 13
 CDR 1
 14 P G R S L R L S C S S G F I F S S Y A 33
 CDR 2
 34 M Y W V R Q A P G K G L E W V A I I W D 53
 54 D G S D Q H Y A D S V K C R F T I S R D 73
 74 N S K N T L F L Q M D S L R P E D T G V 93
 CDR 3
 94 Y F C A R D G G H G F C S S A S C F G P 113
 114 D Y W C Q G T P V T V S S 126

Fig. 9

*Hind*III

1 AAGCTTATGAATATGCAAAATCCTCTGAATCTACATGGTAAATATAGTTTGTCTATACC 59
 60 ACAACAGAAAACATGAGATCAGATTCTCTCTACAGTTACTCAGCACACAGGACCTCA 119
 -19 M G W S C I I L F L V A T A T -5
 120 CCATGGATGGAGCTGTATCACTCTCTTCTGGTAGCAACAGCTACAGGTAAGGGCTCA 179
 180 CAGTAGCAGGCTTGAGGCTCGACATATATATGGGTGACAAATGACATCCACTTTGCCTTT 239
 -4 G V H S Q V Q L V E S G G V V Q 13
 240 CTCICACAGGTGTCCACTCCAGGTCCAACTGGTGGAGTCTGGTGGAGGCGTGGTGCAG 299
 CDR1
 14 P G R S L R L S C S S G F I F S N Y G 33
 300 CCTGGAAGTCCCTGAGACTCTCCCTGTTCCCTCTGGATTTCATCTTCAGTAACTATGGC 359
 CDR2
 34 M A W V R Q A P G K G L E W V A T I S H 53
 360 ATGGCCCTGGTCCGCCAGGCTCCAGGCAAGGGCTGGAGTGGTCCCAACCAATTAGTCAT 419

Fig. 9A

54 D G S D T Y F R D S V K G R F T I S R D 73
 420 GATGGTAGTGACACTTACTTTCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGAT 479

74 N S K N T L F L Q M D S L R P E D T G V 93
 480 AATAGCAAAAACCCCTATTCTGCAAAATGGACAGTCTGAGGCCCGAGGACACGGGGGTG 539

CDR 3

94 Y F C A R [Q G T I A G I R H] W G Q G T P 113
 540 TATTCTGTGCAAGACAAGGACTATAGCAGGTATACGTCACCTGGGCCAAGGACCCCC 599

114 V T V S S 118
 600 GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTCTTCTATTTCAGCTTAAATAGATT 659

660 TTACTGCATTGTGGGGGAAATGTGTATCTCAATTTCAGTCAAGCACTAGG 719

720 GACACCTTGGGAGTCAGAAAGGTCATTTGGAGCCCGGCTGATGCACACATCCTC 779

BanHI

780 ACCTCCACACTTCATGCCCAGAGATTATAGGGATCC 817

Fig. 10

-19 *Hind*III M G W S C I I L F -11
1 AAGCTTACAGTTACTCAGCACACAGGACCTCACCATGGGATGGAGCTGATCATCCTCT 60

-10 L V A T A T -5
61 TCTTGGTAGCAACAGCTACAGGTAAGGGCTCACAGTAGCAGGCTTGAGGCTTGACATA 120

-4 G V H S Q V 2
121 TATAGGTGACAATGACATCCACTTTCCTTTCCTCCACAGGCTGCTCCAGGTC 180

3 Q L V E S G G V Q P G R S L R L S C 22
181 CAACTGGTGAGTCTGGTGAGCGGCTGGTGCAGCCTGGAGGCTCCCTGAGACTCTCCTGT 240

CDR 1
23 S S S G F I F S N Y G M A W V R Q A P G 42
241 TCCCTCCTGGATTTCATCTTCAGTAACATAAGGCAATGGCCCTGGGTCGCCAGGCTCCAGGC 300

CDR 2
43 K G L E W V A T I S H D G S D T Y F R D 62
301 AAGGGCTGGAGTGGTCCGAACCAATTAGTCATGATGGTAGTGACACTTACTTTCGAGAC 360

Fig. 10A

S V K G R F T I S R D N S K N T L F L Q 82
 361 TCCGTGAAGGCCGATTCACTATCTCCAGAGATATAGCAAACACCCCTATTCTCGCAA 420

 M D S L R P E D T G V Y F C A R Q C T I 102
 421 ATGGACAGTCTGAGGCCCGAGGACACGGCGGTGTTCTGTGCAAGACAAGGGACTATA 480

A G I R H W G Q G T P V T V S S 122
 481 GCAGGTATACGTCACCTGGGCCAAGGACCCCGTCACCGTCTCCTCAGGTGAGTCCTTA 540

 541 CAACCTCTCTTCTATTACGCTTAAATAGATTTTACTGCATTTGTGGGGGAAATGT 600

 601 GTGATCTGAATTCAGGTCATGAAGGACTAGGACACCTTGGGAGTCAGAAAGGTCAT 660

 661 TGGAGCCCGGCTGATGCAGACAGACATCCTCAGCTCCAGACTTCATGGCCAGAGATT 720

 BamHI
 721 TATAGGGATCC 731

Fig. 11

HindIII

1 AAGCTTATGAATATGCAAAATCCTCTGAATCTACATGTAATAGGTTTGTCTATACC 59
60 ACAACAGAAAACATGAGATCACAGTTCTCTACAGTTACTCAGCACACAGGACCTCA 119
-19 M G W S C I I L F L V A T A T -5
120 CCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGCTCA 179
180 CAGTAGCAGGCTTGAGGTCIGGACATATATATGGTGACAATGACATCCACTTTGCCCTTT 239
-4 G V H S Q V Q L V E S G G V Q 13
240 CTCCTCACAGGTGTCACCTCCAGGTCCAACTGGTGGAGTCTGGTGGAGGGGTTGGTGCAG 299
14 P G R S L R L S C S S G F I F S N Y G 33
300 CCTGGAAGGTCCTGAGACTCTCCTGTTCTCCTCTGGATTTCATCTTCAGTAACTATGCC 359
34 M A W V R Q A P G K G L E W V A F I S H 53
360 ATGGCTGGTCCGCCAGGCTCCAGGCAAGGGCTGGAGTGGTCCCAACCATTAGTCAT 419

CDR 1

CDR 2

Fig. 11A

54 D G S D T Y F R D S V K G R F T I S R D 73
 420 GATG TAGT GACACTTACTTTCGAGACTCCGCGAAGGCCCATTCACATACTCCAGAGAT 479

74 N S K N T L F L Q M D S L R P E D T G V 93
 480 AATAGCAA AACACCCCTATTCCTGCAAAATGGACAGTCTGAGGCCCGAGGACACGGCCGTCG 539

CDR 3

94 Y F C A R Q G T I A G I R H W G Q G T T 113
 540 TATTCTGTC AAGGACTATAGCAGGTATACCTACTGGGCCAAGGACCACG 599

114 V T V S S 118
 600 GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTCTTCTATTTCAGCTTAAATAGATT 659

660 TTACTGCATTGTGGGGGAAATGIGTATCTGAATTCAGGTCATGAAGGACTAGG 719

720 GACACCTTGGAGTCAGAAAGGTCATTTGGAGCCCGGCTGATGCCAGACAGACATCCTC 779

*Bam*HI

780 AGCTCCAGACTTCATGGCCAGAGATTTATAGGGATCC 817

Fig. 12

```

-19 HindIII           M G W S C I I L F -11
   1 AAGCTTACAGTACTCAGCACACAGGACCTCACAGTGGAGCTGTATCATCTCT 60

-10 L V A T A T           -5
   61 TCTTGGTAGCAACAGCTACAGGTAAGGGCTCACAGTAGCAGGCTTGAGGCTGGACATA 120

-4           G V H S Q V 2
  121 TATATGGTGACAATGACATCCACTTTGCCCTTCTCTCCACAGGTTGCCACTCCAGGTC 180

   3 Q L V E S G G V V Q P G R S L R L S C 22
  181 CAACTGGTGGAGTCTGGTGGAGGGGCTGAGCCTGGAAGTCCCTGAGACTCTCCTGT 240

           CDR 1
   23 S S S G F I F S N Y G M A W V R Q A P G 42
  241 TCCTCCTCGGATTCATCTTCAGTAACTATGGCATGGCCCTGGTCCCGCCAGGCTCCAGGC 300

           CDR 2
   43 K G L E W V A T I S H D G S D T Y F R D 62
  301 AAGGGCTGGAGTGGTCCGAACCCATTAGTCATGATGGTAGTGACACTTACTTTCCGAGAC 360

   63 S V K G R F T I S R D N S K N T L F L Q 82
  361 TCCGTGAAGGGCGGATTCACTATCTCCAGAGATAATAGCAAAAACACCCCTATCTCTGCAA 420
    
```

Fig. 12A

CDR 3

83 M D S L R P E D T G V Y F C A R Q G T I 102
 421 ATGACAGTCTGAGGCCCGAGGACACGGGGTATTCTGTCCAAGACAAGGGACTATA 480

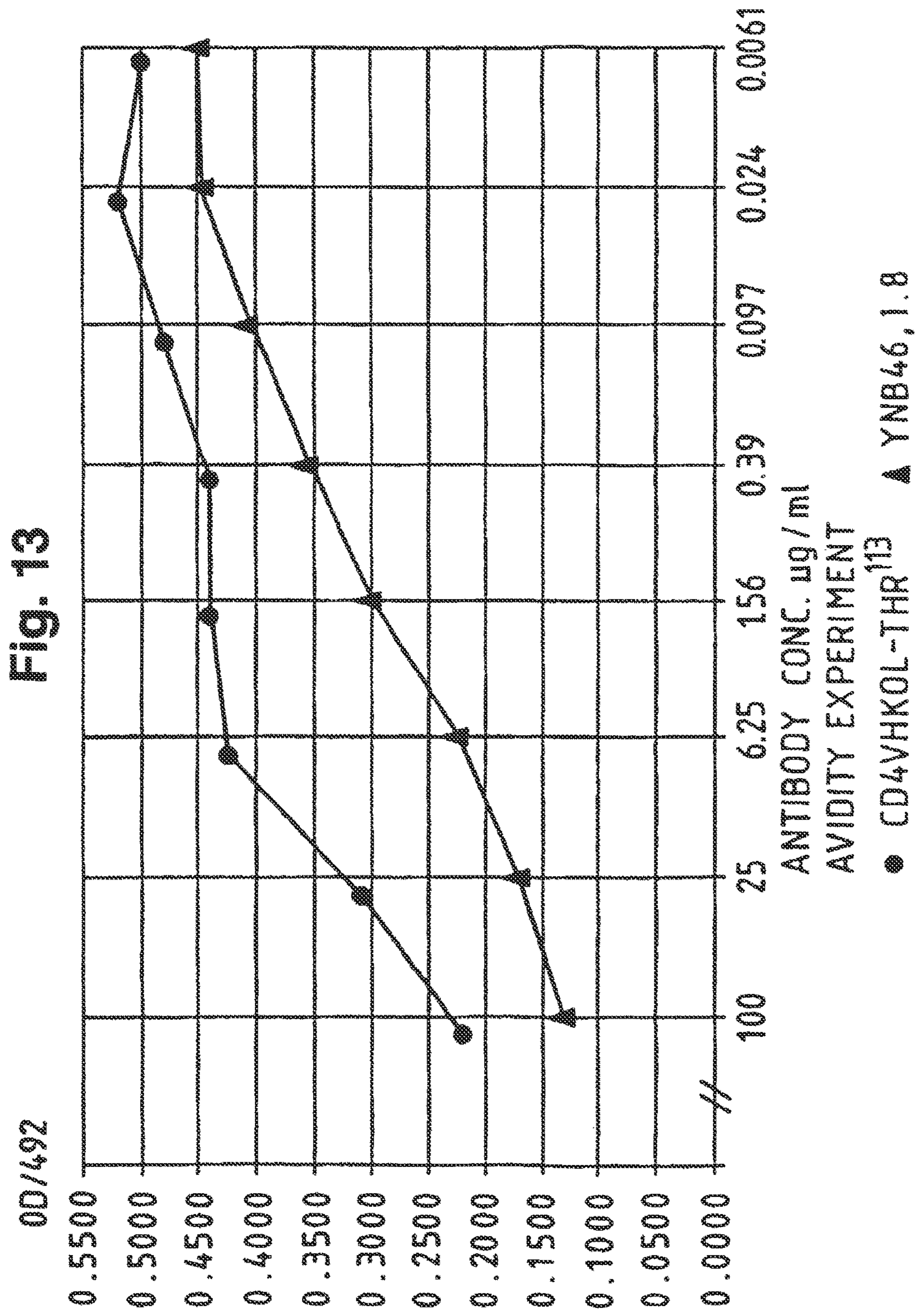
 103 A G I R H W G Q G T T V T V S S 122
 481 GCAGGTATACGTCACCTGGGCCAAGGACCACGGTCACCGTCTCCTCAGGTGAGTCCTTA 540

 541 CAACCTCTCTTCTATTACGCTTAAATAGATTTTACTGCATTTGTTGGGGGAAATGT 600

 601 GTGATCTGAATTCAGGTGATGAAGGACTAGGACACCTTGGGAGTCAGAAGGGTCAT 660

 661 TGGAGCCCGGCTGATGCAGACAGACATCCTCAGCTCCAGACTTCATGCCCAGAGATT 720

 BamHI
 721 TATAGGGATCC 731



ALTERED ANTIBODIES AND THEIR PREPARATION

Matter enclosed in heavy brackets [] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue; a claim printed with strikethrough indicates that the claim was canceled, disclaimed, or held invalid by a prior post-patent action or proceeding.

[This application is a 371 of PCT/GB91/01578, filed Sep. 16, 1991.] *More than one reissue application has been filed for the reissue of U.S. Pat. No. 6,767,996. This application (Ser. No. 13/683,113) is a reissue application of U.S. Pat. No. 6,767,996 and is a continuation reissue of U.S. Reissue application Ser. No. 11/493,016 (now RE43,898), which is also a reissue of U.S. Pat. No. 6,767,996. U.S. Pat. No. 6,767,996 is issued from U.S. application Ser. No. 08/030,175 filed on May 17, 1993, which is the U.S. national stage application of PCT/GB91/01578, filed 16 Sep. 1991. PCT/GB91/01578 claims priority to UK Application No. 9020282.1 filed 17 Sep. 1990, all of which are incorporated herein in their entireties.*

The present invention relates to altered antibodies and their preparation. The invention is typically applicable to the production of humanised antibodies.

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.

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.

We have now devised a new way of preparing an altered antibody. In contrast to previous proposals, this involves altering the framework of a variable domain rather than the

CDRs. This approach has the advantages that it does not require a pre-existing cDNA encoding, for example, a human framework to which to reshape and that it is technically easier than prior methodologies.

Accordingly, the present invention provides a process for the preparation of an antibody chain in which the CDRs of the variable domain of the antibody chain are derived from a first mammalian species and the framework of the variable domain and, if present, the or each constant domain of the antibody chain are derived from a second different mammalian species, which process comprises:

- (i) mutating the framework-encoding regions of DNA encoding a variable domain of an antibody chain of the said first species such that the mutated framework-encoding regions encode the said framework derived from the said second species; and
- (ii) expressing the said antibody chain utilising the mutated DNA from step (i).

A variable domain of either or both chains of an antibody can therefore be altered by:

- (a) determining the nucleotide and predicted amino acid sequence of a variable domain of a selected antibody chain of the said first species;
- (b) determining the antibody framework to which the framework of the said variable domain is to be altered;
- (c) mutating the framework-encoding regions of DNA encoding the said variable domain such that the mutated framework-encoding regions encode the framework determined upon in step (b);
- (d) linking the mutated DNA obtained in step (c) to DNA encoding a constant domain of the said second species and cloning the DNA into an expression vector; and
- (e) introducing the expression vector into a compatible host cell and culturing the host cell under such conditions that antibody chain is expressed.

The antibody chain may be co-expressed with a complementary antibody chain. At least the framework of the variable domain and the or each constant domain of the complementary chain generally are derived from the said second species also. A light chain and a heavy chain may be co-expressed. Either or both chains may have been prepared by the process of the invention. Preferably the CDRs of both chains are derived from the same selected antibody. An antibody comprising both expressed chains can be recovered.

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. The antibody may be an IgG such as an IgG1, IgG2, IgG3 or IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may be a chimaeric antibody of the type described in WO 86/01533.

A chimaeric 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 or heavy chain variable domain. Typically, the chimaeric antibody comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused at its C-terminus to 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 two regions of the chimaeric antibody may be connected via a cleavable linker sequence.

The invention is preferably employed to humanise an antibody, typically a monoclonal antibody and, for example,

a rat or mouse antibody. The framework and constant domains of the resulting antibody are therefore human framework and constant domains whilst the CDRs of the light and/or heavy chain of the antibody are rat or mouse CDRs. Preferably all CDRs are rat or mouse CDRs. The antibody may be a human IgG such as IgG1, IgG2, IgG3, IgG4; IgM; IgA; IgE or IgD carrying rat or mouse CDRs.

The process of the invention is carried out in such a way that the resulting antibody retains the antigen binding capability of the antibody from which it is derived. An antibody is reshaped according to the invention by mutating the framework-encoding regions of DNA coding for the variable domains of the antibody. This antibody and the reshaped antibody should both be capable of binding to the same antigen.

The starting antibody is typically an antibody of a selected specificity. In order to ensure that this specificity is retained, the variable domain framework of the antibody is preferably reshaped to about the closest variable domain framework of an antibody of another species. By "about the closest" is meant about the most homologous in terms of amino acid sequences. Preferably there is a homology of at least 50% between the two variable domains.

There are four general steps to reshape a monoclonal antibody. These are:

- (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy chain variable domains;
- (2) designing the reshaped antibody, i.e. deciding which antibody framework region to use during the reshaping process;
- (3) the actual reshaping methodologies/techniques; and
- (4) the transfection and expression of the reshaped antibody.

These four steps are explained below in the context of humanising an antibody. However, they may equally well be applied when reshaping to an antibody of a non-human species.

Step 1: Determining the Nucleotide and Predicted Amino Acid Sequence of the Antibody Light and Heavy Chain Variable Domains

To reshape 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.

Step 2: Designing the Reshaped Antibody

There are several factors to consider in deciding which human antibody sequence to use during the reshaping. The reshaping 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 spacial 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 spacial orientation if the human variable domain 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. This can be easily accomplished with a program called FASTA but other suitable programs are available. 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 customized sub-databases are first created that only include human immunoglobulin sequences. This has two benefits. First, the actual computational time is greatly reduced because analyses are restricted to only those sequences of interest rather than all the sequences in the databases. The second benefit is that, by restricting analyses to only human immunoglobulin sequences, the output will not be cluttered by the presence of rodent immunoglobulin sequences. There are far more rodent immunoglobulin sequences in databases than there are human.
2. List the human antibody variable domain sequences that have the most overall homology to the rodent antibody variable domain (from above). Do not make a distinction between homology within the framework regions and CDRs. Consider the overall homology.
3. Eliminate from consideration those human sequences that have CDRs that are a different length than those of the rodent CDRs. This rule-does not apply to CDR 3, because the length of this CDR is normally quite variable. Also, there are sometimes no or very few human sequences that have the same CDR lengths as that of the rodent antibody. If this is the case, this rule can be loosened, and human sequences with one or more differences in CDR length can be allowed.
4. From the remaining human variable domains, the one is selected that is most homologous to that of the rodent.
5. The actual reshaped antibody (the end result) should contain CDRs derived from the rodent antibody and a variable domain framework from the human antibody chosen above.

Step 3: The Actual Reshaping Methodologies/techniques

A cDNA encoding the desired reshaped antibody is preferably made beginning with the rodent cDNA from which the rodent antibody variable domain sequence(s) was originally determined. The rodent variable domain amino acid sequence is compared to that of the chosen human antibody variable domain sequence. The residues in the rodent variable domain framework are marked that need to be changed to the corresponding residue in the human to make the rodent framework identical to that of the human framework. There may also be residues that need adding to or deleting from the rodent framework sequence to make it identical to that of the human.

Oligonucleotides are synthesised that can be used to mutagenize the rodent 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.

The advantages of this method of reshaping as opposed to splicing CDRs into a human framework are that (1) this method does not require a pre-existing cDNA encoding the human framework to which to reshape and (2) splicing CDRs is technically more difficult because there is usually a large region of poor homology between the mutagenic oligonucleotide and the human antibody variable domain. This is not so much a problem with the method of splicing human framework residues onto a rodent variable domain because there is no need for a pre-existing cDNA encoding the human variable domain. The method starts instead with the rodent cDNA sequence. Also, splicing framework regions is technically easier because there is a high degree of homology between the mutagenic oligonucleotide and the rodent variable domain framework. This is true because a human antibody variable domain framework has been selected that is most homologous to that of the rodent.

The advantage of the present method of reshaping as opposed to synthesizing the entire reshaped version from scratch is that it is technically easier. Synthesizing a reshaped variable domain from scratch requires several more oligonucleotides, several days more work, and technical difficulties are more likely to arise.

Step 4: The Transfection and Expression of the Reshaped Antibody

Following the mutagenesis reactions to reshape the antibody, the cDNAs are linked to the appropriate DNA encoding light or heavy chain constant region, cloned into an expression vector, and transfected into 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 first antibody and CDRs comprising at least parts of the CDRs from a second antibody of different specificity;
- b) if necessary, 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 antibody chain, the or each constant domain being derived from the first antibody. The antibody can be recovered and purified. 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.

Although the cell line used to produce the altered 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.

It is known that some immortalised lymphoid cell lines, such as myeloma cell lines, in their normal state secrete isolated Ig light or heavy chains. If such a cell line is transformed with the vector prepared in step (a) it will not be necessary to carry out step (b) of the process, provided that the normally secreted chain is complementary to the variable domain of the Ig chain encoded by the vector prepared in step (a).

However, where the immortalised cell line does not secrete or does not secrete a complementary chain, it will be necessary to carry out step (b). This step may be carried out by further manipulating the vector produced in step (a) so that this vector encodes not only the variable domain of an altered antibody light or heavy chain, but also the complementary variable domain.

Alternatively, step (b) is carried out by preparing a second vector which is used to transform the immortalised cell line. This alternative leads to easier construct preparation, but may be less preferred than the first alternative in that it may not lead to as efficient production of antibody.

In the case where the immortalised cell line secretes a complementary light or heavy chain, the transformed cell line may be produced for example by transforming a suitable bacterial cell with the vector and then fusing the bacterial cell with the immortalised cell line by spheroplast fusion. Alternatively, the DNA may be directly introduced into the immortalised cell line by electroporation or other suitable method.

An antibody is consequently produced in which CDRs of a variable domain of an antibody chain are homologous with the corresponding CDRs of an antibody of a first mammalian species and in which the framework of the variable domain and the constant domains of the antibody are homologous with the corresponding framework and constant domains of an antibody of a second, different, mammalian species. Typically, all three CDRs of the variable domain of a light or heavy chain are derived from the first species.

The present process has been applied to obtain an antibody against human CD4 antigen. Accordingly, the invention also provides an antibody which is capable of binding to human CD4 antigen, in which the CDRs of the light chain of the antibody have the amino acid sequences:

CDR1: LASEDIYSDLA (SEQ ID NO:13)

CDR2: NTDTLQN (SEQ ID NO:14)

CDR3: QQYNNYPWT (SEQ ID NO:15),

in which the CDRs of the heavy chain of the antibody have the amino acid sequences:

CDR1: NYGMA (SEQ ID NO:16)

CDR2: TISHDGSPTYFRDSVKG (SEQ ID NO:17)

CDR3: QGTIAGIRH (SEQ ID NO:18), and

in which the framework of the variable domain and, if present, the or each constant domain of each chain are derived from a mammalian non-rat species.

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.

The antibody may be an IgG such as IgG1, IgG2, IgG3 or IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may be a chimaeric antibody of the type described in WO 86/01533.

A chimaeric 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 or heavy chain variable domain. Typically the chimaeric antibody comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused at its C-terminus to 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 two regions of the chimaeric antibody may be connected via a cleavable linker sequence.

The invention is preferably employed to humanise a CD4 antibody such as a rat or mouse CD4 antibody. The framework and the constant domains of the resulting antibody are therefore human framework and constant domains whilst the CDRs of the light and/or heavy chain of the antibody are rat or mouse CDRs. Preferably all CDRs are rat or mouse CDRs. The antibody may be a human IgG such as IgG1, IgG2, IgG3, IgG4; IgM; IgA; IgE or IgD carrying rat or mouse CDRs.

Preferably the framework of the antibody heavy chain is homologous to the corresponding framework of the human antibody KOL (Schmidt et al, Hoppe-Seyler's Z. Physiol. Chem., 364 713-747, 1983). The sixth residue of framework 4 in this case is suitably Thr or Pro, preferably Thr. This residue is the 121st residue in the KOL antibody heavy chain variable region (Schmidt et al, 1983), and is identified as residue 108 by Kabat (Kabat et al, "Sequences of proteins of immunological interest", US Dept of Health and Human Services, US Government Printing Office, 1987). Alternatively, the framework of the antibody heavy chain is homologous to the corresponding framework of the human antibody NEW (Saul et al, J. Biol. Chem. 2: 585-597, 1978). The final residue of framework 1 in this case is suitably Ser or Thr, preferably Ser. This residue is at position 30 (Kabat et al, 1987). Preferably the framework of the antibody light chain is homologous to the variable domain framework of the protein REI (Epp et al, Eur. J. Biochem., 45, 513-524, 1974).

The framework regions of one or both chains of a CD4 antibody can be reshaped by the present process. Alternatively, one or both chains of a CD4 antibody may be reshaped by the procedure described in EP-A-0239400. The procedure of EP-A-0239400 involves replacing CDRs rather than the replacement of frameworks. The CDRs are grafted onto a framework derived from a mammalian non-rat species, typically a human. This may be achieved by oligonucleotide-directed in vitro mutagenesis of the CDR-encoding regions of an antibody chain, light or heavy, from a mammalian non-rat species. The oligonucleotides in such an instance are selected so that the resulting CDR-grafted antibody has the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 shown above.

The reshaped CD4 antibody can be used to induce tolerance to an antigen. It can be used to alleviate autoimmune diseases such as rheumatoid arthritis. It can be used to prevent graft rejection. Tolerance to a graft such as an organ graft or a bone marrow transplantation can be achieved. Also, the reshaped CD4 antibody might be used to alleviate allergies. Tolerance to allergens could be achieved.

The CD4 antibody may be depleting or non-depleting. A depleting antibody is an antibody which depletes more than 50%, for example from 90 to 99%, of target cells in vivo. A non-depleting antibody depletes fewer than 50%, for example, from 10 to 25% and preferably less than 10% of target cells in vivo. A CD4 antibody may be administered alone or may be co-administered with a non-depleting or depleting CD8 antibody. The CD4 antibody, depleting or non-depleting, and CD8 monoclonal antibody, depleting or non-depleting, may be administered sequentially in any order or may be administered simultaneously. An additional antibody, drug or protein may be administered before, during or after administration of the antibodies.

A CD4 antibody and, indeed, a CD8 antibody as appropriate are given parenterally, for example intravenously. The antibody may be administered by injection or by infusion. For this purpose the antibody is formulated in a pharmaceutical composition further comprising a pharmaceutically acceptable carrier or diluent. Any appropriate carrier or diluent may be employed, for example phosphate-buffered saline solution.

The amount of non-depleting or depleting CD4 and, if desired, CD8 antibody administered to a patient depends upon a variety of factors including the age and weight of a patient, the condition which is being treated and the antigen(s) to which it is desired to induce tolerance. In a model mouse system from 1 µg to 2 mg, preferably from 400 µg to 1 mg, of a mAb is administered at any one time. In humans from 3 to 500 mg, for example from 5 to 200 mg, of antibody may be administered at any one time. Many such doses may be given over a period of several weeks, typically 3 weeks.

A foreign antigen(s) to which it is desired to induce tolerance can be administered to a host before, during, or after a course of CD4 antibody (depleting or non-depleting) and/or CD8 antibody (depleting or non-depleting). Typically, however, the antigen(s) is administered one week after commencement of antibody administration, and is terminated three weeks before the last antibody administration.

Tolerance can therefore be induced to an antigen in a host by administering non-depleting or depleting CD4 and CD8 mAbs and, under cover of the mAbs, the antigen. A patient may be operated on surgically under cover of the non-depleting or depleting CD4 and CD8 mAbs to be given a tissue transplant such as an organ graft or a bone marrow transplant. Also, tolerance may be induced to an antigen already possessed by a subject. Long term specific tolerance can be induced to a self antigen or antigens in order to treat autoimmune disease such as multiple sclerosis or rheumatoid arthritis. The condition of a patient suffering from autoimmune disease can therefore be alleviated.

The following Example illustrates the invention. In the accompanying drawings:

FIGS. 1-1A: shows the nucleotide and predicted amino acid sequence of rat CD4 antibody light chain variable region. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. Base pairs 1-269 (HindIII-PvuII) and 577-620 ([Bg1II/Bc1I]-BamHI) are part of the vector M13V_KPCR3, while base pairs 270-576 are from the PCR product of the CD4 antibody light chain variable region (V_L). CDRs (boxes) were identified by comparison to known immunological sequences (Kabat et al, "Sequences of proteins of immunological interest, US Dept of Health and Human Services, US Government Printing Office, 1987). The nucleotide sequence of FIG. 1 corresponds to SEQ ID NO:1.

FIGS. 2-2A: shows the nucleotide and predicted amino acid sequence of the reshaped CAMPATH-1 antibody light chain cDNA. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. The nucleotide sequence of FIG. 2 corresponds to SEQ ID NO:2.

FIGS. 3-3A: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody light chain cDNA CD4V_LREI. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. The nucleotide sequence of FIG. 3 corresponds to SEQ ID NO:3.

FIGS. 4-4A: shows the nucleotide and predicted amino acid sequence of rat CD4 antibody heavy chain variable region. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. Base pairs 1-272 (HindIII-PstI) and 603-817 (BstEII-BamHI) are part of the vector M13V_HPCR1, while base pairs 273-602 are from the PCR product of the CD4 antibody heavy chain variable region (V_H). The nucleotide sequence of FIG. 4 corresponds to SEQ ID NO:4.

FIGS. 5, 5A-D: shows the nucleotide and predicted amino acid sequence of the reshaped CAMPATH-1 antibody heavy chain cDNA. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. The nucleotide sequence of FIG. 5 corresponds to SEQ ID NO:5.

FIGS. 6, 6A-D: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain cDNA CD4V_HNEW-Thr³⁰. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. The nucleotide sequence of FIG. 6 corresponds to SEQ ID NO:6.

FIGS. 7, 7A-D: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain cDNA CD4V_HNEW-Ser³⁰. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. The nucleotide sequence of FIG. 7 corresponds to SEQ ID NO:7.

FIG. 8: shows the heavy chain variable (V) region amino acid sequence of the human myeloma protein KOL. CDRs are identified by boxes. This sequence is taken from the Swiss-Prot protein sequence database. The nucleotide sequence of FIG. 8 corresponds to SEQ ID NO:8.

FIGS. 9-9A: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Pro¹¹³. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. The nucleotide sequence of FIG. 9 corresponds to SEQ ID NO:9.

FIGS. 10-10A: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Pro¹¹³ without immunoglobulin promoter. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. The nucleotide sequence of FIG. 10 corresponds to SEQ ID NO:10.

FIGS. 11-11A: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Thr¹¹³. The number of the first and last amino acid or nucleotide in each line is indicated in the left

and right margins, respectively. CDRs are identified by boxes. The nucleotide sequence of FIG. 11 corresponds to SEQ ID NO:11.

FIGS. 12-12A: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Thr¹¹³ without immunoglobulin promoter. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. The nucleotide sequence of FIG. 12 corresponds to SEQ ID NO:12.

FIG. 13: shows the results of an ELISA that compares the avidity of YNB46.1.8 and CD4V_HKOL-Thr¹¹³ antibodies. The X-axis indicates the concentration (μg/ml) of YNB46.1.8 (triangles) or CD4V_HKOL-Thr¹¹³ (circles) antibody. The Y-axis indicates the optical density at 492 nanometers.

EXAMPLE

1. Materials and Methods

Isolation of monoclonal antibody. The rat-derived anti-human CD4 antibody, clone YNB46.1.8 (IgG_{2b}, kappa light chain serotype), was the result of fusion between a rat splenocyte and the Lou strain rat myeloma cell line Y3-Ag 1.2.3 (Galfré et al, Nature, 277: 131-133, 1979) and was selected by its binding to a rat T cell line NB2-6TG stably transfected with an expression vector containing a complementary DNA (cDNA) encoding the human CD4 antigen (Madden et al, Cell, 42: 93-104, 1985). Antibody was purified by high pressure liquid chromatography (HPLC).

Isolation of Antibody Variable Regions. cDNAs encoding the V_L and V_H regions of the CD4 antibody were isolated by a polymerase chain reaction (PCR)-based method (Orlandi et al, PNAS USA, 86: 3833-3837, 1989) with some modifications. Total RNA was isolated from hybridoma cells by the guanidine thiocyanate method (Chirgwin et al, Biochemistry, 18: 5294, 1979), and poly(A)⁺ RNA was isolated by passage of total RNA through and elution from an oligo (dT)-cellulose column (Aviv and Leder PNAS USA 69: 1408, 1972). Poly(A)⁺ RNA was heated at 70° C. for 5 minutes and cooled on ice just prior to use. A 25 μl first strand synthesis reaction consisted of 5 μg poly(A)⁺ RNA, 250 μM each dNTP, 50 mM Tris.HCl (pH 8.2 at 42° C.), 10 mM MgCl₂, 100 mM KCl, 10 mM dithiothreitol, 23 units reverse transcriptase (Anglian Biotec, Colchester, U.K.), 3.5 pmoles of the V_L region-specific oligonucleotide primer V_K1FOR [5'-d(GTT AGA TCT CCA GCT TGG TCC C)SEQ ID NO:19] or the V_H region-specific primer V_H1FOR-B [5'-d(TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC)SEQ ID NO:20], and incubated for 5 minutes at 20° C. and then 90 minutes at 42° C.

Subsequent 50 μl PCR amplifications consisted of 5 μl of the first strand synthesis reaction (unpurified), 500 μM each dNTP, 67 mM Tris-HCl (pH 8.8 at 25° C.), 17 mM (NH₄)₂ SO₄, 10 mM MgCl₂, 20 μg/ml gelatin, 5 units TAQ DNA polymerase (Koch-Light, Haverhill, U.K.), and 25 pmoles (each) of primer V_K1FOR and V_K1BACK [5'-d(GAC ATT CAG CTG ACC CAG TCT)SEQ ID NO:21] for the V_L region or V_H1FOR-B and the mixed primer V_H1BACK [5'-d(AG GT(CG) (CA)A(GA) CTG CAG (GC)AG TC(TA) GG)SEQ ID NO:22] for the V_H region. Reactions were overlaid with mineral oil and subjected to 30 cycles of 1.5 minutes at 95° C. (denaturation), 1.5 minutes at 37° C. (V_L) or 50° C. (V_H; annealing), and 3 minutes at 72° C. (exten-

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sion) with a Techne PHC-1 programmable cyclic reactor. The final cycle contained a 10 minute extension time.

The samples were frozen at -20°C . and the mineral oil (a viscous liquid at -20°C .) was removed by aspiration. The aqueous phases were thawed, and PCR products were purified by electrophoresis in 2% agarose gels, and then double digested with either PvuII and BglIII (V_L) or PstI and BstEII (V_H) restriction enzymes, and cloned into the PvuII and BclI restriction sites of the vector M13 V_K PCR3 (for V_L region; Orlandi et al, 1989) or the PstI and BstEII restriction sites of the vector M13 V_H PCR1 (for V_H region). As described in the results, V_L region clones were first screened by hybridisation to a ^{32}P -labeled oligonucleotide probe [5'-d(GTT TCA TAA TAT TGG AGA CA)SEQ ID NO:23] for the CDR2 of the Y3-Ag 1.2.3 V_L region. V_L region clones not hybridising to this probe and V_H region clones were sequenced by the dideoxy chain termination method (Sanger et al, PNAS USA 74: 5463, 1977).

Reshaped Light Chain Variable Region and Expression Vector Construct

The reshaped light chain was constructed by oligonucleotide-directed in vitro mutagenesis in an M13 vector by priming with three oligonucleotides simultaneously on a 748 base single-stranded cDNA template encoding the entire V_L and kappa constant (C_K) regions of the reshaped CAMPATH-1 antibody (Reichmann et al, Nature 332: 323-327, 1988). The three oligonucleotides [5'-d(AGA GTG ACC ATC ACC TGT CTA GCA AGT GAG GAC ATT TAC AGT GAT TTA GCA TGG TAC CAG CAG AAG CCA)SEQ ID NO:24, 5'-d(CTG CTG ATC TAC AAT ACA GAT ACC TTG CAA AAT GGT GTG CCA AGC AGA TTC)SEQ ID NO:25, 5'-d(ATC GCC ACC TAC TAC TGC CAA CAG TAT AAC AAT TAT CCG TGG ACG TTC GGC CAA GGG ACC)SEQ ID NO:26] were designed to replace each of the three CDRs in the REI-based human antibody V_L region framework that is part of the reshaped CAMPATH-1 antibody V_L region (Reichmann et al, 1988). A clone containing each of the three mutant oligonucleotides was identified by nucleotide sequencing and was subcloned into the HindIII site of the expression vector pH β APr-1 (Gunning et al, PNAS, 4: 4831-4835, 1987) which also contained a dihydrofolate reductase gene (Ringold et al, J. Mol. Appl. Genet. 1: 165-175, 1981) driven by a truncated SV40 promoter.

Reshaped Heavy Chain Variable Regions Based on the Variable Region Framework of the Human Antibody NEW, and Expression Vector Constructs

Two versions of the NEW-based reshaped heavy chain were created, CD4 V_H NEW-Thr 30 and CD4 V_H NEW-Ser 30 . The CD4 V_H NEW-Thr 30 version (FIG. 6) encodes a threonine residue at position 30 while the CD4 V_H NEW-Ser 30 version (FIG. 7) encodes a Ser residue at position 30. As a matter of convenience, CD4 V_H NEW-Thr 30 was created first by oligonucleotide-directed in vitro mutagenesis in the vector M13mp18 by priming with three oligonucleotides simultaneously on a 1467 base single-stranded cDNA template (FIG. 5) encoding the entire heavy chain of the reshaped CAMPATH-1 antibody (Reichmann et al, 1988). The three oligonucleotides [5'-d(TCT GGC TTC ACC TTC ACC AAC TAT GGC ATG GCC TGG GTG AGA CAG CCA CCT)SEQ ID NO:27, 5'-d(GGT CTT GAG TGG ATT GGA ACC ATT AGT CAT GAT GGT AGT GAC ACT TAC TTT CGA GAC TCT GTG AAG GGG AGA GTG)SEQ ID NO:28, 5'-d(GTC TAT TAT TGT GCA AGA CAA GGC ACT ATA

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GCT GGT ATA CGT CAC TGG GGT CAA GGC AGC CTC)SEQ ID NO:29] were designed to replace each of the three complementarity determining regions (CDRs) in the NEW-based V_H region that is part of the reshaped CAMPATH-1 antibody (Reichmann et al, 1988). A clone (FIG. 6) containing each of the three mutant oligonucleotides was identified by nucleotide sequencing. CD4 V_H NEW-Ser 30 was created second by oligonucleotide-directed in vitro mutagenesis in the vector M13mp18 by priming with a single oligonucleotide on the 1458 base single-stranded cDNA template (FIG. 6) encoding CD4 V_H NEW-Thr 30 . The oligonucleotide [5'-d(GCT TCA CCT TCA GCA ACT ATG GCA T)SEQ ID NO:30] was designed to mutate the residue at position 30 from threonine [ACC] to serine [AGC]. A clone (FIG. 7) containing this mutant oligonucleotide was identified by nucleotide sequencing. Double-stranded forms of the clones CD4 V_H NEW-Thr 30 and CD4 V_H NEW-Ser 30 were subcloned as HindIII fragments into the HindIII site of the expression vector pNH316. The vector pNH316 is a modified version of the vector pH β APr-1 (Gunning et al, PNAS, 84: 4831-4835, 1987) which was engineered to contain a neomycin resistance gene driven by a metallothioneine promoter.

Reshaped Heavy Chain Variable Regions Based on the Variable Region Framework of the Human Antibody KOL, and Expression Vector Constructs

Two versions of the KOL-based reshaped heavy chain were created, CD4 V_H KOL-Thr 113 and CD4 V_H KOL-Pro 113 . The CD4 V_H KOL-Thr 113 version encodes a threonine residue at position 113 (FIG. 11) while the CD4 V_H KOL-Pro 113 version encodes a proline residue at position 113 (FIG. 9). As a matter of convenience, CD4 V_H KOL-Thr 113 was created first by oligonucleotide-directed in vitro mutagenesis of single-stranded DNA template containing the 817 base HindIII-BamHI fragment encoding the V_H region of the rat CD4 antibody (FIG. 4) cloned into M13mp18 by priming simultaneously with five oligonucleotides [5'-d(CAC TCC CAG GTC CAA CTG GTG GAG TCT GGT GGA GGC GTG GTG GAG CCT GG)SEQ ID NO:31, 5'-d(AAG GTC CCT GAG ACT CTC CTG TTC CTC CTC TGG ATT CAT CTT CAG TAA CTA TGG CAT G)SEQ ID NO:32, 5'-d(GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG)SEQ ID NO:33, 5'-d(ACT ATC TCC AGA GAT AAT AGC AAA AAC ACC CTA TTC CTG CAA ATG G)SEQ ID NO:34, 5'-d(ACA GTC TGA GGC CCG AGG ACA CGG GCG TGT ATT TCT GTG CAA GAC AAG GGA C)SEQ ID NO:35] which were designed to replace the rat framework regions with the human framework regions of KOL. A clone containing each of the five mutant oligonucleotides was identified by nucleotide sequencing. CD4 V_H KOL-Pro 113 was created second by oligonucleotide-directed in vitro mutagenesis of single-stranded DNA template containing the 817 base HindIII-BamHI fragment encoding CD4 V_H KOL-Thr 113 cloned into M13mp18 by priming with the oligonucleotide [5'-d(TGG GGC CAA GGG ACC CCC GTC ACC GTC TCC TCA)SEQ ID NO:36]. A clone containing this mutant oligonucleotide was identified by nucleotide sequencing.

The immunoglobulin promoters were removed from the double-stranded DNA forms of clones encoding CD4 V_H KOL-Thr 113 (FIG. 11) and CD4 V_H KOL-Pro 113 (FIG. 9) by replacing (for both versions) the first 125 bp (HindIII-NcoI) with a HindIII-NcoI oligonucleotide linker fragment [5'-d(AGC TTT ACA GTT ACT GAG CAC ACA GGA CCT CAC)SEQ ID NO:37 and its overlapping

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complement 5'-d(CAT GGT GAG GTC CTG TGT GCT CAG TAA CTG TAA)SEQ ID NO:38]. The resultant clones, CD4V_HKOL-Thr¹¹³ (FIG. 12) and CD4V_HKOL-Pro¹¹³ (FIG. 10), now 731 bp HindIII-BamHI fragments, were separately subcloned into the HindIII and BamHI cloning sites of the expression vector pHβAPr-1-gpt (Gunning et al, PNAS USA 76, 1373, 1987) into which had been cloned the human IgG1 constant region gene (Bruggemann et al, J. Exp. Med. 166, 1351-1361, 1987) at the BamHI site. Thus, when transfected and expressed as antibody heavy chains (see below), these reshaped V_H regions are linked to human IgG1 constant regions.

Fluorescence Activated Cell Sorter (FACS) Analysis

The relative affinities of the reshaped antibodies to bind the CD4 antigen were estimated by FACS analysis. The CD4-expressing cells used in this analysis were a cloned rat T cell line NB2-6TG stably transfected with an expression vector containing a complementary DNA (cDNA) encoding the human CD4 antigen (Maddon et al, Cell, 42, 93-104, 1985). Cells were stained with the appropriate reshaped antibody followed by fluorescein-conjugated sheep anti-human antibodies (Binding Site Ltd., Birmingham, UK). Control staining (see Table 1) consisted of no antibody present during the first stage of cell staining. Mean cellular fluorescence was determined with an Ortho FACS.

Antibody Avidity Analysis

The relative avidities of the rat YNB46.1.8 antibody and the reshaped CD4V_HKOL-Thr¹¹³ antibody were estimated by an enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with soluble recombinant CD4 antigen (Byrn et al, Nature, 344: 667-670, 1990) at 50 ul/well, 10 ug/ml, and then blocked with 100 ul/well phosphate buffered saline (PBS) containing 1.0% bovine serum albumin (BSA). Antibodies were diluted in PBS containing 0.1% BSA, and added to wells (50 ul/well) for 45 minutes at room temperature. Biotinylated CD4V_HKOL-Thr¹¹³ antibody (10 ul/well; 20 ug/ml final concentration) was then added to each well for an additional 45 minutes. Wells were washed with PBS containing 0.1% BSA, and then 50 ul streptavidin-biotinylated horseradish peroxidase complex (Amersham; Aylesbury, UK) diluted 1:1,000 was added to each well for 30 minutes. Wells were washed with PBS containing 0.1% BSA, and 100 ul substrate (25 mM citric acid, 50 mM disodium hydrogen phosphate, 0.1% (w/v) o-phenylene diamine, 0.04% (v/v) 30% hydrogen peroxide) was added to each well. Reactions were stopped by the addition of 50 ul/well 1.0 M sulfuric acid. Optical densities at 492 nanometers (OD₄₉₂) were determined with an ELISA plate reader.

Transfections

Dihydrofolate reductase deficient chinese hamster ovary (CHO^{DHFR-}) cells (10⁶/T-75 flask) were cotransfected as described (Wigler et al, PNAS USA 76, 1373, 1979) with 9 μg of heavy chain construct and 1 μg of the light chain construct. Transfectants were selected in medium containing 5% dialysed foetal bovine serum for 2 to 3 weeks, and antibody-secreting clones were identified by ELISAs of conditioned media. Antibody was concentrated and purified by protein-A Sepharose (Trade Mark) column chromatography.

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2. Results

Cloning of Light and Heavy Chain Variable Region cDNAs

cDNAs encoding the V_L and V_H regions from CD4 antibody-secreting hybridoma cells were isolated by PCR using primers which amplify the segment of mRNA encoding the N-terminal region through to the J region (Orlandi et al, 1989). V_L and V_H region PCR products were subcloned into the M13-based vectors M13V_KPCR3 and M13V_HPCR1, respectively. Initial nucleotide sequence analysis of random V_L region clones revealed that most of the cDNAs encoded the V_L region of the light chain expressed by the Y3-Ag 1.2.3 rat myeloma cell line (Crowe et al, Nucleic Acid Research, 17: 7992, 1989) that was used as the fusion partner to generate the anti-CD4 hybridoma. It is likely that the expression of the Y3-Ag 1.2.3 light chain mRNA is greater than that of the CD4 antibody light chain, or the Y3-Ag 1.2.3 light chain mRNA is preferentially amplified during the PCR.

To maximize the chance of finding CD4 V_L region cDNAs, we first screened all M13 clones by hybridisation to a ³²P-labeled oligonucleotide probe that is complementary to the CDR 2 of Y3-Ag 1.2.3 (Crowe et al, Nucleic Acid Research, 17: 7992, 1989). Subsequent sequence analysis was restricted to M13 clones which did not contain sequence complementary to this probe. In this manner, two cDNA clones from independent PCR amplifications were identified that encoded identical V_L regions. Nucleotide sequence analysis of random V_H region PCR products revealed a single species of V_H region cDNA. Two V_H cDNA clones from independent PCR amplifications were found to contain identical sequences except that the codon of residue 14 encoded proline [CCT] in one clone while the second clone encoded leucine [CTT] at the same position.

According to Kabat et al 1987, 524 of 595 sequenced V_H regions contain a proline residue at this position, while only 6 contain leucine. We have therefore chosen the proline-encoding clone for illustration (see below). As residue 14 lies well within the first V_H framework region and not in a CDR, it is unlikely to contribute directly to antigen binding, and the ambiguity at this position did not affect the subsequent reshaping strategy. Thus, we have not investigated this sequence ambiguity further.

The cDNA sequences and their predicted amino acid sequences are shown in FIGS. 1 and 4. As no additional V_L or V_H region-encoding clones were found, it was assumed that these sequences were derived from the CD4 antibody genes.

Construction of Reshaped Antibodies

Our goal was to investigate the importance of selecting the appropriate human V region framework during reshaping. Two reshaping strategies were employed.

First Reshaping Strategy

In the first strategy, we created a reshaped antibody that incorporated the CDRs from the rat-derived CD4 antibody and the same human V region framework sequences that we had previously successfully used for the reshaped CAM-PATH-1 antibody, namely an REI-based framework for the V_L region and a NEW-based framework for the V_H region (Reichmann et al, 1988). This was accomplished by oligonucleotide-directed in vitro mutagenesis of the six CDRs of

the reshaped CAMPATH-1 antibody light and heavy chain cDNAs shown in FIGS. 2 and 5, respectively. The resultant reshaped CD antibody light chain (FIG. 3) is called CD4V_LREI. Two versions of the NEW-based reshaped CD4 antibody heavy chain were created: CD4V_HNEW-Thr³⁰ (FIG. 6) encoding a threonine residue at position 30 (in framework 1) and CD4V_HNEW-Ser³⁰ (FIG. 7) encoding a serine residue at position 30. These two different versions were created because the successfully reshaped CAMPATH-1 antibody heavy chain bound antigen well whether position 30 encoded a threonine or serine residue (Reichmann et al, 1988), and we chose to test both possibilities in this case as well.

Second Reshaping Strategy

In the second reshaping strategy, we have reshaped the CD4 antibody V_H region to contain the V_H region framework sequences of the human antibody KOL. Of all known human antibody V_H regions, the overall amino acid sequence of the V_H region of KOL is most homologous to the rat CD4 antibody V_H region. The V_H regions of the human antibodies KOL and NEW are 66% and 42% homologous to the rat CD4 antibody V_H region, respectively.

Two versions of the KOL-based reshaped CD4 antibody heavy chain V region were created that differ by a single amino acid residue within the fourth framework region: CD4V_HKOL-Pro¹¹³ (FIG. 10) encodes a proline residue at position 113 and CD4V_HKOL-Thr¹¹³ (FIG. 12) encodes a threonine residue at position 113. CD4V_HKOL-Pro¹¹³ is "true to form" in that its framework sequences are identical to those of the KOL antibody heavy chain V region (FIG. 8).

Of all known human antibody V_L regions, the overall amino acid sequence of the V_L region of the human light chain NEW is most homologous (67%) to the rat CD4 antibody V_L region. Thus, the identical reshaped light chain, CD4V_LREI (described above), that was expressed with the NEW-based reshaped CD4 antibody heavy chains CD4V_HNEW-Thr³⁰ and CD4V_HNEW-Ser³⁰, is also expressed with the KOL-based reshaped CD4 antibody heavy chains CD4V_HKOL-Pro¹¹³ and CD4V_HKOL-Thr¹¹³. This is advantageous because expression of the same reshaped light chain with different reshaped heavy chains allows for a direct functional comparison of each reshaped heavy chain.

To summarise, four different reshaped antibodies were created. The reshaped light chain of each antibody is called CD4V_LREI. The reshaped heavy chains of the antibodies are called CD4V_HNEW-Thr³⁰, CD4V_HNEW-Ser³⁰, CD4V_HKOL-Pro¹¹³, and CD4V_HKOL-Thr¹¹³, respectively. Each of the reshaped heavy chains contain the same human IgG1 constant region. As each reshaped antibody contains the same reshaped light chain, the name of a reshaped antibody's heavy chain shall be used below to refer to the whole antibody (heavy and light chain combination).

Relative Affinities of the Reshaped Antibodies

The relative affinities of the reshaped antibodies were approximated by measuring their ability to bind to CD4 antigen-expressing cells at various antibody concentrations. FACS analysis determined the mean cellular fluorescence of the stained cells (Table 1).

It is clear from this analysis that the reshaped CD4 antibodies bind to CD4 antigen to varying degrees over a broad concentration range. Consider Experiment 1 of Table 1 first. Comparing CD4V_HKOL-Thr¹¹³ antibody to CD4V_HNEW-Thr³⁰ antibody, it is clear that both antibodies bind CD4⁺ cells when compared to the control, reshaped CAMPATH-1 antibody. However, CD4V_HKOL-Thr¹¹³ antibody binds CD4⁺ cells with far greater affinity than CD4V_HNEW-Thr³⁰ antibody. The lowest concentration of CD4V_HKOL-Thr¹¹³ antibody tested (2.5 ug/ml) gave a mean cellular fluorescence nearly equivalent to that of the highest concentration of CD4V_HNEW-Thr³⁰ antibody tested (168 ug/ml). Experiment 2 demonstrates that CD4V_HNEW-Ser³⁰ antibody may bind CD4⁺ cells somewhat better than CD4V_HNEW-Thr³⁰. Only 2.5 ug/ml CD4V_HNEW-Ser³⁰ antibody is required to give a mean cellular fluorescence nearly equivalent to 10 ug/ml CD4V_HNEW-Thr³⁰ antibody. Experiment 3 demonstrates that CD4V_HKOL-Thr¹¹³ antibody may bind CD4⁺ cells somewhat better than CD4V_HKOL-Pro¹¹³ antibody.

From these assays, it is clear that the KOL-based reshaped antibodies are far superior to the NEW-based reshaped antibodies with regards to affinity towards CD4⁺ cells. Also, there is a lesser difference, if any, between CD4V_HNEW-Thr³⁰ antibody and CD4V_HNEW-Ser¹¹³ antibody, and likewise between CD4V_HKOL-Thr¹¹³ antibody and CD4V_HKOL-Pro¹¹³ antibody. A ranking of these reshaped antibodies can thus be derived based on their relative affinities for CD4⁺ cells:



It should be restated that each of the reshaped CD4 antibodies used in the above experiments have the identical heavy chain constant regions, and are associated with identical reshaped light chains. Thus observed differences of binding to CD4⁺ cells must be due to differences in their heavy chain V regions.

Relative Avidities of the Rat YNB46.1.8 Antibody and the Reshaped CD4V_HKOL-Thr¹¹³ Antibody

The relative avidities of the rat YNB46.1.8 antibody and the reshaped CD4V_HKOL-Thr¹¹³ antibody were estimated by ELISA. In this assay, the ability of each antibody to inhibit the binding of biotinylated CD4V_HKOL-Thr¹¹³ antibody to soluble recombinant CD4 antigen was determined. Results of an experiment are shown in FIG. 13. The inhibition of binding of biotinylated CD4V_HKOL-Thr¹¹³ antibody was linear for both the unlabeled CD4V_HKOL-Thr¹¹³ and YNB46.1.8 antibodies near the optical density of 0.3. The concentrations of CD4V_HKOL-Thr¹¹³ and YNB46.1.8 antibodies that give an optical density of 0.3 are 28.7 and 1.56 ug/ml, respectively. Thus the avidity of the YNB46.1.8 antibody can be estimated to be 28.7/1.56 or about 18 times better than that of CD4V_HKOL-Thr¹¹³ antibody. It should be noted that this experiment only provides a rough approximation of relative avidities, not affinities. The rat YNB46.1.8 antibody contains a different constant region than that of the CD4V_HKOL-Thr¹¹³ antibody, and this could affect how well the antibodies bind CD4 antigen, irrespective of their actual affinities for CD4 antigen. The actual affinity of the reshaped antibodies for CD4 antigen may be greater, lesser, or the same as the YNB46.1.8 antibody. The other reshaped antibodies CD4V_HKOL-Pro¹¹³, CD4V_HNEW-Ser³⁰, and CD4V_HNEW-Thr³⁰ have not yet been tested in this assay.

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TABLE 1

Mean cellular fluorescence of CD4 ⁺ cells stained with reshaped antibodies		
Reshaped Antibody	Concentration (µg/ml)	Mean cellular Fluorescence
<u>Experiment 1.</u>		
CD4V _H KOL-Thr ¹¹³	113	578.0
CD4V _H KOL-Thr ¹¹³	40	549.0
CD4V _H KOL-Thr ¹¹³	10	301.9
CD4V _H KOL-Thr ¹¹³	2.5	100.5
CD4V _H NEW-Thr ³⁰	168	97.0
CD4V _H NEW-Thr ³⁰	40	40.4
CD4V _H NEW-Thr ³⁰	10	18.7
CD4V _H NEW-Thr ³⁰	2.5	10.9
CAMPATH-1	100	11.6
CAMPATH-1	40	9.4
CAMPATH-1	10	9.0
CAMPATH-1	2.5	8.6
CONTROL	—	9.0
<u>Experiment 2.</u>		
CD4V _H NEW-Thr ³⁰	168	151.3
CD4V _H NEW-Thr ³⁰	40	81.5

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TABLE 1-continued

Mean cellular fluorescence of CD4 ⁺ cells stained with reshaped antibodies		
Reshaped Antibody	Concentration (µg/ml)	Mean cellular Fluorescence
CD4V _H NEW-Thr ³⁰	10	51.0
CD4V _H NEW-Thr ³⁰	2.5	39.3
CD4V _H NEW-Ser ³⁰	160	260.2
CD4V _H NEW-Ser ³⁰	40	123.5
CD4V _H NEW-Ser ³⁰	10	68.6
CD4V _H NEW-Ser ³⁰	2.5	49.2
CONTROL	—	35.8
<u>Experiment 3.</u>		
CD4V _H KOL-Pro ¹¹³	100	594.9
CD4V _H KOL-Pro ¹¹³	40	372.0
CD4V _H KOL-Pro ¹¹³	10	137.7
CD4V _H KOL-Pro ¹¹³	2.5	48.9
CD4V _H KOL-Thr ¹¹³	100	696.7
CD4V _H KOL-Thr ¹¹³	40	631.5
CD4V _H KOL-Thr ¹¹³	10	304.1
CD4V _H KOL-Thr ¹¹³	2.5	104.0
CONTROL	—	12.3

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 43

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 620 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (G) CELL TYPE: Hybridoma
 (H) CELL LINE: YNB46.1.8

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

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AAGCTTATGA ATATGCAAAT CCTCTGAATC TACATGGTAA ATATAGGTTT GTCTATACCA    60
CAAACAGAAA AACATGAGAT CACAGTTCTC TCTACAGTTA CTGAGCACAC AGGACCTCAC    120
CATGGGATGG AGCTGTATCA TCCTCTTCTT GGTAGCAACA GCTACAGGTA AGGGGTGCAC    180
AGTAGCAGGC TTGAGGTCTG GACATATATA TGGGTGACAA TGACATCCAC TTTGCCTTTC    240
TCTCCACAGG TGCCACTCC GACATCCAGC TGACCCAGTC TCCAGTTTCC CTGTCTGCAT    300
CTCTGGGAGA AACTGTCAAC ATCGAATGTC TAGCAAGTGA GGACATTTAC AGTGATTTAG    360
CATGGTATCA GCAGAAGCCA GGGAAATCTC CTCAACTCCT GATCTATAAT ACAGATACCT    420
TGCAAAATGG GGTCCCTTCA CGGTTTAGTG GCAGTGGATC TGGCACACAG TATTCTCTAA    480
AAATAACAG CCTGCAATCT GAAGATGTCG CGACTTATTT CTGTCAACAA TATAACAATT    540
ATCCGTGGAC GTTCGGTGA GGGACCAAGC TGGAGATCAA ACGTGAGTAG AATTTAAACT    600
TTGCTTCCTC AGTTGGATCC                                         620

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 748 base pairs

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(B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

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AAGCTTGGCT CTACAGTTAC TGAGCACACA GGACCTCACC ATGGGATGGA GCTGTATCAT      60
CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCGAC ATCCAGATGA CCCAGAGCCC      120
AAGCAGCCTG AGCGCCAGCG TGGGTGACAG AGTGACCATC ACCTGTAAAG CAAGTCAGAA      180
TATTGACAAA TACTTAAACT GGTACCAGCA GAAGCCAGGT AAGGCTCCAA AGCTGCTGAT      240
CTACAATACA AACAATTTGC AAACGGGTGT GCCAAGCAGA TTCAGCGGTA GCGGTAGCGG      300
TACCGACTTC ACCTTCACCA TCAGCAGCCT CCAGCCAGAG GACATCGCCA CCTACTACTG      360
CTTGACAGCAT ATAAGTAGGC CGCGCACGTT CGGCCAAGGG ACCAAGGTGG AAATCAAACG      420
AACTGTGGCT GCACCATCTG TCTTCATCTT CCCGCCATCT GATGAGCAGT TGAAATCTGG      480
AACTGCCTCT GTTGTGTGCC TGCTGAATAA CTTCTATCCC AGAGAGGCCA AAGTACAGTG      540
GAAGGTGGAT AACGCCCTCC AATCGGGTAA CTCCCAGGAG AGTGTACACAG AGCAGGACAG      600
CAAGGACAGC ACCTACAGCC TCAGCAGCAC CCTGACGCTG AGCAAAGCAG ACTACGAGAA      660
ACACAAAGTC TACGCCTGCG AAGTCACCCA TCAGGGCCTG AGCTCGCCCG TCACAAAGAG      720
CTTCAACAGG GGAGAGTGTT AGAAGCTT                                         748
  
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 748 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 41..742

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

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AAGCTTGGCT CTACAGTTAC TGAGCACACA GGACCTCACC ATG GGA TGG AGC TGT      55
                                         Met Gly Trp Ser Cys
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ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC GAC ATC      103
Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly Val His Ser Asp Ile
          10           15           20

CAG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT GAC AGA      151
Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
          25           30           35

GTG ACC ATC ACC TGT CTA GCA AGT GAG GAC ATT TAC AGT GAT TTA GCA      199
Val Thr Ile Thr Cys Leu Ala Ser Glu Asp Ile Tyr Ser Asp Leu Ala
          40           45           50

TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG CTG CTG ATC TAC AAT      247
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asn
          55           60           65

ACA GAT ACC TTG CAA AAT GGT GTG CCA AGC AGA TTC AGC GGT AGC GGT      295
Thr Asp Thr Leu Gln Asn Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
          70           75           80           85
  
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AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC CTC CAG CCA GAG GAC	343
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90 95 100	
ATC GCC ACC TAC TAC TGC CAA CAG TAT AAC AAT TAT CCG TGG ACG TTC	391
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GGC CAA GGG ACC AAG GTG GAA ATC AAA CGA ACT GTG GCT GCA CCA TCT	439
Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser	
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GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC	487
Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala	
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TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA	535
Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val	
150 155 160 165	
CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT	583
Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser	
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GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC	631
Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr	
185 190 195	
CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC	679
Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys	
200 205 210	
GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC	727
Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn	
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Arg Gly Glu Cys	
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(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 817 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

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CATGGGATGG AGCTGTATCA TCCTCTTCTT GGTAGCAACA GCTACAGGTA AGGGGCTCAC	180
AGTAGCAGGC TTGAGGTCTG GACATATATA TGGGTGACAA TGACATCCAC TTTGCCTTTC	240
TCTCCACAGG TGTCCACTCC CAGGTCCAAC TGCAGGAGTC TGGTGGAGGC TTAGTGCAGC	300
CTGGAAGGTC CCTGAAACTC TCCTGTGCAG CCTCTGGACT CACTTTCAGT AACTATGGCA	360
TGGCCTGGGT CCGCCAGGCT CCAACGAAGG GGCTGGAGTG GGTGCAACC ATTAGTCATG	420
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GCTCCAGAC TTCATGGCCA GAGATTTATA GGGATCC 817

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1467 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

AAGCTTTACA GTTACTGAGC ACACAGGACC TCACCATGGG ATGGAGCTGT ATCATCCTCT 60
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 GTCTTGTGAG ACCTAGCCAG ACCCTGAGCC TGACCTGCAC CGTGTCTGGC TTCACCTTCA 180
 CCGATTTCTA CATGAACTGG GTGAGACAGC CACCTGGACG AGGTCTTGAG TGGATTGGAT 240
 TTATTAGAGA CAAAGCTAAA GGTTACACAA CAGAGTACAA TCCATCTGTG AAGGGGAGAG 300
 TGACAATGCT GGTAGACACC AGCAAGAACC AGTTCAGCCT GAGACTCAGC AGCGTGACAG 360
 CCGCCGACAC CGCGGTCTAT TATTGTGCAA GAGAGGGCCA CACTGCTGCT CCTTTTGATT 420
 ACTGGGGTCA AGGCAGCCTC GTCACAGTCT CCTCAGCCTC CACCAAGGGC CCATCGGTCT 480
 TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG 540
 TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG 600
 GCGTGACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTA CTCCCTC AGCAGCGTGG 660
 TGACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC 720
 CCAGCAACAC CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT 780
 GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC TTCCCCCAA 840
 AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG 900
 TGAGCCACGA AGACCCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG GAGGTGCATA 960
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 TCACCGTCTT GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA 1080
 AAGCCCTCCC AGCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG CCCCAGAGAAC 1140
 CACAGGTGTA CACCCTGCCC CCATCCCGGG ATGAGCTGAC CAAGAACCAG GTCAGCCTGA 1200
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 TCTACAGCAA GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT 1380
 CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG 1440
 GTAAATGAGT GCGACGGCCC CAAGCTT 1467

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1458 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 36..1439

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

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Ile Leu Phe Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln	
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CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG ACC CTG AGC	149
Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln Thr Leu Ser	
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Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Asn Tyr Gly Met Ala	
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Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly Thr Ile	
55 60 65 70	
AGT CAT GAT GGT AGT GAC ACT TAC TTT CGA GAC TCT GTG AAG GGG AGA	293
Ser His Asp Gly Ser Asp Thr Tyr Phe Arg Asp Ser Val Lys Gly Arg	
75 80 85	
GTG ACA ATG CTG GTA GAC ACC AGC AAG AAC CAG TTC AGC CTG AGA CTC	341
Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg Leu	
90 95 100	
AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT TAT TGT GCA AGA CAA	389
Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gln	
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Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro	
135 140 145 150	
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Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu	
265 270 275	

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CCG	CGG	GAG	GAG	CAG	TAC	AAC	AGC	ACG	TAC	CGT	GTG	GTC	AGC	GTC	CTC	1013
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ACC	GTC	CTG	CAC	CAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG	1061
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	
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GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	CCC	ATC	GAG	AAA	ACC	ATC	TCC	AAA	1109
Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	
		345					350					355				
GCC	AAA	GGG	CAG	CCC	CGA	GAA	CCA	CAG	GTG	TAC	ACC	CTG	CCC	CCA	TCC	1157
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	
	360					365					370					
CGG	GAT	GAG	CTG	ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC	CTG	GTC	AAA	1205
Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	
	375				380					385					390	
GGC	TTC	TAT	CCC	AGC	GAC	ATC	GCC	GTG	GAG	TGG	GAG	AGC	AAT	GGG	CAG	1253
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	
				395					400					405		
CCG	GAG	AAC	AAC	TAC	AAG	ACC	ACG	CCT	CCC	GTG	CTG	GAC	TCC	GAC	GGC	1301
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	
			410					415					420			
TCC	TTC	TTC	CTC	TAC	AGC	AAG	CTC	ACC	GTG	GAC	AAG	AGC	AGG	TGG	CAG	1349
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	
		425					430					435				
CAG	GGG	AAC	GTC	TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG	GCT	CTG	CAC	AAC	1397
Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	
	440					445					450					
CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT	AAA	TGAGT	TGCGAC		1446
His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys				
				455		460				465						
GGCCCAAGC	TT															1458

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1458 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 36..1439

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

AAGCTTTACA	GTTACTGAGC	ACACAGGACC	TCACC	ATG	GGA	TGG	AGC	TGT	ATC							53
				Met	Gly	Trp	Ser	Cys	Ile							
				1					5							
ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC	TCC	CAG	GTC	CAA	101
Ile	Leu	Phe	Leu	Val	Ala	Thr	Ala	Thr	Gly	Val	His	Ser	Gln	Val	Gln	
			10					15					20			

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CTG	CAG	GAG	AGC	GGT	CCA	GGT	CTT	GTG	AGA	CCT	AGC	CAG	ACC	CTG	AGC	149
Leu	Gln	Glu	Ser	Gly	Pro	Gly	Leu	Val	Arg	Pro	Ser	Gln	Thr	Leu	Ser	
		25					30					35				
CTG	ACC	TGC	ACC	GTG	TCT	GGC	TTC	ACC	TTC	AGC	AAC	TAT	GGC	ATG	GCC	197
Leu	Thr	Cys	Thr	Val	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Tyr	Gly	Met	Ala	
	40					45					50					
TGG	GTG	AGA	CAG	CCA	CCT	GGA	CGA	GGT	CTT	GAG	TGG	ATT	GGA	ACC	ATT	245
Trp	Val	Arg	Gln	Pro	Pro	Gly	Arg	Gly	Leu	Glu	Trp	Ile	Gly	Thr	Ile	
	55				60					65					70	
AGT	CAT	GAT	GGT	AGT	GAC	ACT	TAC	TTT	CGA	GAC	TCT	GTG	AAG	GGG	AGA	293
Ser	His	Asp	Gly	Ser	Asp	Thr	Tyr	Phe	Arg	Asp	Ser	Val	Lys	Gly	Arg	
			75						80					85		
GTG	ACA	ATG	CTG	GTA	GAC	ACC	AGC	AAG	AAC	CAG	TTC	AGC	CTG	AGA	CTC	341
Val	Thr	Met	Leu	Val	Asp	Thr	Ser	Lys	Asn	Gln	Phe	Ser	Leu	Arg	Leu	
			90					95					100			
AGC	AGC	GTG	ACA	GCC	GCC	GAC	ACC	GCG	GTC	TAT	TAT	TGT	GCA	AGA	CAA	389
Ser	Ser	Val	Thr	Ala	Ala	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Gln	
		105					110					115				
GGC	ACT	ATA	GCT	GGT	ATA	CGT	CAC	TGG	GGT	CAA	GGC	AGC	CTC	GTC	ACA	437
Gly	Thr	Ile	Ala	Gly	Ile	Arg	His	Trp	Gly	Gln	Gly	Ser	Leu	Val	Thr	
	120					125					130					
GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG	GTC	TTC	CCC	CTG	GCA	CCC	485
Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	
	135				140					145					150	
TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG	GGC	TGC	CTG	GTC	533
Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	
			155					160					165			
AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	GGC	GCC	581
Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	
			170				175						180			
CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA	629
Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	
		185					190					195				
CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	677
Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	
	200					205					210					
ACC	CAG	ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	725
Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	
	215				220					225					230	
GTG	GAC	AAG	AAA	GTT	GAG	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	773
Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	
			235					240						245		
CCA	CCG	TGC	CCA	GCA	CCT	GAA	CTC	CTG	GGG	GGA	CCG	TCA	GTC	TTC	CTC	821
Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	
		250						255					260			
TTC	CCC	CCA	AAA	CCC	AAG	GAC	ACC	CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	869
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	
		265					270					275				
GTC	ACA	TGC	GTG	GTG	GTG	GAC	GTG	AGC	CAC	GAA	GAC	CCT	GAG	GTC	AAG	917
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	
	280					285					290					
TTC	AAC	TGG	TAC	GTG	GAC	GGC	GTG	GAG	GTG	CAT	AAT	GCC	AAG	ACA	AAG	965
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	
	295				300					305					310	
CCG	CGG	GAG	GAG	CAG	TAC	AAC	AGC	ACG	TAC	CGT	GTG	GTC	AGC	GTC	CTC	1013
Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	
			315						320					325		
ACC	GTC	CTG	CAC	CAG	GAC	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG	1061
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	
		330						335						340		

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GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA	1109
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys	
345 350 355	
GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC	1157
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser	
360 365 370	
CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA	1205
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys	
375 380 385 390	
GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG	1253
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln	
395 400 405	
CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC	1301
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly	
410 415 420	
TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG	1349
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln	
425 430 435	
CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC	1397
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn	
440 445 450	
CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGAGTGCGAC	1446
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	
455 460 465	
GGCCCCAAGC TT	1458

(2) INFORMATION FOR SEQ ID NO:8:

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

(ii) MOLECULE TYPE: protein

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

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg	
1 5 10 15	
Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe Ser Ser Tyr	
20 25 30	
Ala Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
35 40 45	
Ala Ile Ile Trp Asp Asp Gly Ser Asp Gln His Tyr Ala Asp Ser Val	
50 55 60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe	
65 70 75 80	
Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys	
85 90 95	
Ala Arg Asp Gly Gly His Gly Phe Cys Ser Ser Ala Ser Cys Phe Gly	
100 105 110	
Pro Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser	
115 120 125	

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 817 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

AAGCTTATGA ATATGCAAAT CCTCTGAATC TACATGGTAA ATATAGGTTT GTCTATACCA 60
 CAAACAGAAA AACATGAGAT CACAGTTCTC TCTACAGTTA CTCAGCACAC AGGACCTCAC 120
 CATGGGATGG AGCTGTATCA TCCTCTTCTT GGTAGCAACA GCTACAGGTA AGGGGCTCAC 180
 AGTAGCAGGC TTGAGGTCTG GACATATATA TGGGTGACAA TGACATCCAC TTTGCCTTTC 240
 TCTCCACAGG TGTCCACTCC CAGGTCCAAC TGGTGGAGTC TGGTGGAGGC GTGGTGCAGC 300
 CTGGAAGGTC CCTGAGACTC TCCTGTTTCT CCTCTGGATT CATCTTCAGT AACTATGGCA 360
 TGGCCTGGGT CCGCCAGGCT CCAGGCAAGG GGCTGGAGTG GGTCGCAACC ATTAGTCATG 420
 ATGGTAGTGA CACTTACTTT CGAGACTCCG TGAAGGGCCG ATTCACTATC TCCAGAGATA 480
 ATAGCAAAAA CACCCTATTC CTGCAAATGG ACAGTCTGAG GCCCGAGGAC ACGGGCGTGT 540
 ATTTCTGTGC AAGACAAGGG ACTATAGCAG GTATACGTCA CTGGGGCCAA GGGACCCCCG 600
 TCACCGTCTC CTCAGGTGAG TCCTTACAAC CTCTCTCTTC TATTCAGCTT AAATAGATTT 660
 TACTGCATTT GTTGGGGGGG AAATGTGTGT ATCTGAATTT CAGGTCATGA AGGACTAGGG 720
 ACACCTTGGG AGTCAGAAAG GGTCATTGGG AGCCCGGGCT GATGCAGACA GACATCCTCA 780
 GCTCCAGAC TTCATGGCCA GAGATTTATA GGGATCC 817

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 731 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

AAGCTTTACA GTTACTCAGC ACACAGGACC TCACCATGGG ATGGAGCTGT ATCATCCTCT 60
 TCTTGGTAGC AACAGCTACA GGTAAGGGGC TCACAGTAGC AGGCTTGAGG TCTGGACATA 120
 TATATGGGTG ACAATGACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC 180
 CAACTGGTGG AGTCTGGTGG AGGCGTGGTG CAGCCTGGAA GGTCCCTGAG ACTCTCCTGT 240
 TCCTCCTCTG GATTCATCTT CAGTAACTAT GGCATGGCCT GGGTCCGCCA GGCTCCAGGC 300
 AAGGGGCTGG AGTGGGTTCG AACCATTAGT CATGATGGTA GTGACTTA CTTTCGAGAC 360
 TCCGTGAAGG GCCGATTCAC TATCTCCAGA GATAATAGCA AAAACACCCT ATTCTGCAA 420
 ATGGACAGTC TGAGGCCCGA GGACACGGGC GTGTATTTCT GTGCAAGACA AGGGACTATA 480
 GCAGGTATAC GTCACTGGGG CCAAGGGACC CCCGTCACCG TCTCCTCAGG TGAGTCCTTA 540
 CAACCTCTCT CTTCTATTCA GCTTAAATAG ATTTTACTGC ATTTGTTGGG GGGGAAATGT 600
 GTGTATCTGA ATTTAGGTC ATGAAGGACT AGGGACACCT TGGGAGTCAG AAAGGGTCAT 660
 TGGGAGCCCG GGCTGATGCA GACAGACATC CTCAGCTCCC AGACTTCATG GCCAGAGATT 720
 TATAGGGATC C 731

(2) INFORMATION FOR SEQ ID NO:11:

-continued

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 817 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

```
AAGCTTATGA ATATGCAAAT CCTCTGAATC TACATGGTAA ATATAGGTTT GTCTATACCA    60
CAAACAGAAA AACATGAGAT CACAGTTCTC TCTACAGTTA CTCAGCACAC AGGACCTCAC    120
CATGGGATGG AGCTGTATCA TCCTCTTCTT GGTAGCAACA GCTACAGGTA AGGGGCTCAC    180
AGTAGCAGGC TTGAGGTCTG GACATATATA TGGGTGACAA TGACATCCAC TTTGCCTTTC    240
TCTCCACAGG TGTCCACTCC CAGGTCCAAC TGGTGGAGTC TGGTGGAGGC GTGGTGCAGC    300
CTGGAAGGTC CCTGAGACTC TCCTGTTTCT CCTCTGGATT CATCTTCAGT AACTATGGCA    360
TGGCCTGGGT CCGCCAGGCT CCAGGCAAGG GGCTGGAGTG GGTCGCAACC ATTAGTCATG    420
ATGGTAGTGA CACTTACTTT CGAGACTCCG TGAAGGGCCG ATTCACTATC TCCAGAGATA    480
ATAGCAAAAA CACCCTATTC CTGCAAATGG ACAGTCTGAG GCCCGAGGAC ACGGGCGTGT    540
ATTTCTGTGC AAGACAAGGG ACTATAGCAG GTATACGTCA CTGGGGCCAA GGGACCACGG    600
TCACCGTCTC CTCAGGTGAG TCCTTACAAC CTCTCTCTTC TATTCAGCTT AAATAGATTT    660
TACTGCATTT GTTGGGGGGG AAATGTGTGT ATCTGAATTT CAGGTCATGA AGGACTAGGG    720
ACACCTTGGG AGTCAGAAAG GGTCATTGGG AGCCCGGGCT GATGCAGACA GACATCCTCA    780
GCTCCCAGAC TTCATGGCCA GAGATTTATA GGGATCC                                817
```

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 731 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

```
AAGCTTTACA GTTACTCAGC ACACAGGACC TCACCATGGG ATGGAGCTGT ATCATCCTCT    60
TCTTGGTAGC AACAGCTACA GGTAAGGGGC TCACAGTAGC AGGCTTGAGG TCTGGACATA    120
TATATGGGTG ACAATGACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC    180
CAACTGGTGG AGTCTGGTGG AGGCGTGGTG CAGCCTGGAA GGTCCCTGAG ACTCTCCTGT    240
TCCTCCTCTG GATTCATCTT CAGTAACTAT GGCATGGCCT GGGTCCGCCA GGCTCCAGGC    300
AAGGGGCTGG AGTGGGTGCG AACCATTAGT CATGATGGTA GTGACACTTA CTTTCGAGAC    360
TCCGTGAAGG GCCGATTCAC TATCTCCAGA GATAATAGCA AAAACACCCT ATTCTGCAA    420
ATGGACAGTC TGAGGCCCGA GGACACGGGC GTGTATTTCT GTGCAAGACA AGGGACTATA    480
GCAGGTATAC GTCACTGGGG CCAAGGGACC ACGGTCACCG TCTCCTCAGG TGAGTCCTTA    540
CAACCTCTCT CTTCTATTCA GCTTAAATAG ATTTTACTGC ATTTGTTGGG GGGGAAATGT    600
GTGTATCTGA ATTCAGGTC ATGAAGGACT AGGGACACCT TGGGAGTCAG AAAGGGTCAT    660
TGGGAGCCCG GGCTGATGCA GACAGACATC CTCAGCTCCC AGACTTCATG GCCAGAGATT    720
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Thr Ile Ser His Asp Gly Ser Asp Thr Tyr Phe Arg Asp Ser Val Lys
 1 5 10 15

Gly

(2) INFORMATION FOR SEQ ID NO:18:

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

(ii) MOLECULE TYPE: peptide

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

Gln Gly Thr Ile Ala Gly Ile Arg His
 1 5

(2) INFORMATION FOR SEQ ID NO:19:

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

(ii) MOLECULE TYPE: cDNA

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

GTTAGATCTC CAGCTTGGTC CC

22

(2) INFORMATION FOR SEQ ID NO:20:

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

(ii) MOLECULE TYPE: cDNA

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

TGAGGAGACG GTGACCGTGG TCCCTTGGCC

30

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

GACATTCAGC TGACCCAGTC TCCA

24

(2) INFORMATION FOR SEQ ID NO:22:

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

(ii) MOLECULE TYPE: cDNA

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

-continued

AGGTSMARCT GCAGSAGTCW GG 22

(2) INFORMATION FOR SEQ ID NO:23:

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

(ii) MOLECULE TYPE: cDNA

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

GTTTCATAAT ATTGGAGACA 20

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 69 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

AGAGTGACCA TCACCTGTCT AGCAAGTGAG GACATTTACA GTGATTTAGC ATGGTACCAG 60

CAGAAGCCA 69

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 51 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

CTGCTGATCT ACAATACAGA TACCTTGCAA AATGGTGTGC CAAGCAGATT C 51

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATGCCACCT ACTACTGCCA ACAGTATAAC AATTATCCGT GGACGTTCCG CCAAGGGACC 60

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 51 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTGGCTTCA CCTTACCAA CTATGGCATG GCCTGGGTGA GACAGCCACC T 51

-continued

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 75 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGTCTTGAGT GGATTGGAAC CATTAGTCAT GATGGTAGTG ACACTTACTT TCGAGACTCT 60

GTGAAGGGGA GAGTG 75

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 63 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTCTATTATT GTGCAAGACA AGGCACTATA GCTGGTATAC GTCACTGGGG TCAAGGCAGC 60

CTC 63

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCTTCACCTT CAGCAACTAT GGCAT 25

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 50 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CACTCCCAGG TCCAAGTGGT GGAGTCTGGT GGAGGCGTGG TGCAGCCTGG 50

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 58 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AAGGTCCCTG AGACTCTCCT GTTCCTCCTC TGGATTCATC TTCAGTAACT ATGGCATG 58

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(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTCCGCCAGG CTCCAGGCAA GGGGCTGGAG TGG 33

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 46 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACTATCTCCA GAGATAATAG CAAAAACACC CTATTCCTGC AAATGG 46

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 52 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ACAGTCTGAG GCCCGAGGAC ACGGGCGTGT ATTTCTGTGC AAGACAAGGG AC 52

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TGGGGCCAAG GGACCCCGT CACCGTCTCC TCA 33

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AGCTTTACAG TTACTGAGCA CACAGGACCT CAC 33

(2) INFORMATION FOR SEQ ID NO:38:

-continued

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CATGGTGAGG TCCTGTGTGC TCAGTAACTG TAA

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(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 137 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15
 Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
 20 25 30
 Pro Gly Arg Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe
 35 40 45
 Ser Asn Tyr Gly Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 50 55 60
 Glu Trp Val Ala Thr Ile Ser His Asp Gly Ser Asp Thr Tyr Phe Arg
 65 70 75 80
 Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
 85 90 95
 Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val
 100 105 110
 Tyr Phe Cys Ala Arg Gln Gly Thr Ile Ala Gly Ile Arg His Trp Gly
 115 120 125
 Gln Gly Thr Pro Val Thr Val Ser Ser
 130 135

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 137 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15
 Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln
 20 25 30
 Pro Gly Arg Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe
 35 40 45
 Ser Asn Tyr Gly Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 50 55 60
 Glu Trp Val Ala Thr Ile Ser His Asp Gly Ser Asp Thr Tyr Phe Arg
 65 70 75 80
 Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn

-continued

	85		90		95
Thr	Leu Phe	Leu Gln Met Asp Ser	Leu Arg Pro Glu Asp	Thr Gly Val	
	100		105	110	
Tyr	Phe Cys	Ala Arg Gln Gly Thr	Ile Ala Gly Ile Arg His	Trp Gly	
	115		120	125	
Gln	Gly Thr	Thr Val Thr Val Ser Ser			
	130		135		

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 467 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met	Gly Trp	Ser Cys Ile Ile	Leu Phe Leu Val	Ala Thr Ala Thr	Gly
1		5	10		15
Val	His Ser	Gln Val Gln Leu Gln	Glu Ser Gly Pro Gly	Leu Val Arg	
	20		25	30	
Pro	Ser Gln	Thr Leu Ser Leu Thr	Cys Thr Val Ser Gly	Phe Thr Phe	
	35		40	45	
Thr	Asn Tyr	Gly Met Ala Trp Val	Arg Gln Pro Pro Gly	Arg Gly Leu	
	50		55	60	
Glu	Trp Ile	Gly Thr Ile Ser His	Asp Gly Ser Asp Thr	Tyr Phe Arg	
	65		70	75	80
Asp	Ser Val	Lys Gly Arg Val Thr	Met Leu Val Asp Thr	Ser Lys Asn	
		85	90	95	
Gln	Phe Ser	Leu Arg Leu Ser Ser	Val Thr Ala Ala Asp	Thr Ala Val	
	100		105	110	
Tyr	Tyr Cys	Ala Arg Gln Gly Thr	Ile Ala Gly Ile Arg	His Trp Gly	
	115		120	125	
Gln	Gly Ser	Leu Val Thr Val Ser	Ser Ala Ser Thr Lys	Gly Pro Ser	
	130		135	140	
Val	Phe Pro	Leu Ala Pro Ser Ser	Lys Ser Thr Ser Gly	Gly Thr Ala	
	145		150	155	160
Ala	Leu Gly	Cys Leu Val Lys Asp	Tyr Phe Pro Glu Pro	Val Thr Val	
		165	170	175	
Ser	Trp Asn	Ser Gly Ala Leu Thr	Ser Gly Val His Thr	Phe Pro Ala	
	180		185	190	
Val	Leu Gln	Ser Ser Gly Leu Tyr	Ser Leu Ser Ser Val	Val Thr Val	
	195		200	205	
Pro	Ser Ser	Ser Leu Gly Thr Gln	Thr Tyr Ile Cys Asn	Val Asn His	
	210		215	220	
Lys	Pro Ser	Asn Thr Lys Val Asp	Lys Lys Val Glu Pro	Lys Ser Cys	
	225		230	235	240
Asp	Lys Thr	His Thr Cys Pro Pro	Cys Pro Ala Pro Glu	Leu Leu Gly	
		245	250	255	
Gly	Pro Ser	Val Phe Leu Phe Pro	Pro Lys Pro Lys Asp	Thr Leu Met	
	260		265	270	
Ile	Ser Arg	Thr Pro Glu Val Thr	Cys Val Val Val Asp	Val Ser His	
	275		280	285	
Glu	Asp Pro	Glu Val Lys Phe Asn	Trp Tyr Val Asp Gly	Val Glu Val	
	290		295	300	

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His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 305 310 315 320
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 325 330 335
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
 340 345 350
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 355 360 365
 Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
 370 375 380
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 385 390 395 400
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 405 410 415
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 420 425 430
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 435 440 445
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 450 455 460
 Pro Gly Lys
 465

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 467 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15
 Val His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
 20 25 30
 Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe
 35 40 45
 Ser Asn Tyr Gly Met Ala Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 50 55 60
 Glu Trp Ile Gly Thr Ile Ser His Asp Gly Ser Asp Thr Tyr Phe Arg
 65 70 75 80
 Asp Ser Val Lys Gly Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn
 85 90 95
 Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
 100 105 110
 Tyr Tyr Cys Ala Arg Gln Gly Thr Ile Ala Gly Ile Arg His Trp Gly
 115 120 125
 Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
 130 135 140
 Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
 145 150 155 160
 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
 165 170 175

-continued

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 180 185 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
 195 200 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
 210 215 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
 225 230 235 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
 245 250 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 260 265 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
 275 280 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 290 295 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 305 310 315 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 325 330 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
 340 345 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 355 360 365

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
 370 375 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 405 410 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 420 425 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 450 455 460

Pro Gly Lys
 465

(2) INFORMATION FOR SEQ ID NO:43:

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

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

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

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

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

Tyr Ser Asp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys

-continued

50	55	60
Leu Leu Ile Tyr Asn Thr Asp Thr Leu Gln Asn Gly Val Pro Ser Arg 65	70	75 80
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser 85	90	95
Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Asn 100	105	110
Tyr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr 115	120	125
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu 130	135	140
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro 145	150	155 160
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly 165	170	175
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr 180	185	190
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His 195	200	205
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val 210	215	220
Thr Lys Ser Phe Asn Arg Gly Glu Cys 225	230	

What is claimed is:

[1. An antibody which is capable of binding to human CD4 antigen, in which the CDRs of the light chain of the antibody have the amino acid sequences:

CDR1: LASEDIYSDLA (SEQ ID NO:13)

CDR2: NTDTLQN (SEQ ID NO:14)

CDR3: QQYNNYPWT (SEQ ID NO:15)

in which the CDRs of the heavy chain of the antibody have the amino acid sequences:

CDR1: NYGMA (SEQ ID NO:16)

CDR2: TISHDGSPTYFRDSVKG (SEQ ID NO:17)

CDR3: QGTIAGIRH (SEQ ID NO:18), and

in which the framework of the variable domain of each chain and any constant domain present in said chain are derived from a mammalian non-rat species.]

[2. An antibody according to claim 1, in which the mammalian non-rat species is human.]

[3. An antibody according to claim 2, in which the variable domain framework region of the heavy chain consists essentially of the heavy chain variable domain framework region of the protein KOL.]

[4. An antibody according to claim 3, in which the heavy chain variable domain has the amino acid sequence shown in the upper line in FIG. 10 (SEQ ID NO:39) or 12 (SEQ ID NO:40).]

[5. An antibody according to claim 2, in which the variable domain framework region of the heavy chain consists essentially of the heavy chain variable domain framework region of the protein NEW.]

[6. An antibody according to claim 5, in which the heavy chain variable domain has the amino acid sequence shown in the upper line of FIG. 6 (SEQ ID NO:41) or 7 (SEQ ID NO:42).]

[7. An antibody according to claims 2, 3, 4, 5 or 6, in which the variable domain framework of the light chain consists essentially of the variable domain framework of the protein REL.]

[8. An antibody according to claim 7, in which the light chain has the amino acid sequence shown in the upper line of FIG. 3 (SEQ ID NO:43).]

[9. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an antibody as claimed in claim 1.]

[10. An antibody which is capable of binding to human CD4 antigen, in which the CDRs of the light chain of the antibody have the amino acid sequences:

CDR1: LASEDIYSDLA (SEQ ID NO:13)

CDR2: NTDTLQN (SEQ ID NO:14)

CDR3: QQYNNYPWT (SEQ ID NO:15), and

in which the CDRs of the heavy chain of the antibody have the amino acid sequences:

CDR1: NYGMA (SEQ ID NO:16)

CDR2: TISHDGSPTYFRDSVKG (SEQ ID NO:17)

CDR3: QGTIAGIRH (SEQ ID NO:18), and

in which the framework of the variable domain of each chain and the constant region of said chain are derived from a human.]

[11. An antibody according to claim 1, wherein the antibody has glycosylation characteristic of CHO cells.]

12. A humanized antibody, or an antigen-binding fragment of said humanized antibody, wherein the humanized antibody comprises (1) a light chain variable domain comprising a framework and three complementarity determining regions (CDRs) followed by constant regions and (2) a heavy chain variable domain comprising a framework and three CDRs followed by constant regions, wherein:

the amino acid sequence of the CDRs consists of the amino acid sequence of the CDRs of an antibody of a first species, wherein the first species is a non-human mammal;

the amino acid sequence of the light chain framework, of the heavy chain framework, or of both the light and the

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heavy chain frameworks consists of the amino acid sequence of a selected human antibody variable region, wherein the light chain framework consists of four framework regions and the heavy chain framework consists of four framework regions;

wherein the humanized antibody differs from the antibody of a first species at least in that, for the humanized antibody:

i.) the sequence of all four framework regions of the light chain is identical to the sequence of all four framework regions of a single selected human antibody variable region; or

ii.) the sequence of all four framework regions of the heavy chain is identical to the sequence of all four framework regions of a single selected human antibody variable region; or

iii.) both i.) and ii); and

wherein:

iv.) the selected human antibody variable region of i.) is selected from the human light chain antibody variable regions with the most overall homology to the light chain variable region of the antibody of the first species; or

v.) the selected human antibody variable region of ii.) is selected from the human heavy chain antibody variable regions with the most overall homology to the heavy chain variable region of the antibody of the first species; or

vi.) both iv.) and v.); and

vii.); wherein the most overall homology is determined on the basis of the respective variable domains, considering the overall homology, without making a distinction between homology within the framework regions and CDRs, and wherein only CDR3 of either the selected human heavy chain and/or selected

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human light chain variable region has a different length as that of the corresponding CDRs of the first species antibody;

wherein the humanized antibody is capable of binding the same human antigen as the antibody of the first species, wherein said antigen is not a CD antigen;

wherein at least one of the constant regions is from a human immunoglobulin different from the antibody or antibodies from which the human frameworks are selected; and

wherein the antibody of the first species is not an antibody that binds a CD antigen.

13. A humanized antibody according to claim 12, or an antigen-binding fragment of said humanized antibody, wherein the non-human mammal is rat.

14. A humanized antibody according to claim 12, or an antigen-binding fragment of said humanized antibody, wherein the non-human mammal is mouse.

15. A humanized antibody according to claim 12, or an antigen-binding fragment of said humanized antibody, wherein the humanized antibody or fragment thereof is a (Fab')₂ fragment.

16. A humanized antibody according to claim 12, or an antigen-binding fragment of said humanized antibody, wherein the humanized antibody or fragment thereof is a Fab fragment.

17. A humanized antibody according to claim 12, or an antigen-binding fragment of said humanized antibody, wherein the antibody has glycosylation characteristic of CHO cells.

18. A pharmaceutical composition comprising a humanized antibody according to claim 12, or an antigen-binding fragment of said humanized antibody, and a pharmaceutically acceptable carrier or diluent.

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