DOCKET NO.: CARP-0057 **PATENT**

APPENDIX A

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are so identified in Figure 4.
49. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy chain framework	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 41.

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APPENDIX B

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 18, lines 11-17, and lines 33-37, and Figs. 20-21 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are identified in Figure 21.
49. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

DOCKET NO.: CARP-0057 **PATENT**

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1},	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy chain framework	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 20.

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SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/743,329	09/17/91	ADAIR	J CARP-0009

EXAMINER
BENNETT, L

ART UNIT | PAPER NUMBER
1807 | 19

18N1/0907

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DATE MAILED: 09/07/93

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☑ This application has been examined ☑ Responsive to communication filed on April 9, 1993 ☑ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ___ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892, ~~2 pages~~
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☑ Notice of Art Cited by Applicant, PTO-1449, 4 pages
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☑ Claims 67-119 are pending in the application.
   Of the above, claims Ø are withdrawn from consideration.
2. ☑ Claims 1-66 have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☑ Claims 67-119 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☑ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable, ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner, ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved, ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received
    ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

PTOL-326 (Rev. 9-93)

Carter Exhibit 2028
Carter v. Adair
Interference No. 105,744

15. This Action is in response to the paper filed April 21, 1993. Claims 1-66 have been cancelled, and claims 67-119 have been newly added. All of Applicant's arguments have been thoroughly reviewed but are deemed non-persuasive for the reasons which follow. This Action is made FINAL.

The current status of the pending claims is as follows:

Claims 67-119 are rejected under 35 U.S.C. 112, first paragraph, for introducing new matter.

Claims 67-119 stand rejected under 35 U.S.C. 112, first paragraph scope.

Claims 67-117 stand rejected under 35 U.S.C. 103.

16. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> "The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention."

The specification is objected to under 35 U.S.C. 112, first paragraph, as the specification, as originally filed, does not provide support for the invention as is now claimed. Claims 67-119 have been amended to include the limitation that "in said composite heavy chain, amino acid residues 5,8,10,12-17,19,21,22,40,42-44,66,68,70,74,77,79,81,83-85,90,92, 105,109,111-113 at least are acceptor residues". However, nowhere in the specification is the invention described as containing these particular acceptor amino acid residues. Applicant points to the specification as teaching a number of residues which can be considered for changing from acceptor to donor residues and alleges that this teaching is support for the amendment on the grounds that "it follows that if a residue has not been considered for changing, that it must remain as in the acceptor chain". This

argument is not convincing because it does not necessarily follow that the unmentioned residues were originally contemplated as only being acceptor residues. That is, by not specifically describing whether particular residues are to acceptor or donor, can be interpreted to mean that the source of these residues was irrelevant, i.,e. they could be whether acceptor or donor residues. Therefore, this amendment introduces new matter into the specification which is not supported by the original specification.

Claims 71, 78, 85, 92, ,99,106,118 have been further amended to include a limitation which is not supported by the original specification. These claims have been amended to recite that the amino acid residues 2,4,6,38,48, 67 and 69 as being donor residues is supported by the passage on page 21, lines 13-16 of the specification. However, these pages teach that amino acid residues 2,4,6,38,46, 67 and 69 can be additionally changed to donor residues but does not teach that amino acid 48 is changed to a donor residue. Therefore, this amendment introduces new matter which is not supported by the original specification.

Claims 72, 79, 86, 93, 100,107 have also been further amended to include a limitation which is not supported by the original specification. These claims recite that amino acid residues 7,9,11,18,20,25,37,37,41, 45, 47,48,72,75,80,82,86-89,91,93,103,108,110 and 112 are additionally donor residues. However, the specification does not teach the concept that these particular amino acid residues are limited to being only acceptor amino acids. Applicant argues that this limitation was derived by taking all the donor residues mentioned in claims 67 to 71 and specifying that all other residues are acceptor residues. This rationale is not convincing because the original specification does not describe the invention as encompassing antibodies in which the amino acid residues which remain acceptor residues are specifically identified as these particular amino acid residues recited in claims 72, 79, 86, 93, 100,107. Therefore, this amendment introduces new matter into the specification which is not supported by the original specification.

Claims 108-113 have been further amended to specifically recite particular light chain amino acid residues which are limited to being only acceptor amino acids residues, i.e. residues 5,7-9,11, 13-18,20,22,23,39,41-43,57,59,61,72,74-79,81,82,84,86,88, 100,104,106 and 107. The specification does not teach that these particular positions in the disclosed antibodies are limited to being only acceptor residues. The specification does not discuss these amino acid position and therefore the original specification appears to teach that the source of these amino acids, i.e. from acceptor or donor, is not important to the invention. Therefore, this amendment introduces new matter which is not supported but the original specification.

Claims 118 and 119, drawn to a method for producing recombinant antigen binding molecule, are not supported by the original specification. Applicant points to now cancelled claims 66 and 67 submitted in the amendment filed February 9, 1993, has providing support for claims 118 and 199 respectively. However, the February 9, 1993 amendment does not appear to point to a passage in the originally filed specification which supports the particulars of the claimed method. The specification does not appear to disclose a method having steps in the specific order as claimed. The specification also does not describe the list of amino acid positions which are at least maintained as the acceptor amino acids as previously discussed. The specification appears to discuss amino acid positions which may be important in the structural and functional integrity of the humanized antibodies. The specification does not describe the particular order of making amino acid changes as is now claimed in the steps of claims 118 and 119. Specifically the specification does not appear to teach that the affinity of a generated humanized antibody is measured in order to determine if additional amino acid substitutions to the acceptor sequence are to be made. Therefore the amendment of these claims introduces new matter which is not supported by the original specification.

17. Claims 67-119 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

18. The objection to the disclosure because of the use of terms such as "humanised" and "humanisation" is withdrawn in light of Applicant's convincing arguments.

19. The objection of claims 5,11-16,22 and 23 made under 37 CFR 1.75(c) as being in improper form has been obviated by the cancellation of these claims.

20. The objection of claims 1-23 made over the recitation of "CDR-grafted" has been obviated by the cancellation of these claims.

21. The rejection of claims 1-12 made under 35 U.S.C. 101 because the claimed invention is inoperative and therefore lacks patentable utility has been obviated by the cancellation of these claims.

22. The rejection of claims 17 made under 35 U.S.C. 101 because the claimed invention is drawn to non-statutory subject matter has been obviated by the cancellation of claim 17.

23. The rejection of claims 22-23 made under 35 U.S.C. 101 because the invention was inoperative and therefore lacked patentable utility, has been obviated by the cancellation of these claims drawn to therapeutic compositions.

24. The objection to the specification and the rejection of claims 1-12 made under 35 U.S.C. 112, first paragraph, as failing to adequately teach how to use the isolated heavy and light chains antibodies fragments for the disclosed utility, has been obviated by the cancellation of claims 1-12.

25. The objection to the specification and the rejection of claims 22-23 made under 35 U.S.C. 112, first paragraph, as failing to adequately teach how to use the claimed compositions as therapeutic or diagnostic agents, has been obviated by the cancellation of claims 22-23.

26. The rejection of claims 13-16 made under 35 U.S.C. 112, first paragraph, as the disclosure is enabling only for claims limited to specific

CDR-grafted antibodies disclosed in the specification as having effective binding affinities for their specific antigen, has been obviated by the cancellation of claims 13-16. **However**, the rejection now applies to newly added claims 67-117. The claims are not commensurate in scope with the present disclosure. Insufficient guidance and working examples are provided in the specification to support the broad claims drawn to any CDR-grafted antibodies which contain donor residues at the recited framework amino acid positions for the heavy and light chains. The specification does not sufficiently develop the concept that there are certain framework amino acids which when changed in the acceptor sequence to be the same as in the donor sequence result in an increase in antigen binding affinity. The specification does describe several examples where particular framework amino acid changes result in increased antigen binding affinity, such as an for OKT-3, OKT-4, and anti-ICAM. However, the specification does not clearly establish that every time the recited amino acid positions are the same between the donor and the acceptor, "good" binding to antigen is observed. The specification does not provide actual biding values for most of the examples, but instead qualitatively describes the binding of the humanized antibody to antigen, Furthermore, in light of the prior art (for instance, Reichmann et al., Queen et al., and Chothia et al.) such a universal property appears to be unpredictable since different antibodies will have different amino acids in the framework which are important for antigen binding and stability. The prior art does not teach that a standardized principle of which amino acids must always be changed is possible, but instead appears to teach that three dimensional structures of the antibodies and an understanding of protein folding properties, is necessary to be able to reasonably predict which amino acids will always be effective in increasing or retaining antigen binding ability. Therefore, this analysis shows that undue experimentation would be required of the skilled artisan in order to practice the invention as claimed.

Applicant traverses the rejection on the following grounds. First, Applicant states that Queen et al. provides little guidance for making recombinant antibodies but acknowledges that Queen et al. does teach to first select a human chain which is as closely comparable to the murine chain

as possible, followed by computer modelling to determine which residues outside of the CDRs are important for antigen binding. Applicant states that Queen et al. does not provide guidance as to which residues are critical for improving affinity. Applicant argues that the teachings in the present application in contrast to the teachings of Queen et al. can be applied to any antibody. Applicant asserts that computer modelling is not necessary in the present method. Applicant argues that the specification refers to nine different antibodies which have been successfully humanized, and therefore Applicant that the skilled artisan would readily predict that the concept is applicable to other antibodies. Applicant points to Figures 7-13 as showing data and page 60 as teaching binding affinities of the humanized anti-ICAM.

Applicant's arguments have been thoroughly reviewed but are deemed non-persuasive for the following reasons. First, as amended, the claims are broadly drawn to all antibodies having the specified amino acid donor and acceptor amino acids. However, the specification does not teach an antibody which possesses all of the recited amino acids as claimed. The specification teaches antibodies which have been altered at some of these positions, but does not teach antibodies in general which retain binding affinity for antigen every the acceptor residues are changed or the same as the recited donor residues in the claims. Therefore, although the specification does describe nine different CDR grafted antibodies, the specification does not teach variants of these antibodies which have been additionally modified as recited in the claims. Since the specification does not teach a representative number of the antibodies which are encompassed by the broadly written claims, the specification does not appear to have established the generality of the recited amino acid positions being important for antigen binding and stability. Because no standard and reproducible rules are available for predicting protein folding, the ability to predict that all the recited amino acid positions will always produce functional antibodies regardless of antigen binding specificity and source of antibody acceptor and donor is not reliable. Therefore this rejection is maintained and made FINAL.

27. The rejection of claims 1-23 made under 35 U.S.C. 112, second paragraph, as being indefinite has been obviated by the cancellation of claims 1-23.

28. The rejection of claims 1,5,6-8,12-22 made under 35 U.S.C. 102(b) as being anticipated by Reichmann et al. has been obviated by the cancellation of these claims.

29. The rejection of claims 1-6 and 12-22 made under 35 U.S.C. 102(b) as being anticipated by Queen et al. has been obviated by the cancellation of these claims.

30. The rejection of claims 1-21 made under 35 U.S.C. 103 as being unpatentable over Reichmann et al. and Queen et al. has been obviated by the cancellation of claims 1-21. **However**, this rejection now applies to newly added claims 67-117.

Both Reichmann et al. and Queen et al. teach how to make humanized antibodies using a human antibody variable domain framework as an acceptor and a rat antibody (in the case of Reichmann et al.) or a murine antibody (in the case of Queen et al.) as the complementarity determining region donor. Both of these references also teach how to identify framework amino acids which are important for retaining the binding effective conformation of the CDRs. Specifically, Queen et al. teach that the more homologous the human antibody is to the murine antibody reduces the likelihood of producing distortions in the CDRs. Furthermore, Queen et al. teach making a database comparison of all known human antibodies with the donor antibody to determine the most similar human antibody to use as the framework (page 10031, col. 2, paragraph 2). Queen et al. also teach making a molecular model of the donor variable domain (in this case the anti-Tac V domain) based upon homology to other antibody V domains whose crystal structure is known. By doing so, Queen et al. teach that amino acids outside of the CDRs which are close enough to the CDRs to influence the CDR

conformation or to directly interact with the antigen. When the residues were different between the human and the donor murine antibodies, the human framework amino acid was changed to the corresponding murine amino acid (page 10031, col. 2 paragraph 3). Finally, when the human acceptor antibody contains unusual amino acids with respect to consensus sequences in homologous antibodies, Queen et al. recommends changing these amino acids to the consensus amino acid (page 10032, col. 1) Reichmann et al. and Queen et al. further teach that different changes will be necessary depending on the specific donor and acceptor antibodies which are used. Both references each the cDNA encoding the heavy and light antibody chains which are the templates for making the specific changes in the sequences of CDR-grafted antibodies. The references also both teach the insertion of the cDNAs into vectors, transfection of host cells and co-expression of the heavy and light chains to result in the expression of a complete CDR-grafted antibody molecule.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the guidelines taught by Reichmann et al. and Queen et al. to reshape any given antibody to "humanize" that antibody by making the changes in the framework regions of the human acceptor sequence to the donor residue when those residues are close to the CDR's and when the amino acids would be expected to affect the conformation of the CDRs. One of ordinary skill would have been motivated to make the changes in the framework regions from the human amino acid to the donor amino acid in order to achieve the expected benefit of increasing the binding affinity of the humanized antibody for the specific antigen over the binding affinity observed in the humanized antibodies which do not contain the framework changes as taught by Queen et al. (page 10032, col. 1, para. 3 through col. 2) and Reichmann et al. (Figure 4).

Applicants traverse the rejection on the following grounds. First, applicants argue that Reichmann et al. does not go beyond the original idea of Winter et al. WO-A 89/07452 which teaches transferring only the CDRs to a human framework. Applicant further argues that Reichmann et al. only changed residues 27 and 30 because the at donor sequence was found to be

unusual. Also, Applicant points out that Reichmann et al. did not make any framework residue changes to the light chain of the antibody outside of the CDRs. Applicant argues that Reichmann et al. do not teach that these changes are generally applicable to other antibodies. Also, Applicant states that Reichmann et al. do not suggest that altering residues remote from the CDRs might be effective in improving affinity nor that there might by a hierarchy of residues which should be considered.

Second Applicant argues that Queen et al. teach the amino acid sequence of the donor antibody chain should be determined and then compared to that of known acceptor chains and an acceptor chain chosen which is as homologous as possible to the donor chain. Applicant further states that the next step in Queen et al. is to carry out a computer modelling exercise to determine the residues which may be involved in antigen binding. Applicant alleges that this step may not always lead to the same results. Applicant also alleges that the fact that the donor sequence is compared to a number of possible acceptor sequences and that a computer model of the donor must be made, shows that the procedure is specific to one antibody at a time. Applicant asserts that Queen et al. does not suggest that the changes taught for reshaping the anti-TAC antibody could be expected to be the same necessary in another recombinant antibody. Applicant also states that Queen et al. doe snot teach an antibody containing all the donor residues recited in the claims.

Applicant's arguments have been thoroughly reviewed but are deemed non-persuasive for the following reasons. First, the claims have not been rejected as obvious over Reichmann et al. alone nor over Queen et al. alone. Instead, the claims have been rejected over the combined teachings of both Reichmann et al. and Queen et al. Consequently, Applicant's arguments do not address the rejection made. Second, Applicant's arguments are directed to a procedure of making recombinant antibodies but claims 67-117 are drawn to recombinant antibodies not to a method of making those antibodies. Therefore, when the prior art teaches an antibody which is encompassed by the broadly written claims which is made by a different method than the procedure disclosed in the specification, the prior

art still reads on the claims. Therefore, while Reichmann et al. and Queen et al. do not specifically teach that certain non-CDR framework amino acids must always be either acceptor or donor residues, these references do teach that best antigen binding affinities would be expected when the overall sequence the donor is most similar to the acceptor and that amino acids which come into contact with the CDRs should be donor residues. How these residues are identified is irrelevant when the claims are drawn to the antibodies themselves. Furthermore, the claims as written are not limited to antibodies in which the donor is non-human or that all the "donor residues are from the same donor. Many of the specific residues recited in the claims as being donor residues, are identical in the acceptor and the donor. Consequently, Th references teach many of the specific amino acid limitations without teaching that these amino acids need to be changed. Therefore, for all of these reasons, this rejection is maintained and made FINAL.

31. No claims are allowable.

32. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a). The practice of automatically extending the shortened statutory period an additional month upon the filing of a timely first response to a final rejection has been discontinued by the Office. See 1021 TMOG 35.

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE

PURSUANT TO 37 CFR 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

33. Papers relating to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the P.T.O. Fax Center located in Crystal Mall 1. The CM1 Fax Center number is (703) 308-2730. Papers may be submitted Monday-Friday between 8:00 am and 4:45 pm (EST). Please note that the faxing of such papers must conform with the Notice to Comply in the Official Gazette, 1096 OG 30 (November 15, 1989).

34. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lisa Bennett Arthur (nee Lisa T. Bennett) whose telephone number is (703) 308-3988. Any inquiry of a general nature or relating to the status of an application should be directed to the Group 180 receptionist whose telephone number is (703) 308-0196.

LBA
Lisa Bennett Arthur
September 2, 1993

M. Parr
MARGARET PARR
SUPERVISORY PATENT EXAMINER
GROUP 1800

DOCKET NO.: CARP-0046 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **John R. Adair, Diljeet S. Athwal and John S. Emtage**

Serial No.: **08/485,686** Group No.: **1642**

Filed: **June 7, 1995** Examiner: **J. Burke Reeves**

For: **Humanized Antibodies**

I, Doreen Yatko Trujillo, Registration No.35,719 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

On August 29, 2000

Doreen Yatko Trujillo, Reg. No. 35,719

#23H 09/22/00

Assistant Commissioner for Patents
Washington, D.C. 20231.

Dear Sir,

REQUEST FOR RECONSIDERATION

This responds to the Office Action dated February 29, 2000. A petition for a three-month extension of time and the appropriate fee accompanies this response.

Claims 56-73 were pending. All pending claims were rejected in the Office Action. In view of the arguments and amendments that follow, Applicants respectfully request withdrawal of all rejections upon reconsideration.

In the specification:

Please amend the specification as follows:

Page 1, line 1, after "9/07/94", replace "copending" with -- issued as

U.S. 5,859,205 --.

Page 23, line 20, please delete "Figure 1 shows" and insert -- Figures 1 a and b show --.

At page 23, line 21, insert --(SEQ ID NO: 4 and 5)--between "chain" and ";".

Page 23, line 22, please delete "Figure 2 shows" and insert -- Figures 2 a and b show --.

At page 23, line 23, insert --(SEQ ID NO: 6 and 7)--between "chain" and ";".

At page 23, line 26, insert --(SEQ ID NO: 5, 8, and 9)--between "REI" and ";".

At page 23, line 29, insert --(SEQ ID NO: 7 and 10) – between "KOL" and ";".

At page 23, line 30, please delete "Figure 5 shows" and insert -- Figures 5 a - c show --.

At page 23, line 32, insert --(SEQ ID NO: 7 and 11-24) – between "grafts" and ";".

At page 23, line 35, insert --(SEQ ID NO: 5, 8, 9, and 25-28) -- between "grafts" and ";".

At page 24, line 6, please delete "Figure 10 shows" and insert -- Figures 10 a and b show --.

At page 24, line 8, please delete "Figure 11 shows" and insert -- Figures 11 a and b show --.

At page 30, line 31, insert --(SEQ ID NO: 1) -- between "TCCAGATGTTAACTGCTCAC" and "for".

At page 30, line 33, insert --(SEQ ID NO: 2) -- after "CAGGGGCCAGTGGATGGATAGAC".

At page 33, line 26, insert --(SEQ ID NO: 3) -- after

"Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala".

At page 40, line 14, after "5", please insert -- a - c --.

At page 41, between lines 29 and 30, insert -- (SEQ ID NO: 8-28) -- .

At page 50, line 24, please insert – (SEQ ID NO: 7, 10, and 11-24) –.

At page 50, line 36, please insert – (SEQ ID NO: 5, 8, 9, and 25-28) –.

At page 51, line 13, after "10", please insert -- a and b --.

At page 51, line 15, after "11", please insert -- a and b --.

In the claims:

Please amend the claims as follows:

56. (Twice Amended) An antibody molecule having affinity for [a predetermined] an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions ([CDRS] CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody having binding affinity for said [predetermined] antigen, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 31 to 35, 50 to 65 and 95 to 102 in [(] the [CDRS] CDRs [)] and at least residues 23, 24, 49, 71, and 73 [(] in the framework regions [)] correspond to the equivalent residues in said donor antibody.

62. (Twice Amended) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including [CDRS] CDRs, said variable

domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, in said composite light chain at least residues 24 to 34, 50 to 56 and 89 to 97 in [(] the CDRs [)] and at least residues 46, 48, 58 and 71 [(] in the framework regions [)] correspond to the equivalent residues in said donor antibody.

63. The antibody molecule of claim 62, wherein additionally at least one of residues 2, 4, 6, 35, 38, 44, 47, 49, 62, [64 to 69] 64, 65, 66, 67, 68, 69, 85, 87, 98, 99, 101 and 102 in said composite light chain corresponds to the equivalent residue in said donor antibody.

In claim 64, line 1, please delete "is specific" and insert – has specificity –.

In claim 65, line 1, please delete "is specific" and insert – has specificity –.

In claim 66, line 1, please delete "is specific" and insert – has specificity –.

In claim 67, line 1, please delete "is specific" and insert – has specificity --.

In claim 68, line 2, please delete "is specific" and insert – has specificity –.

In claim 69, line 1, please delete "is specific" and insert – has specificity –.

In claim 70, line 1, please delete "is specific" and insert – has specificity –.

In claim 71, line 1, please delete "is specific" and insert – has specificity –.

In claim 72, line 1, please delete "is specific" and insert – has specificity –.

In claim 73, line 1, delete "therapeutic"; and

replace "an antibody" with -- the antibody molecule --.

Remarks

Preliminarily, Applicants note with appreciation the Examiner's observation that the claims are free of the prior art.

The specification has been objected to because, *inter alia*, the first line of the specification needs to be updated to reflect the status of any parent applications, and to reflect the parent international application. Applicants direct the Examiner to the transmittal letter of the present application in which the latter amendment was effected; the former amendment has been effected herein.

The Brief Description of the Drawings was objected to as not conforming with the labelling of the figures. The specification has been amended herein to place the Brief Description of the Drawings in conformity with the figures. No new matter was added thereby.

The specification was objected to as not complying with the Sequence Rules and Regulations. Specifically, the Examiner suggested that the specification be checked for missing sequence identifiers. The specification has been amended herein to add sequence identifiers. No new matter is added thereby.

The specification was further objected to for missing text on pages 53 and 62. A substitute specification with the complete information is enclosed. Because the missing text was simply a copying error, Applicants have not submitted a marked-up copy showing the addition. If the Examiner so requires, one will be forwarded upon request.

I. Rejections under 35 USC § 112, Second Paragraph

Claims 56-73 have been rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite in the recitation of "CDRS." Claims 56 and 62 have been amended to replace "CDRS" with "CDRs."

Claims 56-73 have been rejected as allegedly indefinite in reciting parentheses around the phrases "in the framework regions" and "the CDRs." The parentheses have been removed from claims 56 and 62 and an appropriate preposition added.

Claims 56-73 have been rejected as allegedly indefinite for reciting "predetermined antigen." Claim 56 has been amended to remove "predetermined." Applicants respectfully submit that the claim as amended covers predetermined antigens.

Claims 56-73 have been rejected as allegedly indefinite for reciting "an antibody molecule having affinity." Claim 56 has been amended to recite "binding affinity." Support for this amendment can be found, *inter alia*, on page 6, lines 21-22, of the application as filed.

Claims 56-73 have been rejected as allegedly indefinite for reciting "the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody." In claim 56 and 62, "using the Kabat numbering system" has been inserted after "equivalent residues." Support for this amendment can be found, *inter alia*, on page 8, lines 24-26, of the application as filed.

Claim 58 has been rejected as allegedly indefinite for the inclusion of a comma after "corresponds." In claim 58, the comma after "corresponds" as been deleted.

Claim 63 has been rejected as allegedly indefinite for reciting "at least one of residues 2, 4. . . 64 to 69. . ." Claim 63 has been amended to recite the residues individually.

Claims 64-72 have be rejected as allegedly indefinite for reciting "which is specific for." The Examiner alleged that is unclear whether the antibody molecule binds to the specific antigen or is otherwise "specific." Applicants respectfully disagree. Nonetheless, claims 64-72 have been amended

to recite "which has specificity for" in replace of "which is specific for." Support for this amendment can be found, *inter alia*, in the paragraph bridging pages 15-16 of the application as originally filed. It is clear from the discussion therein that the reference to "specificity" means that the antibody molecule binds the particular antigen.

Claim 73 has been rejected as allegedly indefinite for recitation of "a therapeutic composition." The Examiner suggests that deleting the term "therapeutic" would obviate the rejection. Accordingly, the term "therapeutic" has been deleted from claim 73. Compositions with therapeutic applications are included in the scope of claim 73.

Claim 73 has been rejected as allegedly indefinite in the recitation of "an antibody." Claim 73 has been amended to recite "the antibody molecule." Support for this amendment can be found, *inter alia*, in claim 13 as originally filed. (This rejection was apparently levied twice – see sub-paragraphs j and n.)

Claims 56-73 have been rejected as allegedly indefinite in the recitation of "said variable domain comprising predominantly human acceptor..." Applicants respectfully disagree and note that this term is present in the claims of issued U.S. Patent No. 5,859,205, the parent of the present application. The term is used to distinguish the claims from chimeric antibodies in which the entire variable domain is from the donor antibody. Clearly, since the claims recite that the variable domain comprises predominantly human acceptor framework residues, the Examiner's query whether only framework residues are counted is correct. Further, the Applicants respectfully submit that it is clear to one skilled in the art that, if the donor and acceptor residues are identical for a particular position, they are counted

as acceptor. Applicants respectfully submit this term is definite and request that this rejection should be withdrawn.

Claims 56-73 have been rejected as allegedly indefinite in the recitation of "the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen." The Examiner alleges that the CDRs appear to be outside the scope of the phrase "remaining heavy chain residues." This allegation is based upon a clear misreading of the claims. Clearly, a correct reading of the claims reveals that the CDRs correspond to the equivalent residues in the donor antibody. See, for example, claim 56.

Claims 58-61 and 63 have been rejected as allegedly indefinite. The Examiner improper alleges that it is unclear whether the claims intend to recite the residues in the alternative or in Markush grouping. Both means of claiming, however, accomplish the same end -- alternative claiming. The Examiner is directed to MPEP 2173.05(h). Applicants respectfully request that this rejection be withdrawn.

In view of the foregoing, Applicants respectfully request that the rejections under 35 U.S.C. § 112, second paragraph be withdrawn.

II. Rejections under 35 USC § 112, First Paragraph

Claims 56-73 have been rejected under 35, U.S.C. §112, first paragraph, as containing subject matter which allegedly was not described in the specification in such a way as to allow one skilled in the art to make and/or use the invention. The Examiner alleges that, in particular, the specification is lacking in guidance in choosing the donor-acceptor antibody pair (Office Action pp. 8-9). The Examiner

indicates that it could not have been expected that antibody molecules of the present invention would be functional because a number of criteria, such as homology between the donor and acceptor antibodies and the identity of packing residues near the CDRs, do not form part of the claims.

Applicants respectfully submit that the Examiner misapprehends Applicants' invention and, indeed, is inappropriately reading disclosure from the specification into the claims. Applicants' invention is based upon the identity of a hierarchy of residues that are of universal import without the need to compare antibodies or identify packing residues. The invention enables one skilled in the art to make an antibody molecule having a composite heavy chain wherein the heavy chain CDRs are from a donor antibody and at least framework residues 23, 24, 49, 71 and 73 are from the same donor antibody. Based on this information, one skilled on the art can obtain functional antibody molecules.

In order to demonstrate that antibody molecules having the claimed features are functional, Applicants enclose the Declaration of Geoffrey T. Yarranton, which was forwarded in the parent application, 08/303,569, on September 18, 1995. Dr. Yarranton's Declaration contains three tables relating to a number of antibodies having the claimed features, i.e. wherein at least residues 31 to 35, 50 to 65, 95 to 102, 23, 24, 49, 71 and 73 of the heavy chain variable domain correspond to residues from a donor antibody. The first table relates to the heavy chain and the second table relates to the light chain. The third table sets out the degree of affinity recovered as a percentage of the affinity of the donor antibody. A comparison with antibodies which have been produced by other methods is also provided. B1.8, D1.3, CAMPATH, and anti-TAC are such antibodies. As is evident therefrom, the subject matter of the present application enables one skilled in the art to obtain functional antibody molecules.

The Examiner further alleges that the claims recite CDR residues 31-35, 50-65, and 95-102 as numbered by the Kabat system, while the specification teaches different boundaries of the CDRs (Office Action p. 9). Furthermore, the Examiner points to constructs 121-141 of the specification to show that resides 26-35 are required for binding activity of the hybrid antibody (Office Action, p. 10). The Examiner also indicates that the range of the CDRs needs to be determined by structural analysis. Applicants respectfully disagree. The specification is clearly in agreement with the claims. See, for example, the disclosure bridging pages 19-20 of the application as filed, under the heading "The extent of the CDRs." As is clear therefrom, the CDRs are as defined by Kabat; the structural loops corresponded to the CDRs and, indeed, are completely encompassed within the CDRs, with the exception of CDR1 of the heavy chain. In the case of CDR1 of the heavy chain, the structural loop corresponds to residues 26-32; residues 26-30, thus, are part of the structural loop not contained within the Kabat CDR. Regarding the constructs referred to in Table 1, Applicants note that the table reports the changes made and remind the Examiner that no change is necessary if the donor and acceptor residues are the same at a particular position.

The Examiner alleges that while the claims recite the limitation of heavy chain residues 23, 24, 49, 71 and 73, other required residues are taught by the specification. In particular, the Examiner alleges that the specification specifically teaches that residues 71, 73 and 78 will always be all donor or all acceptor (Office Action, p. 10). This allegation is not correct. The specification clearly indicates that residue 78 is optional (see specification page 6, line 34: "residues at at least one of positions . . ."). Further, it is stated that

> "The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

(Specification page 7, lines 3-5, emphasis added.). Page 17, section 2.1 of the specification is cited by the Examiner. This section is part of a protocol. On page 16, fifth paragraph, it is stated that:

> "This protocol and rationale are give without prejudice to the generality of the invention as hereinbefore described...." (Emphasis added)

The present application clearly indicates that it is merely preferred that residues 71, 73 and 78 are either all donor or all acceptor residues, not that it is required. Claim 56 therefore does not have to specify that residue 78 is a donor residue. Furthermore, in Example 5 of the application, antibody molecules 61E71 and hTNF1 are described. Neither of these antibodies have a donor residue at position 78. This can clearly be seen from the enclosed tables. Furthermore, both antibodies were found to have 100% of the potency of the parent antibody.

The Examiner also alleges that the specification teaches that residue 6 is necessary to retain binding functions. The previous argument also applies to residue 6, which is referred to as being important to binding affinity in the protocol given in the specification. As indicated above, the protocol is without prejudice to the generality of the invention. Again, in Example 5 of the application, antibody molecules 61E71 and hTNF1 are described. Neither of these antibodies have a donor residue at position 6. This can clearly be seen from the enclosed tables. Furthermore, both antibodies were found to have 100% of the potency of the parent antibody.

The Examiner alleges that the unpredictability in the art is high and undue experimentation would be required to make the invention. Rudikoff et al., Panka et al., and Amit et al. are cited as

examples in which one amino acid change in a CDR or framework region dramatically affected antigen binding (Office Action, p 11). None of these documents suggest that the antibody molecules as defined in the present claims do not function to bind antigen.

Rudikoff et al. describes amino acid changes to CDRs. The finding that by changing the sequence of a CDR, which is known to determine the binding affinity of the antibody, actually results in a decreased binding affinity is not relevant to the presently claimed subject matter. The presently claimed subject matter recites that the residues in the CDRs (defined by Kabat numbering) entirely correspond to residues in the CDRs of the donor antibody. There are no alterations in the sequence of the CDRs of the donor antibody molecule and therefore Rudikoff et al. is irrelevant.

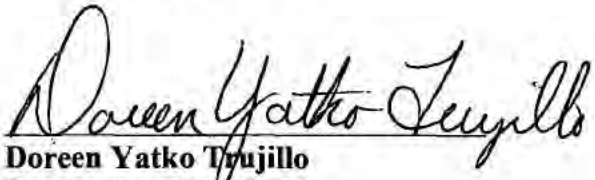
Panka et al. describes a single amino acid substitution at position 94. Although this amino acid substitution alters the binding affinity of the antibody, the antibody still binds the antigens digoxin and digitoxin (see Abstract). Panka et al. thus reports that amino acid changes made in the framework region can alter the binding affinity of antibodies. The same is clearly taught in the present application. See, for example, pages 20 to 21 wherein non-CDR, i.e. framework, residues which contribute to antigen binding are discussed. In particular, on page 21, lines 10-12, it is dislcosed that residue 94 should be changed if it is not arginine. The subject matter of the present application enables one skilled in the art to produce antibody molecules having affinity for a predetermined antigen. The fact that the antibody molecules may not have the optimum binding affinity for the antigen is not relevant to the claimed subject matter.

Amit et al. is said to indicate that at least one amino acid in the framework region of an antibody is involved in antigen binding. We assume that the Examiner is referring to residue 30 in the heavy chain of the antibody. All that is said concerning this residue is that it contacts the antigen. There is no indication that this residue is required for affinity binding of an antigen. There is no disclosure of substituting residue 30 for another amino acid and therefore no evidence to suggest that the binding affinity will change. The disclosure of Amit et al. is therefore not relevant to the presently claimed subject matter.

In view of the foregoing, Applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph be withdrawn.

For the foregoing reasons, Applicants submit that the present claims meet all the requirements for patentability. The Examiner is respectfully requested to allow all the present claims. If the Examiner is of a contrary view, it is requested that she contact the undersigned at (215) 557-5948.

Respectfully submitted,



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Date: 29 August 2000

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00035

DATE: September 14, 2000

Please deliver this and the following pages to:

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Burke**

For: **Humanised Antibodies**

FACSIMILE

CERTIFICATE OF FACSIMILE TRANSMISSION

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being transmitted by facsimile to the U.S. Patent and Trademark Office, Washington, D.C 20231, ATTENTION: Examiner Julie E Burke, née Reeves, Ph.D., Group/Art Unit No. 1642, Facsimile Number (703) 305-7401, on the date shown below.

On September 14, 2000

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Reg. No. 35,719

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

SUPPLEMENTAL AMENDMENT AND REQUEST FOR RECONSIDERATION

Pursuant to 37 C.F.R. § 1.111, please amend the above-identified application as a follows.

In the claims:

24. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the

variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

28. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

REMARKS

This paper is being filed to supplement the amendment referred to in the Request for Continued Examination filed June 1, 2000 ("the RCE"). No extension of time is believed to be necessary. To the extent this belief is in error, Applicants hereby request the necessary extension and the undersigned authorizes charging any such fee to Deposit Account 23-3050.

The amendment referred to in the RCE referenced certain claim amendments that had, inadvertently, not been included in the amendment. Specifically, the foregoing amendments to claims 24 and 28 were referenced but not effected in the amendment referred to in the RCE. Applicants respectfully request entry of the foregoing amendments and respectfully submit that, upon entry of these amendments, Applicants will have allowable subject matter.

In view of the foregoing, Applicants respectfully request that the Examiner declare an interference between the present application and the Queen patent. The Examiner is

DOCKET NO.: CARP-0057 **PATENT**

requested to contact the undersigned at (215) 564-8352 if she feels a telephonic discussion will be helpful.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. 35,719

Date: September 14, 2000

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DOCKET NO.: CARR0046 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Adair et al.

Serial No.: **08/485,686**

Group Art Unit: **1642**

Filed: **June 7, 1995**

Examiner: **M. Davis**

For: **Humanised Antibodies**

#30
KD
2-6-02

I, Paul K. Legaard, Registration No. 38,534 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On November 12, 2001

Paul K. Legaard

Paul K. Legaard Reg. No. 38,534

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

In response to the Office Action mailed August 10, 2001 in connection with the above-identified patent application, Applicants respectfully request that the application be amended as follows.

In the Application:

Please delete pages 67-89 of the application as filed containing the Sequence Listing and insert substitute pages 1-22 enclosed herewith, which contain the amended Sequence Listing formatted under the new rules for the Sequence Listing. In addition, please renumber the remaining pages of the application, containing the claims and Abstract, accordingly.

Carter Exhibit 2033
Carter v. Adair
Interference No. 105,744

In the Claims:

Please amend claims 56, 58 and 62 to read as follows:

56. (Amended four times) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, wherein said framework regions of said variable domain comprise predominantly human acceptor antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and at least residues 23, 24, 49, 71, and 73 in the framework regions correspond to the equivalent residues in said donor antibody.

58. (Amended three times) The antibody molecule of claim 56, wherein additionally at least one of the residues selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

62. (Amended four times) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework regions, wherein said framework regions of said light chain variable domain comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, in said composite light chain at least residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and at least residues 46, 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

REMARKS

Claims 56-73 are pending in the present application. Claims 56, 58 and 62 have been amended herein. No new matter has been added. Upon entry of the present amendment, claims 56-73 will remain pending.

As a preliminary matter, claim 58 has been amended as suggested in the Office Action to delete the comma inside brackets.

Applicants acknowledge receipt of the "Attachment for PTO-948" outlining changes for prosecution of applications containing drawings. In addition, Applicants enclose herewith a Drawing Change Authorization Request in which changes to Figures 5c and 6 are proposed. In particular, the changes in regard to Figure 5c are directed to reciting the correct sequence identifier. No new matter is added. In addition, the changes to Figure 6 are directed to replacing the "RW" amino acids with "LL" amino acids, support for which can be found, for example, in Table 2 at page 50 of the specification where positions 46 and 47 are both indicated to be "L" amino acids. Thus, no new matter is being added. The drawings have also been amended to incorporate sequence identifiers. Formal drawings have been filed on date even herewith under separate cover to the Draftsperson, including formal drawings of Figures 5c and 6, in order to be completely responsive to the Office Action.

Applicants have amended the Sequence Listing to correct the typographical error in SEQ ID NO:27 set forth above (e.g., replacement of "RW" with "LL"). New pages are provided to comply with the Sequence Rules set forth in 37 CFR §§ 1.821-1.825. In addition, enclosed herewith is a Statement to Support Filing and Submission of DNA/Amino Acid Sequences in Accordance with 37 CFR §§ 1.821-1.825, and a computer readable form (CRF). No new matter has been added. In addition, the contents of the paper copy of the Sequence Listing and computer readable copy of the Sequence Listing, submitted in accordance with 37 CFR §§ 1.821(c) and (e), are the same.

I. There Is No Obviousness-Type Double Patenting

Claims 56 and 62 remain rejected under the doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 3 and 7 of U.S. Patent No. 5,859,205 (the "'205 patent").

Applicants traverse the rejection and request reconsideration thereof because a proper *prima facie* case of obviousness has not been made.

The only reasoning provided in the Office Action for the rejection is that claims 56 and 62 of the present application and claims 3 and 7 of the '205 patent relate to the same inventive concept and that claims 56 and 62 are **generic** to claims 3 and 7 because they have all of the characteristics of the claimed humanised antibody. These assertions, however, are insufficient to support a proper *prima facie* case of obviousness-type double patenting. Rather, an obviousness-type double patenting rejection is analogous to a failure to meet the non-obviousness requirement of 35 U.S.C. § 103. *In re Braithwaite*, 154 U.S.P.Q. 29, 34 (C.C.P.A. 1967) and *In re Longi*, 225 U.S.P.Q. 645, 648 n.4 (Fed. Cir. 1985). Thus, under the law, the pivotal question in an obviousness-type double patenting analysis is: Does any claim in the application define merely an obvious variation of an invention disclosed and claimed in the patent? *In re Vogel*, 164 U.S.P.Q. 619 (C.C.P.A. 1970). If the answer to this question is no, there can be no double patenting.

In making the obviousness-type double patenting analysis, then, the proper inquiry is as taught in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). See, M.P.E.P. § 804. A determination whether one patent is generic to another patent is not the appropriate inquiry. The following quotation from *In re Kaplan*, 229 U.S.P.Q. 678, 681 (Fed. Cir. 1986) is instructive:

> By domination we refer ... to that phenomenon ... whereunder one patent has a broad or "generic" claim which "reads on" an invention defined by another narrower or more specific claim in another patent, the former "dominating" the latter because the more narrowly claimed invention cannot be practiced without infringing the broader claim ... In possibly simpler terms, one patent dominates another if a claim of the first patent reads on a device built or process practiced according to the second patent disclosure. This commonplace situation is not, *per se*, double patenting as the board seems to think. (citations omitted).

Thus, that some of Applicants' antibodies claimed in the present patent application may also meet limitations of claims in the '205 patent is not, alone, grounds for an obviousness-type double patenting rejection. It may simply be a case of one patent application dominating another patent application. Domination by itself cannot support a double patenting rejection. The obviousness-type

double patenting rejection is, therefore, misplaced. Further, the law requires more than a mere overlap in claim scope when concluding that particular compounds are obvious variants. Indeed, as stated by the Federal Circuit:

> The fact that a claimed compound may be encompassed by a disclosed generic formula does not by itself render that compound obvious. (citation omitted)

In re Baird, 29 U.S.P.Q.2d 1550, 1552 (Fed. Cir. 1994). As stated in § 804 of the M.P.E.P., the analysis employed in an obviousness-type double patenting determination parallels the guidelines for analysis of a 35 U.S.C. § 103 rejection, which requires analysis of the factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). No such factual inquiries have, in fact, been set forth in the Office Action. In view of the foregoing, Applicants request that the obviousness-type double patenting rejection be withdrawn.

II. The Claims Are Clear And Definite

Claims 56-73 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Applicants traverse the rejection and request reconsideration thereof because the claims are clear and definite.

The Office Action acknowledges that the terms "predominantly" and "remaining" are terms commonly used in English and that the term "predominantly" is defined in dictionaries. The Office Action, quite remarkably, asserts that the "metes and bounds" of these terms are not defined in the specification or the dictionary. As set forth in the previous response, "predominantly" means "having numerical superiority or advantage" (as defined in *Random House Webster's Dictionary*, 2nd ed., Random House, New York, 1997, p.1026). Thus, for a particular antibody having human acceptor antibody heavy chain framework residues (acceptor residues) and also having residues corresponding to residues in a donor antibody (donor residues), such antibody has "predominantly" acceptor residues if there is a greater number of acceptor residues than donor residues. For example, if a heavy chain has 51 acceptor residues and 50 donor residues, then it has "predominantly" acceptor

residues. Regarding the term "remaining," Applicants respectfully submit that this term must not be viewed in a vacuum. Claim 56 recites that the variable domain comprises predominantly human acceptor antibody heavy chain framework residues and that the "remaining" heavy chain residues correspond to equivalent residues in a donor antibody. Thus, the term "remaining" refers to those residues that are not the "human acceptor antibody heavy chain framework region residues." To be even more clear, Applicants have amended claims 56 and 62 to recite "remaining heavy chain framework region residues" and "remaining light chain framework region residues," respectively. Claims 56 and 62 have also been amended to provide antecedent basis for these recitations. Persons of ordinary skill would have no difficulty in determining whether a particular antibody meets these criteria. Thus, the claims are definite within the meaning of § 112. *In re Mercier*, 185 U.S.P.Q. 774 (C.C.P.A. 1975) (claims sufficiently define an invention so long as one skilled in the art can determine what subject matter is or is not within the scope of the claims). Because claims 56-73 are clear and definite, Applicants request that the rejection under 35 U.S.C. § 112, second paragraph be withdrawn.

III. The Claimed Invention Is Sufficiently Described

Claims 56-73 are rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to provide a sufficient written description. The Office Action mistakenly asserts that Applicants' specification fails to adequately describe the heavy chain CDR ranges. Applicants traverse the rejection and request reconsideration because Applicants' specification permits a person skilled in the art to clearly recognize that Applicants had possession of the claimed invention.

As stated in the "Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶1, 'Written Description' Requirement,":

> A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. The Examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In rejecting a claim, the

> examiner must set forth express findings of fact regarding the above analysis which support the lack of written description conclusion. These findings should: (1) Identify the claim limitation at issue; and (2) Establish a *prima facie* case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed.

In accordance with these standards, Applicants have indeed, provided a sufficient written description of the claimed inventions. The Office Action fails to establish a *prima facie* case, let alone show sufficient evidence to maintain this rejection.

In stark contrast to the mistaken assertion in the Office Action that Applicants did not contemplate nor disclose use of the CDRs defined by Wu and Kabat for humanising antibodies, Applicants provide ample written description regarding the heavy chain CDR ranges for not only the Kabat CDRs, but also for all claimed inventions. Applicants teach, for example, at page 8, lines 8-16 of the specification, that the antibody molecules of the present invention can comprise three donor CDRs that can be: 1) the Kabat CDRs; 2) the structural loop CDRs; 3) a composite of the Kabat and structural loop CDRs; and 4) any combination of any of these. Applicants teach, for example, at page 19, lines 19-23 of the specification, that the Kabat CDRs comprise residues 31-35, 50-65, and 95-102 of the heavy chain. Thus, Applicants ***clearly*** teach the ranges of heavy chain CDRs recited in claim 56 (*i.e.*, 31 to 35, 50 to 65 and 95 to 102). At page 19, lines 24-31 of the specification, Applicants teach that the structural loop CDRs comprise residues 26-32 of the heavy chain. Residues 26 to 35, thus, represent a composite of the Kabat CDR H1 and the structural loop CDR H1. To make this CDR composite, residues 26-30, in addition to residues 31-35, are donor in the heavy chain. Indeed, page 17, lines 6-11 of the specification, ***expressly teaches*** that donor residues that are substituted for acceptor residues in the CDRs include regions defined as residues 26-35, 50-65 and 95-102. Claim 57 recites that, in addition to residues 31 to 35 (see claim 56 from which claim 57 depends), residues 26 to 30 also come from the donor antibody. Thus, in effect, claim 57 encompasses antibodies that comprise the composite CDR (*i.e.*, residues 26 to 35). The effective range of the particular heavy chain CDR recited in claim 57 (*i.e.*, 26 to 35) is clearly supported by

Applicants' specification. Thus, Applicants' specification ***clearly*** provides written description of heavy chain CDRs having the recited residues, which are ***clearly*** taught as regions that can be substituted. Accordingly, Applicants request that the written description rejection under 35 U.S.C. § 112, first paragraph be withdrawn.

IV. The Claimed Invention Is Enabled

Claims 56-73 are rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to provide an enabling disclosure. Applicants traverse the rejection and request reconsideration because one skilled in the art would be able to practise the claimed invention without being required to perform undue experimentation.

A. Residues 23, 24, 49, 71 and 73

The Office Action mistakenly asserts that it would require undue experimentation for one skilled in the art to retain antigen binding in an antibody wherein at least residues 23, 24, 49, 71 and 73 in the framework region correspond to the equivalent residues in the donor antibody. In particular, the Examiner doubts whether substitution of residues 23, 24, 49, 71 and 73, without also substituting position 48, would result in an antibody that retained antigen binding. As will be recognized, however, the enablement requirement of §112 is satisfied so long as a disclosure contains sufficient information that persons of ordinary skill in the art having the disclosure before them would be able to make and use the invention. *In re Wands*, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988) (the legal standard for enablement under §112 is whether one skilled in the art would be able to practice the invention without undue experimentation). In this respect, the following statement from *In re Marzocchi*, 169 U.S.P.Q. 367, 369-370 (C.C.P.A. 1971), is noteworthy:

> The only relevant concern of the Patent Office under these circumstances should be over the truth of any such assertion. The first paragraph of §112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance.

> As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented **must** be taken as in compliance with the enabling requirements of the first paragraph of §112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied upon for enabling support. (emphasis added)

Any assertion by the Patent Office that an enabling disclosure is not commensurate in scope with the protection sought must be supported by evidence or reasoning substantiating the doubts so expressed. *In re Dinh-Nguyen*, 181 U.S.P.Q. 46 (C.C.P.A. 1974); *In re Bowen*, 181 U.S.P.Q. 48 (C.C.P.A. 1974).

The reasoning provided in the Office Action in support of the enablement rejection is three-fold: 1) humanised antibodies 61E71 and hTNF3 in Applicants' specification require substitution at position 48; 2) U.S. Patent No. 5,530,101 shows that different humanised antibodies require different combination of mouse residues, and 3) Applicants' specification is alleged to teach that residues 71, 73 and 78 will "always" be all donor or all acceptor residues. Each of these reasons will be addressed separately below.

First, what may be required for a particular antibody is not necessarily required for all antibodies. Applicants teach a hierarchy of residues which can, if necessary, be changed in sequence. Depending on the antibody, different residues may need to be changed. Applicants teach, at page 20, line 25 of the specification, that particular key residues near the CDR contribute to antigen binding, *i.e.*, residues 23, 71 and 73. Each of these residues are recited in claim 56. Applicants also teach, at page 21, line 9 of the specification, that particular key packing residues near the CDR contribute to antigen binding, *i.e.*, residues 24, 49 and 78. Residues 24 and 49 are recited in claim 56. Thus, five of the six residues identified as being key residues are recite in claim 56. Residue 48, identified in the Office Action and alleged to be necessary, is not among these. If the Examiner maintains that residue 48 is required to be a donor residue, Applicants request that the Examiner provide an affidavit containing evidence substantiating this position. 37 C.F.R. § 1.104(d)(2).

U.S. Patent No. 5,530,101 (the "'101 patent") is alleged in the Office Action to show that different humanised antibodies require different combination of mouse residues for antigen binding. Applicants respectfully submit that the '101 patent is irrelevant. Regardless, Applicants cannot find, nor did the Office Action point out, any portion of the '101 patent that teaches that an antibody molecule that comprises donor residues at positions 23, 24, 49, 71 and 73 will be unable to bind an antigen. Rather, the Office Action supports such an erroneous conclusion by attempting to show that a change in one amino acid in the OKT3 antibody disclosed in Applicants' specification "could drastically change the antibody affinity," referring to antibody constructs JA207 and JA197. Applicants cannot find, however, where their specification shows a "drastic" change in affinity between JA207 and JA197. Indeed, when one skilled in the art examines Figures 7 and 10a, it is quite clear that JA197 and JA207 have binding affinities that are very close to the binding affinity of JA185, which is the "fully grafted" product that has a binding affinity very similar to that of the OKT3 murine reference antibody (see, page 51, lines 29-31 of the specification). In the absence of any specific teaching that an antibody molecule that comprises donor residues at positions 23, 24, 49, 71 and 73 will be unable to bind an antigen, Applicants' statements that such antibodies have affinity for an antigen must be taken as in compliance with the enabling requirements.

Finally, the section of the specification referred to in the Office Action (page 17, section 2.1) for supporting the allegation that residues 71, 73 and 78 are either all donor or all acceptor merely points to a "preferred protocol" for practicing the invention. Indeed, the Summary of the Invention states that these residues are "preferably either all donor or all acceptor" (page 7, lines 3-5 of the specification). Further, Applicants teach, for example, at page 6, lines 28-35 of the specification, that the framework comprises donor residues at at least **one** of positions "71 and/or 73, 75 and/or 76 and/or 78..." Thus, Applicants' specification clearly teaches that residues at positions 71, 73 and 78 can independently be substituted by donor residues.

Thus, in view of the foregoing, there is no reason to believe that one skilled in the art would be required to perform any amount of experimentation, let alone an undue amount, in order to make and use the claimed invention wherein the antibody molecules comprise donor residues at positions 23, 24, 49, 71 and 73. Accordingly, Applicants respectfully request that the rejection under 35

U.S.C. § 112, first paragraph, in regard to donor residue positions 23, 24, 49, 71 and 73 be withdrawn.

B. Framework Regions

The Office Action mistakenly asserts that it would require undue experimentation for one skilled in the art to use a humanised antibody "wherein its framework region is from any human framework region." In particular, the Examiner asserts that "one necessary criteria for choosing these frameworks [EU, REI, KOL, LAY, HIL, SGI, and SGIII] is that they are substantially analogous to the donor framework." This assertion is, however, wholly unsupported by any evidence and is, in fact, explicitly contrary to the teachings in Applicants' specification.

Applicants teach at, for example page 11, lines 21-33 of the specification:

> However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences.

Thus, contrary to the erroneous assertions in the Office Action, substantial homology between the acceptor and donor framework is not a necessary criteria. If the Examiner maintains that a particular level of homology is a necessary criteria, Applicants request that the Examiner provide an affidavit containing evidence substantiating this position. 37 C.F.R. § 1.104(d)(2).

The Office Action also asserts that Applicants' specification teaches that human KOL and NEWM heavy chain frameworks "could not be used for humanizing antibody B72.3" because of poor homology as allegedly disclosed on page 56 of the specification. Applicants' specification, however, does **not** teach that KOL and NEWM "could not be used for humanizing antibody B72.3." Rather, Applicants teach that the EU heavy chain was chosen for B72.3 to determine **whether** "transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology

between the donor and the receptor frameworks was maximised." There is **no** discussion or suggestion that KOL and NEWM would not work. Indeed, the contrary is suggested for B72.3; Applicants were trying to see whether the human framework selection had to be of a known crystal structure, or could be based on another criteria. That some experimentation may be required (and Applicants maintain that no further experimentation is required) does not preclude enablement so long as the amount of experimentation is not undue. *W. L. Gore & Associates, Inc. v. Garlock, Inc.*, 220 U.S.P.Q. 303, 316 (Fed. Cir. 1983). Further, the Office Action fails to establish that if any experimentation is required, it is anything other than routine experimentation. Indeed, routine experimentation does not constitute undue experimentation.

> The test [for undue experimentation] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the claimed invention.

PPG Indus., Inc. v. Guardian Indus. Corp., 75 F.3d 1558, 1564, 37 U.S.P.Q.2d 1618, 1623 (Fed. Cir. 1996) (quotation and citation omitted). Thus, the Office Action fails to establish that any experimentation, let alone undue experimentation, is required to practice the claimed invention.

The Office Action asserts that the binding data in Table 2 of the Yarranton Declaration is confusing, and appears to allege that the data conflicts. Applicants submit there is no conflict between the data provided therein. The Examiner continues to misread the data. For 61E71, the first data point for 61E71, *i.e.*, 100, falls under the heading "POTENCY RELATIVE TO RODENT ANTIBODY" and represents the potency relative to the rodent antibody as measured by the relative ability to compete with the murine antibody for binding to the antigen. Thus, 61E71 is as potent in antigen binding as the murine antibody. The second data point for 61E71, *i.e.*, <1, falls under the heading "ANTI-CYTOKINE" and represents the score using a cytokine neutralization assay in which antibody binds to the cytokine and the resultant complex is tested for the ability to affect growth of L929 cells, which are dependent on TNFα. Thus, 61E71 is a potent inhibitor of L929 cell growth, demonstrating that the antibody not only binds TNFα but also has biological effectiveness.

Thus, in view of the foregoing, there is no reason to believe that one skilled in the art would be required to perform any amount of undue experimentation in order to make and use the claimed invention wherein the antibody framework region is from any human framework region. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 112, first paragraph, in regard to framework regions be withdrawn.

V. Conclusion

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at (215) 564-8906 if there are any questions regarding Applicants' claimed invention. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,

Paul K. Legaard
Registration No. **38,534**

Date: **November 12, 2001**

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 56, 58 and 62 have been amended as follows:

56. (Amended four times) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, [said] wherein said framework regions of said variable domain comprise [comprising] predominantly human acceptor antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and at least residues 23, 24, 49, 71, and 73 in the framework regions correspond to the equivalent residues in said donor antibody.

58. (Amended three times) The antibody molecule of claim 56, wherein additionally at least one of the residues selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds [,] to the equivalent residue in said donor antibody.

62. (Amended four times) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework regions, [said] wherein said framework regions of said light chain variable domain [comprising] comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, in said composite light chain at least residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and at least residues 46, 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

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DATE: March 18, 2002 JOB CODE: 27136/6

Please deliver this and the following pages to:

Name:	Examiner M. Davis
Company/Firm:	U.S.P.T.O.
Telecopier No.:	(703) 746-7145
Client/Matter No.:	U.S. Serial No. 08/485,686; Our Docket No. CARP-0046
Sender's Name:	Paul K. Legaard
Pages to Follow:	3

If transmission is not complete, please call (215) 568-3100
COVER MESSAGE:

Examiner Davis, attached is a copy of proposed amendments to the claims which corresponds with our discussion last week. Please call me to discuss them. If you concur with these amendments, I will prepare a formal amendment to be faxed to you later this afternoon. Best regards, Paul.

Serial No.: 08/485,686
Filed: June 7, 1995

CARP-0046 PENDING CLAIMS

56. (AMEND) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, wherein said framework regions of said variable domain comprise predominantly human acceptor antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system ~~in a donor antibody~~, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, [in] said composite heavy chain [at least] comprises residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and [at least] comprises residues 23, 24, 49, 71, [and] 73, and 78 in the framework regions that correspond to the equivalent residues in said donor antibody

are donor residues.

Key ? p. 20 surface residues near CDR

57. (AMEND) The antibody molecule of claim 56, wherein additionally residues 26 to 30 [and 78] in said composite heavy chain correspond to the equivalent residues in said donor antibody.

58. (AMEND) The antibody molecule of claim 56, wherein additionally [at least one of the residues] a residue selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

59. (AMEND) The antibody molecule of claim 57, wherein additionally [at least one of residues] a residue selected from the group consisting of 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

60. (AMEND) The antibody molecule of claim 58, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

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Filed: June 7, 1995

CARP-0046 PENDING CLAIMS

61. (AMEND) The antibody molecule of claim 59, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

62. (AMEND) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework regions, wherein said framework regions of said light chain variable domain comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, [in] said composite light chain [at least] comprises residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and [at least] comprises residues [46], 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

63. (AMEND) The antibody molecule of claim 62, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 6, 35, 38, 44, 47, 49, 62, 64, 65, 66, 67, 68, 69, 85, 87, 98, 99, 101 and 102 in said composite light chain corresponds to the equivalent residue in said donor antibody.

64. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a T-cell antigen.

65. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a lymphokine.

66. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a growth factor.

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67. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for interferon.

68. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for an adhesion molecule.

69. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a hormone.

70. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a cancer marker.

71. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for a TNF-α.

72. The antibody molecule of any one of claims 56, 57 or 62 which has specificity for mucin.

73. (AMEND) A [therapeutic] composition comprising the antibody molecule of any one of claims 56, 57 or 62 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

DOCKET NO.: CARP-0046 **PATENT-DRAFT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Adair et al.

Serial No.: **08/485,686** Group Art Unit: **1642**

Filed: **June 7, 1995** Examiner: **M. Davis**

For: **Humanised Antibodies**

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On August 9, 2002

Doreen Yatko Trujillo, Reg. No. 35,719

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

In response to the Office Action mailed April 9, 2002 in connection with the above-identified patent application, Applicants respectfully request that the application be amended as follows. The period for responding to the Office Action has been extended, by enclosure of a petition and fee, to and through August 9, 2002.

In the Claims:

Please amend claims 56-63 and 73 to read as follows:

56. (Amended five times) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, wherein said framework regions of said variable domain comprise predominantly human acceptor

antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, said composite heavy chain comprises residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and comprises residues 23, 24, 49, 71, 73, and 78 in the framework regions that correspond to the equivalent residues in said donor antibody.

57. (Amended twice) The antibody molecule of claim 56, wherein additionally residues 26 to 30 in said composite heavy chain correspond to the equivalent residues in said donor antibody.

58. (Amended four times) The antibody molecule of claim 56, wherein additionally a residue selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

59. (Amended) The antibody molecule of claim 57, wherein additionally a residue selected from the group consisting of 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

60. (Amended) The antibody molecule of claim 58, wherein additionally a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

61. (Amended) The antibody molecule of claim 59, wherein additionally a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

62. (Amended five times) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework

regions, wherein said framework regions of said light chain variable domain comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, said composite light chain comprises residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and comprises residues 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

63. (Amended twice) The antibody molecule of claim 62, wherein additionally a residue selected from the group consisting of 2, 4, 6, 35, 38, 44, 47, 49, 62, 64, 65, 66, 67, 68, 69, 85, 87, 98, 99, 101 and 102 in said composite light chain corresponds to the equivalent residue in said donor antibody.

73. (Amended twice) A composition comprising the antibody molecule of any one of claims 56, 57 or 62 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

REMARKS

Claims 56-73 are pending in the present application. Claims 56-63 and 73 have been amended herein. No new matter has been added. Upon entry of the present amendment, claims 56-73 will remain pending.

As a preliminary matter, Applicants thank the Examiner for taking the time and effort to engage Applicants' representative in an interview on April 3, 2002, as well as subsequent discussions thereafter. The following remarks are based upon the substance of the interview.

In addition, the Interview Summary provided along with the present Office Action requests that Applicants provide a "comparison between the sequences having substituted residues of Queen et al as shown for example in Table I and the claimed sequences after adjusting for differences in numbering systems." During the interview, the Examiner indicated a desire to see a comparison similar to the one filed in connection with application Serial No. 08/116,247. Although Applicants contend that such a showing is not required, Applicants submit herewith a comparison similar to the

comparison submitted in application Serial No. 08/116,247.

All four sequences presented in the attachments were reported in Queen *et al.*, *PNAS-USA*, *86*:10029-10033, **1989**. More specifically, the sequences are found at the bottom of page 10031 of the reference. Applicants duplicated the sequences in the attachments to facilitate comparison of linear numbering with Kabat numbering. All sequences in the attachments are presented in single letter amino acid code.

The first sequence on the first page of the attachments is the top sequence in Panel A on page 10031 and represents the amino acid sequence of the light chain variable domain of the human Eu antibody. The second sequence on the first page is the bottom sequence in Panel A and represents the amino acid sequence of the light chain variable domain of an anti-Tac antibody. The first sequence on the second page of the attachments is the top sequence in Panel B on page 10031 and represents the amino acid sequence of the heavy chain variable domain of the human Eu antibody. The second sequence on the second page is the bottom sequence in Panel B and represents the amino acid sequence of the heavy chain variable domain of an anti-Tac antibody.

The amino acids of the sequences presented in the attachments are numbered using two different numbering systems. The numbers above each sequence are according to the numbering system used in Queen *et al.*, which represents the linear numbering system. The numbers below each sequence are according to the Kabat numbering system. As is evident from the attachments, the two numbering systems result in the assignment of the same residue number to a particular amino acid in some instances - *i.e.*, the first sequence on the first page of the attachments. However, in other instances - *i.e.*, the remaining sequences of the attachments - the two numbering systems do not result in the assignment of the same residue number to a particular amino acid.

The differences between the two approaches is clearly evident from the comparison. The residues which are specified to be donor residues are indicated by horizonal bars. The blue bars above the sequences depict the residues which are specified as donor in Queen *et al.* The red bars below the sequences depict the ***minimum*** number of residues specified as donor in Applicants' invention (as set forth in, for example, claims 56 and 62).

I. No New Matter Has Been Introduced Into The Claims

Claims 56-73 were rejected under 35 U.S.C. § 112, first paragraph as allegedly containing new matter. The Office Action objects to the phrase "at least" in claims 56 and 62. Applicants request reconsideration in view of the amended claims.

Although Applicants provide ample written description of an antibody molecule having, *inter alia*, a composite heavy chain in which at least residues 23, 24, 49, 71, and 73 in the framework regions correspond to the equivalent residues in a donor antibody (referring to claim 56) and an antibody molecule having, *inter alia*, a composite light chain in which at least residues 46, 48, 58 and 71 in the framework regions correspond to the equivalent residues in a donor antibody (referring to claim 62), to advance prosecution of the present application Applicants have amended the claims as suggested during the teleconference with the Examiner on April 3, 2002. In particular, the Examiner suggested deleting "at least" and replacing it with "comprises." In addition, the Examiner suggested that Applicants insert "78" in claim 56 and delete "46" in claim 62. Applicants have also amended claims 57-61, 63 and 73 to be consistent with the language of the claims from which they depend. In addition, claim 73 has been amended, as suggested by the Examiner, to provide additional clarity.

In view of the forgoing, Applicants respectfully request that the new matter rejection under 35 U.S.C. § 112, first paragraph be withdrawn.

II. There Is No Obviousness-Type Double Patenting

Claims 56 and 62 remain rejected under the doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 3 and 7 of U.S. Patent No. 5,859,205 (the "'205 patent"). Applicants, again, traverse the rejection and request reconsideration thereof because **a proper *prima facie* case of obviousness has not been made**.

The only reason of record provided by the Examiner (see, the Office Action mailed August 10, 2001) for the rejection is that claims 56 and 62 of the present application and claims 3 and 7 of the '205 patent relate to the same inventive concept and that claims 56 and 62 are **generic** to claims 3 and 7 because they have all of the characteristics of the claimed humanised antibody. **These assertions, however, are insufficient to support a proper *prima facie* case of obviousness-type**

double patenting. Rather, an obviousness-type double patenting rejection is analogous to a failure to meet the non-obviousness requirement of 35 U.S.C. § 103. *In re Braithwaite*, 154 U.S.P.Q. 29, 34 (C.C.P.A. 1967) and *In re Longi*, 225 U.S.P.Q. 645, 648 n.4 (Fed. Cir. 1985). Thus, under the law, the pivotal question in an obviousness-type double patenting analysis is: Does any claim in the application define merely an obvious variation of an invention disclosed and claimed in the patent? *In re Vogel*, 164 U.S.P.Q. 619 (C.C.P.A. 1970). If the answer to this question is no, there can be no double patenting.

In making the obviousness-type double patenting analysis, then, the proper inquiry is as taught in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). See, M.P.E.P. § 804. **A determination whether one patent is generic to another patent is not the appropriate inquiry.** The following quotation from *In re Kaplan*, 229 U.S.P.Q. 678, 681 (Fed. Cir. 1986) is instructive:

> By domination we refer ... to that phenomenon ... whereunder one patent has a broad or "generic" claim which "reads on" an invention defined by another narrower or more specific claim in another patent, the former "dominating" the latter because the more narrowly claimed invention cannot be practiced without infringing the broader claim ... In possibly simpler terms, one patent dominates another if a claim of the first patent reads on a device built or process practiced according to the second patent disclosure. This commonplace situation is not, *per se*, double patenting as the board seems to think. (citations omitted).

Thus, that some of Applicants' antibodies claimed in the present patent application may also meet limitations of claims in the '205 patent is not, alone, grounds for an obviousness-type double patenting rejection. It may simply be a case of one patent application dominating another patent application. **Domination by itself cannot support a double patenting rejection.** The obviousness-type double patenting rejection is, therefore, misplaced. Further, the law requires more than a mere overlap in claim scope when concluding that particular compounds are obvious variants. Indeed, as stated by the Federal Circuit:

> The fact that a claimed compound may be encompassed by a disclosed generic formula does not by itself render that compound obvious. (citation omitted)

In re Baird, 29 U.S.P.Q.2d 1550, 1552 (Fed. Cir. 1994). As stated in § 804 of the M.P.E.P., the analysis employed in an obviousness-type double patenting determination parallels the guidelines for analysis of a 35 U.S.C. § 103 rejection, which requires analysis of the factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1 (1966). No such factual inquiries have, in fact, been set forth in the Office Action.

The only rebuttal offered by the Examiner in response to the above arguments in Applicants previous response is that because "the instant claims 56 and 62 are narrower than claims 3 and 7 of PN=5,859,205" claims 56 and 62 "would be subjected to obviousness-type double patenting." Applicants understand that the Examiner may want to "subject" the claims of the present application to an obviousness-type double patenting "analysis." The Examiner, however, fails to carry out such an analysis, let alone provide any reasoning or evidence supporting the obviousness of the 56 and 62. **Thus, the Examiner has not established a *prima facie* case of obviousness.** Again, merely because some of Applicants' antibodies claimed in the present patent application may also meet limitations of claims in the '205 patent is not, alone, grounds for an obviousness-type double patenting rejection. If the present rejection is not withdrawn, Applicants respectfully request that the Examiner call Applicants' undersigned representative so that an interview can be scheduled with the Examiner and the Examiner's supervisor.

In view of the foregoing, Applicants request that the obviousness-type double patenting rejection be withdrawn.

III. The Amendments to the Drawings are Supported by the Specification

The Office Action objects to the proposed changes in Figure 6 and instructs Applicants to correct the same. In particular, the Office Action asserts that the drawing changes have not been granted because "it seems that changing the amino acid residues RW to LL of the sequence gL221B would be new matter." Applicants respectfully request that this objection be withdrawn and the formal drawings accepted because the specification provides ample written description supporting the changes to the drawings.

As pointed out in the previously filed response, **the proposed change in Figure 6, in**

which the "RW" amino acids are replaced with "LL" amino acids, find support, for example, in Table 2 at page 50 of the specification. In particular, Table 2 provides explicit written description showing that amino acids at positions 46 and 47 of gL221B are both "L" (*i.e.*, leucine). Thus, the specification provides explicit written description support for the proposed changes to Figure 6. No new matter is being added to Figure 6.

In view of the foregoing, Applicants request that the objections to the proposed changes to the drawings be withdrawn and that the formal drawings be accepted.

IV. Conclusion

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is respectfully requested to contact Applicants' undersigned representative at (215) 564-8352 if a Notice of Allowance is not forthcoming so that an interview can be scheduled. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 56-63 and 73 have been amended as follows:

56. (Amended five times) An antibody molecule having affinity for an antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs) and framework regions, wherein said framework regions of said variable domain comprise predominantly human acceptor antibody heavy chain framework region residues, the remaining heavy chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in a donor antibody, said donor antibody having binding affinity for said antigen, wherein, according to the Kabat numbering system, [in] said composite heavy chain [at least] comprises residues 31 to 35, 50 to 65 and 95 to 102 in the CDRs and [at least] comprises residues 23, 24, 49, 71, [and] 73, and 78 in the framework regions that correspond to the equivalent residues in said donor antibody.

57. (Amended twice) The antibody molecule of claim 56, wherein additionally residues 26 to 30 [and 78] in said composite heavy chain correspond to the equivalent residues in said donor antibody.

58. (Amended four times) The antibody molecule of claim 56, wherein additionally [at least one of the residues] a residue selected from the group consisting of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

59. (Amended) The antibody molecule of claim 57, wherein additionally [at least one of residues] a residue selected from the group consisting of 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

60. (Amended) The antibody molecule of claim 58, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

61. (Amended) The antibody molecule of claim 59, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

62. (Amended five times) The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRs and framework regions, wherein said framework regions of said light chain variable domain comprise predominantly human acceptor antibody light chain framework region residues, the remaining light chain framework region residues corresponding to the equivalent residues using the Kabat numbering system in said donor antibody, wherein, according to the Kabat numbering system, [in] said composite light chain [at least] comprises residues 24 to 34, 50 to 56 and 89 to 97 in the CDRs and [at least] comprises residues [46], 48, 58 and 71 in the framework regions correspond to the equivalent residues in said donor antibody.

63. (Amended twice) The antibody molecule of claim 62, wherein additionally [at least one of residues] a residue selected from the group consisting of 2, 4, 6, 35, 38, 44, 47, 49, 62, 64, 65, 66, 67, 68, 69, 85, 87, 98, 99, 101 and 102 in said composite light chain corresponds to the equivalent residue in said donor antibody.

73. (Amended twice) A [therapeutic] composition comprising the antibody molecule of any one of claims 56, 57 or 62 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

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RECEIPT FOR DOCUMENTS FOR A PATENT APPLICATION

Documents for a patent application have been received and recorded under the provisions of the Patents Act 1977 in the name(s) shown below.

The documents bear this number which should be used on all correspondence concerning them: 8928874 -O

The filing date provisionally given to the application is: 21 DEC 1989

Applicants:

Celltech Limited

RECEIVED 10 JAN 1990

THE DOCUMENTS RECEIVED PURPORT TO BE:

REQUEST FOR GRANT OF A PATENT	✓
DESCRIPTION	✓
CLAIMS	X
DRAWINGS (No of Sheets)	35+35
ABSTRACT	X
STATEMENT OF INVENTORSHIP (Form 7/77)	X
REQUEST FOR SEARCH (Form 9/77)	X
PRIORITY DOCUMENTS	X
TRANSLATION OF PRIORITY DOCUMENTS	X
REQUEST FOR EXAMINATION (Form 10/77)	X
OTHER (Specify)	X
	X

Address for service.

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Ms. I. Summersby 4/J
Signature & Date

Agent's Reference

PA 259

NB — ISSUE OF THIS RECEIPT DOES NOT CONFIRM THAT THE DOCUMENTS RECEIVED ARE SUFFICIENT TO MEET THE FILING CONDITIONS UNDER SECTION 15(1) OR THE RELEVANT CONDITIONS UNDER SECTION 89(4).

8928874.0 PA 259

FILED 21 DEC 1989

HUMANISED ANTIBODIES

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

In the present application, the term "recombinant antibody molecule" (RAM) is used to describe an antibody produced by an process involving the use of recombinant DNA technology, including any analogues of natural immunoglobulins or their fragments. The term "humanised antibody molecule" (HAM) is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise either complete variable domains fused onto constant domains or complementarity determining regions grafted onto appropriate framework regions in the variable domains. The abbreviation "MAb" is used to indicate a monoclonal antibody.

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

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

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of techniques for the preparation of monoclonal antibodies of defined specificity (1). However, most MAbs are produced by fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. Thus, in practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions.

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

Some early methods for carrying out such a procedure are described in EP-A-0 171 496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), EP-A-0 194 276 (Celltech Limited) and WO-A-8 702 671 (Int. Gen. Eng. Inc.). The Celltech application discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. It also shows the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin.

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable regions. The present invention relates to HAMs prepared according to this alternative approach, i.e. CDR-grafted HAMs.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells respectively were humanised by CDR-grafting are shown by Verhoeyen et al (2) and Riechmann et al (3).

In the latter case (Riechmann et al) it was found that transfer of the CDR regions alone (as defined by Kabat refs. 4 and 5) was not sufficient to provide

satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having satisfactory antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens.

In recent years a number of rodent MAbs have been developed for therapeutic applications. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in the USA as an immunosuppressant in the treatment of acute allograft rejection (Chatenond et al (1986) J. Immunol., 137, 830-838, and Jeffers et al (1986) Transplantation, 41, 572-578). However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, builds up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response by suitable humanisation or other recombinant DNA manipulation of these very useful antibody and thus enlarge their areas of use.

We have further investgated the preparation of CDR-grafted HAMs and have identified residues within the framework of the variable region (i.e. outside both the Kabat CDRs and structural loops of the variable regions) the amino acid identities of which are important for obtaining CDR-grafted products with satisfactory binding affinity.

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising human framework and non-human (rodent) antigen binding regions wherein the human framework comprises non-human (rodent) residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDR at CDR2 (residues 50-65), the structural loop residues at CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

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

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34) and CDR2 (residues 50-56) and the structural loop residues at CDR3 (residues 91-96).

The invention further provides a CDR-grafted HAM comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second aspects of the invention.

The residue designations given above and elsewhere in the present specification are numbered according to the Kabat numbering (refs. 4 and 5).

Preferably the CDR-grafted heavy chain comprises non-human (rodent) residues at positions 23 and/or 24, 48 and/or 49 and 71 and/or 73. Preferably, the CDR-grafted light chain comprises non-human (rodent) residues at positions 46 and/or 47.

Preferably the CDR-grafted antibody heavy and light chains and HAM are produced by recombinant DNA technology.

The HAM of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as the Fab or $(Fab')_2$ fragment; a light chain or heavy chain monomer or dimer; or any other molecule with the same specificity as the original non-human (rodent) antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Alternatively, the heavy or light chains or HAM of the present invention may have attached to them an effector or reporter molecule. For instance, they may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used

to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by an enzyme or toxin molecule.

For CDR-grafted products of the invention, appropriate variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of human framework used is of the same/similar class/type as the donor antibody. Advantageously, the framework is chosen to maximise/optimise homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. It will be appreciated that in some cases that the non-human and human amino acid residues, identified above in connection with the first and second aspects of the invention, may be the same and thus no change of the human framework to the corresponding non-human framework residue is required.

Also human constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domain. In particular, IgG human constant region domains may be used especially of the IgG1 and IgG3 isotypes, when the HAM is intended for therapeutic uses.

However, the remainder of the HAM need not comprise only protein sequences from the human immunoglobulin. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence

encoding the amino acid sequence of a polypeptide effector or reporter molecule.

Thus, according to a further aspect the present invention provides a process for producing an anti-CD3 HAM which process comprises:

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

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

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

(d) culturing the transfected cell line to produce the HAM.

The cell line may be transfected with two vectors, the first vector containing an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical except in so far as the coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed.

Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both.

The present invention also includes cloning and expression vectors and transfected cell lines used in the process of the invention, therapeutic and diagnostic compositions comprising the variable domains or the HAM of the invention and uses of such compositions in therapy and diagnosis.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 6 and 7.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 29.

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882-1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL. of supernatant was sent to Ortho to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as Maniatis et al.(ref. 6) with, in some cases minor modifications. DNA sequencing was performed as described in Sanger et al.(ref. 7) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al.(ref.8)and the Anglian Biotechnology Ltd handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al. (ref. 9)

3. RESEARCH ASSAYS

3.1 ASSEMBLY ASSAYS

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

3.1.1 COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

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

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

UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2 COS CELLS TRANSFECTED WITH CHIMAERIC OR CDR GRAFTED OKT3 GENES

The assembly assay for intact humanised OKT3 in COS cell supernatants was an ELISA with the following format:-

96 well microtitre plates were coated with $F(ab')_2$ goat anti-human IgG Fc. The plates were washed and samples added for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and $F(ab')_2$ goat anti mouse IgG Fc (HRPO conjugated) was added. Substrate was added to reveal the reaction.

Chimaeric B72.3 (IgG4) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimaeric standard.

3.2 ASSAY FOR OKT3 ANTIGEN BINDING ACTIVITY

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

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

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

The negative control for the cell-based assay was chimaeric B72.3. The positive control was mouse Orthomune OKT3 or chimaeric OKT3, when available. This cell-based assay was difficult to perform and gave poorly reproducible results with a high background.

4. cDNA LIBRARY CONSTRUCTION

4.1 mRNA PREPARATION AND cDNA SYNTHESIS

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

4.2 LIBRARY CONSTRUCTION

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

5. SCREENING

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

5' TCCAGATGTTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5' CAGGGGCCAGTGGATGGATAGAC for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNA s were obtained (Figs 1 and 2).

ANALYSIS OF SEQUENCES

DNA sequences from cDNA's were compared with RNA sequences

provided by Ortho. The cDNA sequences included 5' untranslated region sequence as well as signal peptide sequence. The 3' untranslated region was also sequenced. A single coding difference was observed at position 9 in the heavy chain where the mRNA suggested a Proline but the cDNA sequence read as an Alanine. The cDNA sequence was used for further analysis.

The light chain is a member of the mouse V_L subgroup VI and uses a J_K4 minigene. The heavy chain is probably a member of the mouse V_H subgroup II, most probably IIb, although it also has significant homology to the consensus for group Va which itself is very homologous to subgroup II. The D region is currently unclassified and the JH region is J_H2 (Figs 3 and 4).

The light chain shows a high degree of homology to the Ox-1 germline gene and to the published antibodies 45.21.1, 14.6b.1 and 26.4.1. The heavy chain shows reasonable homology to a subgroup of the J558 family including 14.6b.1. These combinations of light and heavy chain genes have previously resulted in antibodies with affinity for alpha-1-6 dextran (Sikder et al.(ref.10)Wallick et al. (ref.11)).

The heavy chain has the sequence Asparagine (Asn)- Proline (Pro)- Serine (Ser) in CDR2. Normally Asn-X-Ser would be a potential glycosylation site, but when X is Pro these sites tend not to be glycosylated.

8. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (Fig. 5) (ref. 12)A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamHI cassettes in the unique BamHI site of pEE6 hCMV. It is usual practice to insert the *neo* and *gpt* markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoRI sites in the cassette.

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

The mouse sequences were excised as EcoRI fragments and cloned into either EE6-hCMV-neo for the heavy chain (Fig 6) and into EE6-hCMV-gpt

for the light chain (Fig 7).

9. EXPRESSION OF cDNA'S IN COS CELLS

Plasmids pJA135 (Fig 7) and pJA136 (fig 6) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched peripheral blood lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

10. CONSTRUCTION OF CHIMAERIC GENES

Construction of chimaeric genes followed a previously described strategy (Whittle et al (ref. 9)) A restriction site near the 3' end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

10.1 LIGHT CHAIN GENE CONSTRUCTION- VERSION 1

The mouse light chain cDNA sequence showed an AvaI site near the 3' end of the variable region (Fig 8). The majority of the sequence of the variable region was isolated as a 376 bp. EcoRI-AvaI fragment. An oligonucleotide adapter was designed to replace the remainder of the 3' region of the variable region from the AvaI site and to include the 5' residues of the human constant region up to and including a unique NarI site which had been previously engineered into the constant region.

TOP STRAND 5' TCGGGGACAAAGCTTGAAATAAACAGAACTGTGGCGG 3'

BOTTOM STRAND 3' CCTGTTTCGAACTTTATTTGTCTTGACACCGCCGC 5'

A Hin$_d$III site, shown in bold type within the oligonucleotide sequence above, was introduced to act as a marker for insertion of the linker.

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

The constant region was isolated as an NarI-BamHI fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoRI/BamHI/CIP pSP65 treated vector in a three way reaction. Clones were isolated after transformation into *E.coli* and the linker and junction sequences were confirmed by the presence of the HinDIII site and by DNA sequencing (Fig 9).

10.2 LIGHT CHAIN GENE CONSTRUCTION- VERSION 2

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

.....Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala

VARIABLE CONSTANT

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

TOP STRAND 5' TCGGGGACAAAGTTGGAAATAAACAGAGCTGTGGCGG 3'

BOTTOM STRAND 3' CCTGTTTCAACCTTTATTTGTCTCGACACCGCCGC 5'

The internal HinDIII site present in the version 1 adapter was not included to differentiate the two chimaeric light chain genes.

The variable region fragment was isolated as a 376 bp. EcoRI-AvaI fragment. The oligonucleotide linker was ligated to NarI cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoRI. The variable region fragment and the modified constant region fragment were ligated directly into EcoRI/CIP treated pEE6hCMVneo.

Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing (Fig10).

10.3 HEAVY CHAIN GENE CONSTRUCTION

10.3.1 CHOICE OF HEAVY CHAIN GENE ISOTYPE

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

10.3.2 GENE CONSTRUCTION

The heavy chain cDNA sequence showed a BanI site near the 3' end of the variable region (Fig 11). The majority of the sequence of the variable region was isolated as a 426bp. EcoRI/CIP/BanI fragment. An oligonucleotide adapter was designed to replace the remainder of the

3' region of the variable region from the BanI site up to and including a unique HinDIII site which had been previously engineered into the first two amino acids of the constant region.

TOP STRAND 5' GCACCACTCTCACCGTGAGCTC3'

BOTTOM STRAND 3'GTGAGAGTGGCACTCGAGTCGA 5'

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

The variable region was ligated to the constant region by cutting mJA91with EcoRI and Hind III removing the intron fragment and replacing it with the V_H (Fig 12). Clones were isolated after transformation into *E.coli* JM101 and the linker and junction sequences were confirmed by DNA sequencing. (NB. The Hind III site is lost on cloning).

11. CONSTRUCTION OF CHIMAERIC EXPRESSION VECTORS

11.1 neo AND gpt VECTORS

The chimaeric light chain (version 1) was removed from pJA143 (Fig 9) as an EcoRI fragment and cloned into EcoRI/CIP treated pEE6hCMVneo expression vector. Clones with the insert in the correct orientation were identified by restriction mapping (Fig 13).

The chimaeric light chain (version 2) was constructed as described above (see Fig 10).

The chimaeric heavy chain gene was isolated as a 2.5Kbp EcoRI/BamHI fragment and cloned into the EcoRI/BclI/CIP treated vector fragment of pJA97, a derivative of pEE6hCMVgpt (Fig 14).

11.2 GS SEPARATE VECTORS

GS versions of pJA141 (Fig 10) and pJA144 (Fig 14) were constructed by replacing the neo and gpt cassettes by BamHI/SalI/CIP treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 (Figs 15 and 16)

11.3 GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL, cH and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS and with transcription of the genes being head to tail eg. cL>cH>GS were constructed. These plasmids were made by treating pJA179 (Fig 15) or pJA180 (Fig 16) with BamHI/CIP and ligating in a BglII/HinDIII hCMV cassette from pJA146 along with either the HinDIII/BamHI from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 (Fig17), or the HinDIII/BamHI from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181 (Fig 18).

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12. EXPRESSION OF CHIMAERIC GENES

12.1 EXPRESSION IN COS CELLS

The chimaeric antibody plasmids pJA145 (cL) and pJA144 (cH) were cotransfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels (Fig 19) suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimaeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin (Fig 19). This second version of the chimaeric light chain, when expressed in association with chimaeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

12.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines are being prepared from plasmids pJA141/pJA144 and from pJA179/pJA180, pJA181, and pJA182 by transfection into CHO cells.

13. CDR GRAFTING

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

13.1 VARIABLE REGION ANALYSIS

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

heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

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

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

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

13.1.1 LIGHT CHAIN

Figure 20 shows an alignment of sequences for the human framework region REI and the OKT3 light variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1C. REI was chosen as the human framework because the light chain is a Kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region eg KOL (see below). REI was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

13.1.2 HEAVY CHAIN

Figure 21 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1C. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also

the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

13.2 DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage (Grantham and Perrin(ref.13))and used the B72.3 signal sequences (Whittle et al.(ref.9))The sequences were designed to be attached to the constant region in the same way as for the chimaeric genes described above. Some constructs contained the "Kozak consensus sequence" (Kozak,(ref.14))directly linked to the 5' of the signal sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

13.3 GENE CONSTRUCTION

To build the variable regions two strategies are available. Either to assemble the sequence using oligonucleotides in a manner similar to Jones et al. (ref. 15)or to simultaneously replace all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al. (ref. 2) Both strategies were used and a list of constructions is set out in Table 1. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides. Figs 22a and b and 23a and b show by way of example the nucleotide sequences and procedures required to construct gH341 by site directed mutagenesis and kgH341A by oligonucleotide assembly.

14 CONSTRUCTION OF EXPRESSION VECTORS

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

15 EXPRESSION OF CDR GRAFTED GENES

A number of points should be noted.

1. There is no standard for the antigen binding assay when chimaeric or CDR grafted antibody are being measured, except when the heavy chain of the antibody is murine when murine OKT3 can be used as standard with an anti-murine Fc antibody as revealing antibody. Therefore all comparisons of antigen binding assays with chimaeric (c) or CDR grafted (g) genes can only be made within an individual

TABLE 1 CDR GRAFTED GENE CONSTRUCTIONS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE -	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK REI			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive +46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive +46, 47	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly Gene assembly	+ 	 +
341	26-35, 50-65, 95-100B inclusive	SDM Partial gene assembly	+ 	 +
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63=human)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive +48, 49, 71, 73, 76, 78, 88, 91 (+63=human)	Gene assembly	n.d.	+

KEY

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

experiment.

2. The cell-based antigen binding assay is not robust and resulting data varies depending on cell binding to the plates and the amount of antibody used. Therefore several experiments are needed to confirm marginal results.

3. The COS cell expression system can give batch to batch variation in antibody yield which has a direct bearing on the results obtained in the antigen binding assay.

Bearing these factors in mind the data can be divided into three groups. Table 2 shows a summary of data for the various constructs.

15.1 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMAERIC HEAVY (cH) CHAINS.

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression (Fig 24a and b). Over an extended series of experiments expression levels were raised from approx 200ng/mL to approx. 500 ng/mL for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) demonstrated some weak binding in association with mH or cH (Fig 25B). However when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 13.1 antigen binding can be demonstrated when both of the new constructs, which were termed 121A and 221A ,are coexpressed with cH (Fig 25A and B). When the effects of these residues are examined in more detail it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene shows little detectable binding activity in association with cH (Fig 25B). The light chain product of gL221C, in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH (Fig 25 B).

15.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMAERIC LIGHT(cL) CHAINS.

Expression of the gH genes has proven to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appears to have

had no marked effect on expression of gH genes (Fig 26). Expression may be slightly improved but not to the same degree as seen for the grafted light chain.

Second, it has proven difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used eg. gH121, 131, 141 (Fig 27) and no conclusions can be drawn about these constructs. Further, in experiments where low antibody production was seen it has not been possible to detect free light chain expression and secretion which would be expected if heavy chain expression was not occurring at all. Therefore the data suggests, but does not confirm, that in these cases the heavy chain is being expressed but the processing of the chain once it has become associated with light chain is aberrant leading to degradation of assembled or partially assembled antibody inside the cell. Experiments to determine gH mRNA levels, or to attempt to demonstrate the presence of antibody within the cells have not been done.

Third, coexpression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 to produce gH341A and gH341B appear to lead to improved levels of expression (Fig 27 lanes h-k). This may partly be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 are expressed in association with cL, antibody is produced but antibody binding activity has not been detected (Table 2). When the more conservative gH341 gene is used antigen binding can be detected in association with cL or mL, but the activity is only marginally above the background level (Fig 28). When further mouse residues are substituted based on the arguments in 13.1 antigen binding can be clearly demonstrated for the antibody produced when kgH341A and kgH341B are expressed in association with cL (Fig 29).

15.3 PRODUCTION OF FULLY CDR GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A, or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression experiment (Fig 29A ancd C). For the

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combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH or cL/cH was produced (Fig 29A and C).

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations (see for example Fig 29), although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed (Fig 29B). In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimaeric antibody (Fig 29B).

The objectives of the programme were to produce both a chimaeric mouse variable-human constant IgG4/K antibody and a fully humanised antibody retaining the antigen binding activity of the murine monoclonal antibody OKT3.

Cells were obtained from Ortho and mRNA prepared. A cDNA library was screened for heavy and light chain cDNAs using oligonucleotide probes. Full length cDNAs were obtained and the variable regions were sequenced (Figs 1 and 2). The cDNAs showed a high level of homology with sequences of antibodies which have specificity for alpha-1-6-dextrans. It would be of interest to test OKT3 to determine whether it recognises and binds to dextran antigens.

The cDNAs were transferred to expression vectors (Figs 6 and 7) and expressed in COS cells. Antibody was produced which bound to an enriched T-cell population from peripheral blood cells.

Two versions of the chimaeric antibody were produced, differing in the light chain at the first amino acid of the constant region. In version 1 (Figs 9 and 13) the amino acid sequence which resulted at the V-C junction when the chimaeric light chain was constructed generates a potential N-linked glycosylation site at the "elbow' region. This region is an extended sequence of peptide between the V and C domains and is potentially accessible to the enzymes of the glycosylation process. Fig 19 shows that the version 1 chimaeric light chain is glycosylated demonstrating that the secondary structure generated at the elbow is sufficient for the Asn-Arg-Thr motif to be used for glycosylation.

A second version of the chimaeric light chain was constructed (Fig 10) in which the first amino acid of the human constant region (Thr) was returned to the mouse amino acid (Ala), so removing the glycosylation site. Antibody was produced by coexpression with chimaeric heavy chain (Fig 13) and in both versions the chimaeric material was equivalent in binding to the mouse OKT3 (see Fig 19). These observations have been confirmed by Ortho staff (L Jolliffe pers. comm.).

Vectors for the expression of chimaeric OKT3 using neo/gpt or glutamine synthetase (GS) selection were prepared, including vectors in which both genes were on the same plasmid (Figs 15 to18).

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

For the light chain the regions defining the loops known from

structural studies of other antibodies to contain the antigen contacting residues, and those hypervariable sequences defined by Kabat et al. as Complementarity Determining Regions (CDRs) are equivalent for CDR 2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework REI has glutamine. For CDR 3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and REI (Fig 20). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and coexpressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions, 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W, see Fig 20 and Table 1) was made, cloned in EE6hCMVneo and coexpressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity(Fig 25 and Table 2). When the related genes gL221B (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when coexpressed with cH, only the gL221C/cH combination showed good antigen binding (Fig 25). When the gl 121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and coexpressed with cH antibody was produced which also bound to antigen (Fig 25).

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 incusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various

combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were coexpressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 eg. gH121, gH131, gH141 very little antibody was produced in the culture supernatants (see Fig 27). As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was being degraded internally. In some experiments trace amounts of antibody could be detected in ^{35}S labelling studies (see Fig 27). As no net antibody was produced, analysis of these constructs was not pursued further.

When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residue to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when coexpressed with cL (Fig 27). Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5' to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production (compare Figs 24 and 26). However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated (see Fig 28 and Table 2). When the kgH341 gene was coexpressed with kgL221A, the net yield of antibody was too low (see Figs 29A column 6 and 29C laneE) to give a signal above the background level in the antigen binding assay (see Fig 29A column 5).

As in the case of the light chain the heavy chain frameworks were reexamined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes, kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to improve domain packing. Both showed good levels of expression with cL or kgL221A (Fig 29A) and both showed antigen binding with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice (Fig 29B).

It has been demonstrated here for OKT3 that to transfer antigen binding ability to the humanised antibody mouse residues outside the

CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human Kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has already been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generates activity without the presence of the 6 and 23 changes. It would be of interest to determine by further study the individual contribution of the other 8 mouse residues of the kgH341A gene compared to kgH341 and to determine whether the 7 extra mouse surface residues in the antibody produced by the kgH341A/kgL221A combination contribute to idiotypic epitopes which can be detected by sera from patients treated with murine OKT3.

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  1 GAATTCCCAA AGACAAAatg gattttcaag tgcagatttt cagcttcctg
 51 ctaatcagtg cctcagtcat aatatccaga ggacaaattg ttctcaccca
101 gtctccagca atcatgtctg catctccagg ggagaaggtc accatgacct
151 gcagtgccag ctcaagtgta agttacatga actggtacca gcagaagtca
201 ggcacctccc ccaaaagatg gatttatgac acatccaaac tggcttctgg
251 agtccctgct cacttcaggg gcagtgggtc tgggacctct tactctctca
301 caatcagcgg catggaggct gaagatgctg ccacttatta ctgccagcag
351 tggagtagta acccattcac gttcggctcg gggacaaagt tggaaataaa
401 ccgggctgat actgcaccaa ctgtatccat cttcccacca tccagtgagc
451 agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
501 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa
551 tggcgtcctg aacagttgga ctgatcagga cagcaaagac agcacctaca
601 gcatgagcag caccctcacg ttgaccaagg acgagtatga acgacataac
651 agctatacct gtgaggccac tcacaagaca tcaacttcac ccattgtcaa
701 gagcttcaac aggaatgagt gtTAGAGACA AAGGTCCTGA GACGCCACCA
751 CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC
801 CCACAAGCGC tTACCACTGT TGCGGTGCTC tAAACCTCCT CCCACCTCCT
851 TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA
901 AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA
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SEQUENCE LENGTH 943 RESIDUES
INITIATOR MET AT 18
MATURE SEQUENCE BEGINS AT 84
CODING SEQUENCE 639 RESIDUES

NB. KAPPA CHAIN SEQUENCE OBTAINED FROM PUBLISHED SEQUENCE. ONLY THE JUNCTION WITH VARIABLE REGION AND 3' UNTRANSLATED REGION HAS BEEN CHECKED.

OKT3 LIGHT CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

```
  1 MDFQVQIFSF LLISASVIIS RGQIVLTQSP AIMSASPGEK VTMTCSASSS
 51 VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT SYSLTISGME
101 AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG
151 ASVVCFLNNF YPKDINVKWK IDGSERQNGV LNSWTDQDSK DSTYSMSSTL
201 TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC*
```

FIGURE 1

The DNA sequence of the OKT3 light chain as deduced from DNA sequencing of cDNA's and, for the Kappa constant region, from known sequence.
Untranslated regions are shown in uppercase type and the signal sequence is underlined. Also shown is the protein sequence translated from the major open reading frame.

```
   1  GAATTCCCCT CTCCACACAC ACTGAAAACT CTGACTCAAC ATGGAAGGGC
  51  ACTGGATCTT TCTACTCCTG TTGTCAGTAA CTGCAGGTGT CCACTCCCAG
 101  GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCAGT
 151  GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
 201  ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAATGGAT TGGATACATT
 251  AATCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
 301  CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
 351  GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
 401  GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
 451  CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
 501  GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
 551  TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
 601  TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
 651  GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
 701  AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
 751  CAGAGGGCCC ACAATCAAGC CCTGTCCTCC ATGCAAATGC CCAGCACCTA
 801  ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
 851  GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
 901  GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
 951  AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001  CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051  CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101  AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151  GTCTTGCCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201  CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251  ACAACGGGAA AACAGAGCTA AACTACAAGA ACACTGAACC AGTCCTGGAC
1301  TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351  CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401  ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451  CAGCACCCAC AAAACTCTCA GGTCCAAAGA GACACCCACA CTCATCTCCA
1501  TGCTTCCCTT GTATAAATAA AGCACCCAGC AATGCCTGGG ACCATGTAAA
1551  AAAAAAAAAA AAAGGAATTC
```

SEQUENCE LENGTH 1570 RESIDUES
INITIATOR MET AT 41
SIGNAL SEQUENCE UNDERLINED
MATURE SEQUENCE BEGINS AT 98
CODING SEQUENCE 1407 RESIDUES
5' UNTRANSLATED REGION 40 RESIDUES 3' UNTRANSLATED REGION 123 RESIDUES

OKT3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE


```
  1  MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTR
 51  YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101  QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTAPSVYPLA
151  PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201  TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251  PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED DPDVQISWFV
301  NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351  APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401  EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451  EGLHNHHTTK SFSRTPGK*
```

FIGURE 2

The DNA sequence of the OKT3 heavy chain chain as deduced from DNA sequencing of cDNA's and, for the constant regions, from known sequence.

The signal sequence is underlined.

Also shown is the protein sequence translated from the major open reading frame.

```
  1 QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDT 50
    |||||||||||||||||||||||||||||||| |||||||||||||||||
  1 QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDT 50

 51 SKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNP..FTFG 98
    ||||||||| | |||||||||||| ||||||||||||||||||   ||| 
 51 SKLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPPMLTFG 100

 99 SGTKLEINR 107
    .|||||  |
101 AGTKLELKR 109
```

HOMOLOGY 92.5%

UPPER LINE OKT3 V_L

LOWER LINE MOUSE V_L SUB GROUP 6

FIGURE 3

The protein sequence comparison of the OKT3 light chain variable region with the Kabat mouse sub group 6 consensus sequence (Kabat et al. 1987).

```
   OKT3 QVQLQQSGaELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY 50
        ||||||| ||  |||||||||||||||||  |   ||||  |  |||||
2A CONS evqlqqsgpelvkpgasvkmsckasgytftdyymkwvkqshgkslewigd 50

     51 INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCAR.. 98
        |||  | | ||||||  |||||  ||||||||||| ||||||||||||
     51 inpnnggtsynqkfkgkatltvdkssstaymqlnsltsedsavyycards 100

     99 .YYDDHYCLDYWGQGTTLTVss 119
                ||||||||  ||||
    101 ywyfxyywfdywgqgttvtvss 122     Percent Similarity: 78.151


   OKT3 QVQLQQSGaELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY 50
        ||||||  ||||  ||||||  |||||||||| | ||||||||||||||||
2B CONS qvqlqqpgaelvkpgasvklsckasgytftsywmhwvkqrpgqglewigr 50

     51 INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARY. 99
        | |  | | || ||| ||||| ||||||||||||||||||||||||||
     51 idpnsggtkynekfkskatltvdkssstaymqlssltsedsavyycaryx 100

    100 ......YDDHYCLDYWGQGTTLTVss 119
                  ||||||||||||||
    101 yygsssxgyyxyfdywgqgttltvss 126 Percent Similarity: 81.513


   OKT3 QVQLQQSGaELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY 50
        |||||||||  ||  ||||||  ||  |||       ||||||||  |||||||
2C CONS qvqlqqsgaelvkpgasvklsctasgfnikdtymhwvkqrpeqglewigr 50

     51 INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYY 100
        | |  | | |  ||  |||  | |  ||  |||  |||||||||  |||||||  |
     51 idpangntkydpkfqgkatitadtssntaylqlssltsedtavyycargy 100

    101 D..DHYCLDYWGQGTTLTVss 117
           | |  |||||||  ||||
    101 xxydyyamdywgqgtsvtvss 121     Percent Similarity: 69.231


   OKT3 QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY 50
        ||||||||||  |  |  ||||||||||||||  |   ||||||||||||||
5A CONS evqlqqsgaelvragssvkmsckasgytftsyginwvkqrpgqglewigy 50

     51 INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCAR.. 98
        |||  |||  ||  |||  |  |||  ||||||||||  ||||||||||  |||
     51 inpgngytkynekfkgkttltvdkssstaymqlrsltsedsavyfcarsn 100

     99 YYDDHYC..LDYWGQGTTLTVSS 119
        ||   |    ||||||||||||
    101 yyggsyyfffdywgqgttltvss 123     Percent Similarity: 81.513
```

FIGURE 4

The protein sequence comparison of the OKT3 heavy chain variable region with the Kabat mouse sub groups 2A, 2B, 2C and 5A (Kabat et al. 1987)

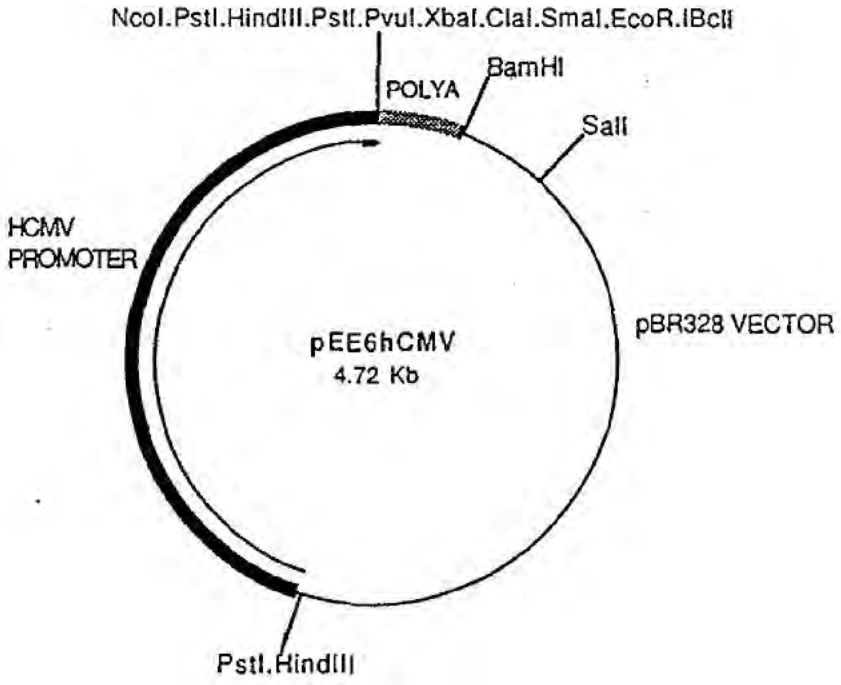


FIGURE 5

A map for the EE6hCMV expression vector used in this study
Only necessary sites are shown.

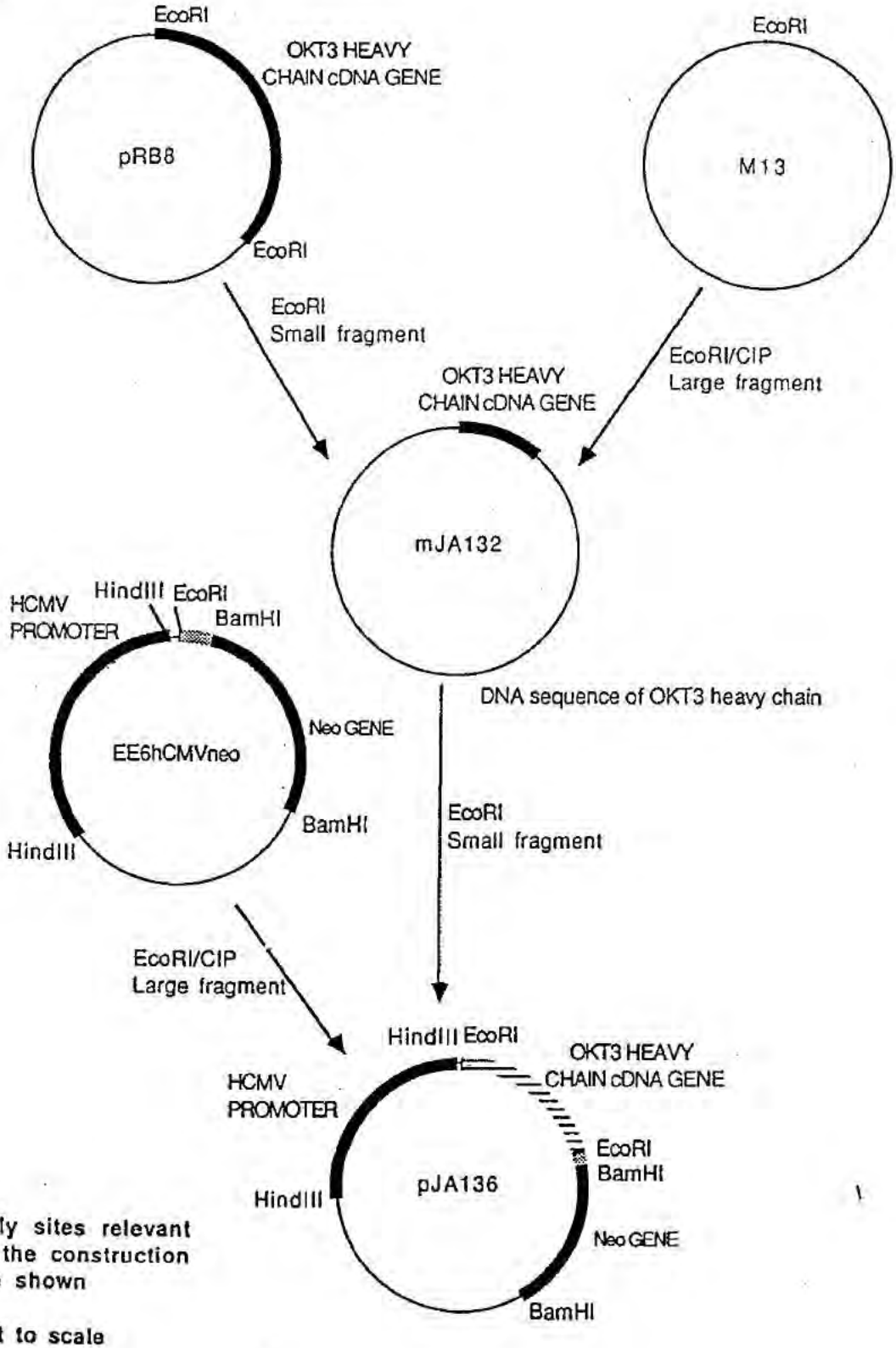


FIGURE 6
An outline schematic of the procedures involved in the construction of pJA136, a vector for the expression in eukaryotic cells of the OKT3 heavy chain cDNA gene.

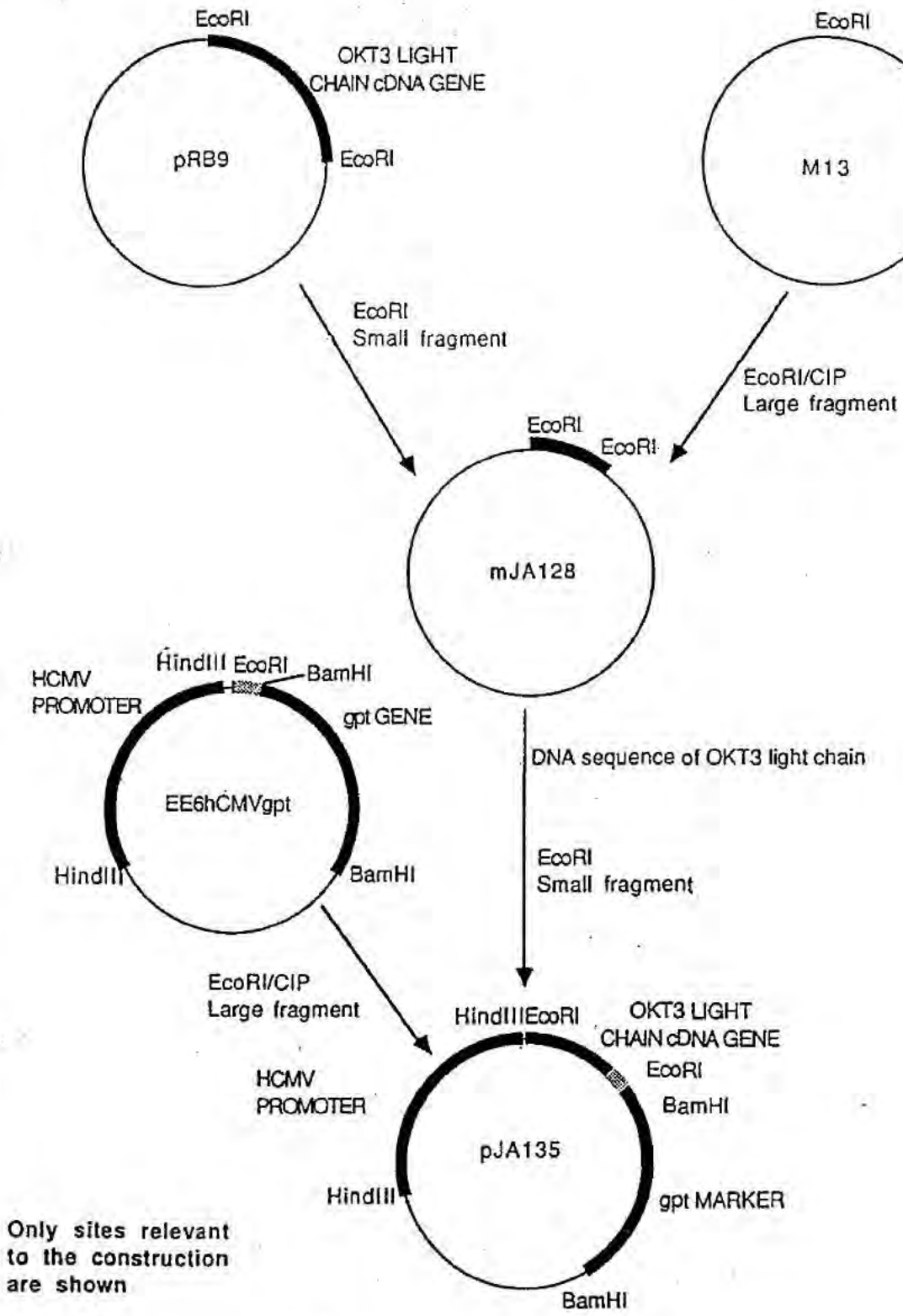


FIGURE 7
An outline schematic of the procedures involved in the construction of pJA135, a vector for the expression in eukaryotic cells of the OKT3 light chain cDNA gene.

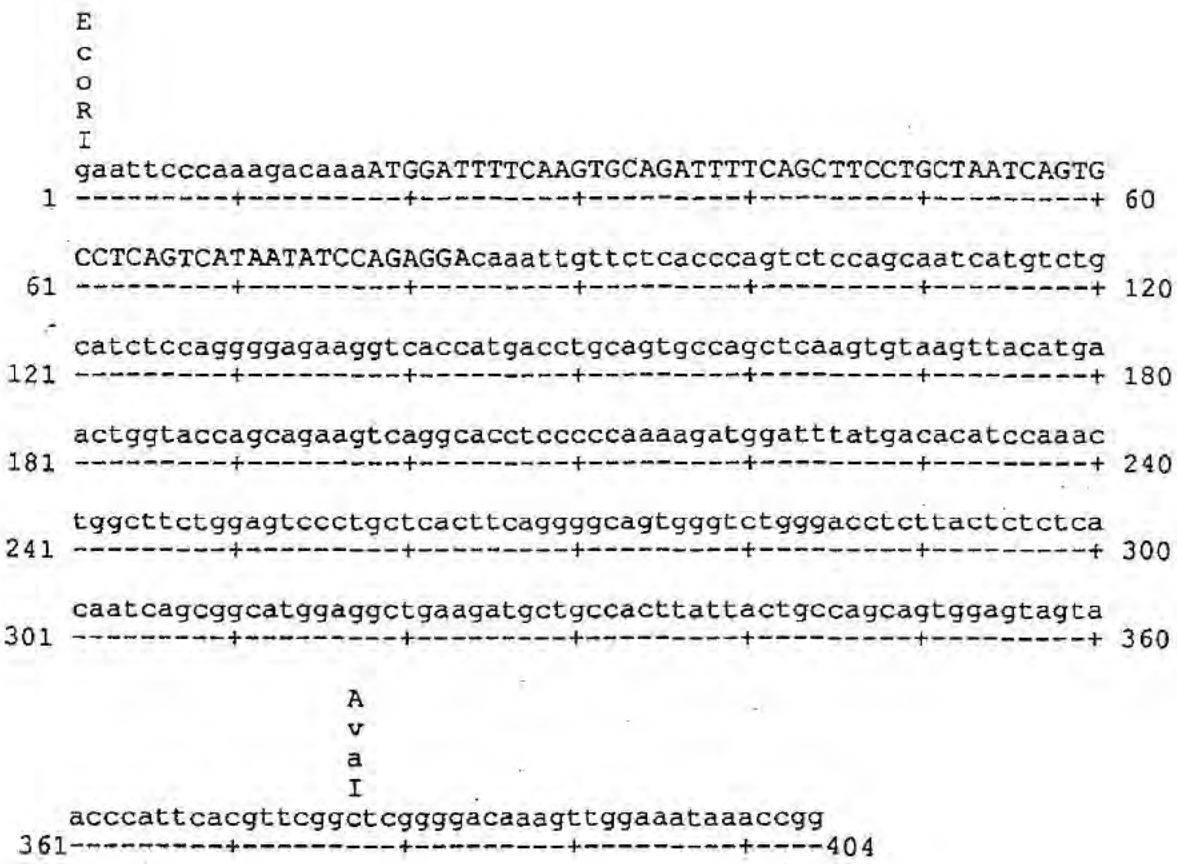
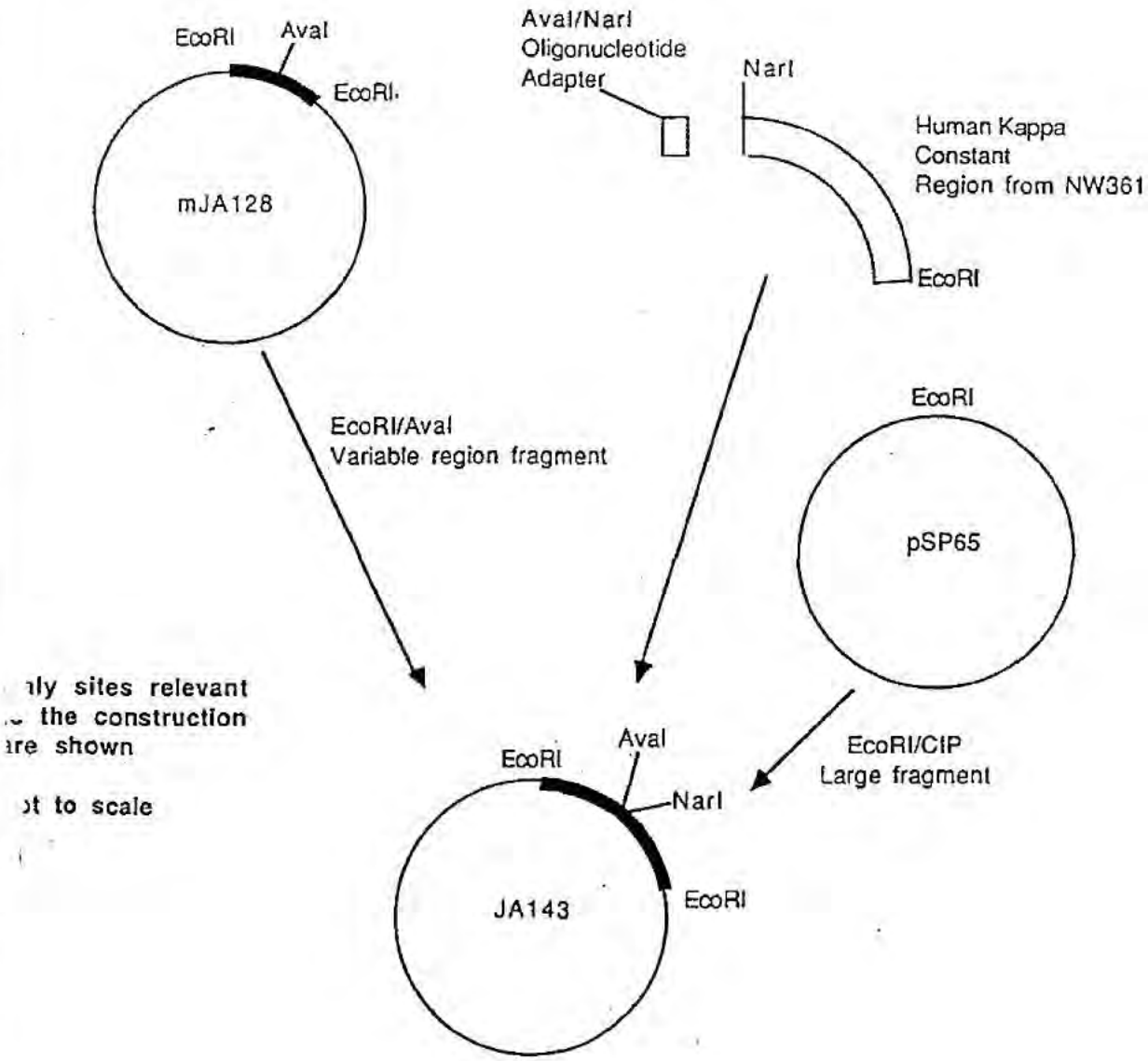


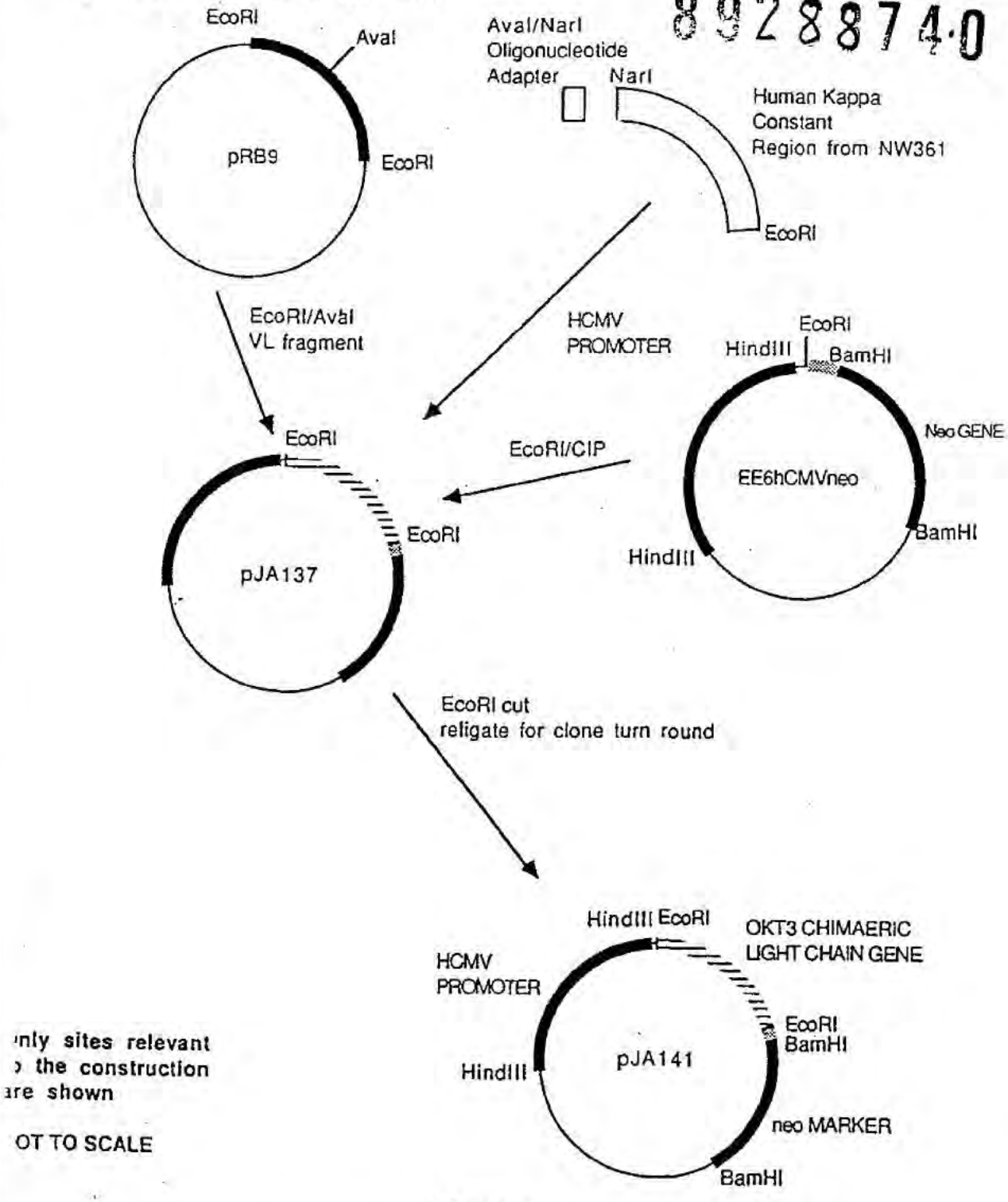
FIGURE 8
The nucleotide sequence for the OKT3 light chain variable region and the location of the EcoRI and AvaI sites used in the construction of the chimaeric OKT3 light chain gene.



ıly sites relevant
.. the construction
ıre shown

ɔt to scale

FIGURE 9
An outline schematic of the procedures involved in the construction of pJA143, an MI3 vector, including the OKT3 chimaeric light chain gene (Version1).



nly sites relevant
the construction
are shown

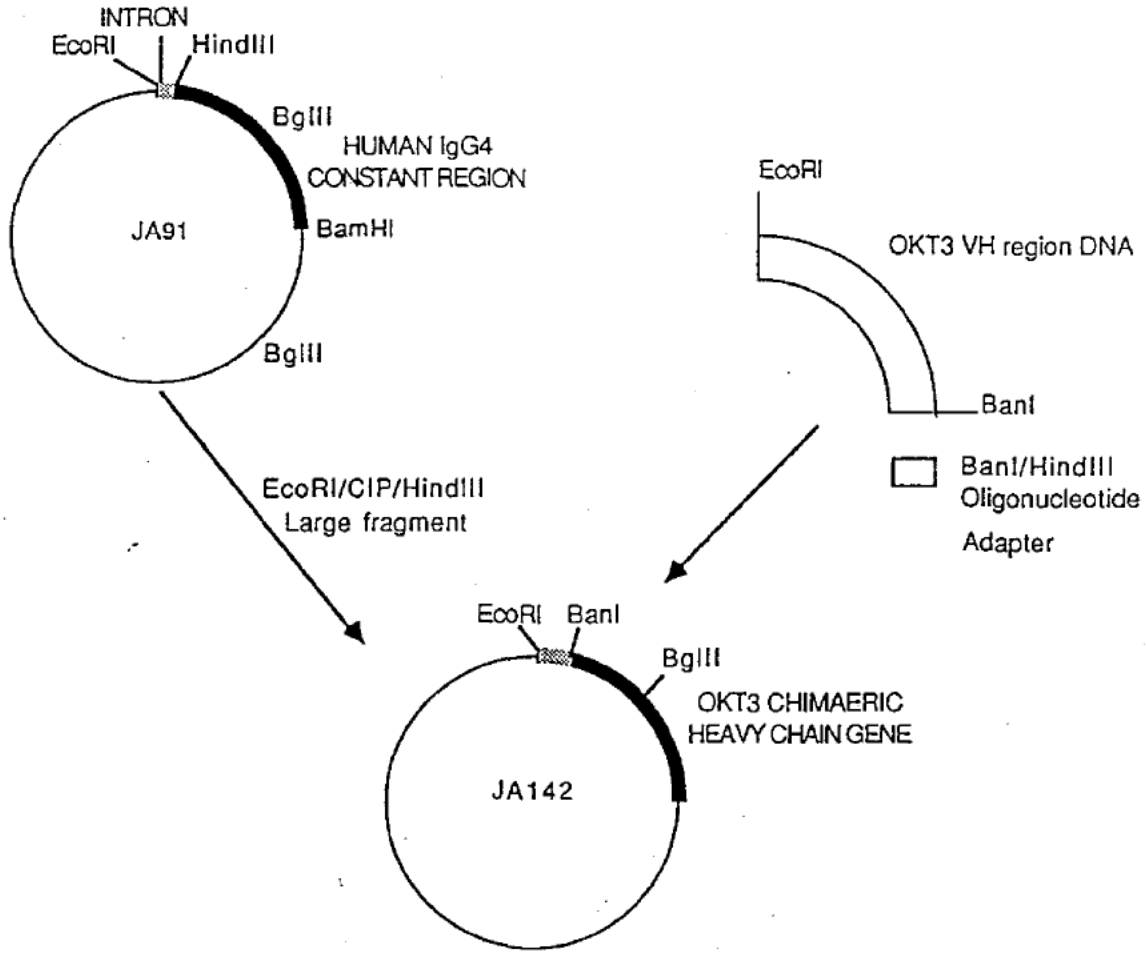
OT TO SCALE

FIGURE 10

An outline schematic of the procedures involved in the construction of pJA141, a vector for the expression in eukaryotic cells of the OKT3 chimaeric light chain gene (Version 2).

```
    E
    c
    o
    R
    I
    GAATTCCCCTCTCCACAGACACTGAAAACTCTGACTCAACATGGAAAGGCACTGGATCTT
1   ---------+---------+---------+---------+---------+---------+ 60

    TCTACTCCTGTTGTCAGTAACTGCAGGTGTCCACTCCCAGGTCCAGCTGCAGCAGTCTGG
61  ---------+---------+---------+---------+---------+---------+ 120

    GGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGgCTTCTGGCTACAC
121 ---------+---------+---------+---------+---------+---------+ 180

    CTTTACTAGGTACACGATGCACTGGGTAAAACAGAGGCCTGGACAGGGTCTGGAATGGAT
181 ---------+---------+---------+---------+---------+---------+ 240

    TGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGC
241 ---------+---------+---------+---------+---------+---------+ 300

    CACATTGACTACAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATC
301 ---------+---------+---------+---------+---------+---------+ 360

    TGAGGACTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTA
361 ---------+---------+---------+---------+---------+---------+ 420

        B
        a
        n
        I
    CTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
421 ---------+---------+---------+---- 454
```

FIGURE 11
The nucleotide sequence for the OKT3 heavy chain variable region and the location of the EcoRI and BanI sites used in the construction of the chimaeric OKT3 heavy chain gene.



Only sites relevant to the construction are shown

FIGURE 12

An outline schematic of the procedures involved in the construction of pJA142, an MI3 vector including the OKT3 chimaeric heavy chain gene.

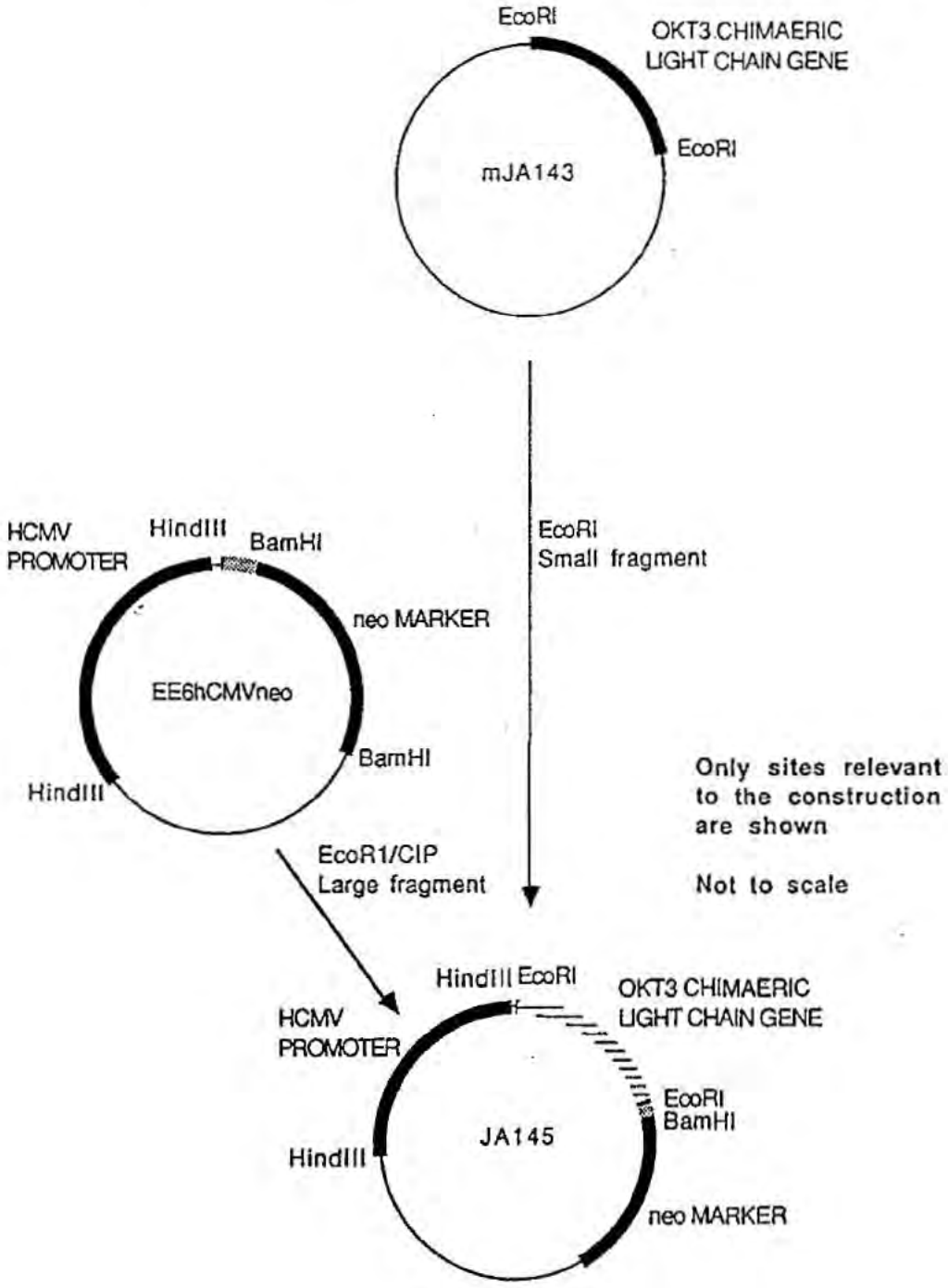


FIGURE 13
An outline schematic of the procedures involved in theconstruction of pJA145, a vector for the expression in eukaryotic cells of the OKT3 chimaeric light chain gene (Version 1).

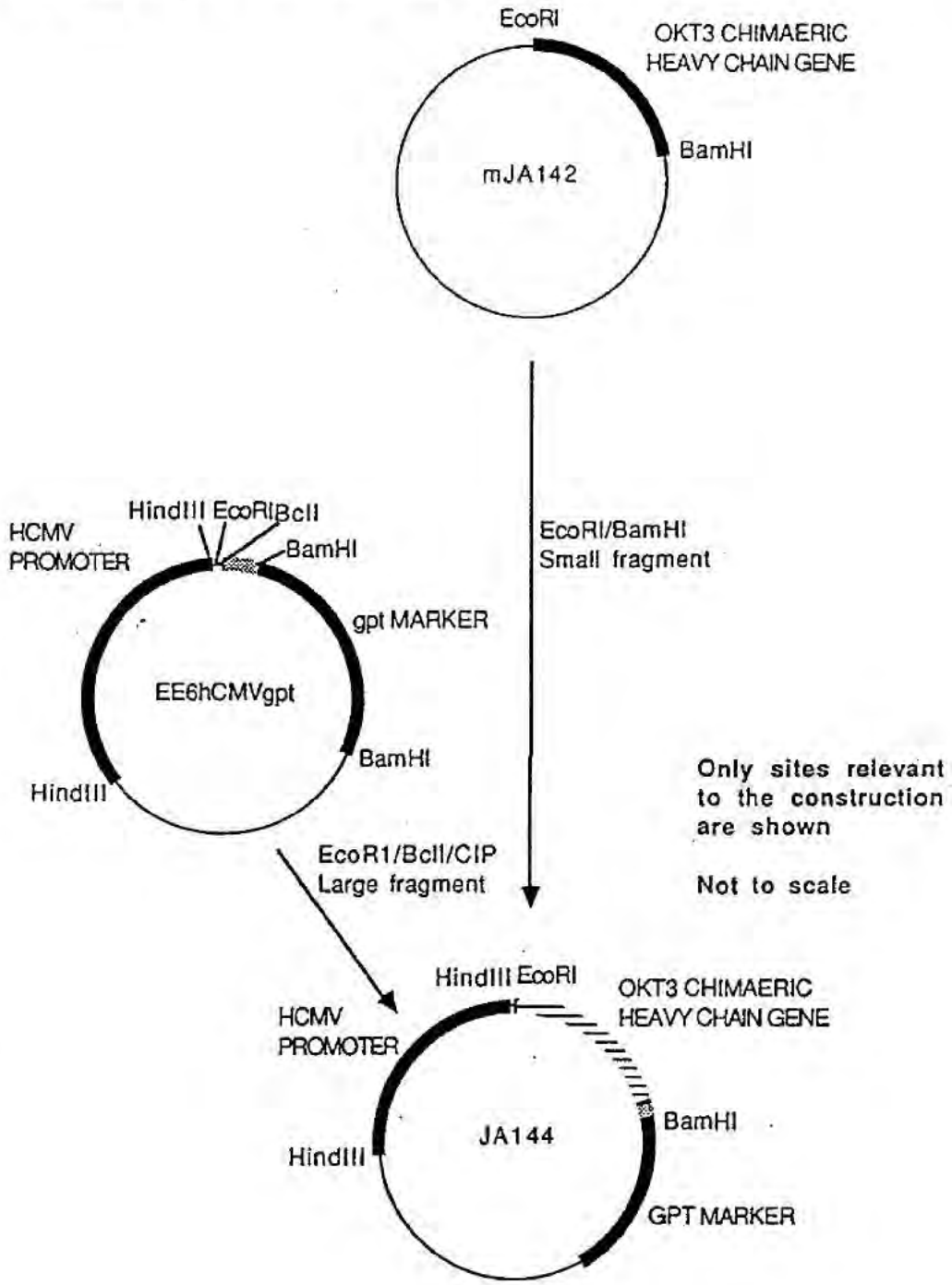


FIGURE 14
An outline schematic of the procedures involved in the construction of pJA144, a vector for the expression in eukaryotic cells of the OKT3 chimaeric heavy chain gene.

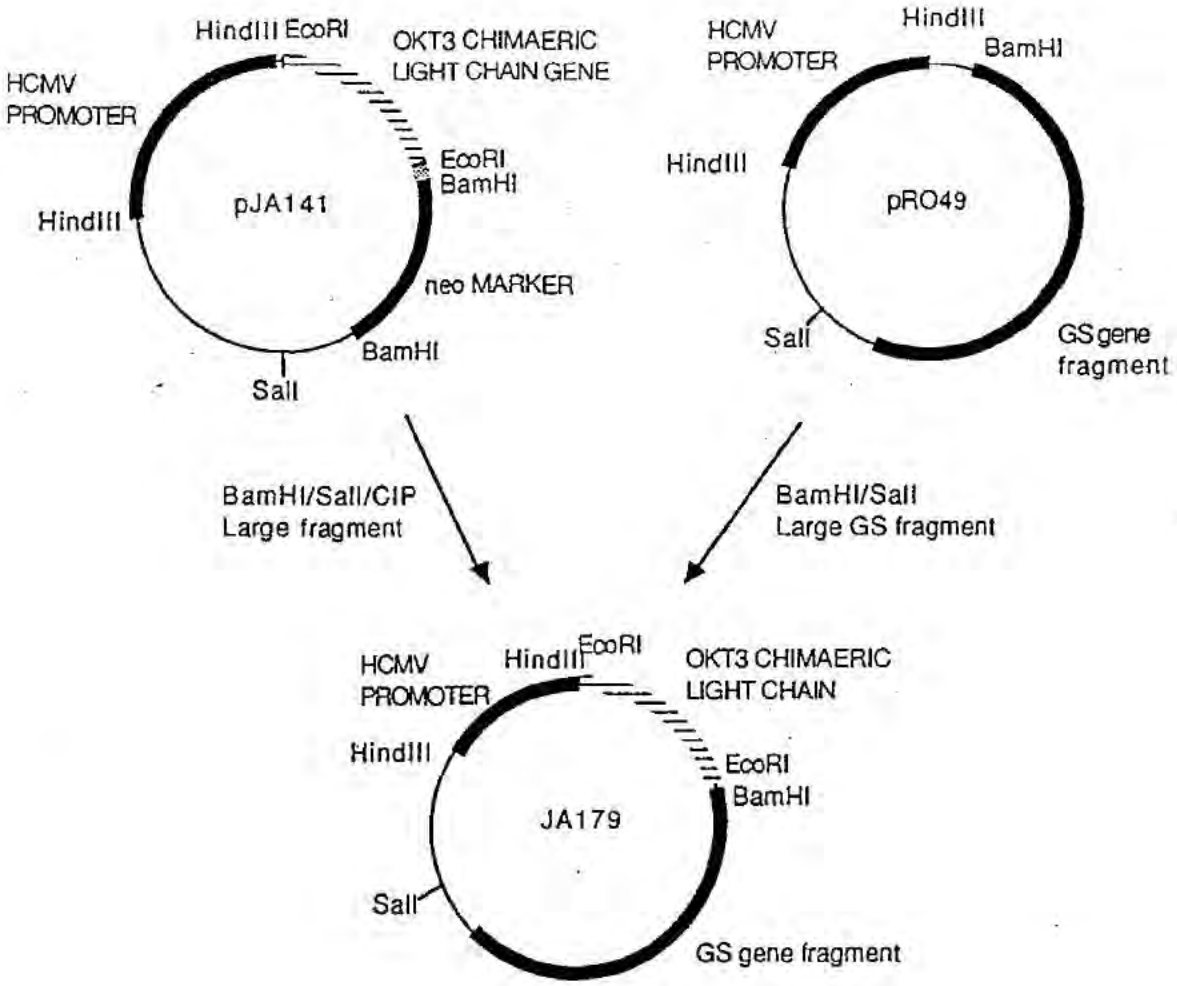


FIGURE 15
An outline schematic of the procedures involved in the construction of pJA179, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric light chain gene (Version 2).

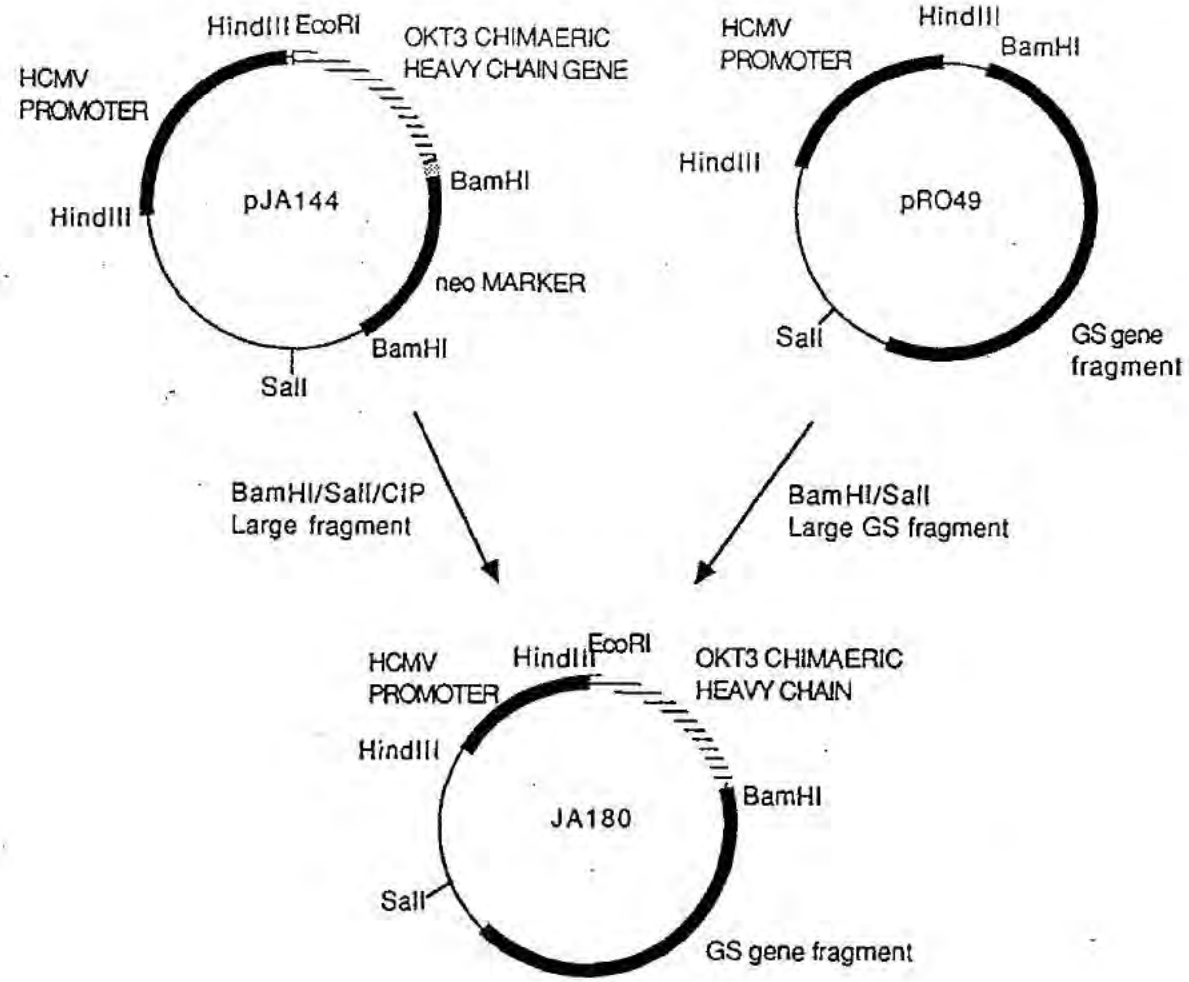


FIGURE 16
An outline schematic of the procedures involved in the construction of pJA179, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric heavy chain gene.

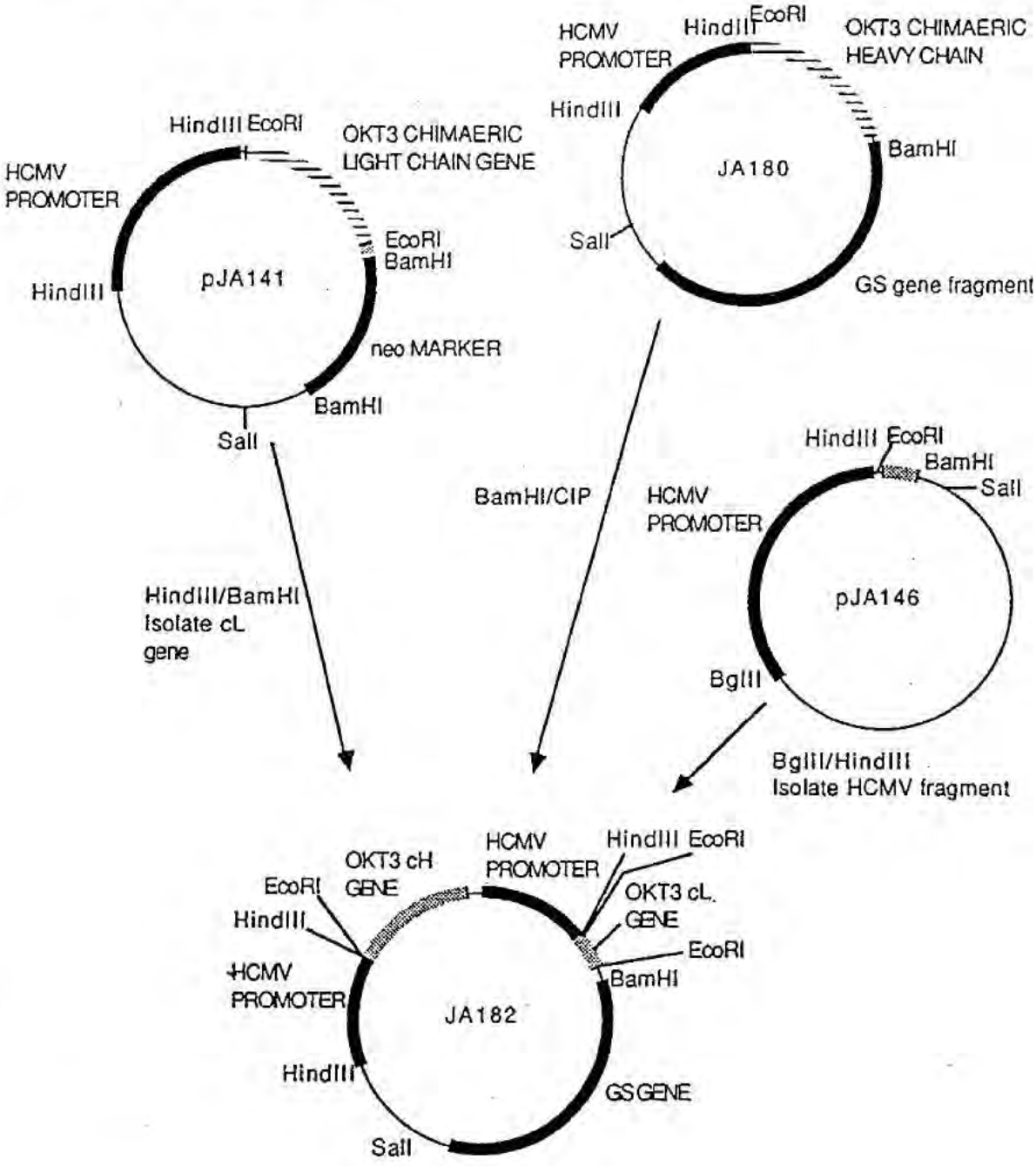


FIGURE 17
An outline schematic of the procedures involved in the construction of pJA182, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric heavy chain gene and chimaeric light chain gene (Version 2) in the transcription order cH>cL>GS.

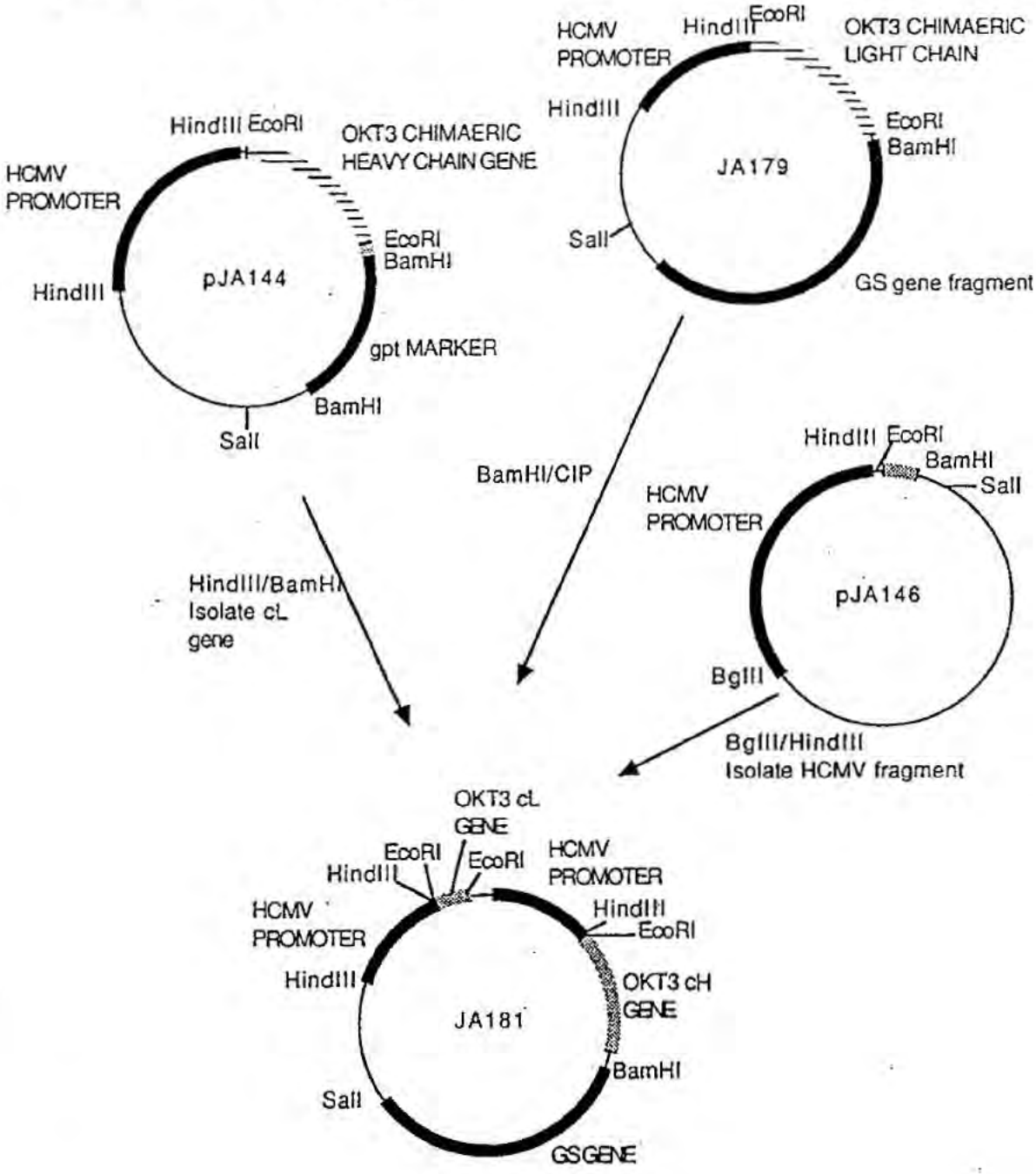


FIGURE 18
An outline schematic of the procedures involved in the construction of pJA181, a vector for the expression in eukaryotic cells using the GS amplification system, of the OKT3 chimaeric light chain gene (Version 2) and chimaeric heavy chain gene in the transcription order cL>cH>GS.

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8928874.0

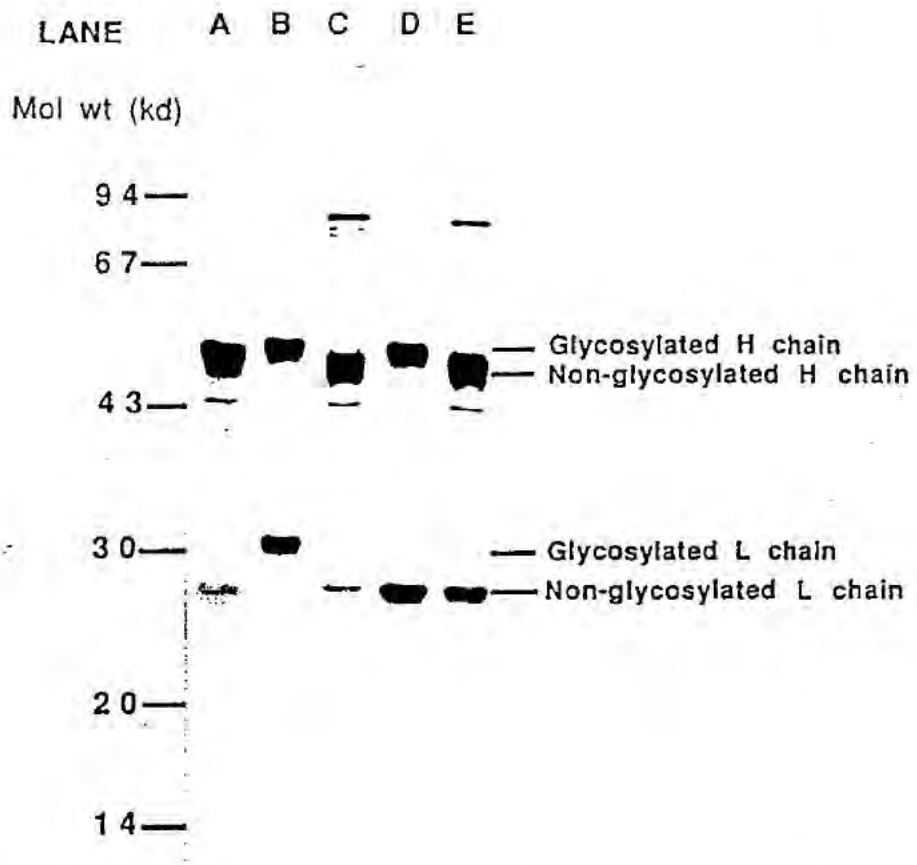


Fig 19. Effect on glycosylation of the presence of tunicamycin during cell growth

Reducing SDS-PAGE of ^{35}S labelled antibody produced in the absence (lanes A ,B, D) or presence (lanes C & E) of tunicamycin. COS cells were transfected and medium replaced after 24hrs by medium with or without tunicamycin. Antibody was recovered from culture supernatants by protein-A Sepharose precipitation. after 48hrs further incubation.

Key:

A. - cLcH B72.3 control
B. - cL*cH - Tunicamycin
C. - cL*cH + Tunicamycin
D. - cLcH - Tunicamycin
E. - cLcH + Tunicamycin

NB: cL* - chimaeric light chain version 1
cL - chimaeric light chain version 2

```
                1                   23                  42
                NN      N           N       N       N
 RES TYPE   SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3v1      QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI         DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
            ? ?
               CDR1   (LOOP)          *******
               CDR1   (KABAT)        ***********

                          56                            85
            N  NN
 RES TYPE   *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3v1      SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI         APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
            ?  ??                          ?  ?
                    ******* CDR2   (LOOP/KABAT)

                          102   108
RES TYPE    PiPIPies**iPIIsPPSPSPSS
Okt3v1      YYCQQWSSNPFTFGSGTKLEINR
REIv1       YYCQQYQSLPYTFGQGTKLQITR
                         ?     ?
                  ******        CDR3   (LOOP)
                 *********      CDR3   (KABAT)
```

KEY TO RES TYPE

N NEAR TO CDR (FROM X RAY STRUCTURES)

P PACKING — B BURIED NON PACKING

S SURFACE — E EXPOSED

I INTERFACE — * INTERFACE/PART EXPOSED

^ PACKING/PART EXPOSED

? NON-CDR RESIDUES WHICH MAY REQUIRE TO BE LEFT AS MOUSE SEQUENCE

FIGURE 20

The alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI. Above the sequence the residue type (defined in the key) notes the spatial location of each residue side chain (derived by examination of resolved structures from Xray crystallography analysis). Residues in bold type refer to amino acids which differ from the residue found at that position in the consensus sequence for the species subgroup to which the antibody belongs ie. mouse sub group 6 for the OKT3 sequence and human sub group 1 for the REI sequence.

```
          NN N                  23 26    32 35 N39  43
RES TYPE  SESPs^SBssS^sSSsSpSpSPsPSEbSBssBePiPIpiesss
Okt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
KOL       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
               ?                ??
                                   ******     CDR1 (LOOP)
                                      *****   CDR1 (KABAT)

                52a      60  65     N N  N    82abc     89
RES TYPE  IIeIppp^ssssssss^ps^pSSsbSpseSsSseSp^pSpsSBssS^ePb
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
KOL       GLEWVAIIWDDGSDQHYADSVKGRFTISRDNSKNTLFLQMDSLRPEDTGV
             ??                      ? ?  ?  ?            ?
                ************        CDR2  (LOOP)
                ******************* CDR2  (KABAT)

           92 N                  107    113
RES TYPE  PiPIEissssiiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.......CLDYWGQGTTLTVSS
KOL       YFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS
               ***************** CDR3 (KABAT/LOOP)
```

KEY TO RES TYPE

N NEAR TO CDR (FROM X RAY STRUCTURES)

P PACKING — B BURIED NON PACKING

S SURFACE — E EXPOSED

I INTERFACE — * INTERFACE/PART EXPOSED

^ PACKING/PART EXPOSED

? AMINO ACIDS WHICH MAY NEED TO REMAIN AS MOUSE IN CDR GRAFT

FIGURE 21

The alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL. Above the sequence the residue type (defined in the key) notes the spatial location of each residue side chain (derived by examination of resolved structures from Xray crystallography analysis). Residues in bold type refer to amino acids which differ from the residue found at that position in the consensus sequence for the species subgroup to which the antibody belongs ie. mouse sub group 2B for the OKT3 sequence and human sub group 3 for the KOL sequence.

```
  1  AATTCATGGA ATGGAGCTGG GTCTTTCTCT TCTTCCTGTC AGTAACTACA
 51  GGTGTCCACT CCCAGGTTCA GCTGGTGGAG TCTGGAGGAG GAGTCGTCCA

                                                26  27 28 29 30
                                                G   Y  T  F  T
101  GCCTGGAAGG TCCCTGAGAC TGTCTTGTTC TTCTTCTGGA TACACATTCA
                                  oligo JA88-44 cct atgtgtaagt...
                                                 PROBE JA88-45
                                                 G   Y  T  F

      31 32 33  34 35
       D  H  A   M  Y  W
151  CAGACCACGC TATGTACTGG GTCAGACAGG CTCCTGGAAA GGGACTGGAG
       ****  *      *                          oligo JA88-40    c...
  ...gttctatgtg atacgtgacc cagtctgtcc 5' R1198
     gttctatgtg atacgt 5' R1197
     T  R  Y  T   M  H  W

              50  51 51 52a 53 54 55  56 57 58  59 60 61 62
               Y   I  S  P   G  N  D   D  I  K   Y  N  E  K
201  TGGGTCGCTT ACATCTCTCC TGGAAATGAC GACATCAAGT ACAATGAGAA
                  ***     * * ** ** *   **  *
  ...acccagcgaa tgtaattagg atcgtctcct atgtgtttaa tgttagtctt...
       PROBE JA88-41  gg atcgtctcct atgtgttta 5' R1153
            Y   I  N  P   S  R  G   Y  T  N  Y   N  Q  K

      63 64 65  66
      F  K  G   R
251  GTTCAAGGGA AGATTCACAA TTTCTAGAGA CAATTCTAAG AATACACTGT
           **
  ...caagttcctg tctaagtgtt aaagatc 5' R1152
      F  K  D   R

301  TCCTGCAGAT GGACTCACTC AGACCTGAGG ACACAGGAGT CTACTTCTGT
                                  oligo JA88-42 tgaagaca

           95 96  97 98 99  100  a  b 101102
           S  Y   Y  G  H             D  Y
351  GCTAGATCCT ACTACGGCCA C......... GACTACTGGG GCCAAGGTAC
           *     *   *    *********
     cgatctatga tgctgctggt gatgacagac ctgatgaccc cggtt 5' R1154
PROBE JA88-43 a tgctgctggt gatg 5' R1155
           Y  Y   D  D  H   Y  C  L   D  Y

401  CCCGGTCACC GTGAGCTC
```

KEY

LINE 1 AMINO ACID SEQUENCE NUMBERS (KABAT NOMENCLATURE)
LINE 2 AMINO ACID SEQUENCE OF B72.3 GH341V_H REGION (PARENT)
LINE 3 NUCLEOTIDE SEQUENCE OF JA148 (B72.3 GH341 PARENT SEQ)
LINE 4 * LOCATION OF POINT MUTATIONS
LINE 5 NUCLEOTIDE SEQUENCE OF MUTAGENIC OLIUGONUCLEOTIDES
LINE 6 NUCLEOTIDE SEQUENCE OF PROBE OLIGONUCLEOTIDES
LINE 7 AMINO ACID SEQUENCE OF MUTATED SEQUENCE (GH341 OKT3)

FIGURE 22A

The DNA sequence of the B72.3 grafted heavy (gH341) sequence (J Adair and A Docherty unpublished) and the sequences of oligonucleotides necessary to replace the CDR regions with OKT3 CDRs and to act as specific probes for the desired alterations.

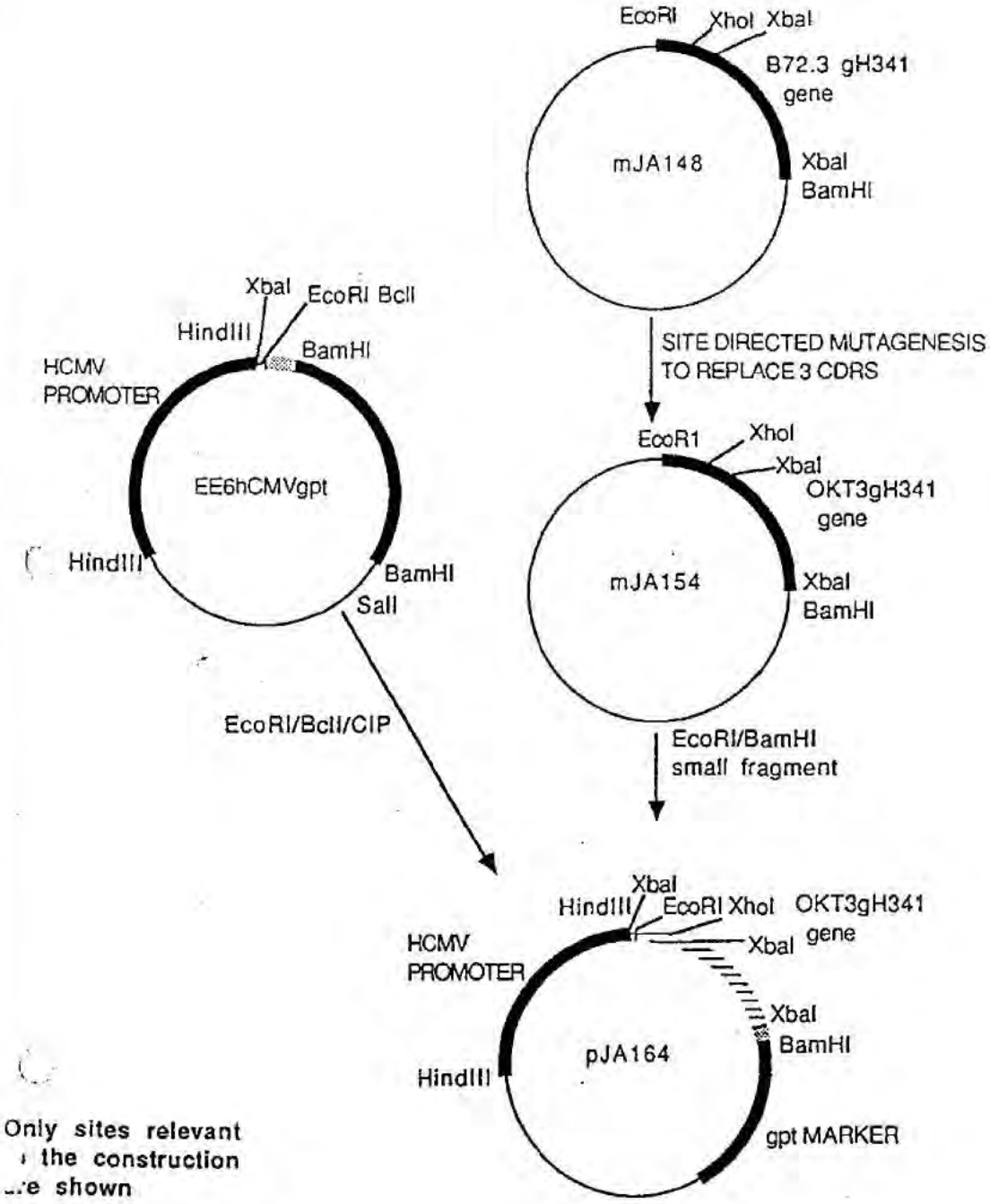
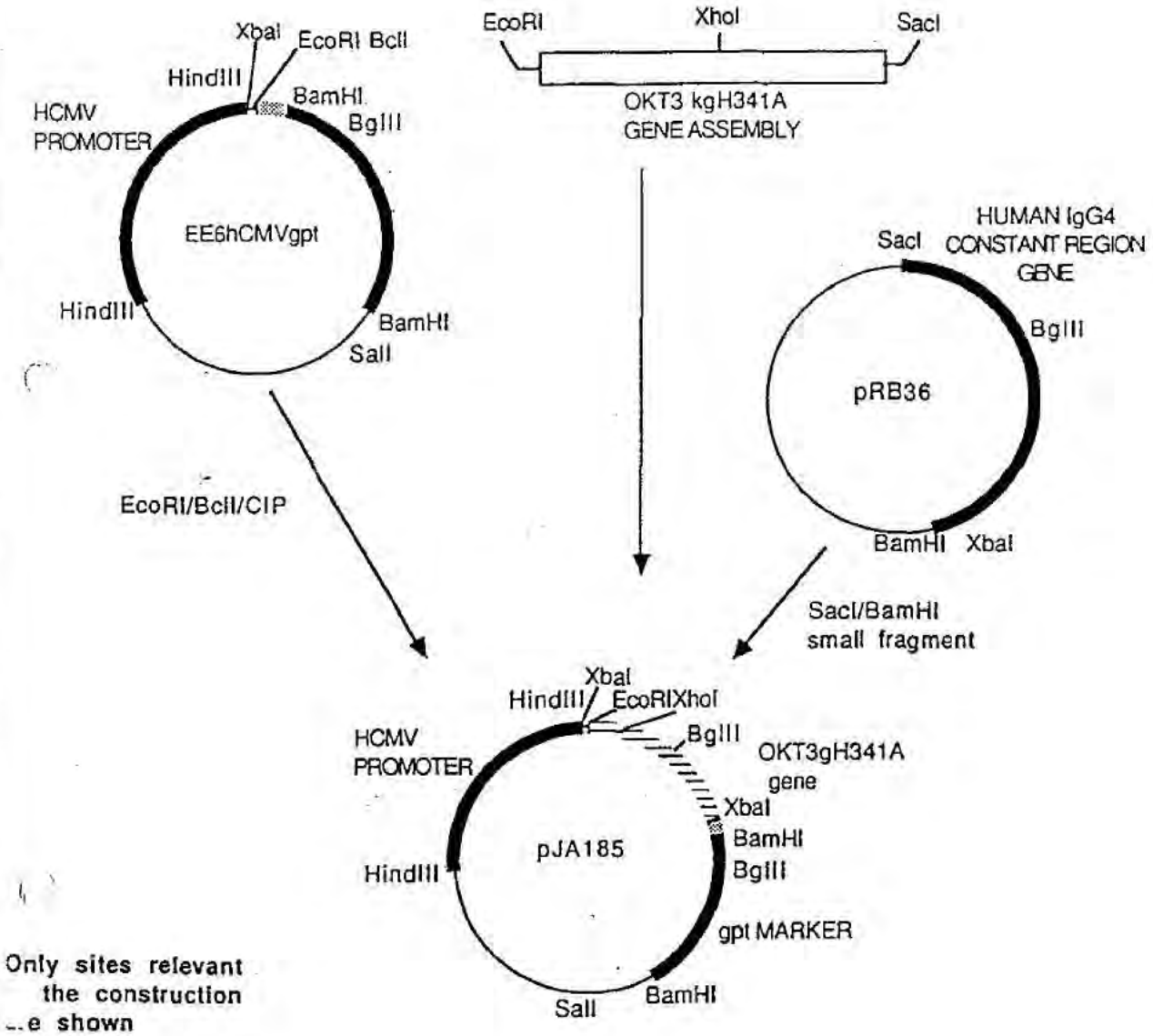


FIGURE 22B
An outline schematic of the procedures involved in the construction of pJA164, a vector for the expression in eukaryotic cells of the OKT3 gH341 CDR grafted heavy chain gene.

```
                    KOZAK SEQ   SIGNAL SEQ
                                M  E  W  S  W  V  F  L  F  F  L  S  V
R1387   1  AATTCGCCGCCACCATGGAATGGAGCTGGGTCTTTCTCTTCCTGTCAGTA      33
R1388   2     GCGGCGGTGGTACCTTACCTCGACCCAGAAAGAGAAGAAGGAC          43

                                MATURE V_H
                    T  T  G  V  H  S  Q  V  Q  L  V
R1089   3           ACTACAGGTGTCCACTCCCAGGTTCAGCTGGTG              33
R1091   4  AGTCATTGATGTCCACAGGTGAGGGTCCAAGTC                      33

                    q  S  G  G  G  V  V  Q  P  G
R1594   5           cAGTCTGGAGGAGGAGTCGTCCAGCCTGGA                 30
R1595   6  GACCACgTCAGACCTCCTCCTCAGCAGGTC                         30

                    R  S  L  R  L  S  C  k  a
R1540   7           AGGTCCCTGAGACTGTCTTGTaaggct                    27
R1095   8  GGACCTTCCAGGGACTCTGACAGAACA                            27

                    S  G  Y  T  F  T  R  Y  T  M  H                CDR1
R1385   9           TCTGGATACACCTTCACTAGATACACAATGCAC               33
R1590   10 ttccgaAGACCTATGTGGAAGTGATCTATGTGTTACGTGACCCAG            45

                 W  V  R  Q  A  P  G  K  G  L  E  W  i
R1591   11       TGGGTCAGACAGGCTCCTGGAAAGGGACTCGAGTGGatt            39
R1258   12             TCTGTCCGAGGACCTTTCCCTGAGCTC                  27
                                          -XhoI-

                    g  Y  I  N  P  S  R  G  Y  T  N  Y
R1586   13          ggaTACATTAATCCTAGCAGAGGTTATACTAACTAC
R1587   14  ACCtaacctATGTAATTAGGATCGTCTCCAATATGATTGATG

                 N  Q  K  v  K  D  R                            CDR2
                 AATCAGAAGgtgAAGGACAGA                          57
                 TTAGTCTTCcacTTCCTGTCTAAGTGT                    69

                 F  T  I  S  t  D  k  S  K  s  T  a
R1599   15       TTCACAATTTCTactGACaaaTCTAAGagcACAgcc            36
R1600   16             TAAAGAtgaCTGtttAGATTCtcg                  24

                    F  L  Q  M  D  S  L  R  P
R1106   17          TTCCTGCAGATGGACTCACTCAGACCT                    27
R1601   18  TGTcggAAGGACGTCTACCTGAGTGAG                            27

                    E  D  T  a  V  Y  y  C  A
R1680   19          GAGGATACCGccGTCTAtTatTGTGCT                    27
R1681   20  TCTGGACTCCTATGGCggCAGATaAta                            27

                    R  Y  Y  D  D  H  Y  C  L  D  Y  W             CDR3
R1426   21          AGATATTACGATGACCACTACTGTCTGGACTACTGG            36
R1427   22  ACACGATCTATAATGCTACTGGTGATGACAGACCTGATGACCCCGGTT       48

                 G  Q  G  T  P  V  T  V  S  S  a
R1114   23       GGCCAAGGTACCCCGGTCACCGTGAGCTC                  29
R1115   24             CCATGGGGCCAGTGGCACTCGAGTCGA               27
                                             >CH1 DOMAIN
```

FIGURE 23A

The sequences of oligonucleotides necessary to construct, by oligonucleotide assembly procedures, the OKT3 CDR grafted kgH341A gene. Above the nucleotide sequences are shown the peptide sequences coded by the oligonucleotides. Lower case nucleotide and amino acid residues show differences compared to the gH341 sequence (see Fig 22A).



Only sites relevant
the construction
..e shown

OT TO SCALE

FIGURE 23B
An outline schematic of the procedures involved in theconstruction of pJA185,
a vector for the expression in eukaryotic cells of the OKT3
gH341 CDR grafted heavy chain gene.

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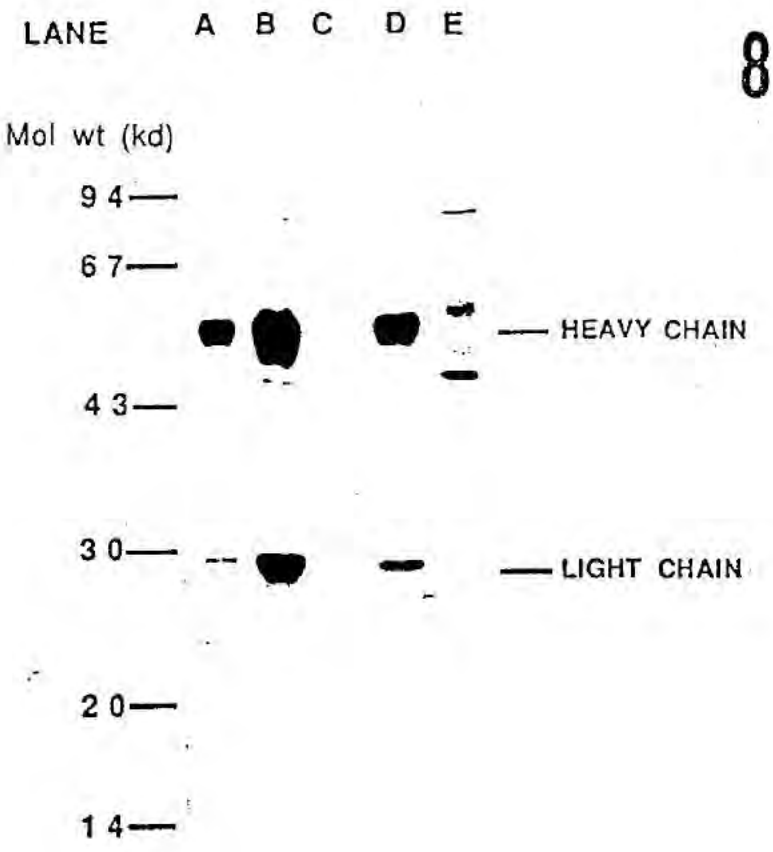

Fig 24a. Effect on antibody expression of the presence of a "Kozak consensus sequence" immediately preceding the gL gene.

Reducing SDS-PAGE of ^{35}S labelled antibody purified from a COS cell transient expression experiment by Protein A-Sepharose precipitation.

KEY:
A. - gL221 cH
B. - gLK221 cH
C. - gL221A cH
D. - gLK221A cH
E. - Mock transfection

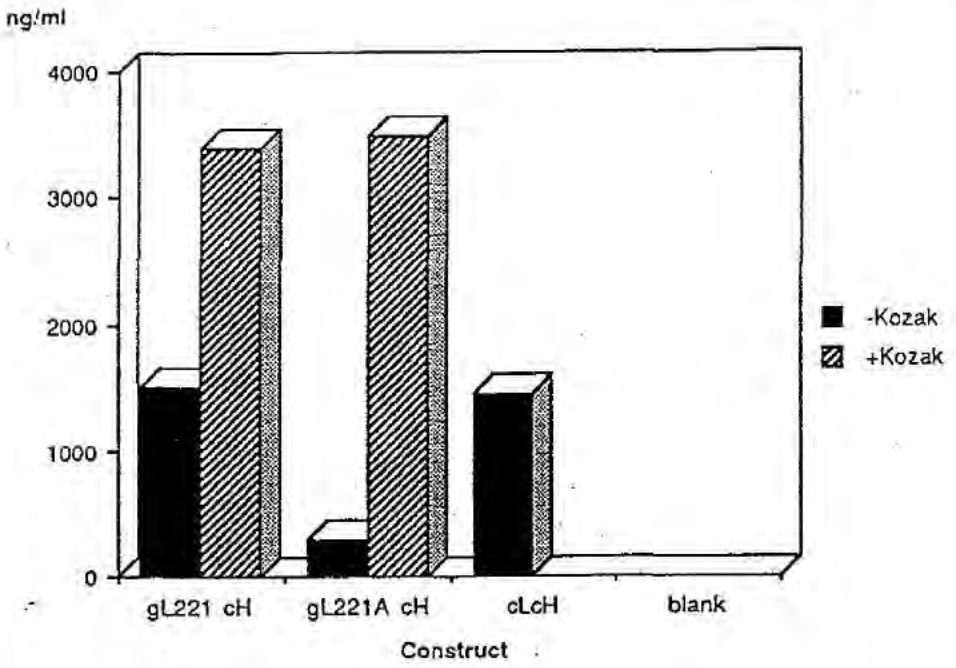

Fig 24b. Effect on antibody expression in the presence of the Kozak consensus sequence immediately preceding the gL gene.

Yield of antibody (ng/ml) from COS cell transient expression experiment.

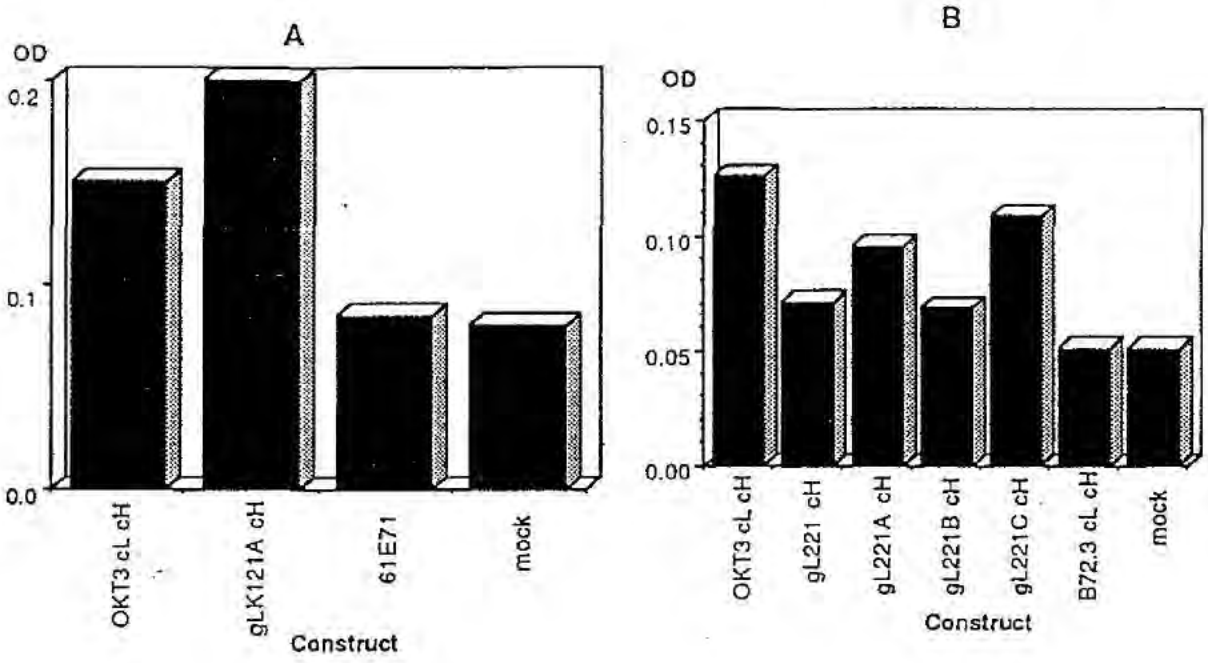

Fig 25. Antigen binding data for gL series genes

Culture supernatants from COS cell transient expression experiments. Various combinations of gL and cH genes were tested for binding to Hut 78 cells.

Chimaeric B72.3 or chimaeric 61E71 was used as a negative control

For codes to genes see table 1.

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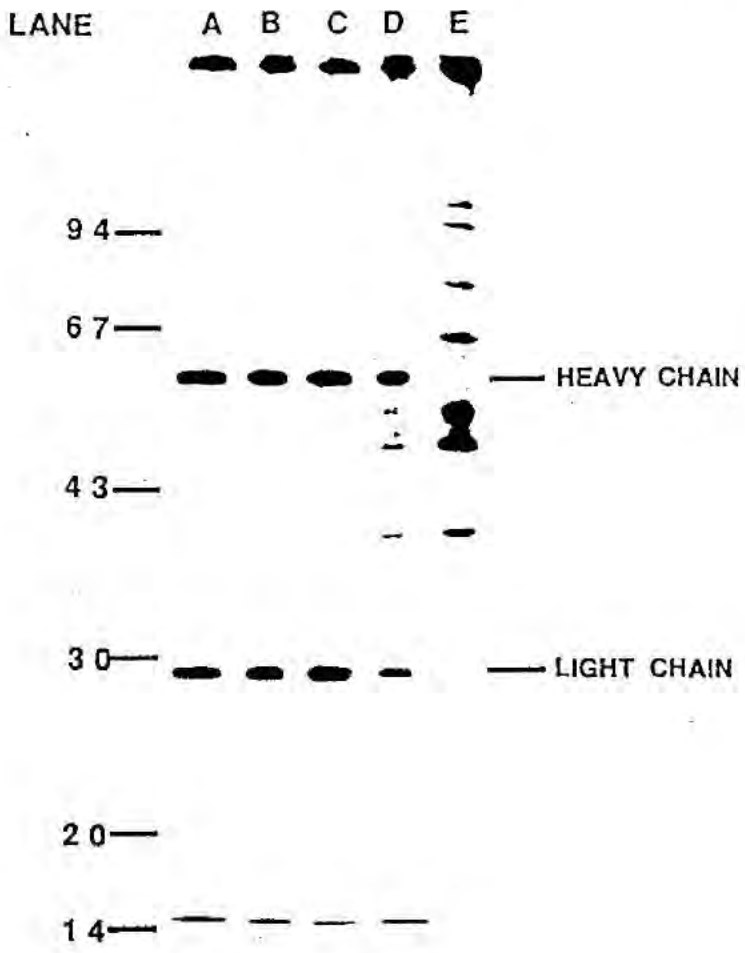

Fig 26. Effect on antibody expression of the presence of a "Kozak consensus sequence" immediatly preceding the gH gene.

Reducing SDS-PAGE of ^{35}S labelled antibody purified from a COS cell transient expression experiment by Protein A-Sepharose precipitation.

KEY:
A. - gH331 cL
B. - gHK331 cL
C. - gH341 cL
D. - gHK341 cL
E. - Mock transfection

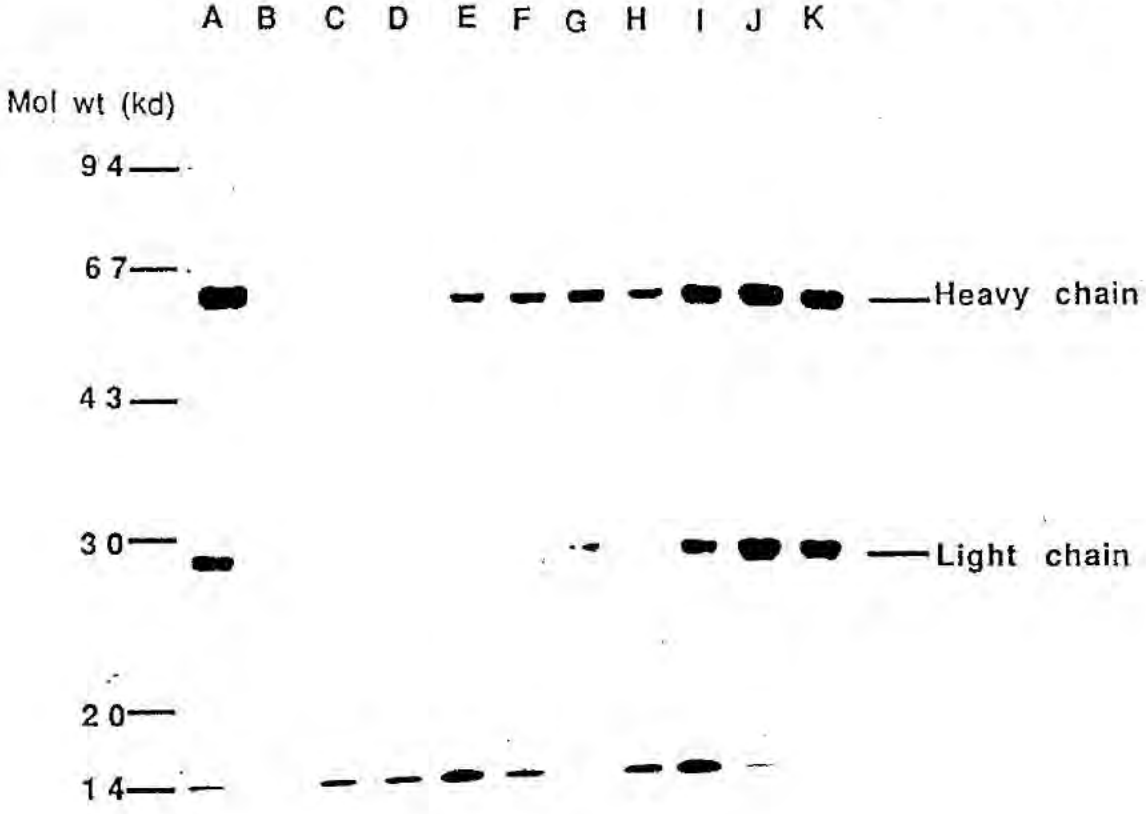

Fig 27. Expression of gH chain genes with cL chain

Reducing SDS-PAGE of ^{35}S labelled antibody produced from COS cell transient expression experiment. Antibody was recovered from culture supernatant by binding to polyclonal anti-human $F(ab')_2$ and then by precipitation with Protein A-Sepharose.

KEY:

A. cLcH OKT3
B. gHK121 cL
C. gHK131 cL
D. gH141 cL
E. gH321 cL
F. gH331 cL
G. gHK331 cL
H. gH341 cL
I. gHK341 cL
J. gHK341B cL
K. gHK341A cL

1. CDR GRAFTED LIGHT (gL) WITH MOUSE (mH) OR CHIMAERIC (cH) HEAVY CHAIN GENES

GENE COMBINATIONS		ANTIGEN BINDING	EXPRESSION
gL121	cH	-	+
KgL121A	cH	+	+
gL221	cH	+/-	+
KgL221	cH	-	++
gL221A	cH	+	+
KgL221A	cH	+	++
gL221B	cH	-	+
KgL221B	cH	-	++
gL221C	cH	+	+
KgL221C	cH	+	++

2. CDR GRAFTED HEAVY (gH) WITH MOUSE (mL) OR CHIMAERIC (cL) LIGHT CHAIN GENES

GENE COMBINATIONS		ANTIGEN BINDING	EXPRESSION
KgH121	mL	not det.	-
KgH121	cL	not det.	-
KgH131	mL	not det.	-
KgH131	cL	not det.	-
gH141	mL	-	+/-
gH141	cL	-	+/-
gH321	cL	-	+
gH331	cL	-	+
KgH331	cL	-	+
gH341	mL	+	+
gH341	cL	+/-	+
KgH341	cL	+/-	+
KgH341A	cL	+	+
KgH341B	cL	+	+

3. FULLY CDR GRAFTED ANTIBODY

GENE COMBINATIONS		ANTIGEN BINDING	EXPRESSION
KgL221A	KgH121	not det.	-
KgL221A	KgH131	not det.	-
KgL221A	gH141	not det.	-
KgL221A	KgH331	not det.	-
KgL221A	gH341	not det.	-
KgL221A	KgH341	not det.	-
KgL221A	KgH341A	+	+
KgL221A	KgH341B	+	+

KEY		
	L	LIGHT CHAIN GENE (SEE TABLE 1 FOR NUMBER CODE)
	H	HEAVY CHAIN GENE
	m	MOUSE
	c	CHIMAERIC
	g	CDR GRAFTED
	K	PRESENCE OF KOZAK CONSENSUS SEQUENCE
	not det.	NOT DETERMINED (EXPRESSION LEVELS TOO LOW)

TABLE 2
A summary of the expression and antigen binding data for the CDR grafted genes constructed in this study

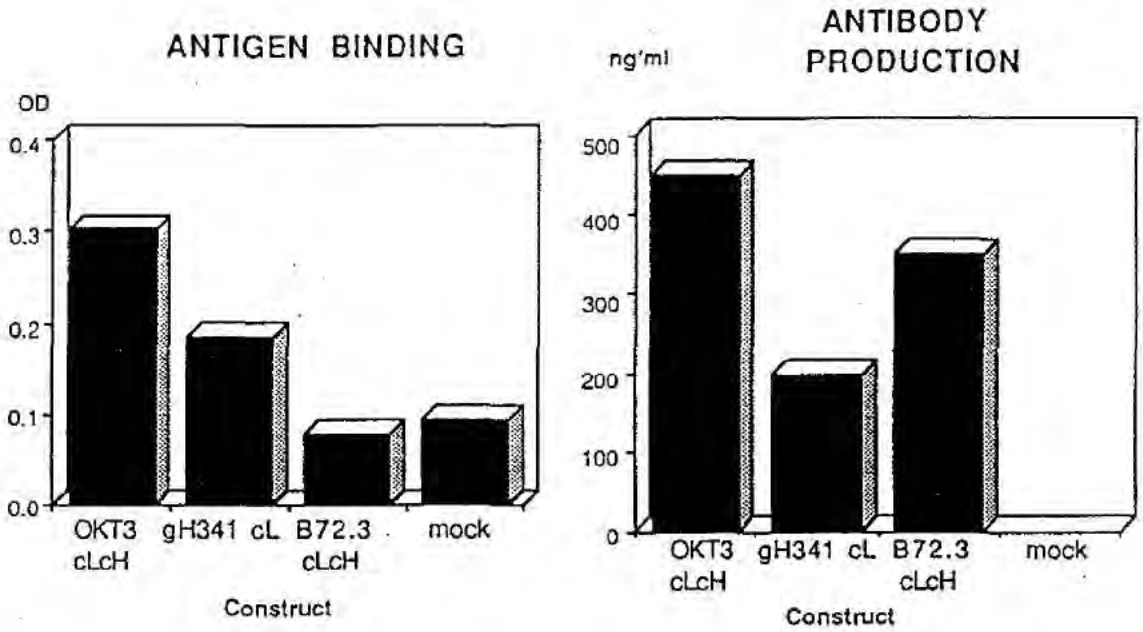

Fig 28 Antigen binding assay for gH341 cL gene combination.

Culture supernatants from COS cell transient expression experiments were tested for binding to Hut-78 cells (panel A) and for yield of assembled antibody (panel B). Chimaeric B72.3 was included as a negative control.

Note. Poor expression of gH341 cL gene combination.

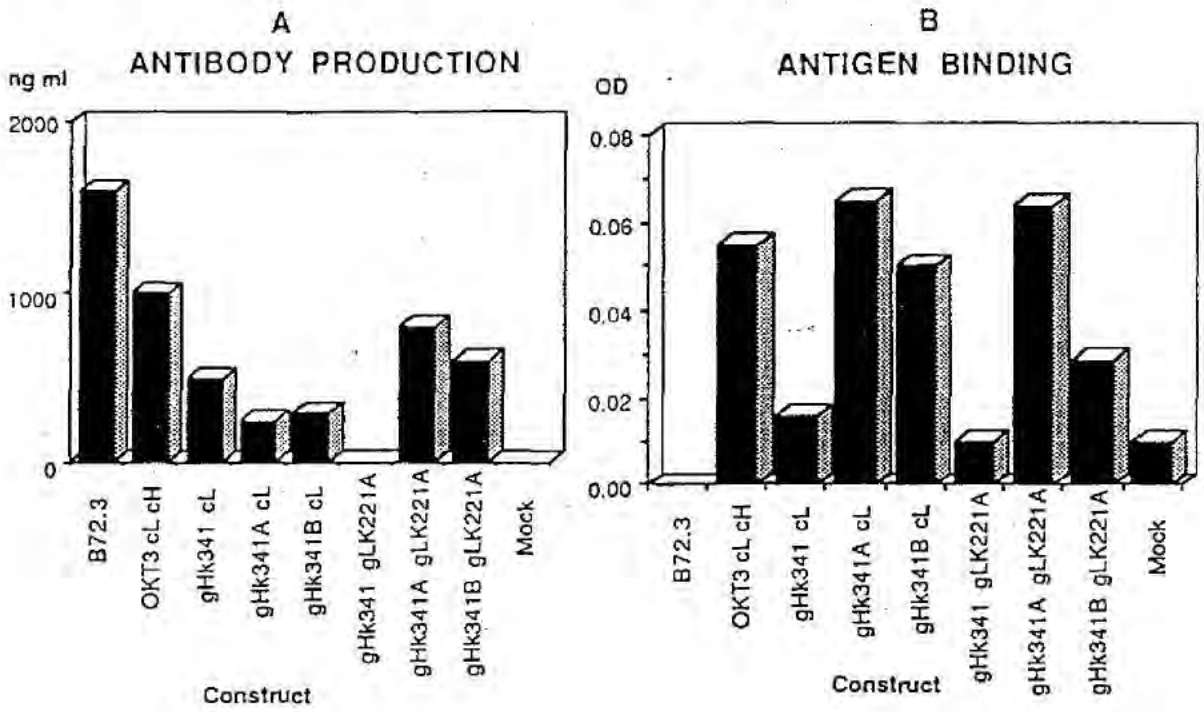

Fig 29 Antigen Binding assay for grafted OKT3 combinations

Culture supernatant from COS cell transient expression experiments were tested for yield of assembled antibody (Panel A) and for binding to Hut 78 cells (Panel B). Chimaeric B72.3 was included as a negative control. Panels show level of antibody produced and resultant antigen binding for various combinations of heavy and light chain genes cotransfected into COS cells.
NB: In panel B binding data has been normalised so that the level of binding B72.3 is set to zero.

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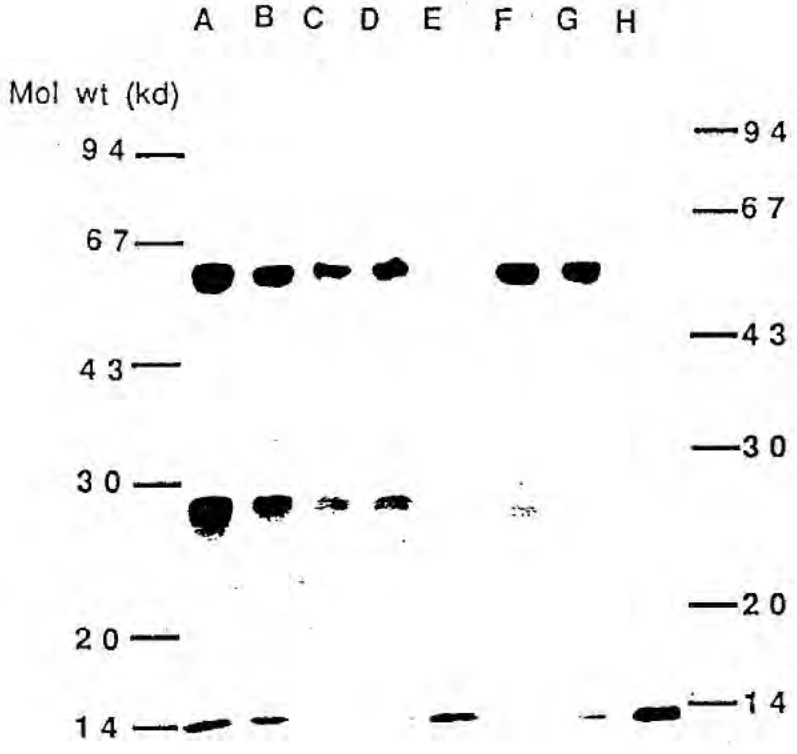

Fig 29c. gH341 series cotransfected with cL and gLK221A

Key:
A. cL cH OKT3
B. gHK341 cL
C. gHK341A cL
D. gHK341B cL
E. gHK341 gLK221A
F. gHK341A gLK221A
G. gHK341B gLK221A
H. Mock transfection