composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

4. An anti-CD3 antibody molecule having affinity for the CD3 antigen comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said CD3 antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

5. An anti-CD4 antibody molecule having affinity for the CD4 antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said CD4 antigen, wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

6. An anti-adhesion molecule antibody molecule having affinity for an adhesion molecule and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human

acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having affinity for said adhesion molecule wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues.

7. The antibody molecule of any one of claims 2 to 6 wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

8. The antibody molecule of any one of claims 1 to 7, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

9. The antibody molecule of any one of claims 1 to 8, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

10. The antibody molecule of any one of claims 1 to 9, wherein at least one of amino acid residues 36, 94, 104, 106 and 107 in said composite heavy chain are additionally donor residues.

11. The antibody molecule of claim 10, wherein at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 in said composite heavy chain are additionally donor residues.

12. The antibody molecule of any one of claims 1 to 11 wherein amino acid residues 7, 9, 11, 18, 20, 25, 37, 39, 41, 45, 47, 48, 72, 75, 80, 82, 86 to 89, 91, 93, 103, 108, 110 and 112 in said composite heavy chain are additionally acceptor residues.

13. The antibody molecule of any one of claims 1 to 12,

wherein said complementary light chain is a composite light chain having a variable domain comprising acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79, 81, 82, 84, 86, 88, 100, 104, 106 and 107 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

14. The antibody molecule of claim 13, wherein amino acid residues 1, 3 and 47 in said composite light chain are additionally donor residues.

15. The antibody molecule of claim 13 or claim 14, wherein amino acid residues 36, 44, 47, 85 and 87 in said composite light chain are additionally donor residues.

16. The antibody molecule of any one of claims 13 to 15, wherein at least one of amino acid residues 2, 4, 6, 49, 62, 64 to 69, 98, 99, 101 and 102 in said composite lightly chain are additionally donor residues.

17. The antibody molecule of any one of claims 13 to 16, wherein at least one of amino acid residues 1, 3, 10, 12, 21, 40, 60, 63, 70, 73, 80, 103 and 105 in said composite light chain are additionally donor residues.

18. A therapeutic or diagnostic composition comprising the antibody molecule of any one of claims 1 to 17 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

19. A method for producing a recombinant antigen binding molecule having affinity for a predetermined antigen comprising the steps of:

[1] determining the amino acid sequence of the variable domain of the heavy chain of a donor antibody which has affinity for said predetermined antigen;

[2] determining the amino acid sequence of the variable domain of the heavy chain of a non-specific acceptor antibody;

[3] providing a composite heavy chain for an antibody molecule, said composite heavy chain having acceptor framework residues and donor antigen binding residues wherein, according to the Kabat numbering system, amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues and amino acid residues 23, 24, 31 to 35, 49 to 58 and 95 to 102 at least are donor residues;

[4] associating the heavy chain produced in step [3] with a complementary light chain to form an antibody molecule;

[5] determining the affinity of the antibody molecule formed in step [4] for said predetermined antigen;

[6] if the affinity determined in step [5] is not equivalent to that of the donor antibody, providing a heavy chain as described in [3] above but in which amino acid residues 71, 73 and 78 are additionally donor residues;

[7] associating the heavy chain produced in step [6] with a complementary light chain to form an antibody molecule;

[8] determining the affinity of the antibody molecule formed in step [7] for said predetermined antigen;

[9] if the affinity determined in step [8] is not equivalent to that of the donor antibody, providing a heavy chain as described in [6] above but in which amino acid residues 26 to 30 are additionally donor residues;

[10] associating the heavy chain produced in step [9] with a complementary light chain to form an antibody molecule;

[11] determining the affinity of the antibody molecule formed in step [10] for said predetermined antigen;

[12] if the affinity determined in step [11] is not equivalent to that of the donor antibody, providing a heavy chain as described in [9] above but in which at least one of amino acid residues 1, 3, and 76 are additionally donor residues;

[13] associating the heavy chain produced in step [12] with a complementary light chain to form an antibody molecule;

[14] determining the affinity of the antibody molecule formed in step [13] for said predetermined antigen;

[15] if the affinity determined in step [14] is not equivalent to that of the donor antibody, providing a heavy chain as described in [12] above but in which at least one of amino acid residues 36, 94, 104, 106, 107 are additionally donor residues;

[16] associating the heavy chain produced in step [15] with a complementary light chain to form an antibody molecule.

[17] determining the affinity of the antibody molecule formed in step [16] for said predetermined antigen;

[18] if the affinity determined in step [17] is not equivalent to that of the donor antibody, providing a heavy chain as described in [15] above but in which at least one of amino acid residues 2, 4, 6, 38, 48, 67 and 69 are additionally donor residues; and

[19] associating the heavy chain produced in step [18] with a complementary light chain to form an antibody molecule.

20. The method of claim 19, further comprising the steps of:

[1] determining the amino acid sequence of the variable domain of the light chain of said donor antibody which has affinity for said predetermined antigen;

[2] determining the amino acid sequence of the variable domain of the light chain of a non-specific acceptor antibody;

[3] providing a composite light chain for an

antibody molecule, said composite light chain having acceptor framework residues and donor antigen binding residues wherein, according to the Kabat numbering system, amino acid residues 5, 7 to 9, 11, 13 to 18, 20, 22, 23, 39, 41 to 43, 57, 59, 61, 72, 74 to 79 to 79, 81, 82, 84, 86, 88, 100, 104 and 106 to 109 at least are acceptor residues and amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues;

[4] associating the light chain produced in step [3] with a complementary heavy chain to form an antibody molecule;

[5] determining the affinity of the antibody molecule formed in step [4] for said predetermined antigen;

[6] if the affinity determined in step [5] is not equivalent to that of the donor antibody, providing a light chain as described in [3] above but in which amino acid residues 1, 2, 3 and 47 are additionally donor residues;

[7] associating the light chain produced in step [6] with a complementary heavy chain to form an antigen-binding molecule;

[8] determining the affinity of the antigen-binding molecule formed in step [7] for said predetermined antigen;

[9] if the affinity determined in step [8] is not equivalent to that of the donor antibody, providing a light chain as described in [6] above but in which amino acid residues 36, 44, 47, 85 and 87 are additionally donor residues;

[10] associating the light chain produced in step [9] with a complementary heavy chain to form an antibody molecule;

[11] determining the affinity of the antibody molecule formed in step [10] for said predetermined antigen;

[12] if the affinity determined in step [11] is not equivalent to that of the donor antibody, providing a light chain as described in [9] above but in which at least one of amino acid residues 2, 4, 6, 49, 62, 64 to 69, 98, 99, 101 are additionally donor residues; and

[13] associating the light chain produced in step [9]

with a complementary heavy chain to form an antibody molecule.

DOCKET NO.: CARP-0009 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John R. Adair, et al.

Serial No.: 07/743,329 Group Art Unit: 1807

Filed: September 17, 1991 Examiner: L. Bennett

For: HUMANISED ANTIBODIES

Not Enter B.L.

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 February 7, 1994

Doreen Yatko Trujillo
Doreen Yatko Trujillo, Reg. No. 35,719

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

Dear Sir:

AMENDMENT

This amendment is filed in response to the Office Action mailed September 7, 1993. A petition for extension of time and the appropriate fee is attached.

In the claims:

Please cancel claims 73 to 107, 109 to 113 and 115 to 119, without prejudice.

Please amend claims 67 and 71 as follows:

67. (Amended) An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising human acceptor antibody heavy chain [framework] residues and donor antibody heavy chain [antigen-binding] residues, said donor antibody having affinity for said predetermined antigen, said variable domain further comprising complementarity determining regions, wherein, according to the Kabat numbering system, in said composite heavy chain, said complementarity determining regions comprise donor residues at least at residues 31 to 35, 50 to 58 and 95 to 102; amino acid residues 5, 8, 10, 12 to 17, 19, 21, 22, 40, 42 to 44, 66, 68, 70, 74, 77, 79, 81, 83 to 85, 90, 92, 105, 109, 111 and 113 at least are acceptor residues; and amino acid residues 23, 24, [31 to 35,] 49 [to 58], 71, 73[,] and 78 [and 95 to 102] at least are donor residues.

Claim 71, line 2, please delete "48" and insert --46--.

REMARKS

Claims 67-72, 108 and 114 are pending. The number of claims pending in the present application has been reduced in order to expedite the prosecution of the case. The deletion of some claims should not be taken to be an admission that the subject

matter of the deleted claims is unpatentable. The Applicants reserve the right to file continuation applications directed to the deleted subject matter. The Examiner is thanked for bringing the typographical error in Claim 71 to the Applicants' attention.

To the extent the rejections are maintained against amended claim 67, and the remaining claims, Applicants respectfully request reconsideration for the reasons set forth below.

Rejections Under 35 U.S.C. § 112, First Paragraph

In paragraph 16 of the present Office Action, the Examiner contends that the application does not contain any support for the recitation of acceptor residues in the light or heavy chains. It is submitted, for the following reasons, that the Examiner's contention is incorrect.

At a very helpful interview held at the beginning of 1993, there was some discussion of the word "comprising" as used in the claims under consideration at that time. In those claims, it was only specified that certain residues should be donor residues. It was considered that it was not clear whether these were the only residues which could be donor residues. The alternative view was that these were only the minimum number of residues which must be donor but that any of the other residues could also be donor.

If the second line of interpretation were taken, the claims could be read to cover a situation in which all except one of the residues in the variable domain were donor residues. In

this case, the claims could then be interpreted to cover a structure similar to a "chimeric" antibody comprising a donor variable domain and a human constant region. Such chimeric antibodies were already well known at the priority date.

It plainly is not the intention of the Applicants to claim chimeric antibodies or any similar structures. As can be seen from the description, the superhumanised antibodies of the present invention are compared to the prior art chimeric antibodies. Moreover, the present invention was intended to deal with the problem of chimeric antibodies in that chimeric antibodies were believed to be too "foreign" because of the presence of the complete donor variable domain.

For the above reasons, it is clear that the wording of the claims needed to be changed so that the Applicants' intention of excluding chimeric antibodies was made effective. The language now present in the claims puts this intention clearly into effect.

As to support for this wording, the Examiner is referred firstly to page 16, under the heading "Protocol". It can be seen from this paragraph that the first step in the process involves the choice of an appropriate acceptor chain variable domain. This acceptor domain must be of known sequence. Thus, the protocol starts with a variable domain in which <u>all</u> the residues are acceptor residues. In the sentence bridging pages 16 and 17, it is stated that:

> "The CDR-grafted chain is then designed starting from the basis of the acceptor sequence".

On page 17, in the middle paragraph, it is stated that:

> "The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows...."

This again shows that, unless a residue is chosen for substitution, it will remain as in the acceptor sequence.

It must also be borne in mind that the purpose of the invention is to obviate some of the disadvantages of prior art proposals. The proposal of using chimeric antibodies had the disadvantage that they were more "foreign" than desirable. The problem of making CDR-grafted antibodies was that they generally did not provide good recovery of affinity. Thus, the aim of the present invention was to minimise as far as possible the "foreign" nature of the antibody while maximising as far as possible its affinity.

Bearing the passages referred to above and the aim of the invention in mind, it would have been abundantly clear to the skilled person reading the application that as many residues as possible should remain as acceptor residues. If this were not the case, it could hardly be said that the composite chain is based on the acceptor sequence.

The skilled person reading the application can plainly see that certain residues have been considered for changing from acceptor to donor. These are clearly set out in the description.

It would be plain to the skilled person that all other residues should not be considered for changing at all. It would therefore be obvious that any residue which is not specified as being under consideration for changing must remain as in the acceptor chain.

It may be that there is no explicit statement in the description that the specified residues should remain as in the acceptor chain. However, the disclosure in a specification is not limited to the explicit disclosure but also includes that which is implicit. It is implicit, in the recitation that the chain is based on the acceptor and that only certain residues are considered for changing, that all non-specified residues must remain as acceptor residues. Subject matter which might be fairly deduced from the disclosure is not new matter. *Acme Highway Products Corp. v. D.S. Brown Co.*, 431 F.2d 1074, 1080, 167 U.S.P.Q. 129, 132-133 (6th Cir. 1970), *cert denied*, 401 U.S. 956 (1971).

Another way to look at it is to consider a different way in which the claim could be drafted. It could be specified that in the composite chain, at least a certain minimum number of residues are donor residues (as in the present claims) and at most a certain maximum number of residues are donor residues. The maximum number would be derived by listing all the residues which are considered for changing. Such an amendment would have clear explicit basis in the description because all those residues are mentioned as such. However, the effect of such an amendment would be to produce claims of exactly the same scope as the present claims. It can thus be

seen that the present claims do not add subject matter but are plainly properly based on the disclosure in the description.

It is therefore submitted that the claims are fully supported by the description, are commensurate in scope with the disclosure in the description, and are properly delimited over the prior art.

The rejections of Claims 73-107, 109-113, and 115-119 under 35 U.S.C. §112 has been rendered moot by their withdrawal.

In paragraph 26 of the Office Action, the Examiner maintains the rejection of the claims for lack of enablement. It is submitted that this rejection cannot stand for the following reasons.

The Examiner contends that the description does not provide a "representative" number of Examples falling within the scope of the claims. Even if this were the case (and it is not, for reasons set out below) this does not provide a proper basis for rejection under 35 U.S.S. §112. A "representative" number of Examples is not required to obtain a patent. All that is required is that the disclosure be enabling. Enablement does not depend on the number of examples provided. Sufficient disclosure can be provided by illustrative examples or terminology. Further, "It is well settled that patent applications are not required to disclose every species encompassed by their claims, even in an unpredictable art." *In re Vaeck,* 20 U.S.P.Q.2d 1438, 1445 (Fed. Cir. 1991).

The Examiner also appears to be arguing that it may be that some antibodies will not be susceptible to the protocol of the present invention, i.e. that not all embodiments will work. Even if this were the case (and it is not, for reasons set out below). this also does not provide a proper basis for rejection under 35 U.S.C. §112. That inoperative embodiments may be encompassed is not detrimental. "It is not the function of claims or the specification to exclude all inoperative substances." *Ex parte Janin,* 209 U.S.P.Q. 761, 763 (Bd. of App. 1979). "The mere fact that a claim embraces undisclosed or inoperative species or embodiments does not necessarily render it unduly broad." *Horton v. Stevens,* 7 U.S.P.Q.2d 1245, 1247 (Bd. of Pat. App. & Int. 1988).

Apart from the legal points made above, it is submitted that the Examiner is incorrect on the technical facts. Before expanding on this, however, it would be worthwhile to make a few points concerning affinity. There is no absolute value which can be set which defines good affinity. Affinity can be measured, for instance in reciprocal moles (M^{-1}). In this measurement system, affinity can vary from 10^6 to 10^{12}.

Natural antibodies, as produced *in vivo,* do not all have the same affinity, even for the same antigen. Thus, in the normal polyclonal antiserum produced on challenge by an antigen, the body will produce a variety of antibodies having affinities within the range given above. Monoclonal antibodies, as produced by hybridoma

technology, also have varying affinities, again within the range given above. The variation in the affinity may in part be due to the structure of the antibody and in part to the structure of the antigen. It may therefore be that a good antibody directed against antigen X has an affinity of only 10^7 whereas a good antibody against antigen Y may have an affinity of 10^{12}. These are both good antibodies, even though they have very different affinities.

It can be seen that if an engineered antibody is produced against antigen X with an affinity of 10^8, this will be regarded as being exceptional, in that the affinity has gone up 10 fold compared to the good antibody. However, if an engineered antibody recognizing antigen Y is produced with an affinity of 10^8, this will be regarded as being an awful result as the affinity will have been reduced 10,000 fold.

Thus, the only sensible way to determine whether an engineered antibody is successful is to compare its affinity with that of the prototype antibody from which it is derived. It is pointless to look at the absolute value of the affinity because this does not tell you anything about the success or failure of the engineering operation. It is for this reason that the Applicants have provided such qualitative evidence of the success of the protocol described in the application.

Further, in some cases, a residue which is selected for changing according to the protocol described in the application may

not need to be changed. It may be that, fortuitously, it is the same in the donor and acceptor chains. This does not mean that, if the residues had been different, it would not have been changed. It merely means that, in effect, the change had already been made.

As to the number of antibodies which have been shown to have been successfully superhumanised using the protocol of the present invention, the Examiner is requested to look at the sheets attached to the previous response submitted April 7, 1993. Although Applicants are not required to provide a "representative number of examples", the provision of so many antibodies in these attachments should have satisfied any doubts on the part of the Examiner. Yet the Examiner makes no reference to these attachments and the evidence they provide.

The Examiner is also referred to the passage beginning on page 17 through page 19 of the last response. This shows in detail that a representative number of antibodies falling within the terms of the present claims were superhumanised successfully. Again the Examiner has not even referred to these pages. The Examiner has not provided any reasoning as to why these pages are not persuasive. It is submitted that mere allegation is not enough. The Examiner must also provide references or, if based upon personal knowledge, an affidavit, in support of the Examiner's allegations. MPEP § 706.02.

As has been shown by the third sheet attached to the previous response, the successful antibodies are representative not

only in number, but also as regards to antigens recognized. The antigens include cell surface antigens found on both healthy and cancerous cells, soluble cytokines and adhesion molecules. These are all very different in structure and function, yet antibodies against each of them have been successfully superhumanised using the protocol of the present invention.

It is no doubt the case that some of the antibodies referred to in the sheets were more successfully humanised than others. However, the reasons for this were clearly set out in the previous response. Thus, evidence that the replacement scheme is not generally applicable has not been provided.

The Examiner places much reliance on the prior art as, in her view, showing that there would have been no reasonable expectation of success. The Applicants agree that, if there were only the prior art to go on, then there would have been no reasonable expectation of success. However, the skilled person trying to put the present invention into practice does not have to rely on only the prior art. The skilled person has available the teaching of the present application. It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann and Queen, at least. Thus, the skilled person would not rely on Reichmann and Queen as teachings relevant to whether the present description is enabling.

It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is

enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims.

Rejections Under 35 U.S.C. § 103

The Examiner rejected all the claims as being obvious over Reichmann and Queen. However, this rejection appears contrary to her previous assertions. When attacking the enablement of the claims, the Examiner stated that:

> "... 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... The prior art does not teach that standardized principle... is possible."

(emphasis added)

The Applicants agree with the Examiner that the prior art provides no predictability of success and certainly no expectation that a generally applicable principle can be devised. It is submitted that this is a clear indication that the surprising

discovery that there is a generally applicable principle involves an invention.

The Examiner indicated that the arguments previously presented by the Applicants were deemed to be non-persuasive because they did not address the combined effect of Reichmann and Queen. This, of course, assumes that the skilled artisan would have combined Reichmann and Queen in the first place. The Examiner has shown no reason why Reichmann and Queen would have been combined. It is submitted that there is no reason why they should be combined.

The earlier publication is Reichmann. This shows a relatively simple procedure in which the six CDRs from a rat antibody against a leukocyte cell surface antigen are transferred onto human frameworks. The only additional residue change is in the heavy chain at residue 27. The reason that this residue is changed is because it was atypical in the human (acceptor) chain. The change was to replace residue 27 with the more normal acceptor residue. Thus, the teaching of Reichmann is that, as long as you have normal human (acceptor) chain, all that is needed is for the CDRs to be changed.

Queen does in fact refer to Reichmann. Reichmann is reference 24 in Queen. However, this is only referred to in passing on page 10029 as being an example of the work of Winter and his colleagues. The teaching of Queen clearly goes beyond that of

Reichmann. Thus, there is no incentive to try to combine the teachings of Reichmann and Queen.

Even if one could find some motivation for combining Reichmann and Queen, it is submitted that Reichmann would not add to Queen such that Applicants invention would be rendered obvious. Reichmann teaches the skilled person to use a normal acceptor sequence and merely to change the CDRs. If the acceptor sequence is not normal, then the abnormal residues are to be changed to normal acceptor residues. This is all disclosed in Queen. Since, at best, Queen incorporates all the teaching of Reichmann, even if Reichmann and Queen are combined, the total teaching is no more than teaching of Queen by itself. If the Examiner is of the contrary view, she is requested to point out specifically the teaching in Reichmann which is not present in Queen and why she believes this additional teaching, when combined with the teaching in Queen, renders the present claims obvious.

For the reasons set forth in the previous response, it is submitted that Queen, and therefore also a combination of Queen and Reichmann, does not render the present claims obvious.

The Examiner noted that the previously presented arguments concentrated on the process aspects of the disclosure in Queen and contended that this is irrelevant as far as the product claims in the present application are concerned. It is submitted that this is not correct. The only specifically disclosed product in Queen does not fall within the terms of the present claims. It

is therefore necessary for the Examiner to show that a product falling within the terms of the claims would have been produced using the teachings of Queen, alone or in combination with the prior art, or obvious variants thereof. If there is no expectation that such a product would have been produced, then the product claims are not obvious.

To maintain otherwise is to employ impermissible hindsight. Queen only describes the replacement of some acceptor residues outside the CDR for a "specific" antibody. Queen does not disclose, or even suggest, a "general" approach for replacing acceptor residues outside the CDRs with donor residues. In fact, the absence of such disclosure in Queen, or in any of the prior art cited, was previously emphasized in the Examiner's rejection under § 112 for enablement. Therefore, it is respectfully submitted that the Examiner would not have even contemplated that Queen discloses, or suggests, a general superhumanised antibody as claimed without the benefit of Applicants' disclosure. Neither would one skilled in the art.

Further, it is submitted that the Examiner has not established a *prima facie* case of obviousness. The teachings from the prior art *itself* should appear to have suggested the claimed subject matter to a person of ordinary skill in the art to establish a *prima facie* case. *In re Rijckaert*, 28 U.S.P.Q. 2d 1955 (Fed. Cir. 1993). As already admitted by the Examiner in the rejections under § 112, the expectation that a generalized approach

is feasible is nowhere present in the prior art. Thus, the claims as drafted, covering superhumanised antibodies in general (i.e., not limited to a particular antibody) are not obvious over the prior art.

The Examiner also asserts that how the residues are identified is irrelevant. It is submitted that this is not the case when the rejection is one for obviousness. The references do not disclose the residues claimed by Applicants. To render Applicants' claims obvious, the Examiner needs to show that the prior art could lead one skilled in the art to identify the same residues as are identified in the present claims. The Examiner has not shown any reason why the skilled person, carrying out the method described by Queen, alone or in combination with Reichmann, would have expected to identify the specific sets of residues identified in the present claims. In this regard, it is noted that a rejection for obviousness was not levied against the method claims in the Office Action--i.e., claims 118 and 119.

It is again to be pointed out that the present claims cannot be generalized to "an antibody in which the CDRs and some, (unspecified) framework residues have been changed." The present claims relate to "an antibody in which the CDRs and only certain, specific framework residues have been changed." Nothing in the prior art, whatever combination is used, leads the skilled person to the specific set of residues set forth in the present claims.

It is therefore submitted that the present claims are not at all obvious over Queen, combined with Reichmann, or on its own.

Paragraphs 18 to 25 and 27 to 29

The contents of paragraphs 18 to 25 and 27 to 29 are noted with appreciation.

The foregoing represents a bona fide attempt to advance the case to allowance. Applicants respectfully request that all presently pending claims be allowed.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. 35,719

Date: February 7, 1994

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

different paths, and by ensuring that an azimuthally uniform coverage of stations is used in the averaging calculation. To compensate for other factors, such as focal depth, fault geometry and corner frequency would require such a detailed knowledge of the earthquake source that the M_s measurement itself would be redundant.

The results of this analysis can be summarized in five points.

(1) A global average moment-magnitude relationship M_s has been defined which can be used to predict M_0 over a wide range of magnitudes and scalar moments.

(2) The variance of surface wave measurements for an event of a particular scalar moment is ~0.2 magnitude units.

(3) Large regional biases in M_s exist.

(4) Differences in source scaling may explain some of the differences. Specifically, observations show that the transition from a slope of unity to a smaller value occurs at large moments for continental events than for ridge and fracture zone events, suggesting systematic differences in stress drop.

(5) Other systematic factors affecting the calculation of M_s also appear to contribute to the observed regional bias.

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Reshaping human antibodies for therapy

Lutz Riechmann†, Michael Clark*, Herman Waldmann* & Greg Winter‡

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK
* Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK

A human IgG1 antibody has been reshaped for serotherapy in humans by introducing the six hypervariable regions from the heavy- and light-chain variable domains of a rat antibody directed against human lymphocytes. The reshaped human antibody is as effective as the rat antibody in complement and is more effective in cell-mediated lysis of human lymphocytes.

IN 1890 it was shown that resistance to diphtheria toxin could be transferred from one animal to another by the transfer of serum. It was concluded that the immune serum contained an anti-toxin, later called an antibody[1]. For many years animal antisera were used in the treatment of microbial infections and for the neutralization of toxins in man[2]. More recently rodent monoclonal antibodies (mAbs)[3] have been used as 'magic bullets'[4] to kill and to image tumours[5,6]. The foreign immunoglobulin, however, can elicit an anti-globulin response which may interefere with therapy[7] or cause allergic or immune complex hypersensitivity[8]. Thus ideally human antibodies would be used. Human immunoglobulins are widely used as both prophylactic and microbicidal agents[9], but it would be far better to have available human mAbs of the desired specificity. It has proven difficult, however, to make such mAbs by the conventional route of immortalization of human antibody-producing cells[9].

There is an alternative approach. Antibody genes have been transfected into lymphoid cells, and the encoded antibodies expressed and secreted; by shuffling genomic exons, simple chimaeric antibodies with mouse variable regions and human constant regions have been made[10–12]. Such chimaeric antibodies have at least two advantages over mouse antibodies. First, the effector functions can be selected or tailored as desired. For example, of the human IgG isotypes, IgG1 and IgG3 appear to be the most effective for complement and cell-mediated lysis[13–15], and therefore for killing tumour cells. Second, the use of human rather than mouse isotypes should minimize the anti-globulin responses during therapy[16,17] by avoiding anti-isotypic antibodies. The extent to which anti-idiotypic responses to rodent antibodies in therapy are dictated by foreign components of the variable versus the constant region is not known, but the use of human isotypes should reduce the anti-idiotypic response. For example, when mice were made tolerant to rat immunoglobulin constant-region determinants, administration of rat anti-lymphocyte antibodies did evoke anti-idiotypic responses, but these were delayed and weaker than in animals that had not been made tolerant[18]. Nevertheless, it is likely that a chimaeric antibody would provoke a greater immune response than a human mAb.

We have attempted to build rodent antigen binding sites directly into human antibodies by transplanting only the antigen binding site, rather than the entire variable domain, from a rodent antibody. The antigen binding site is essentially encoded by the hypervariable loops at one end of the β-sheet framework. The hypervariable regions of the heavy chain of mouse antibodies against a hapten[19] or a protein antigen[4] were previously transplanted into a human heavy chain, and, in association with the mouse light chain, the antigen binding site was retained.

† Address from April 1988: Department of Molecular Biology, The Research Institute of Scripps Clinic, North Torrey Pines Road, La Jolla, California 02937, USA
‡ To whom correspondence should be addressed.

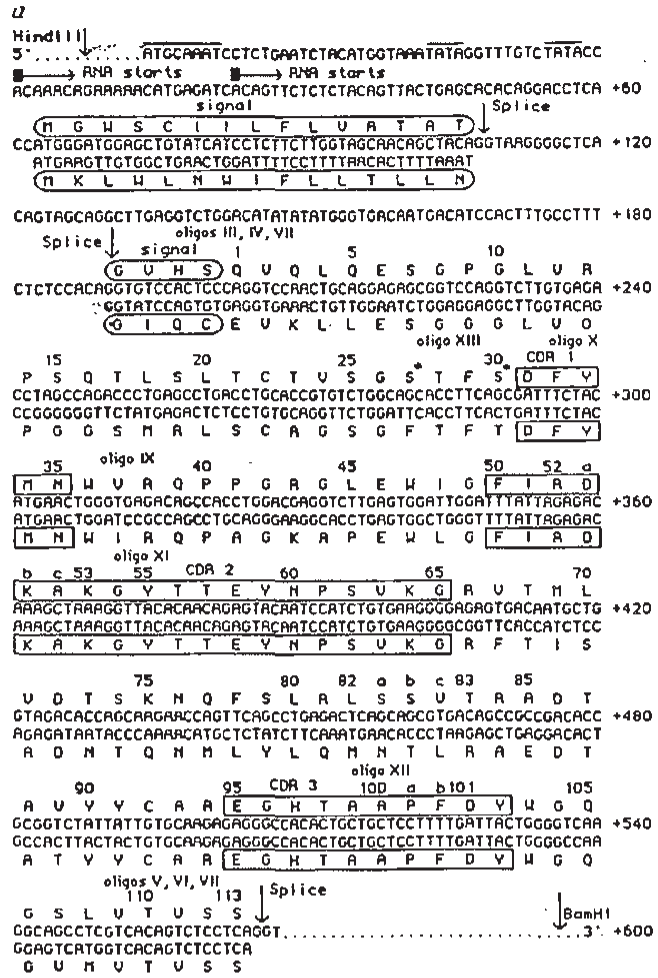

Fig. 1 Heavy-chain (*a*) and light-chain (*b*) sequences of the variable domains of reshaped (upper line) or rat YTH 34.5HL (lower line) antibodies. The reshaped heavy-chain variable domain HuVHCAMP was based on the HuVHNP gene[12,19], with the framework regions of human NEW (see note) alternating with the hypervariable regions of rat YTH 34.5HL. The reshaped light-chain variable domain HuVLCAMP is a similar construct, except with the framework regions of the human myeloma protein REI, with the C-terminal and the 3' non-coding sequence taken from a human J_κ-region sequence[36]. The sequences of oligonucleotide primers are given and their locations on the genes are marked.

Methods. Messenger mRNA was purified[37] from the hybridoma clone YTH 34.5HL ($\gamma 2a$, κ^b). First strand cDNA was synthesized by priming with oligonucleotides complementary to the 5' end of the CH1 (oligonucleotide I) and the Cκ exons (oligonucleotide II), and then cloned and sequenced as described previously[18,19]. Two restriction sites (*Xba*I and *Sal*I) were introduced at each end of the rat heavy-chain variable region RaVHCAMP cDNA clone in M13 using mutagenic oligonucleotides III and V respectively, and the *Xba*I–*Sal*I fragment was excised. The corresponding sites were introduced into the M13-HuVHNP gene using oligonucleotides IV and VI, and the region between the sites was then exchanged. The sequence at the junctions was corrected with oligonucleotides VII and VIII, and an internal *Bam*HI site removed using the oligonucleotide IX, to create the M13-RaVHCAMP gene. The encoded sequence of the mature domain is thus identical to that of YTH 34.5HL. The reshaped heavy-chain variable domain (HuVHCAMP) was constructed in an M13 vector by priming with three long oligonucleotides simultaneously on the single strand containing the M13-HuVHNP gene[12,19]. Each oligonucleotide (X, XI and XIII) was designed to replace each of the hypervariable regions with the corresponding region from the heavy chain of the YTH 34.5HL antibody. Colony blots were probed initially with the oligonucleotide X and hybridization positives were sequenced: the overall yield of the triple mutant was 5%. The (Ser27 → Phe) and (Ser27 → Phe, Ser30 → Thr) mutants of M13mp8-HuVHCAMP were made with the mixed oligonucleotide XIII. The reshaped light-chain variable domain (HuVLCAMP) was constructed in M13 from a gene with framework regions based on human REI (J. Foote, unpublished data). As above, three long oligonucleotides (XIV, XV and XVI) were used to introduce the hypervariable regions of the YTH 34.5HL light chain.

Note: There are discrepancies involving the first framework region and the first hypervariable loop of the NEW heavy chain between the published sequence[37] used here and the sequence deposited in the Brookhaven data base (in parentheses): Ser27 (→Thr), Thr28 (→Ser) and Ser30 (→Asp). Neither version is definitive (R. J. Poljak, personal communication) and the discrepancies do not affect our interpretations.

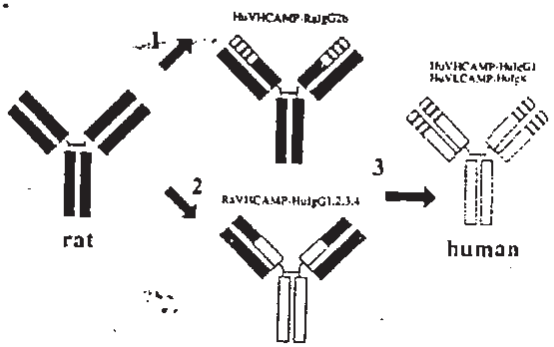

Fig. 2 Strategy for reshaping a human antibody for therapy. Sequences of rat origin are marked in black, and those of human origin in white. The recombinant heavy and light chains are also marked using a systematic nomenclature. See text for description of stages 1, 2 and 3. The genes encoding the variable domains were excised from the M13 vectors as *Hin*dIII–*Bam*HI fragments, and recloned into pSV2gpt[29] (heavy chains) or pSV2neo[30] (light chains), expression vectors containing the immunoglobulin enhancer[12]. The human γ1 (ref. 40), γ2 (ref. 41), γ3 (ref. 42), γ4 (ref. 41) and κ (ref. 36) and the rat γ2b (ref. 43) constant domains were introduced as *Bam*HI fragments. The following plasmids were constructed and transfected into lymphoid cell lines by electroporation[44]. In stage 1, the pSVgpt plasmids HuVHCAMP-RaIgG2B, HuVHCAMP(Ser→Phe)-RaIgG2B, HuVHCAMP-(Ser27→Phe, Ser30→Thr)-RaIgG2B were introduced into the heavy chain loss variant of YTH 34.5HL. In stage 2, the pSVgpt plasmids RaVHCAMP-RaIgG2B, RaVHCAMP-HuIgG1, RaVHCAMP-HuIgG2, RaVHCAMP-HuIgG3, RaVHCAMP-HuIgG4 were transfected as above. In stage 3, the pSV-gpt plasmid Hu(Ser27→Phe, Ser30→Thr)VHCAMP-HuIgG1 was co-transfected with the pSV-neo plasmid HuVLCAMP-HuIgK into the rat myeloma cell line Y0 (Y B2/3.0 Ag 20 (ref. 31). In each of the three stages, clones resistant to mycophenolic acid were selected and screened for antibody production by ELISA assays. Clones secreting antibody were subcloned by limiting dilution (for Y0) or the soft agar method (for the loss variant) and assayed again before 1 litre growth in roller bottles.

Since, to a first approximation, the sequences of hypervariable regions do not contain characteristic rodent or human motifs, such 'reshaped' antibodies should be indistinguishable in sequence from human antibodies.

There are mAbs to many cell-type-specific differentiation antigens, but only a few have therapeutic potential. Of particular interest is a group of rat mAbs directed against an antigen, the 'CAMPATH-1' antigen, which is strongly expressed on virtually all human lymphocytes and monocytes, but is absent from other blood cells including the haemopoietic stem cells[20]. The CAMPATH-1 series contains rat mAb of IgM, IgG2a and IgG2c isotypes[21], and more recently IgG1 and IgG2b isotypes which were isolated as class-switch variants from the IgG2a-secreting cell line YTH 34.5HL[22]. All of these antibodies, except the rat IgG2c isotype, are able to lyse human lymphocytes efficiently with human complement. Also the IgG2b antibody YTH 34.5HL-G2b, but not the other isotypes, is effective in antibody-dependent cell-mediated cytotoxicity (ADCC) with human effector cells[22]. These rat mAbs have important applications in problems of immunosuppression: for example control of graft-versus-host disease in bone-marrow transplantation[20]; the management of organ rejection[23]; the prevention of marrow rejection; and the treatment of various lymphoid malignancies (ref. 24 and M. J. Dyer, Hale, G., Hayhoe, F. G. J. and Waldmann, H., unpublished observations). The IgG2b antibody YTH 34.5HL-G2b seems to be the most effective at depleting lymphocytes *in vivo* but the use of all of these antibodies is limited by the anti-globulin response which can occur within two weeks of the initiation of treatment[24]. Here we describe the reshaping of human heavy and light chains towards binding the CAMPATH-1 antigen and the selection of human effector functions to match the lytic potential of the rat IgG2b isotype.

Table 1 Reshaping the heavy-chain variable domain

Heavy chain variable domain	Concentration of antibody in μg ml^{-1} at 50% antigen binding	50% complement lysis
RaVHCAMP	0.7	2.1
HuVHCAMP	27.3	*
HuVHCAMP (Ser27→Phe)	1.8	16.3
HuVHCAMP (Ser 27→Phe, Ser 30→Thr)	2.0	17.6

Antibodies with the heavy-chain variable domains listed above, rat IgG2b constant domains and rat light chains were collected from supernatants of cells at stationary phase and concentrated by precipitation with ammonium sulphate, followed by ion exchange chromatography on a Pharmacia MonoQ column. The yields of antibody were measured by an enzyme-linked immunosorbent assay (ELISA) directed against the rat IgG2b isotype, and each was adjusted to the same concentration[35]. To measuring binding to antigen, partially purified CAMPATH-1 antigen was coated onto microtitre wells and bound antibody was detected via a biotin-labelled anti-rat IgG2b mAb[35], developed with a streptavidin-peroxidase conjugate (Amersham). Complement lysis of human lymphocytes was with human serum as the complement source[21]. For both binding and complement assays, antibody titres were determined by fitting the data to a sigmoid curve by at least squares iterative procedure[21].

* Complement lysis with the HuVHCAMP variable domain was too weak for the estimation of lytic titre.

Strategy

The amino-acid sequences of the heavy- and light-chain variable domains of the rat IgG2a CAMPATH-1 antibody YTH 34.5HL were determined from the cloned complementary DNA (Fig. 1), and the hypervariable regions were identified according to Kabat[25]. In the heavy-chain variable domain there is an unusual feature in the framework region. In most known heavy-chain sequences Pro41 and Leu45 are highly conserved: Pro41 helps turn a loop distant from the antigen binding site and Leu45 is in the β bulge which forms part of the conserved packing between heavy- and light-chain variable domains[26]. In YTH 34.5HL these residues are replaced by Ala41 and Pro45 and presumably this could have some effect on the packing of the heavy- and light-chain variable domains. Working at the level of the gene and using three large mutagenic oligonucleotides for each variable domain, the rat hypervariable regions were mounted in a single step on the human heavy- or light-chain framework regions taken from the crystallographically solved proteins NEW[27] and REI[28] respectively (Fig. 1). The REI light chain was used because there is a deletion at the beginning of the third framework region in NEW. The reshaped human heavy- and light-chain variable domains were then assembled with constant domains in three stage (Fig. 2). This permits a step-wise check on the reshaping of the heavy-chain variable domain (stage 1), the selection of the human isotype (stage 2), and the reshaping of the light-chain variable domain and the assembly of human antibody (stage 3). The plasmid constructions were genomic, with the sequences encoding variable domains cloned as *Hin*dIII–*Bam*HI fragments and those encoding the constant domains as *Bam*HI–*Bam*HI fragments in either pSVgpt (heavy chain)[29] or pSVneo (light chain)[30] vectors. The heavy-chain enhancer sequence was included on the 5′ side of the variable domain, and expression of both light and heavy chains was driven from the heavy-chain promoter and the heavy-chain signal sequence.

Heavy-chain variable domain

In stage 1, the reshaped heavy-chain variable domain (HuVHCAMP) was attached to constant domains of the rat

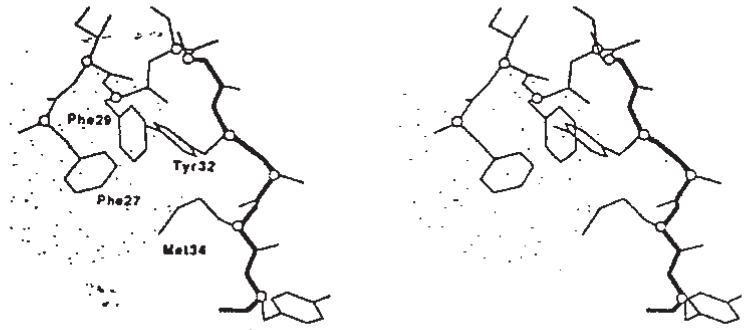

Fig. 3 Loop Phe27 to Tyr35 in the heavy-chain variable domain of the human myeloma protein KOL, which has been solved crystallographically[45]. The backbone of the hypervariable region according to Kabat[25] is highlighted, and a 200% van der Waal surface is thrown around Phe 27 to show the interactions with Tyr 32 and Met 34 of the Kabat hypervariable region. In the rat YTH 34.5HL heavy chain, these three side chains are conserved in character, but in HuVHCAMP, Phe27 is replaced by Ser.

isotype IgG2b and transfected into a heavy-chain loss variant of the YTH 34.5 hybridoma. This variant carries two light chains, one derived from the Y3 fusion partner[31]. The cloned rat heavy-chain variable domain (RaVHCAMP) was also expressed as above, and the antibodies were purified and quantified (Table 1). The HuVHCAMP and RaVHCAMP antibodies, each of the rat IgG2b isotype, were compared to the CAMPATH-1 antigen in a direct binding assay and in complement lysis of human lymphocytes (Table 1). Compared with the original rat antibody, or the engineered equivalent, the antibody with the reshaped heavy-chain domain bound poorly to the CAMPATH-1 antigen and was weakly lytic. This suggested an error in the design of the reshaped domain.

There are several assumptions underlying the transfer of hypervariable loops from one antibody to another[47], in particular the assumption that the antigen binds mainly to the hypervariable regions. These are defined as regions of sequence[25] or structural[32] hypervariability, the locations of hypervariable regions being similar by both criteria except for the first hypervariable loop of the heavy chain. By sequence the first hypervariable loop extends from residues 31–35 (ref. 25) whereas by structure it extends from residues 26–32 (ref. 32). Residues 29 and 30 form part of the surface loop, and residue 27, which is phenylalanine or tyrosine in most sequences, including YTH 35.5HL, helps pack against residues 32 and 34 (Fig. 3). Unlike most human heavy chains, in NEW (see note in Fig. 1) the phenylalanine is replaced by serine, which would be unable to pack in the same way. To restore the packing of the loop, we made both a Ser 27→Phe mutation, and a Ser 27→Phe, Ser 30→Thr double mutation in HuVHCAMP. These two mutants showed a significant increase in binding to CAMPATH-1 antigen and lysed human lymphocytes with human complement (Table 1). Thus the affinity of the reshaped antibody could be restored by a single Ser 27→Phe mutation, possibly as a consequence of an altered packing between the hypervariable regions and the framework. This suggests that alterations in the 'Kabat' framework region can enhance the affinity of the antibody and extends previous work in which an engineered change in the hypervariable region yielded an antibody with increased affinity[33].

Heavy-chain constant domains

In stage 2 (Fig. 2), the rat heavy-chain variable domain was attached to constant domains of the human isotypes IgG1, 2, 3 and 4, and transfected into the heavy-chain loss variant of the YTH 34.5 hybridoma. In complement lysis (Fig. 4*a*), the human IgG1 isotype proved similar to the YTH 34.5HL-G2b, with the human IgG3 isotype being less effective. The human IgG2 isotype was only weakly lytic and the IgG4 isotype was non-lytic. In ADCC (Fig. 4*b*) the human IgG1 was more lytic than the YTH 34.5HL-G2b antibody. The decrease in lysis at higher concentrations of the rat IgG2b and the human IgG1 antibody is due to an excess of antibody, which causes the lysis of effector cells. The human IgG3 antibody was weakly lytic, and the IgG2 and IgG4 isotypes were non-lytic.

We therefore selected the human IgG1 isotype for the reshaped antibody. Other recent work also favours the use of IgG1 isotype for therapeutic application. When the effector functions of human isotypes were compared using a set of chimaeric antibodies with an anti-hapten variable domain, the IgG1 isotype appeared superior to the IgG3 in both complement and cell-mediated lysis[15]. Also, of two mouse chimaeric antibodies with human IgG1 or IgG3 isotypes directed against cell surface antigens as tumour cell markers, only the IgG1 isotype mediated complement lysis[13,14].

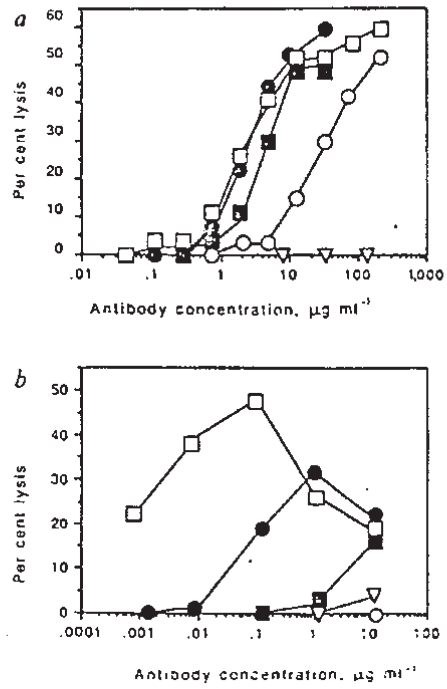

Fig. 4 *a*, Complement lysis and *b*, ADCC for antibodies with rat light-chain and rat heavy-chain variable domain attached to human IgG1 (□), IgG2 (○), IgG3 (■), or IgG4 (▽) isotypes. Lysis with the YTH 34.5HL antibody (●) is also shown.
Methods. Antibody was collected from cells in stationary phase, concentrated by precipitation with ammonium sulphate and desalted into phosphate buffered saline (PBS). Antibodies bound to the CAMPATH-1 antigen-coated on microtitre plates, were assayed in ELISA directed against the rat κ light chain[34], and each adjusted to the same concentration. The antibodies were assayed in complement lysis (Table 1) and ADCC with activated human peripheral blood mononuclear cells[35,46]. Briefly, 5×10^4 human peripheral blood cells were labelled with ^{51}Cr and incubated for 30 min at room temperature with different concentrations of antibody. Excess antibody was removed and a 20-fold excess of activated cells added as effectors. After 4 h at 37 °C cell death was estimated by ^{51}Cr release.

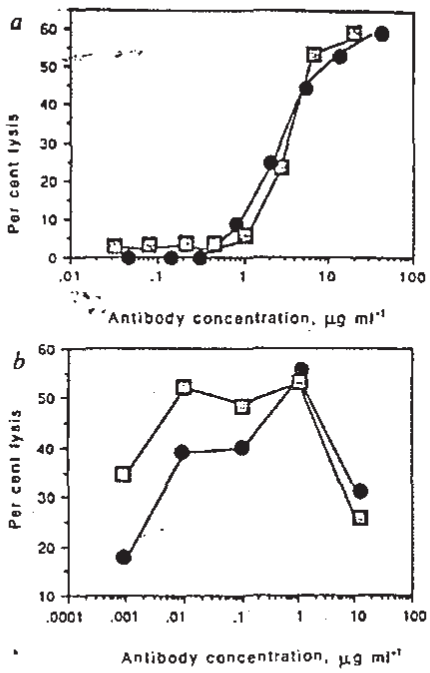

Fig. 5 *a*, Complement lysis and *b*, ADCC of the reshaped human (▨) and rat YTH 34.5HL (●) antibodies. Antibody HuVHCAMP (Ser27→Phe, Thr30→Ser)-HuIGG1, HuVLCAMP-HuIGK was purified from supernatants of cells in stationary phase by affinity chromotography on protein-A Sepharose. The yield (about 10 mg l^{-1}) was measured spectrophotometrically. Complement and ADCC assays were performed as in Fig. 4.

Light chain

In stage 3 (Fig. 2), the reshaped heavy chain was completed by attaching the reshaped HuVHCAMP (Ser27→Phe, Ser30→Thr) domain to the human IgG1 isotype. The reshaped light-chain domain HuVLCAMP was attached to the human Cκ domain. The two clones were co-transfected into the non-secreting rat Y0 myeloma line. The resultant antibody, bound to CAMPATH-1 antigen (data not shown), and proved almost identical to the YTH 34.5HL-G2b antibody in complement lysis (Fig. 5*a*). In cell-mediated lysis the reshaped human antibody was more effective than the rat antibody (Fig. 5*b*). Similar results were obtained with three different donors of target and effector cells (data not shown). Also, the antibody was as effective as YTH 34.5HL-G2b in killing leukaemic cells from three patients with B-cell lymphocytic leukaemia by complement-mediated lysis with human serum. Thus, by transplanting the hypervariable regions from a rodent to a human antibody of the IgG1 subtype, we have reshaped the antibody for therapeutic application.

Prospects

The availability of a reshaped human antibody with specificity for the CAMPATH-1 antigen should permit a full analysis of the *in vivo* potency and immunogenicity of an anti-lymphocyte antibody with wide therapeutic potential. Even if anti-idiotypic responses are eventually observed, considerable therapeutic benefit could be derived from an extended course of treatment. Also, it should be possible to circumvent an anti-globulin response restricted to idiotype by using a series of antibodies with different idiotypes[34]. In principle, the idiotype of the reshaped CAMPATH-1 could be changed by altering the hypervariable regions or the framework regions—evidence from a reshaped antibody specific for the hapten nitrophenyl acetate suggests that recognition by anti-idiotypic antisera and anti-idiotypic mAbs is influenced by residues in the framework region[19]. Thus, recycling the hypervariable regions on different human framework regions should change the idiotype, although ultimately it might focus the response directly onto the binding site for the CAMPATH-1 antigen. Although such focusing would be undesirable for CAMPATH-1 antibodies, it could be an advantage for the development of anti-idiotypic vaccines. It is likely that the answers to some of these questions will emerge from the use of this reshaped antibody in therapy.

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5-18-94 Dr Stone

27/E B. White 5-20-94 (NE)

DOCKET NO.: CARP-0009 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John R. Adair, et al.

Serial No.: 07/743,329 Group Art Unit: 1807

Filed: September 17, 1991 Examiner: L. Bennett Arthur

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

On May 9, 1994

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Reg. No. 35,719

Ltr to enter 5-23-94 B. White 9/28/94 B. White

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

RECEIVED
MAY 13 1994

Dear Sir:

RESPONSE TO ADVISORY ACTION

In the claims:

Please amend claim 67 as follows:

In claim 67, line 4, after "chain", please insert --framework--.

REMARKS

This paper is being filed in response to the Advisory Action mailed February 25, 1994, pursuant to Examiner Arthur's suggestion during a teleconference. This response is being filed in order to reduce the number of issues to be addressed upon

appeal. It is believed that no petition or extension fee is required, since the response is being filed within two months of the "Notice of Appeal". To the extent this belief is erroneous, please charge any fees due to Deposit Account No. 23-3050.

In the Advisory Action, Examiner Arthur stated that the deletion of the recitation "acceptor antibody heavy chain [framework] resides [sic]" changes the scope of the claimed invention to more broadly encompass composite antibodies in which the acceptor contributes more than just the framework. During a teleconference, the Examiner clarified that she was referring to the deletion of the word "framework", and not suggesting that the entire recitation in quotes had been deleted. Accordingly, Applicants have amended claim 67 to reinsert the word "framework" where it had been previously deleted.

The Examiner also stated, in the Advisory Action, that the deletion changed the scope such that the "...donor antibody is no longer limited to donating the antigen binding residues. The specification does not appear to support this broader concept." The Examiner, therefore, asserted that the amendment raised new matter and a new scope rejection under 35 U.S.C. § 112, first paragraph.

During the teleconference, the undersigned explained that the donor antibody was *never* limited to contributing the antigen

binding residues. The Examiner suggested submission of the present response to clarify this point and make the foregoing amendment.

On page 17, line 15, of the application as filed, the following is stated: "The positions at which donor residues are to be substituted for *acceptor* residues are then chosen as follows." (Emphasis added.) The text following this passage discloses which residues in the **framework** are to be changed, in addition to those changes to be made in the CDRs.

In paragraph 2.1 on the same page, residues 23, 24, 49, 71, 73, and 78 in the heavy chain variable domain are listed to be changed. In a previous paragraph, numbered 1, the residues of the CDRs (i.e. antigen binding residues) are listed for the heavy chain. As can be seen, the residues to be changed do not occur in the CDRs and are, therefore, in the framework region. Claim 1 as originally filed recited changing these residues to donor. Contrary to the Examiner's interpretation, the claimed invention was never limited to changes to donor only within the antigen binding residues.

Neither the Advisory Action, nor the teleconference, specifically addressed the remaining rejections. It appears, however, that the rejections stand "for the reasons of record." (See item #4, Advisory Action.) Applicants do not wish to reiterate the whole Amendment here, but would like to emphasize

some points in the interest of possibly reducing issues to be addressed on appeal.

The first point concerns the specification of acceptor residues in the claims. It was previously believed, as a result of an interview, that the Examiner thought the claims as drafted covered the situation in which all the variable domain residues are donor -- i.e., chimeric antibodies. Thus, the Applicants specified residues which are to remain as acceptors. As discussed in the Amendment, this is implicit, if not explicit, from the application as filed.

Further, the claims specify that the variable domain comprises donor and acceptor sequences. This specification distinguishes the antibodies of the claims from chimerics. In chimerics, the variable domain is comprised entirely of donor residues.

Now, however, it appears the Examiner thinks the claims had previously been limited to antibodies in which the residues in the CDRs are donor, and the remaining residues are acceptor. This is inconsistent with the specification and claims as well as the prior Office Actions and suggests further discussion is merited.

Applicants would also like to clarify a point regarding the Queen reference. Panels A and B on page 10003 of Queen refer to the light and heavy chains, respectively, of the acceptor antibody (upper sequences) and the humanized anti-TAC antibody

(lower sequences). The attached panels correspond to Panels A and B of Queen. The upper and lower sequences are further separated in the attached, the lower sequence representing the humanized antibody. The numbers above the sequences utilize the linear numbering system. The numbers below utilize Kabat numbering. Queen donor residues are indicated in red. Applicants' donor residues are indicated in blue. As can be seen, the two approaches are very different particularly for the heavy chain.

During the teleconference, the Examiner also indicated that she would be amenable to conducting an interview with the Applicants upon return from her upcoming leave. It was further indicated that her return would be sometime in May. Applicants still wish to conduct an interview and respectfully request a prompt communication thereafter regarding scheduling so that appropriate arrangements can be made.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. **35,719**

Date: **May 9, 1994**

+8352

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

A

QUEEN ET AL - SEQUENCES FROM FIGURE 2 - NUMBERING IN PAPER ABOVE
NUMBERING ACCORDING TO KABAT BELOW

PANEL A - UPPER SEQUENCE

```
1                   10                  20                  30
D I Q M T Q S P S T L S A S V G D R V T I T C R A S Q S I N T W L A
1                   10                  20  23              30

         40                  50                  60
W Y Q Q K P G K A P K L L M Y K A S S L E S G V P S R F I G S G S G
         40                  50                  60

 70                  80                  90                  100
T E F T L T I S S L Q P D D F A T Y Y C Q Q Y N S D S K M F G Q G I
 70                  80                  88  90              100

      107
K V E V K
      107
```

PANEL A - LOWER SEQUENCE

```
1                   10                  20                  30
Q I V L T Q S P A I M S A S P G E K V T I T C S A S S S I S Y M H W
1                                       23                  35

         40                  50                  60
F Q Q K P G T S P K L W I Y T T S N L A S G V P A R F S G S G S G T
        40                  50                  60

 70                  80                  90                  100
S Y S L T I S R M E A E D A A T Y Y C H Q R S T Y P L T F G S G T K
70                  80                  88  90              100

    106
L E L K
    107
```

B

PANEL B - UPPER SEQUENCE

```
1                 10                  20                  30
Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S R S A I
1                 10                  20  22              30

        40                  50                  60
I W V R Q A P G Q G L E W M G G I V P M F G P P N Y A Q K F Q G R V
 36

 70                  80                  90                  100
T I T A D E S T N T A Y M E L S S L R S E D T A F Y F C A G G Y G I
                          82 a b c            90  92

            110           117
Y S P E E Y N G G L V T V S S
100                 110   113
```

PANEL B - LOWER SEQUENCE

```
1                 10                  20                  30
Q V Q L Q Q S G A E L A K P G A S V K M S C K A S G Y T F T S Y R M
                                                22

        40                  50                  60
H W V K Q R P G Q G L E W I G Y I N P S T G Y T E Y N Q K F K D K A
                            52 a                60

 70                  80                  90                  100
T L T A D K S S S T A Y M Q L S S L T F E D S A V Y Y C A R G G G V
                          82 a b c            92  94

          110           116
F D Y W G Q G T T L T V S S
  103                 113
```

(There is a deletion in the Kabat system between F and D at beginning of last line.)

DOCKET NO.: CARP-0032 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

35/1
093
11/17/94

In re application of:

John Robert Adair, Diljeet Singh Athwal and John Spencer Emtage

Serial No.: N/A Group Art Unit: 1807

Filed: Herewith Examiner: B. Sisson

For: HUMANISED ANTIBODIES

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

Dear Sir:

PRELIMINARY AMENDMENT

Pursuant to 37 C.F.R. § 1.115, prior to examination of the above-identified patent application, please amend the claims as follows.

In the claims:

Please cancel claims 67 - 72, 108, and 114 without prejudice.

Please add claims 120 - 127, as follows:

-- ~~120~~. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in

a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues, ~~provided that the antibody does not have affinity for the p55 chain of the human interleukin 2 receptor~~.

~~121~~. The antibody molecule of claim ~~120~~, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

~~122~~. The antibody molecule of claim ~~120~~, wherein amino acid residues 71, 73, and 78 in said composite heavy chain are additionally donor residues.

~~123~~. The antibody molecule of claim ~~120~~, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

~~124~~. The antibody molecule of claim ~~120~~, wherein at least one of amino acid residues 36, 94, 104, 106, and 107 in said composite heavy chain are additionally donor residues.

125. The antibody molecule of claim 124, wherein at least one of amino acid residues 2, 4, 6, 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

126. The antibody molecule of claim 120, wherein said complementary light chain is a composite light chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain; said CDRs comprise donor residues at least at residues 24 to 34, 50 to 56, and 89 to 97; and amino acid residues 46, 48, 58, and 71 at least are donor residues.

127. The antibody molecule of claim 126, wherein amino acid residues 1, 3, 60 (if this residue can form a salt bridge with residue 54), and 70 (if this residue can form a salt bridge with residue 24) in said composite light chain are additionally donor residues.--

REMARKS

The present application is a continuation of U.S. Application Serial No. 07/743,329 (hereinafter the "'329 application") under 37 C.F.R. § 1.62.

Claims 67 - 72, 108, and 114 were pending in the '329 application. Those claims have been cancelled in the present amendment. Applicants reserve the right to pursue the subject matter of the cancelled claims in continuation or divisional applications, as may be appropriate.

Claims 120 - 127 are being submitted in the present amendment. Support for the amendments is found in the application as originally filed as indicated below. In claims 120 and 126, the specification that the variable domain "predominantly" comprises "human acceptor framework residues" is supported by, *inter alia*, page 3, lines 16 - 21, of the application as originally filed. The problems attendant to chimerics are therein described. It is also clear from the number of framework residues discussed as being changed to donor that the framework residues remain, predominantly, acceptor. Support for the residues specified as donor in claim 120 can be found, *inter alia*, on page 7, first full paragraph, page 19, Section 1, and page 46, Section 15.2.1. Support for the residues specified as donor in claim 126 can be found, *inter alia*, on page 17, lines 12 to 14 and page 18, Section 3.1.

As to the remaining claims, support for the residues specified as donor in claim 121 can be found, *inter alia*, on page 8, middle paragraph and page 17, Section 1. Support for the residues specified as donor in claim 122 can be found, *inter alia*, on page 7, first full paragraph. Support for the residues specified as donor in claim 123 can be found, *inter alia*, on page 20, Section 2.1.1. Support for the residues specified as donor in claim 124 can be found, *inter alia*, on page 21, lines 10 to 12. Support for the residues specified as donor in claim 125 can be found, *inter alia*, on page 21, lines 13 to 16. Support for the residues specified as donor in claim 127 can be found, *inter alia*, on page 21, lines 3 to 7.

In a helpful telephonic discussion on June 2, 1994 between Examiner Sisson and the undersigned, Examiner Sisson expressed his reservations regarding specification of acceptor residues in the claims. The present claims do not specify acceptor residues. It is expected that this concern has been obviated.

Alternatively, the present claims specify that the variable domain comprises "**predominantly** human acceptor antibody heavy chain framework residues". It is, thus, asserted that any concerns regarding the claims encompassing chimeric antibodies in which the entire variable domain is of donor origin have been obviated.

The Examiner also indicated during that discussion that his concerns were more directed to issues of scope and that, if the claims contained critical limitations not taught in the art, the art rejections would not be a problem.

Applicants believe that, in light of the amendments, the application is now in condition for allowance and request early notification of the same. To the extent this belief is erroneous, Applicants request that the Examiner contact the undersigned at (215) 564-8352 to discuss the same.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. 35,719

Date: **September 7, 1994**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

DOCKET NO.: CARR-0032 PATENT

39/H
B. Denny
10/20/95

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage

Serial No.: 08/303,569 Group Art Unit: 1806

Filed: 9/07/94 Examiner: D. Adams

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

On September 18, 1995

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Esquire Reg. No. 35,719

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

Dear Sir:

AMENDMENT

Please amend the application as follows.

In the Specification:

Page 1, line 2, after "filed September 17, 1991", please insert -- , now abandoned --.

Please insert new page 94.

In the Claims:

Claim 120, line 12, please delete, ", provided that the antibody does not have affinity for the P55 chain of the human interleukin 2 receptor."

REMARKS

This paper is being filed in response to the Office Action mailed on May 16, 1995. The following comments use the section numbering set forth in the Office Action. Applicants respectfully request reconsideration and withdrawal of all rejections.

CLAIM AMENDMENT

As will be shown below, a reference which the Examiner initially asserted as prior art was not published before the priority date. The "provided that .... " clause in claim 120 was inserted in view of this reference. Since the rejection over this reference is not proper, claim 120 has been amended accordingly.

Sections 15 to 18

The contents of these sections are noted. No action is required.

Section 19

It is believed that this point has been taken care of by the amendment to the description.

Section 20

The Abstract has been added in the required form on new page 94. The text of the abstract is found on the cover page of the PCT publication from which the present application derived and, therefore, does not represent new matter.

Sections 21 to 23

In these sections, the Examiner raises objections to the description and claims under 35 USC § 112, first paragraph. The Examiner alleges that the specification fails to provide an enabling disclosure. However, it appears that the Examiner is actually questioning whether the invention will work over the entire of the scope of the claims.

As regards enablement, the first paragraph of § 112 requires that a person skilled in the art should be able to make and use the invention. In order to make and use the invention, the skilled person needs to be able to carry out the following steps, all of which are clearly set out in the specification.

1. Obtain a donor antibody having affinity for a predetermined antibody. This can best be done either by obtaining a hybridoma, for instance from a culture collection such as the ATCC, or by producing a hybridoma, using the well established Kohler-Milstein procedure.

2. Determine the sequences of the variable domains of the heavy and light chains of the donor antibody. As shown in the specification, this can best be achieved by isolating cDNA from the hybridoma, sequencing the cDNA and decoding the cDNA to give

the amino acid sequence.

3. Number the amino acid sequences from the donor antibody according to the Kabat numbering system. For any person of skill in the art, this is a simple matter, as the Kabat numbering system was well established at the priority date of the application. Thus, the skilled person would readily be able to identify the donor residues specified in the present claims.

4. Determine the amino acid sequences of the variable domains of a human antibody. This is again a simple matter as the sequences of a large number of human antibodies have been published, for instance by Kabat. Thus, this merely requires the skilled person to look in a readily available reference book. Alternatively, the skilled person would look in any one of a large number of papers disclosing the sequences of human antibodies.

5. Number the human amino acid sequences. Quite often, this had already been done. In any event, the comments in 3 above apply.

6. Determine the composite sequence(s) in accordance with the instructions in the claims. This merely requires comparison of the numbered sequences of steps 3 and 5.

7. Produce DNA molecule(s) encoding the composite sequence(s) determined in step 6. This is a matter of standard recombinant DNA technology. The DNA molecule(s) could be produced by total synthesis, partial synthesis or mutagenesis techniques, all of which were well know in the art at the priority date.

8. Transform a suitable host cell with the DNA sequence(s) produced in step 7 such that the host cell expresses the DNA sequence(s). This again is a matter of standard recombinant DNA technology.

It is to be noted that the present specification discloses copious details how to carry out all these steps. For instance, on page 25, there is a description of how to grow

hybridomas. On page 30, there is a description of cDNA preparation and screening, with details of probes to be used. Page 31 discloses details of DNA sequencing and production of expression vectors. Page 36 gives details of expression. Further on in the specification, even more detailed instructions are provided for the production of antibodies as defined in the present claims.

The procedures disclosed in the present specification could have been applied by any skilled person at the priority date for any available donor antibody or for any donor antibody the skilled person could have produced. The skilled person is told exactly what to do and how it can be done. The skilled person is given a number of examples to follow. It cannot be seen that this would require "undue experimentation".

The Examiner has not pointed to any step in the disclosed processes which could not have been carried out by a person of skill in the art. If the Examiner believes that any particular one or more of the steps could not have been carried out by a person of skill in the art, it is requested that the Examiner provide evidence of the same or, if in the Examiner's knowledge, provide an affidavit, both pursuant to 37 C.F.R. §1.107.

It is submitted that, in the absence of any evidence to the contrary, it must be accepted that the skilled person could have started with any donor antibody and followed the instructions in the specification to produce an antibody as now claimed.

The Examiner's real point appears to be that he does not believe that carrying out the steps referred to above would always lead to the production of a useful antibody. However, the Examiner has provided no evidence to support his belief; nor is this a requirement for enablement. *In re Sarrett*, 140 U.S.P.Q. 474 (C.C.P.A., 1964), *reh'g denied*. ". . . the mere *possibility* of inclusion of inoperative substances does not prevent *allowance* of broad claims." (emphasis in original) *Id.*, 140 U.S.P.Q. at 486.

Regardless, as the Examiner has pointed out in Section 22 (A) of the Office Action, the present specification provides a number of examples in which the procedure described in the specification has been applied successfully. The specification has examples relating to OKT3, OKT4A, B72.3 (an anti-mucin MAb), R6-5-D6 (an anti-ICAM-1 MAb) and 61E71 hTNF1, hTNF3 and 101.4 (all anti-TNF MAbs). Thus, the specification by itself provides examples in which MAbs against a variety of different antigens have been successfully humanised.

The Examiner is also referred to the enclosed Declaration executed by Dr. G. T. Yarranton (who the Examiner met during interviews on other cases last year). This declaration provides further evidence that the Applicants' employers have successfully used the procedure disclosed in the specification to humanise 17 antibodies.

The Examiner cannot point to a single example where anyone has tried to use the procedure set forth in the present specification and has failed to produce a useful antibody. The Examiner has merely referred to three papers which do not even try to put the Applicants' invention into effect. It is submitted that this does not provide any evidence that the procedure set forth in the present specification cannot be applied to any antibody. It is the *claimed invention* which is relevant to an analysis of enablement. *Ex parte Ehrlich*, 3 U.S.P.Q. 2d 1011 (Bd. Pat. App's. Int. 1987).

The first paper to which the Examiner refers is Reichmann et al. (Nature, 372, 323-327, 1988). This paper shows the results of "reshaping" the rat monoclonal antibody YTH34.5HL. As can be seen from Table 1 in the right hand column on page 325, Reichmann made four heavy chain constructs. The first (RaVHCAMP) is a chimeric heavy chain having the rat heavy chain domain fused to a human constant region. In the other three constructs, the heavy chain variable domain is predominantly of human origin but all three CDRs (residues 31 to 35, 50 to 58 and 95 to 102) correspond to those of the rat antibody. In the second construct, only the CDRs are rat residues. In the third construct, in addition to changing the CDRs, residue 27 has been changed to a more usual human residues (Ser → Phe). In the fourth construct, in addition to changing the CDRS, residues 27 and 30 have been changed to more

usual human residues (27 Ser → Phe, 30 Ser → Thr). In the light chain construct only the CDRs are changed to rat.

It can thus be seen that Reichmann does not disclose a procedure which leads to an antibody as defined in the present claims. As far as the heavy chain is concerned, Reichmann does not even mention Kabat residues 23, 24 and 49, let alone change them to the rat residues. Reichmann did not carry out a procedure as set forth in the present specification and thus Reichmann does not provide any evidence which shows or suggests that the present procedure cannot be put into practice for any antibody.

The second paper to which the Examiner refers is Queen et al. (PNAS-USA, 86 10029-10033, 1989). However, as is discussed in more detail below, Queen et al. is not prior art. Nonetheless, to the extent Queen et al. is contemporaneous, it will be discussed. Queen adopts an entirely different approach than that set forth in the present specification. This can be seen from the passage in the right hand column on page 10031 headed "Construction of a Humanised Anti-Tac Antibody". The first step in this approach is to select human acceptor sequences which are *as homologous as possible* to the mouse donor antibody. The second step is to use the donor CDRs in the human acceptor sequence. The third step is to carry out molecular modelling and then to select donor residues, to be put into the acceptor sequences, on the basis of various criteria. This leads to a composite variable domain sequence which

contains a large number of donor residues. Although the procedure used by Queen is different from that disclosed in the present specification, in the heavy chain, the CDRs and residues 23, 24 and 49 all correspond to those residues in the donor mouse antibody. Thus, the humanised antibody of Queen is, to a certain extent, in accordance with the present invention.

However, the foregoing residues were not the only residues transformed to donor in Queen. This point was acknowledged by the Examiner on page 4, lines 35-36, of the Office Action. It is to be noted that Queen's humanised antibody has only one third of the affinity of the donor mouse antibody. This is not a particularly good result as the aim of any humanisation procedure is to recover the same affinity as that of the donor antibody. It is believed that the reason for such a low recovery of affinity by Queen is due to there being too many donor residues in the acceptor frameworks. It is believed that, had the procedure of the present specification been adopted, certain of these residues would not have been changed, and improved recovery of affinity would have been obtained.

Nevertheless, given the above explanation of the low recovery affinity in Queen, it can be seen that, if anything, Queen shows that following the procedure of the present specification will lead to the production of useful antibodies

In passing, it is to be noted that the Examiner appears to be laboring under a misconception. The Examiner refers in Section 22 (A) to the changes resulting "in increased antigen binding affinity". The present Applicants do not claim that using the procedure of the specification will result in increased affinity. The aim of the invention is to provide an antibody having equivalent affinity to that of the donor antibody, but with increased human compatibility. The problem with mouse or rat antibodies is not that they have low affinity. They generally have very good affinity. The problem is that they are not compatible with humans. Thus, the problem is to retain the affinity but to remove human incompatibility.

It is no doubt true that, in some cases, it is possible to increase the affinity of the antibody by using the procedure of the present specification. However, this is a bonus effect and is not the object of the invention. Thus, while recovering one third of affinity may not be acceptable, recovering close to 100% of activity is more than enough. It is therefore suggested that the Examiner should look at the present invention in terms of recovering, not increasing, affinity. Nonetheless, an increase in affinity is an unexpected result relevant to nonobviousness.

Returning to the main theme of this Section the Examiner finally relies on Chothia et al. (J. Mol. Biol., 196, 901917, 1987). However, this has no bearing at all on whether the present

invention is applicable to any donor antibody.

Chothia describes theoretical work carried out on the structures and sequences of known antibodies or antibodylike molecules. These are all "natural" molecules in that they have not been in any way engineered. Chothia studied the resolved crystallographic structures of some of these molecules and also compared the sequences of the molecules. Most of the work was concerned with the conformation of the antigen-binding loops (L1 to L3 and H1 to H3). Chothia arranged the loops into groups, called "canonical structures".

Chothia also looked at the framework regions to a certain extent and identified certain framework residues which appeared to be involved in positioning the loops. However, Chothia appears to believe that these residues are specific to the loops with which they are associated. There is no indication that it is possible to make any predictions on the basis of these observations.

It can thus be seen that Chothia did not make any composite antibody chains, nor did Chothia produce any antibody chains by recombinant DNA technology. Most importantly, Chothia did not even attempt to produce a composite antibody chain using the procedure of the present specification. Thus, Chothia provides absolutely no evidence that one skilled in the art could not apply the teachings given in the present specification to any donor antibody.

It is submitted that the disclosures in Reichmann and Queen are relevant in one sense, in that they clearly show that, at the priority date, the skilled person was able to carry out all the necessary steps, for instance using recombinant DNA technology, to produce composite antibody chains. Thus, Reichmann and Queen support the Applicants' view that the present specification provides an enabling disclosure.

It is submitted that the Examiner's objection under Section 112 confuses the requirement for an enabling disclosure with the requirement for the invention to be nonobvious. As to enablement, the question to be asked is whether the skilled person, given the teaching in the specification, could have put that teaching into effect. In the present case, it is clear that the skilled person was able to carry out the necessary steps to produce an antibody according to the claims, using any donor antibody. Moreover, the present specification clearly shows that the procedure disclosed therein had been used successfully to produce a number of humanised antibodies having affinity equivalent to that of the donor antibody. Thus, on the basis of the teaching in the specification, the skilled person had every reason to expect that the procedure would be applicable to any donor antibody. As has been shown above, the prior art does not provide any evidence to suggest otherwise.

It is no doubt true, as the Examiner stated in Section 22 (A), that the prior art does not teach that a standardized principle is possible. However, this has no relevance to the question of enablement, because the prior art does not include the disclosure in the present specification. Absent the disclosure in the present specification, the skilled person would not have been taught that there was a standardized principle. This merely shows that the claimed subject matter is non-obvious. It does not show that the teaching in the present description is non-enabling.

In summary, it is submitted that:

(i) the present specification provides all the instructions necessary to enable the procedure disclosed therein to be put into effect for any donor antibody;

(ii) Reichmann and Queen confirm that, at the priority date, the skilled person was able to carry out the disclosed procedure;

(iii) the present specification shows that the disclosed procedure had been applied successfully to a number of donor antibodies, thus providing the skilled person with a reasonable expectation that the procedure is applicable to any donor antibody;

(iv) there is no evidence to show that the procedure is unsuccessful; and

(v) the prior art is not relevant to the skilled person's expectation of success because it does not include the disclosure in the present specification.

It is therefore submitted that the present specification provides an enabling disclosure for the whole scope of the claims.

For the above reasons, it is respectfully requested that the rejection under 35 USC § 112 against the specification and claims be withdrawn.

Section 25

The Applicants confirm the Examiner's presumption that the subject matter of all the claims was commonly owned at the time the inventions covered by the claims were made.

Sections 26 and 27

The Examiner's raising of a provisional obviousness-type double patenting rejection is acknowledged. This will be dealt with, probably by use of a terminal disclaimer, once the Examiner has acknowledged that the claimed subject matters in this and the co-pending application are patentable.

Section 28

In Section 28, the Examiner rejected all the claims as allegedly being obvious over Reichmann (see above), Queen (see above) and Waldmann (sic) (EP-A-0 239 400). It is presumed that the Examiner correctly cited the European patent application number (EP-A-0 239 400) for the third reference. If this is the case, the applicant is Winter. The Applicants therefore assume that the name for the third reference should be Winter. If this is not the case,

the Examiner is requested to identify the Waldmann paper to which he refers.

As a preliminary point, it is submitted that Queen et al. is not part of the prior art. The attached Declaration by the undersigned attorney shows that Queen was not published before the priority date of the present application. Thus, Queen cannot be used to attack the present claims.

The present application is a national phase filing of a PCT application claiming a foreign priority date of December 21, 1989. As indicated in the attached Declaration of the undersigned, the journal volume in which the Queen et al. reference appeared was not mailed until December 20, 1989. The journal was mailed by second class mail. Accordingly, no addressee could have received the journal *before* December 21, 1989.

As the Examiner is aware, magazines are effective as of the date they are received, not the date they are mailed. M.P.E.P. § 715.01(c). As no addressee could have received the reference before the foreign priority date, no addressee could have been in possession of the reference such that the subject matter sought to be patented as a whole would have been obvious "at the time the invention was made."[1] Indeed, in *Protein Foundation, Inc. v. Brenner*, 151 U.S.P.Q. 561 (D.D.C. 1966), the court took judicial

[1] In that regard, the time differential is also emphasized. The priority application was filed in the United Kingdom. The reference volume was mailed in the United States.

notice of the fact that second class mail does not travel and is not distributed as fast as first class mail. The court concluded that no magazine was delivered two days after being mailed in bulk. *Id.*, at 562. Regardless, it is emphasized that Queen et al. discloses that only 1/3 of the affinity of the murine antibody was recovered in their slightly less "human" antibody, and that "further work" was needed.

Since Queen is not part of the prior art, it is presumed that the Examiner's obviousness rejection can only be based on Reichmann and Winter. Before discussing those references in details, the Examiner is referred to Section 22 (A) of the Office Action, wherein the Examiner himself states that:

> 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 reasonable (sic) predict which amino acids will be effective in retaining antigen binding ability for a particular antibody.

It is submitted that this statement completely undermines the Examiner's position on obviousness. If the skilled person is not taught that a standardized principle is possible, it cannot be seen how the present invention, directed to a standardized principle, can be obvious.

It is also to be pointed out that the present invention, as set forth in the claims, is not that it will be necessary to

change some (unspecified) framework residues. The invention is that it will be necessary to change certain, carefully specified framework residues. Thus, the Examiner has to go beyond showing that a standardized principle was possible and has to show that the skilled person would inevitably have been led to the residue changes set forth in the present claims. As the Examiner admits in Section 28 of the Office Action:

> Neither Reichmann et al. or Queen et al. teach all of the exact mutations found in the claimed antibodies.

It thus cannot be seen how it can be obvious to get to the present invention.

This conclusion is reinforced if the prior art references cited by the Examiner not including Queen are properly considered. It is firstly to be pointed out that Winter was published in 1987, whereas Reichmann was published in 1988. Moreover, one of the authors of Reichmann is Dr. Winter, the sole inventor of Winter. It can thus be clearly seen that Reichmann follows on from the work of Winter. It is therefore believed that it is appropriate to discuss Winter first and then Reichmann as this reflects the way in which the art developed.

Winter teaches the basic concept of CDR-grafting. Thus, Winter clearly teaches the production of a grafted antibody in which only the CDRs (as defined by Kabat) from a donor antibody are grafted into an acceptor antibody in place of its natural CDRs.

There are examples in the citation showing this CDR grafting procedure. It is to be noted that in the examples, it is only the CDRs which are changed. No changes at all are made in the framework regions.

It is no doubt true that Winter contains a passage from page 7, line 25 to page 8, line 18 which refers to the possibility that framework residues may need to be changed. However, this passage is entirely devoid of any practical teaching. It does not mention a single residue number, nor does it even mention possible locations for such residues. Thus, Winter provides absolutely no suggestion, much less guidance, as to where to look for framework residues which may need changing.

The Examiner has asserted in Section 22 (A) of the Office Action that:

> ... it would require undue experimentation for a person of ordinary skill in the art to practice applicant's claimed invention from what has been disclosed in the specification.

Although the Applicants believe, for the reasons set forth above, that this is not true of the present specification, it is submitted that the Examiner's assertion applies with full force to Winter. The passage in Winter is merely an invitation to carry out further experiments without providing any directions or even hints as to how to carry out such experiments. It is therefore

submitted that the present claims are not at all obvious over Winter.

Turning now to Reichmann, it can be seen that this follows on from Winter. The first construct made by Reichmann is one in which only the donor CDRs are grafted into the acceptor frameworks. This construct is HUVHCAMP. As can be seen from Table 1, the first construct was nearly 40 times worse at binding antigen than the original rat antibody and was immeasurably worse in a complement binding assay. Reichmann therefore produced two more constructs. HuVHCAMP (Ser 27 → Phe, Ser 30 → Phe) and HuVHCAMP (Ser 27 → Phe, Ser 30 → Thr). The further changes in these constructs significantly improved both antigen binding and complement activation, but not to the level of the rat antibody.

The reason for making the further mutations at positions 27 and 30 is set forth in the left-hand column on page 326 of Reichmann. It can be seen that, at residue 27, the human acceptor sequence was unusual. Residue 27 was therefore changed to the more usual human residue. The change at residue 30 was made to bring the extended CDR, including the surface loop (residues 26 to 32) as well as the Kabat CDRs (residues 31 to 35) into conformity with those of the rat antibody. Thus, one mutation has the effect of making the grafted antibody look more human. The other mutation has the effect of extending CDR1.

It cannot be seen how this can in any way suggest the present invention. At best, it suggests that it would be better to define CDR1 as being residues 26 to 35 in the heavy chain. However, this certainly does not teach a general principle of changing framework residues and in particular it does not suggest that the particular framework residues of the present claims should be altered.

The passage on page 326 of Reichmann ends with the following sentence.

> This suggests that alterations in the 'Kabat' framework region can enhance the affinity of the antibody and extends previous work in which an engineered change in the hypervariable region yielded an antibody with increased affinity. (emphasis added).

Again, this is merely a suggestion for further work. It does not provide any hint or direction as to how the work should proceed. The only teaching is to extend CDR1 to include the surface loop. Thus, this certainly does not even remotely suggest the specific residue changes of the present claims.

The Examiner asserts that:

> ..... it would have been prima facie obvious ..... to apply the teachings of Winter to those of Reichmann .....

However, the Examiner appears to have failed to appreciate that the teaching of Winter is already contained in Reichmann. In fact, the teaching of Reichmann is based on the teaching of Winter. The

teaching of Winter was also published in Jones et al. (Nature, 321, 522-525, 1986), of which Winter is a co-author. Jones is reference 19 in Reichmann and is referred to in the paragraph bridging pages 323 and 325 of Reichmann. This paragraph makes it clear that Jones (and thus Winter) provided the basis for the work reported by Reichmann. Thus, the obvious combination of Winter and Reichmann had already been made in the prior art. However, despite having the expertise of Winter to call on, Reichmann comes nowhere near the present invention. It is therefore submitted that Reichmann by itself proves that a combination of Winter and Reichmann does not lead to the present invention.

Accordingly, Applicants submit that the Examiner has not established a *prima facie* case of nonobviousness of Applicants invention, with or without the Queen et al. reference. In fact, the Examiner's arguments under 35 U.S.C. §112 support this. Alternatively, to the extent a *prima facie case* is believed to be established, it is rebutted by the unexpected results of increased affinity.

For the above reasons, it is requested that the rejection under 35 U.S.C. § 103 be withdrawn.

Section 29

A copy of document AT is enclosed.

Summary

It is submitted that the remarks set forth above and the evidence sent herewith clearly show that the present application is in order for allowance which is hereby respectfully requested.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. 35,719

Date: September 18, 1995

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100
DYT/mb
Enclosures

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

Adair, et al.

Serial No.: **08/485,686** Group No.: **1806**

Filed: **June 7, 1995** Examiner: **Not Yet Assigned.**

For: **Humanised Antibodies**

CERTIFICATE OF FACSIMILE TRANSMISSION
I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being facsimile transmitted to Examiner Cech at (703) 308-4242 at the U.S. Patent and Trademark Office., Washington, D.C. 20231.

On August 23, 1996
Doreen Yatko Trujillo
Doreen Yatko Trujillo, Reg. No. 35,719

Assistant Commissioner for Patents
Washington DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to examination, please amend the above-identified application as follows:

In the Claims:

Please cancel claims 1-23.

Please add the following claims:

24. Antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues in a donor

Carter Exhibit 2015
Carter v. Adair
Interference No. 105,744

antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 100; and amino acid residues 23, 24, 49, 71, 73 and 78 at least are donor residues.

25. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 26 to 35, 50 to 65, and 95 to 102; and amino acid residues 23, 24 and 49 at least are donor residues.

26. An antibody according to claim 24 wherein one or more of residues 1, 3, 46, 48, 58, and 71 are additionally donor residues.

27. An antibody according to claim 25 wherein one or more of residues 1, 3, 46, 47, 48, 58, and 71 are additionally donor residues.

28. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat

Cont

numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 100; and amino acid residues 23, 24 and 49 at least are donor residues, provided that the antibody does not have affinity for the p55 chain of the human interleukin 2 receptor.

29. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 100; and amino acid residues 23, 24, 49, 71, 73 and 78 at least are donor residues, provided that the antibody does not have affinity for the p55 chain of the human interleukin 2 receptor.

30. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 26 to 35, 50 to 65, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues, provided that the antibody does not have affinity for the p55 chain of the human interleukin 2 receptor.

Cont

31. An antibody according to claim 28 wherein one or more of residues 1, 3, 46, 47, 48, 58, and 71 are additionally donor residues.

32. An antibody according to claim 29 wherein one or more of residues 1, 3, 46, 47, 48, 58, and 71 are additionally donor residues.

33. An antibody according to claim 30 wherein one or more of residues 1, 3, 46, 47, 48, 58, and 71 are additionally donor residues.

34. An antibody molecule having affinity for a T-cell antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a T-cell antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

35. An antibody molecule having affinity for a lymphokine and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a lymphokine, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

Cont

36. An antibody molecule having affinity for a growth factor and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a growth factor, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

37. An antibody molecule having affinity for a stimulating factor and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a stimulating factor, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

38. An antibody molecule having affinity for an interferon and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for an interferon, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

39. An antibody molecule having affinity for an adhesion molecule and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for an adhesion molecule, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

40. An antibody molecule having affinity for a hormone and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a hormone, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

41. An antibody molecule having affinity for a cancer marker and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a cancer marker, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

42. An antibody molecule having affinity for TNF-α and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for TNF-α, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

43. An antibody molecule having affinity for mucin and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for mucin, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

44. An antibody molecule having affinity for a receptor and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for a receptor, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

45. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody OKT3 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

46. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody OKT4A having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

47. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody B72.3 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor

residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

48. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody 61E71 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

49. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody hTNF1 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

50. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework

Cont

residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody hTNF3 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

51. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody 101.4 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

52. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in the donor murine antibody A5B7 having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 23, 24, and 49 at least are donor residues.

53. A therapeutic composition comprising an antibody molecule according to

claim 24 or claim 25 in combination with a pharmaceutically acceptable carrier, diluent, or excipient.

54. A method of therapy comprising administering an effective amount of an antibody according to claim 24 or claim 25 to a human or animal subject.

55. A method of diagnosis comprising administering an effective amount of an antibody according to claim 24 or claim 25 to a human or animal subject.

REMARKS

The foregoing amendments are to remove improper multiple dependency and to otherwise advance prosecution. No new subject matter has been added. Support for the amendments can be found, *inter alia*, in claims 1-23 and Examples 1-5, of the application as originally filed. An early notification of allowance is earnestly requested.

Respectfully submitted,

Date: August 23, 1996

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. **35,719**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

5-1-97

DOCKET NO.: CARP-0057 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

3/B
D. Williams
9/11/97

In re application of:

Adair et al

Serial No.: Unassigned Group Art Unit: Unassigned

Filed: Herewith Examiner: Unassigned

For: HUMANIZED ANTIBODIES

I, Francis A. Paintin, Registration No. 19386 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 May 1, 1997
Francis A. Paintin

Assistant Commissioner
for Patents
Washington, D.C. 20231

Dear Sir:

PRELIMINARY AMENDMENT AND REQUEST
FOR INTERFERENCE UNDER 37 CFR §1.607

Please amend the above-identified application as follows:

In the Specification:

At page 1, before line 1, insert the following:

--This application is a continuation of U.S. application Serial No.08/303,569, filed September 7, 1994, now U.S. Patent No. 5,859,205 which is a continuation of Serial No. 07/743,329, filed September 17, 1991, abandoned which is a U.S. national phase application stemming from PCT/GB90/02017, filed December 21, 1990, which PCT application claims priority benefit of GB national application Serial No. 89/28874.0, filed

B1

December 21, 1989 in the United Kingdom, the entire content of each of said applications is incorporated by reference herein.--

Amend the above-identified specification in accordance with the enclosed copy of a preliminary amendment (dated 7/8/92) filed in applicants' Serial No. 07/743,329 application which enters the Sequence Listing as replacement pages 67-89, and renumbers original pages 67-70 as pages 90-93, respectively, and amends the specification to refer to said listing appropriately.

In the claims:

Cancel claim 1 without prejudice and enter the following claims 24-31 in this 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 immunoglogulin 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.

25. A humanized immunoglobulin according to claim 24 which specifically binds to an antigen with an affinity in the range 10^{8}-10^{12} M^{-1}.

26. A humanized immunoglobulin according to claim 24, wherein the antigen is an IL-2 receptor.

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27. A humanized immunoglobulin according to claim 24, wherein the donor immunoglobulin is the anti-CD4 T-cell receptor antibody.

Sub H2

28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglogulin 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.

29. A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity equivalent to that of a chimeric antibody formed from said donor immunoglobulin.

30. A humanized immunoglobulin according to claim 28, wherein the antigen is a human CD3 T-cell receptor.

31. A humanized immunoglobulin according to claim 28, wherein the donor immunoglobulin is the anti-CD3 T-cell receptor antibody.--

REMARKS

Applicants have entered claims 24-31 to request an interference in accordance with 37 CFR §1.607 as follows. It is noted that the Queen patent whose claims present the basis for an interference is classified in Class 424/133.1. MPEP §2306 suggests a transfer to the group where the patent is classified.

Compliance With 37 CFR §1.607(a)

(a) Identification of the Patent

Applicants request that an interference be declared between applicants' above-identified application and Queen et al., U.S. Patent No. 5,585,089 (hereinafter "the Queen patent"), issued December 17, 1996, a copy of which is enclosed herewith. Applicants have in claims 24-27 substantially copied claims 1, 5,

9 and 10 of the Queen patent. Applicants have fully complied with the requirements of 35 USC §135(b) in claiming substantially the same subject matter directed to the same invention as that claimed in the Queen patent prior to one year from the 12/17/96 date the Queen patent was granted.

(b) Presentation of a Proposed Count

Applicants present in Appendix A attached hereto the "Proposed Count." In compliance with 37 CFR §1.606, proposed Count 1 is broader than any of claims 1-4, the broadest claims in the Queen patent, and as broad as any one of claims 24-31 being entered into the instant application.

The proposed count contains disjunctive or alternative language to cover the claim terminology of the two parties. Such counts were expressly approved by the Board in *Hsing v. Myers*, 2 USPQ2d 1861 (Bd, Pat,. App. & Int. 1987). It is clear, however, that both alternatives are directed to the same invention as that claimed in the Queen patent.

For Queen's term, "Chothia CDRs", applicants' claims and the proposed count paragraph (b) use the alternative term "the structural loop CDRs of the variable regions." In the Queen patent (at col. 11, lines 38-44) it is stated that the light or heavy chain variable regions consist of a "framework" region interrupted by three "hypervariable regions, also called CDRs."

In Chothia et al., *J. Mol. Biol.*(1987) 197, pp.901-917,

the authors (at p. 904) define their "loops" as having "somewhat different" limits from those of the CDRs defined by Kabat et al.(1983). Chothia et al. (at page 904) describe six loops in the domains L1, L2, L3, H1, H2, and H3[1], and use the descriptive terms "hairpin loops" (Fig.1), "hairpin turns" (Table 2), and "hypervariable loops" (p.903) to describe their regions which Queen has chosen to call "Chothia CDRs". A copy of the Chothia et al. publication is enclosed.

(c) Identification of Claims Corresponding to the Count

Applicants identify all of the Queen patent claims 1-11 and applicant's claims 24-27 as corresponding to the Count and as being directed to the same patentable invention.

(d) Application of the Terms of Applicants' Disclosure to the Copied Claims

In attached Appendix B, applicants illustrate the representative support in their present application disclosure for the limitations of their claims 24-27, substantially copied from Queen claims 1, 5, 9 and 10. There is, of course, additional support in applicants' application omitted for the sake of brevity.

(e) Applicants' Effective Filing Date

Applicants' present application, being a Rule 60 continuation, has the identical specification and drawings as

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[1] In Fig. 1 of Chothia et al., these six domains are referred to as the "hypervariable regions."

that originally filed in U.S. application Serial No. 08/303.569, filed September 7, 1994, which is a U.S. national phase application stemming from PCT/GB-90/02017, filed December 21, 1990. The latter PCT application claimed priority benefit of GB national application Serial No. 89/28874.0, filed December 21, 1989. Enclosed is a copy of the GB application Serial No. 89/28874.0, a certified copy of which is on file in the aforesaid PCT/GB application.

In attached Appendix C is a diagram of support in applicants' 1989 GB application for each limitation of applicants' claims 28-31, which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

(f) Queen's Effective Filing Date

The Queen patent stems from U.S. Serial No. 08/477,728, filed June 7, 1995, which is a continuation of Serial No. 07/634,278, filed 12/19/90, which is a c-i-p of Serial No. 07/590,274, filed 9/28/90, and Serial No. 07/310,252, filed 2/13/89, which is a c-i-p of Serial No. 07/290,975, filed 12/28/88.

A careful study of Queen's 1988 and 1989 application disclosures reveals that that there is no disclosure therein for

certain critical limitations of the Queen patent claims and the proposed count, e.g., for the count limitation:

> ...said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework **outside: (a) the Kabat and Chothia CDRs, or (b) both the Kabat CDRs and the structural loop CDRs of the variable regions,....**

Neither of the Queen 1988 and 1989 applications contains any disclosure that teaches or suggests the requirement of amino acids from the donor Ig outside both the Kabat CDRs **and** structural loop (or Chothia) CDRs. This is a material limitation that was added to its claims by Queen to overcome the teachings of the prior art.[2]

The most that can be argued is that the 1988 and 1989 disclosures may be read to suggest that such amino acids are outside the Kabat CDRs; but nothing therein suggests that such amino acids also be outside the structural loop (or Chothia) CDRs. Thus, the absence of a disclosure of that presently claimed limitation in the earlier-filed Queen applications is clearly fatal to any attempt by Queen to claim priorty benefit thereof.

While applicants have been unable to locate a copy of the Queen application allegedly filed on 9/28/90 to evaluate its

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[2] In Queen's amendment of May, 31, 1996, at page 5, it was argued that the claims distinguish over the prior art because the immunoglobulins contain donor amino acids "outside the Kabat and Chothia CDRs."

disclosure, that application date is almost nine months later than the 12/21/89 filing date of applicants' GB application.

Queen should not be entitled to priority benefit of any application filed prior to 12/19/90, for the invention of Count 1 or its patent claims. Moreover, applicants do not concede that either the 12/19/90 application, or the application that matured into the Queen patent, contains an adequate disclosure of the invention of the proposed count. However, that issue need not be considered at this time.

Compliance With 37 CFR §1.608

Since applicants have the earlier effective filing date, there is no requirement for them to establish a prima facie case of earlier priority under §1.608.

The Requested Interference Should Be Declared

In applicants' parent application Serial No. 08/303,569, Queen's assignee, Protein Design Labs, Inc. ("PDL") has filed a Protest under 37 CFR §1.248. Therein, PDL specifically states (at page 2):

> [A]n interference analysis should be undertaken by the appropriate Examiner....

Thus, PDL acknowledges that there is interfering subject matter in the paties' respective applications. For that reason, applicants have filed the present application with claims specifically directed to the claimed ssubject matter of the Queen patent. This paper more accurately characterizes the effective

filing dates of the parties and shows that Queen would be the junior party of any interference declared hereon.

Applicants respectfully request that the proposed interference be promptly declared. MPEP §2307 states as follows:

> Examiners should note that 37 CFR 1.607 requires that examination of an application in which applicant seeks an interference with a patent "shall be conducted with special dispatch." See MPEP §708.01 (emphasis added herein).

Applicants wish to point out that in their efforts to provoke the interference, claims 1, 5, 9 and 10 of the Queen patent were substantially copied. Thus, most claim limitations are those that were examined and approved by the Examiner who allowed the Queen patent. Should the present examination involve rejections of applicants' claims that would have been equally applicable against the Queen claims, applicants respectfully note MPEP §2307.02, which requires the approval of the Group Director for such a rejection. Applicants are presumptively the prior inventors of the claimed subject matter and only desire an interference to prove that they are the actual prior inventors. Their opportunity to do so should not be unduly delayed.

Enclosed is a copy of an Information Disclosure Statement filed in applicants' parent Serial No. 08/303,569, filed 9/7/94, and Serial No. 07/743,929. Copies of the references are in said parent applications.

Please contact applicants' attorney, Francis A. Paintin, at 215-568 3100 if he can be of assistance in expediting this request.

Respectfully submitted,

Francis A. Paintin

Francis A. Paintin
Registration No. 19,386

Date: May 1, 1997

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

APPENDIX A

PROPOSED COUNT FOR INTERFERENCE

Count 1:

A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglogulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with:

(i) an effective antigen binding activity, or

(ii) an affinity constant of at least 10^7 M^{-1} and no greater than about four-fold that of the donor immunoglobulin,

wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside:

(a) the Kabat and Chothia CDRs, or

(b) both the Kabat CDRs and the structural loop CDRs of the variable regions,

wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids:

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

(II) contains an atom within a distance of 4 A^0 of a CDR in said humanized immunoglobulin .

APPENDIX B

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglogulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1},	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.

and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence.	See page 11, lines 16-20, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At page 6, lines 25-35, it is indicated that the heavy chain "framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49...." In the heavy chain, Kabat CDR2 together with [Chothia] structural loop H2 extends from residues 50 to 65. Thus, residue 49 is immediately adjacent the beginning of this CDR2/H2 region. In Figs.3-4 residues marked with "N" to indicate near or adjacent a CDR (see p.38, l. 13.)
25. A humanized immunoglobulin according to claim 24 which specifically binds to an antigen with an affinity in the range 10^{8}-10^{12} M^{-1}.	Page 11, lines 27-30.
26. A humanized immunoglobulin according to claim 24, wherein the antigen is an IL-2 receptor.	Page 15, line 37, and page 16, line 2.
27. A humanized immunoglobulin according to claim 24, wherein the donor immunoglobulin is the anti-CD4 T-cell receptor antibody.	Page 53, Example 2.

APPENDIX C

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglogulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity'	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.

and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence.	See page 7, lines 11-14, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At p.5, l. 9-16, reference is made to heavy chain "framework comprises donor at at least one of residues 6, 23 and/or 24, 48 and/or 49...." Residue 49 is immediately adjacent CDR2/H2 loop region. On Figs.20-21 residues marked "N" are near or adjacent a CDR.
29. A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity as binding as a chimeric antibody formed from said donor immunoglobulin.	Page 23, lines 1-10; Fig. 29B.
30. A humanized immunoglobulin according to claim 28, wherein the antigen is a human CD3 T-cell receptor.	Page 11, lines 14-21. Page 17, lines 1-8; page 24, bottom paragraph.
31. A humanized immunoglobulin according to claim 28, wherein the donor immunoglobulin is the anti-CD3 T-cell receptor antibody.	Page 17, lines 1-8; page 24, bottom paragraph.

DOCKET NO.: CARP 0057 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

ADAIR ET AL.

For: HUMANIZED ANTIBODIES

Anticipated Classification of this application:

Class: 424, Subclass: 133.1

Prior Application
Examiner: D. ADAMS
Art Unit: 1816

"Express Mail" Label No. EM405876152US
Date of Deposit May 1, 1997

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Typed Name: Bob Inforzato

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington DC 20231

37 C.F.R. §1.60 TRANSMITTAL LETTER

Sir:

This is a request for filing a

(XX) Continuation ( ) Divisional

application under 37 CFR 1.60, of pending prior application Serial No.08/303,569, filed on September 7, 1994, which is a continuation of Serial No. 07/743,329, filed September 17, 1991.

1. (XX) Enclosed is a copy of prior application Serial No. 07/743,329, including the oath or declaration as originally filed.

I hereby verify that the attached papers comprise a true copy of the prior application Serial No. 07/743,329, as originally filed on September 17, 1991, and that no amendments referred to in the Oath or Declaration filed to complete the prior application introduced new matter therein.

The filing fee is calculated below on the basis of claims as filed in the prior application, less any claims cancelled or including any claims added by amendment listed below:

			SMALL ENTITY			OTHER THAN SMALL ENTITY	
For:	No. Filed	No. Extra	Rate	Fee	OR	Rate	Fee
BASIC FEE				$385	OR		$770
Total Claims	8 - 20 =		x $11=	$	OR	x $22=	$
Indep. Claims	2 - 3 =		x $40=	$	OR	x $80=	$
First Presentation Multiple Dependent Claims			+$130=	$	OR	+$260=	$
			TOTAL	$			$770

2. ( ) Verified Statement Claiming Small Entity Status is enclosed herewith.

3. ( ) Verified Statement Claiming Small Entity Status was filed in the parent case.

4. ( ) Please charge my Deposit Account No. 23-3050 in the amount of $_____. This sheet is attached in triplicate.

5. (XX) A check in the amount of $770.00 is attached. Please charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.

6. (XX) The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 23-3050. This sheet is attached in triplicate.

   (XX) Any additional filing fees required under 37 CFR 1.16 including fees for presentation of extra claims.

   (XX) Any additional patent application processing fees under 37 CFR 1.17 and under 37 CFR 1.20(d).

7. (XX) The Commissioner is hereby authorized to charge payment of the following fees during the pendency of this application or credit any overpayment to Deposit Account No. 23-3050. This sheet is attached in triplicate.

(XX) Any patent application processing fees under 37 CFR 1.17 and under 37 CFR 1.20(d).

( ) The issue fee set in 37 CFR 1.18 at or before mailing of the Notice of Allowance, pursuant to 37 CFR 1.311(b).

(XX) Any filing fees under 37 CFR 1.16 including fees for presentation of extra claims.

8. (XX) Cancel in this application original claims 2-23 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

9. (XX) Amend the specification as set forth in the accompanying preliminary amendment.

10. (XX) Formal drawings/photographs will be submitted when requested by the United States Patent and Trademark Office.

11. ( ) Please abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May only be used if signed by person authorized by 37 CFR 1.138 and before payment of the base issue fee.)

12. (XX) Priority of GB application Serial No. 89/28874.0 filed on December 21, 1989 in United Kingdom (country) is claimed under 35 U.S.C. Section 119.

(XX) The certified copy has been filed in prior PCT application Serial No. PCT/GB90/02017, filed December 21, 1990.

13. (XX) The prior application is assigned of record to Celltech Limited.

14. ( ) Copy of the Assignment(s) of the invention and separate Form(s) 1595 for each Assignment will be submitted upon receipt of the Official Filing Receipt.

15. (XX) The power of attorney in the prior application is to Francis A. Paintin, Registration No. 19,386.

(XX) The power appears in the original papers in the prior application.

(XX) Since the associate power of attorney does not appear in the original papers, a copy of the associate power in the prior application is enclosed.

16. (XX) Address all future communications to:

Francis A. Paintin
WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103

17. (XX) A preliminary amendment is enclosed. Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claim in the prior application.

18. ( ) A Petition for Extension of Time has been filed in the parent application, Serial No. _____ filed _____. A copy of the Petition for Extension of Time is enclosed.

19. (XX) Enclosed is a Statement to Support Filing and Submission of DNA/Amino Acid Sequences in Accordance with 37 CFR §§ 1.821 through 1.825 as filed in Serial No. 08/303,569.

(XX) Enclosed is a copy of a Letter of Reference to Computer Readable Form filed in Serial No. 08/303,569.

Date: May 1, 1997

Francis A. Paintin
Signature
Francis A. Paintin
Attorney of Record
Registration No. **19,386**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

IID
7/98
9/24/97

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF	:	John Robert Adair et al.
SERIAL NO.	:	08/485 686
FILED	:	JUNE 7, 1995
FOR	:	HUMANISED ANTIBODIES
GROUP ART UNIT	:	1816
EXAMINER	:	EVELYN RABIN Ph. D.

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 20, 1997

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Esquire Reg. No. 35,719

Assistant Commissioner for Patents
Washington, DC 20231.

RESPONSE AND AMENDMENT

Dear Sir:

Pursuant to 37 C.F.R. § 1.115, Applicants submit the following in response to the Office Action dated February 20, 1997.

In the Claims:

Please cancel claims 24 to 55, without prejudice.

Please add the following claims.

D'

--56. An antibody molecule having affinity for a predetermined antigen and

comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarily determining regions (CDRS) , said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain at least residues 31 to 35, 50 to 65 and 95 to 102 (the CDRS) and at least residues 23, 24, 49, 71, 73 and 78 (in the framework regions) correspond to the equivalent residues in said donor antibody.

57. 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. The antibody molecule of claim 56, wherein additionally at least one of residues 6, 37, 48 and 94 in said composite heavy chain corresponds, to the equivalent residue in said donor antibody.

59. The antibody molecule of claim 57, wherein additionally at least one of residues 6, 37, 48 and 94 in said composite heavy chain corresponds to the equivalent residue in said donor antibody.

60. The antibody molecule of claim 58, wherein additionally at least one of

residues 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. The antibody molecule of claim 59, wherein additionally at least one of residues 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. The antibody molecule of claim 56, wherein said complementary light chain is a composite light chain having a variable domain including CDRS, said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues 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 69 to 97 (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, 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 ~~is~~ has specificity ~~specific~~ for a T-cell antigen.

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

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

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

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

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

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

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

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

73. A ~~therapeutic~~ composition comprising ~~an~~ the antibody of any one of claims 56, 57 or 62 in combination with a pharmaceutically acceptable carrier, diluent or excipient. --

Remarks

This paper is being filed in response to the Office Action dated February 20, 1997. A petition for a three-month extension of time, and the appropriate fee, accompanies this response.

Claims 24 to 55 were pending. Claims 24 to 55 have been canceled herein without prejudice, and replaced by the new claims 56 to 73.

Support for present claim 56 can be found, *inter alia,* on page 19, lines 24 and 25, of the application as filed which defines the extent of the heavy chain CDRs, and page 7, lines 1 to 3, which defines the preferred basic set of framework residues which correspond between the donor and humanized chains.

Support for claim 57 can be found, *inter alia*, on page 17, lines 11 to 13 of the application as filed, which defines the preferred extent of the "CDRs". This extension, in fact, merely adds the Chothia loop residues to CDR1 in the heavy chain (see also page 8, lines 17 to 22).

Claims 58 and 59 are based on page 7, lines 7 to 9 of the application as filed. Claims 60 and 61 are based on page 7, lines 9 to 14 of the application as filed.

Support for claim 62 can be found, *inter alia*, is based on page 17, lines 1-4 to 16, of

the application as filed, which defines the extent of the light chain CDRs, and page 18, line 21, which defines the preferred basic set of light chain framework residues which correspond between the donor and humanized chains.

Basis for claim 63 is found on page 18, lines 23 to 28 of the application as filed.

Basis for claims 64 to 72 is found on page 15, line 24 through page 16, line 3 and in the Examples of the application as filed. Basis for claim 73 is found on page 16, lines 5 to 8 of the application as filed.

In view of the foregoing amendments, and arguments which follow, Applicants hereby request withdrawal of all rejections upon reconsideration. To the extent the rejections of claims 24 to 55 may be applied to the present claims, Applicants submit the following.

Rejections Under 35 USC § 112

In light of the amendments to the claims, it is submitted that the points raised by the Examiner in Sections 3, 4 and 5 (35 U.S.C. § 112, first paragraph) are rendered moot.

As regards the objection set forth in the last two paragraphs of the Examiner's Section 5, Applicants respectfully submit that such a rejection is unfounded. It is first to be noted that the Examiner has made a number of sweeping assertions which are totally unsupported by any evidence, reference to the statute, or reference to legal precedent. In particular, the Examiner has provided no evidence of a technical nature.

It is to be pointed out that, by the priority date of the present application, a large number of monoclonal antibodies had been successfully used in therapy. In particular, the OKT3

antibody, which is the subject of one of the examples, had been in use since the early 1980's in the treatment of acute rejection episodes in liver transplant patients. Thus, by the priority date, it was well known how to make a therapeutic antibody composition.

As is shown in the present description, the humanized antibodies of the present invention have affinities similar to those of the monoclonal antibodies from which they are derived. It would therefore have been clear to the skilled person that a composition containing a humanized antibody would be very similar to a composition containing the monoclonal antibody. It is therefore submitted that a skilled person would have been able readily to produce a therapeutic composition containing a humanized antibody of the present invention.

It may be that the Examiner has doubts as to the efficacy of the humanized antibodies of the present invention. Again, however, the Examiner has provided no evidence to support any such doubts. The Applicants have a number of humanized antibodies according to the present invention in clinical trials at various stages. These trials have shown that the humanized antibodies are effective. It is therefore submitted that there is no basis for the Examiner's, rejection which should therefore be withdrawn.

It is believed that the point raised in Section 6 by the Examiner is now moot. In any event, it is submitted that it is not necessary to use the particular monoclonal antibodies recited by the Examiner. The procedure described in the application is a general procedure which can be applied to any donor antibody and to any acceptor antibody. The Applicants have shown that this protocol can be applied generally, and have successfully produced a number of humanized antibodies according to the invention. It is therefore submitted that the rejection should be withdrawn.

Rejection Under 35 USC § 102(e)

In her Section 8, the Examiner refers to U.S. Patent No. 5,530,101 issued to Queen et al. The Examiner indicates, almost as a footnote, that the Queen Patent apparently has an effective filing date of December 28, 1988. While this may be true, the disclosures of the earliest Queen applications do not support this rejection. Following on from the initial application filed on December 28, 1988, there was a continuation-in-part application (CIP) filed February 13, 1989. There was then a separate new application filed September 28, 1990. A further continuation-in-part application was filed December 19, 1990, which claims priority to the prior continuation-in-part application and he separate new application. The '101 patent granted on the last continuation-in-part application.

The present application has a priority date of December 21, 1989. Only the initial application and the first continuation-in-part application of the '101 patent were filed before Applicants' priority date. Therefore, in order for the Examiner to be able to show that the present claims lack novelty, it must be shown that any subject matter which may be relevant was disclosed in these two earliest Queen applications.[1] Applicants respectfully submit that the Examiner has not shown this.

Applicants have been able to study the two early Queen applications in connection with the application from which the present application is a continuation. This study shows that there is

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[1] Similarly, the most recently issued patent from the same family, U.S. Patent No. 5,585,089 issued to Queen et al. on December 17, 1996 (the "'089 patent") is to no avail. The claims specify that the changes be made "outside the Kabat *and* Chothia CDRs." (emphasis added) There was no mention of Chothia. CDRs in the two earliest Queen applications. Thus, the '089 patent is not entitled to a filing date of December 28, 1988.

considerably more subject matter in the granted '101 patent than in either of the two early Queen applications. As far as Applicants can tell, the only working example provided in the two early Queen applications is the anti-tac antibody which is referred to in Figures 1A and 1B of the '101 patent.

The Examiner will see from a study of Figure 1B of the Queen Patent that residue 74 (according to the numbering in the Figure) is different between the mouse (top line) and human (bottom line) sequences. It is indicated in the Figure that the places where changes were made are indicated by double underlining. At residue 74, no change was made and so there is a clear difference between the humanized and mouse sequences. In Figure 1B, the sequences are numbered linearly and not according to the Kabat system. If the sequences are numbered according to the Kabat system, as specified in the present claim, linear residue 74 becomes Kabat residue 73. Since it is specified in present claim 56 that Kabat residue 73 should be identical between donor and acceptor sequences, it can be seen that the antibody of Figure 1 of the '101 Patent does not destroy the novelty of claim 56.

It is therefore submitted that there is no specific disclosure in the '101 patent entitled to the date of the two early Queen applications which destroys the novelty of present claim 56.

The general disclosure is equally unavailing. Looking first at the initial Queen application, it can be readily seen that it is not directed to a general process for humanizing antibodies. As is made clear from the introduction on pages 1 to 4 and the Summary of the Invention, the initial application is very specifically directed to the production of a humanized anti-tac antibody. Thus, there is absolutely no disclosure or suggestion of a claim to humanized antibodies in

general; nor is there any disclosure or suggestion to make the specific residues recited in the present claims donor.

Even if (which is denied) such a general teaching for humanizing anitbodies could be derived from the initial Queen application, it must be borne in mind that the teaching in the initial application is not enabling. It will be seen from pages 21 and 22 that there are disclosed three criteria for selecting framework residues outside the Kabat CDRs which can be considered for changing. One of these criteria is that the framework residues should be physically close to the antigen binding region. However, this criterion is so vague as to be meaningless. There is no description as to what "physically close" means. How close is close? How does, a skilled person build a model to determine whether a residue is close? What is the antigen binding region? It can thus be seen that it would be impossible for a skilled person to put this part of the teaching of the initial Queen application into effect.

The second Queen application has a slightly more general teaching. However, the general teaching is still not enabling for the invention recited therein, much less the present claims. It requires the skilled person to put into practice various criteria set out therein. One of these criteria is that residues a certain distance away from the antigen binding region should be identified. In order to identify these residues, it is necessary to build a molecular model of an antibody. Given the lack of detail in the Queen application, it is submitted that this would not have been possible.

By the date of filing of the continuation-in-part application, it was possible to build rough models of antibodies. However, these models could only be built using details which are not provided in this application. Even when such details were available, the models which were built

were not very accurate. The best accuracy was obtained for the main chain conformation of the framework regions, but even this was not very good. The modeled main chain conformations for the CDRs was poor. The results obtained for side chains, whether on framework or CDR residues, were not very accurate at all.

In the Queen applications, it is a requirement that a determination be made either of the physical closeness or of the distance between framework side chain atoms and CDR atoms. These are the two least accurate areas in any model. This clearly shows that it would not have been possible to put the teaching in the early Queen applications into effect in a reproducible manner, even using modern modeling programmes.

It is therefore submitted that there is no disclosure in either of the two early Queen applications, much less an enabling disclosure, of Applicants' invention. Thus, there can be no novelty-destroying disclosure therein.

Even if (which is denied) there were any enabling disclosure in the two Queen applications, it would still not be possible to obtain the subject matter of the present claims by following the teachings therein. Applicants have carried out their own modeling procedures using a current available modeling program. These results have shown that none of residues 23, 24, 71 and 73 in the heavy chain meet the criteria set out in the first Queen continuation-in-part application. A study of 39 solved X-ray crystal structures of antibodies homologous to the OKT3 antibody has shown that in NONE of these real (as opposed to modeled) structures does either residue 23 or residue 24 meet the distance criterion of the first Queen CIP application. Thus, as these residues would never be identified, there is no novelty-destroying disclosure in the two early Queen

applications.

If the Examiner requires, details of any of the studies the Applicants have made, can be submitted.

Accordingly, Applicants respectfully submit that the rejection under 35 U.S.C. §102(e) should be withdrawn.

Rejections Under 35 USC § 103

Regarding the Examiner's Section 10, it is hereby confirmed that the subject matter of the various claims was commonly owned at the time that the inventions covered by these claims were made.

In the remaining sections of the Office Action, the Examiner raises a number of obviousness rejections. These are all based on the Queen '101 patent which was discussed above in connection with novelty, discussion incorporated herein. It is submitted that the secondary references do not overcome the deficiencies of the '101 patent. The secondary references are relied upon for the specific antigen recitations in the dependent claims.

As stated above, even by the priority date of the present application, it was not possible to build accurate models of antibodies. The identification of residues which might be changed in the Queen applications is based on the use of molecular modeling. Since no accurate models could have been built, it would not have been possible to apply the teaching of the two early Queen applications to any antibody except the specific one referred to in the Queen applications. It has been shown above that this does not destroy the novelty of the present claims. Further, applying any teaching

derived from the Queen applications would still not produce anything falling within the present claims.

It should again be stressed that, in the CIP application, it is specified that residues within a certain distance of the CDR atoms should be selected as candidates for changing. Using modern molecular modeling techniques, it is possible to show that residues 23, 24, 71 and 73 in the heavy chain can never meet this distance criterion. As discussed previously, this is confirmed by studies of resolved structures for residues 23 and 24. Thus, even if it had been possible to build a molecular model at the priority date of the present application, doing so would not have identified the above four residues. It would not have been possible to produce anything falling within the present claims based on the teaching in these two early Queen applications.

None of the remaining references, or combination thereof, cited by the Examiner overcomes these deficiencies. Applicants respectfully submit that the rejections under 35 U.S.C. § 103(a) should be withdrawn.

Summary

It is submitted that the new claims submitted for the Examiner's attention are supported by the description as originally filed, are novel over any disclosure in the two early Queen applications, and are nonobvious over the '101 patent, whether taken alone or in combination with any other documents.

It is therefore submitted that the application is in condition for allowance, notice of which is hereby respectfully requested.

Respectfully requested

Date: August 20, 1997

Signature
Doreen Yatko Trujillo
Registration No. **35,719**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

93542

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WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS

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ONE LIBERTY PLACE - 46TH FLOOR
PHILADELPHIA, PA 19103-7301

(215) 568-3100

Facsimile: (215) 568-3439
Cable: WOODCOCK

DATE: January 28, 1998

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COVER MESSAGE: A proposed amendment to the previously allowed claims consistent with our discussions is attached. I look forward to speaking with you.

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PROPOSED CLAIMS

120. An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues, corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 6, 23, 24, and 49 at least are donor residues, provided that said antibody is not the Queen et al. humanized anti-tac antibody.

121. The antibody molecule of claim 120, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

122. The antibody molecule of claim 120, wherein amino acid residues 71, 73, and 78 in said composite heavy chain are additionally donor residues.

123. The antibody molecule of claim 120, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

124. The antibody molecule of claim 120, wherein at least one of amino acid residues 36, 94, 104, 106, and 107 in said composite heavy chain are additionally donor residues.

125. The antibody molecule of claim 124, wherein at least one of amino acid residues 2, 4, [6,] 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

126. The antibody molecule of claim 120, wherein said complementary light chain is a composite light chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain; said CDRs comprise donor residues at least at residues 24 to 34, 50 to 56, and 89 to 97; and amino acid residues 46, 48, 58, and 71 at least are donor residues.

127. The antibody molecule of claim 126, wherein amino acid residues 1, 3, 60 (if this residue can form a salt bridge with residue 54), and 70 (if this residue can form a salt bridge with residue 24) in said composite light chain are additionally donor residues.

DOCKET NO.: CARP-0032 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage**

Serial No.: **08/303,569** Group Art Unit: **1816**

Filed: **9/07/94** Examiner: **L. Feisee**

For: **HUMANISED ANTIBODIES**

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I Doreen Yatko Trujillo, Registration No.: 35,719 hereby certify that this paper is being facsimile transmitted to Examiner Feisee at (703)305-7230 of the U.S. Patents and Trademark Office, Washington, D.C. 20231, on the date shown below

On February 23, 1998

Doreen Yatko Trujillo, Reg. No. 35,719

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Dear Sir:

AMENDMENT PURSUANT TO 37 C.F.R. §1.312

Pursuant to 37 C.F.R. §1.312 (b), please amend the above-identified application as follows. A petition and appropriate fee accompanies this Amendment.

In the claims:

120. (Twice Amended) An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50

to 58, and 95 to 102; and amino acid residues 6, 23, 24, and 49 at least are donor residues, provided that said antibody is not the humanized anti-tac antibody described in WO 90/07861.

125. (Amended) The antibody molecule of claim 124, wherein at least one of amino acid residues 2, 4, [6,] 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

REMARKS

The foregoing amendments are being made to advance the present case to issuance. Although the issue fee had already been paid on December 4, 1996, issuance was delayed following the filing and subsequent entry of a Protest. These amendments are being submitted following the Examiner's consideration of the Protest, Applicants' response thereto, and a personal interview conducted with the Examiner on October 16, 1997. Thus, these amendments do not require additional search or examination, nor could they been submitted ealier. Support for the amendments can be found, *inter alia*, on page 5, line 10 through page 6, line 37, of the application as originally filed. The humanized anti-tac antibody of WO 90/07861 is therein described and distinguished from the present invention. The inclusion of residue 6 as a donor residue is also disclosed therein. As is clear from the foregoing, no new matter is added by these amendments. Applicants respectfully request that they be entered.

Respectfully submitted,

Doreen Yatko Trujillo

Date: February 23, 1998

Doreen Yatko Trujillo
Registration No. **35,719**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

1642 1641

DOCKET NO.: CARP-0032 **PATENT**

Issue Batch No.: D80
Date of Notice
of Allowance : September 4, 1996
Serial No. : 08/303,569

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John Robert Adair, Diljeet Singh Athwal, and John Spencer Emtage

Serial No.: **08/303,569** Group Art Unit: **1642**

Filed: **9/07/94** Examiner: **L. Feisee**

For: **HUMANISED ANTIBODIES**

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

Dear Sir:

AMENDMENT PURSUANT TO 37 C.F.R. §1.312

Pursuant to 37 C.F.R. §1.312 (b), please amend the above-identified application as follows. A petition and the appropriate fee accompanies this Amendment.

In the specification:

Page 6, line 9, after "heavy chain", please insert -- SEQ ID NO:31 --.

Please replace pages 67-92 of the Sequence Listing with the attached substitute Sequence Listing, pages 67-93. Please renumber the pages thereafter accordingly.

In the claims:

120. (Twice Amended) An antibody molecule having affinity for a predetermined antigen and comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody heavy chain framework residues, the remaining heavy chain residues corresponding to the

equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite heavy chain: said CDRs comprise donor residues at least at residues 31 to 35, 50 to 58, and 95 to 102; and amino acid residues 6, 23, 24, and 49 at least are donor residues, provided that said composite heavy chain does not comprise the amino acid sequence of SEQ ID NO: 31.

125. (Amended) The antibody molecule of claim 124, wherein at least one of amino acid residues 2, 4, [6,] 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

REMARKS

The foregoing amendments are being made to advance the present case to issuance. Although the issue fee had already been paid on December 4, 1996, issuance was delayed following the filing and subsequent entry of a Protest in the above-identified application. In the Protest, U.S. Patent No. 5,585,089 issued to Queen et al. on December 17, 1996 was cited as relevant. These amendments are being submitted following the Examiner's consideration of the Protest, Applicants' response thereto, a personal interview conducted with the Examiner on October 16, 1997, and telephonic discussions and communications with the Examiner, as well as telephonic communications with Examiner Schwartz. These amendments do not require additional search or examination.

Support for the proviso in claim 120 can be found, *inter alia*, on page 5, line 10 through page 6, line 37, of the application as originally filed. The heavy chain variable region of the humanized anti-tac antibody of WO 90/07861 is therein described and distinguished from the present invention. The heavy chain variable region of the humanized anti-tac antibody of WO 90/07861 was, thus, incorporated by reference. The inherent amino acid sequence of the heavy chain variable region of the humanized anti-tac antibody of WO 90/07861 was, thus, also incorporated by reference. In view of its recitation in claim 120, the amino acid sequence of the heavy chain variable region of the humanized anti-tac antibody of WO 90/07861 is presumed essential. Accordingly, Applicants have amended the specification to include the sequence of

the heavy chain variable region of the humanized anti-tac antibody described in WO 90/07861. The sequence is included as SEQ ID NO: 31 of the substitute Sequence Listing. A paper and computer-readable copy of the substitute Sequence Listing, and accompanying papers, are included herein. A Declaration by the undersigned that the amendatory material consists of the same material incorporated by reference is included pursuant to M.P.E.P. 608.01(p). Applicants have also corrected typographical errors in the sequence listing inadvertently introduced in the substitute Sequence Listing previously submitted on December 4, 1996.

Support for the recitation of residue 6 as a donor residue can be found, *inter alia*, on page 6, line 35, of the application as filed.

No new matter is added by any of the foregoing.

Applicants request that the foregoing amendments be entered and that, upon their entry, the application be allowed to issue. Pursuant to M.P.E.P. § 1309, Applicants request that the application be tagged to ensure appropriate printing priority in the publishing division. This application is at least entitled to category (2) priority as listed in M.P.E.P. § 1309.

If anything remains outstanding, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo

Date: July 13, 1998

Doreen Yatko Trujillo
Registration No. **35,719**

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
[(215) 568-3100

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ALLOWED CLAIMS

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

121. The antibody molecule of claim 120, wherein amino acid residues 26 to 30 and 59 to 65 in said composite heavy chain are additionally donor residues.

122. The antibody molecule of claim 120, wherein amino acid residues 71, 73, and 78 in said composite heavy chain are additionally donor residues.

123. The antibody molecule of claim 120, wherein at least one of amino acid residues 1, 3, and 76 in said composite heavy chain are additionally donor residues.

124. The antibody molecule of claim 120, wherein at least one of amino acid residues 36, 94, 104, 106, and 107 in said composite heavy chain are additionally donor residues.

125. The antibody molecule of claim 124, wherein at least one of amino acid residues 2, 4, 38, 46, 67, and 69 in said composite heavy chain are additionally donor residues.

126. The antibody molecule of claim 120, wherein said complementary light chain is a composite light chain having a variable domain including complementarity determining regions (CDRs), said variable domain comprising predominantly human acceptor antibody light chain framework residues, the remaining light chain residues corresponding to the equivalent residues in a donor antibody having affinity for said predetermined antigen, wherein, according to the Kabat numbering system, in said composite light chain; said CDRs comprise donor residues at least at residues 24 to 34, 50 to 56, and 89 to 97; and amino acid residues 46, 48, 58, and 71 at least are donor residues.

127. The antibody molecule of claim 126, wherein amino acid residues 1, 3, 60 (if this residue can form a salt bridge with residue 54), and 70 (if this residue can form a salt bridge with residue 24) in said composite light chain are additionally donor residues.

DOCKET NO.: CARP-0057 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Adair et al

Serial No.: 08/846,658 Group Art Unit: 1642

Filed: May 1, 1997 Examiner: J. Reeves

For: HUMANIZED ANTIBODIES

I, Francis A. Paintin, Registration No. 19386 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 Nov. 5, 1998
Francis A. Paintin

Assistant Commissioner
for Patents
Washington, D.C. 20231

Dear Sir:

FOURTH PRELIMINARY AMENDMENT

Please amend the above-identified application as follows:

In the claims:

Please enter the following claims 32-48 in this application:

--32. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin

specifically binds to an antigen with an affinity constant of at least about $10^8\ M^{-1}$ and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework and comprises at least 70 amino acid residues identical to those in the acceptor human immunoglobulin heavy chain variable region framework.

33. A vector comprising first and second polynucleotides according to claim 32.

34. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about $10^8\ M^{-1}$ and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is a

consensus sequence of human immunoglobulin heavy chain variable region frameworks.

35. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about $10^8\ M^{-1}$ and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin heavy chain comprises one or more amino acids from the donor immunoglobulin heavy chain framework outside the Kabat and Chothia CDRs, wherein the donor amino acids substitute for corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids:

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

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

36. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of a least about $10^8\ M^{-1}$ and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin heavy chain comprises one or more amino acids from the donor immunoglobulin heavy chain framework outside the Kabat and Chothia CDRs that substitute for the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, wherein each of these said donor amino acids:

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

(II) is capable of interacting with amino acids in the CDRs, or

(III) is typical at its position for human immunoglobulin sequences, and the substituted amino acid in the acceptor is rare at its position for human immunoglobulin sequences.

37. A cell line transfected with a vector according to claim 33.

38. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant within about four-fold of that of the donor immunoglobulin, wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework and comprises at least 70 amino acid residues identical to those in the acceptor human immunoglobulin heavy chain variable region framework.

39. First and second polynucleotides respectively encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant within about four-fold of that of the donor

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

REMARKS

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

Respectfully submitted,

Francis A. Paintin
Registration No. 19,386

Date: November 5, 1998

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 10029–10033, December 1989
Immunology

A humanized antibody that binds to the interleukin 2 receptor

(chimeric antibody/antibody affinity/autoimmune disease)

CARY QUEEN*, WILLIAM P. SCHNEIDER*, HAROLD E. SELICK*†, PHILIP W. PAYNE*, NICHOLAS F. LANDOLFI*, JAMES F. DUNCAN*‡, NEVENKA M. AVDALOVIC*, MICHAEL LEVITT§, RICHARD P. JUNGHANS¶, AND THOMAS A. WALDMANN¶

*Protein Design Labs, 3181 Porter Drive, Palo Alto, CA 94304; §Department of Cell Biology, Stanford University, Stanford, CA 94305; and ¶Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Contributed by Thomas A. Waldmann, August 30, 1989

ABSTRACT **The anti-Tac monoclonal antibody is known to bind to the p55 chain of the human interleukin 2 receptor and to inhibit proliferation of T cells by blocking interleukin 2 binding. However, use of anti-Tac as an immunosuppressant drug would be impaired by the human immune response against this murine antibody. We have therefore constructed a "humanized" antibody by combining the complementarity-determining regions (CDRs) of the anti-Tac antibody with human framework and constant regions. The human framework regions were chosen to maximize homology with the anti-Tac antibody sequence. In addition, a computer model of murine anti-Tac was used to identify several amino acids which, while outside the CDRs, are likely to interact with the CDRs or antigen. These mouse amino acids were also retained in the humanized antibody. The humanized anti-Tac antibody has an affinity for p55 of 3×10^9 M^{-1}, about 1/3 that of murine anti-Tac.**

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

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

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

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

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

MATERIALS AND METHODS

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

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

†Present address: Biospan, 440 Chesapeake Drive, Redwood City, CA 94063.

‡Present address: Beckman Instruments, 1050 Page Mill Road, Palo Alto, CA 94304.

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

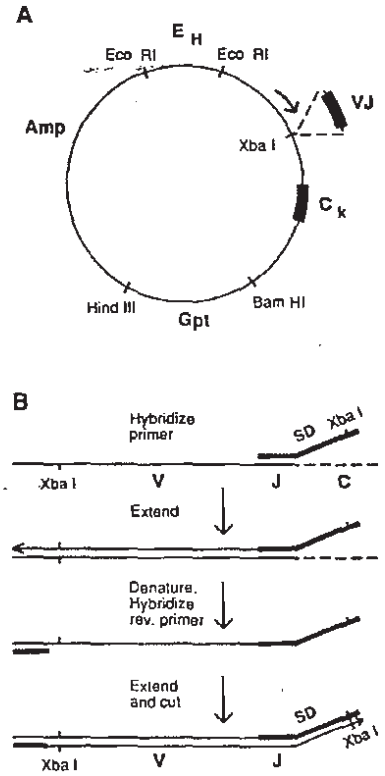

FIG. 1. (*A*) Schematic diagram of the plasmids pVκ1 and pLTac. Light chain exons are shown as boxes. An arrow indicates the direction of transcription from the κ promoter. E_H, heavy chain enhancer. Not drawn to scale. (*B*) Schematic diagram of the method used to excise the *V–J* region. SD, splice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

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

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

We hybridized this oligonucleotide to M13L and extended it with the Klenow fragment of DNA polymerase. The DNA was heat-denatured, hybridized with an excess of the "reverse primer" 5′-AACAGCTATGACCATG-3′, again extended with Klenow DNA polymerase, and digested with *Xba* I. The digested DNA was run on a gel, and an approximately 400-bp fragment was excised and inserted into the *Xba* I site of pVκ1. Sequencing showed that the fragment consisted of the V–J region of the light chain cDNA followed by the splice donor "tail," as expected (Fig. 1*B*), and pLTac, a clone with the appropriate orientation, was chosen. In an analogous fashion, the heavy chain V–J segment, followed by the mouse $J_H 2$ splice donor sequence, was excised from M13H and inserted into the *Xba* I site of pVγ1 to yield pGTac.

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

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

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

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

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

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

RESULTS

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

The V–J segments of the other pair of κ clones were sequenced completely and were identical. This light chain uses the $J_\kappa 5$ segment. Partial sequencing of the four γ2a clones showed they were all from the same gene. The V–J segments of two were sequenced completely and were identical. This heavy chain uses the $J_H 2$ segment and is of subgroup II (38). The DNA sequences have been deposited with GenBank;[11] the deduced protein sequences are shown in Fig. 2. As both alleles of the κ light chain were accounted for and only one heavy chain sequence was detected, we tentatively assigned these sequences to the anti-Tac antibody genes.

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

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

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

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

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

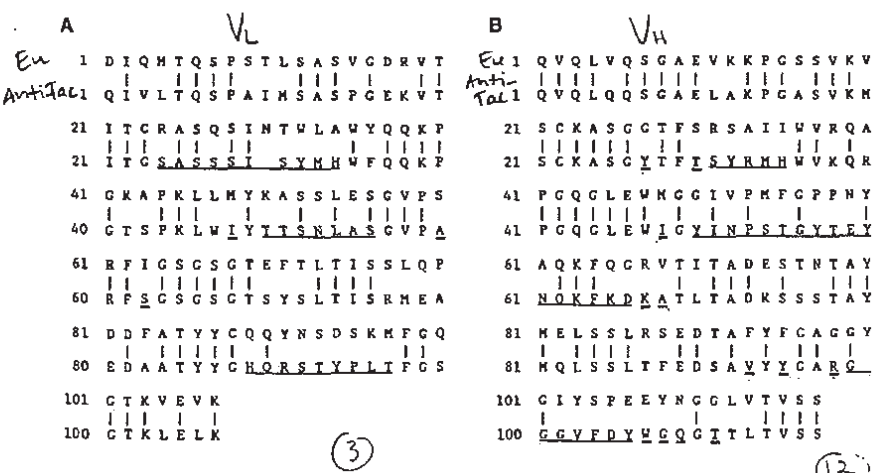

FIG. 2. Amino acid sequences of the humanized anti-Tac light (*A*) and heavy (*B*) chains. The sequences of the Eu antibody light and heavy chains (upper lines) are shown aligned above the mouse anti-Tac light and heavy chain sequences (lower lines), with a | indicating identity of amino acids. The three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

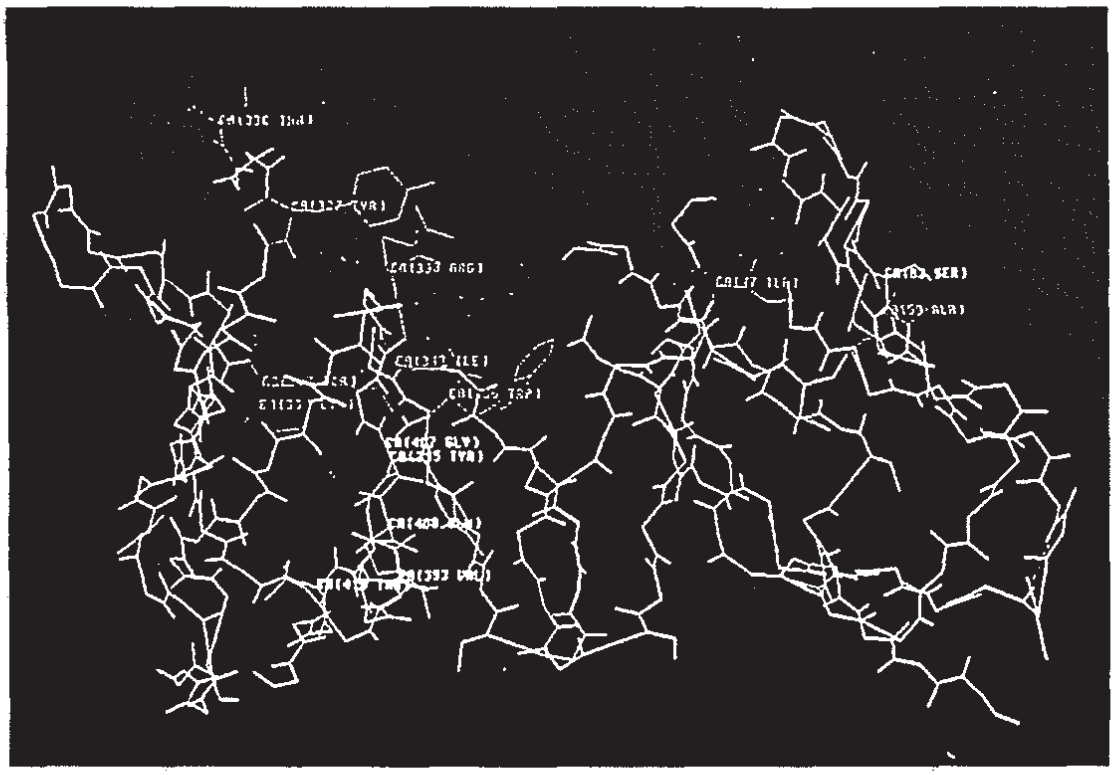

FIG. 3. Model of the mouse anti-Tac antibody V region, generated with the ENCAD program and displayed with the MIDAS program. Amino acids in the CDRs are shown in red; amino acids potentially interacting with the CDRs are shown in blue; other mouse amino acids used in the humanized antibody are shown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

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

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

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

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

DISCUSSION

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

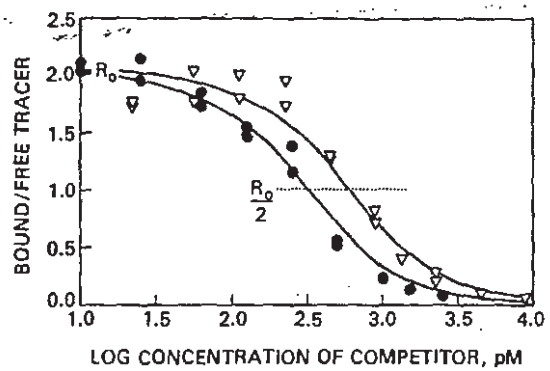

FIG. 4. Competitive binding of labeled anti-Tac tracer to Hut-102 cells. Duplicate samples are shown. ●, Mouse anti-Tac competitor; ▽, humanized anti-Tac competitor.

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

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

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

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United States Patent [19]

Adair et al.

[11] Patent Number: 5,859,205

[45] Date of Patent: Jan. 12, 1999

[54] **HUMANISED ANTIBODIES**

[75] Inventors: **John Robert Adair**, High Wycombe; **Diljeet Singh Athwal**, London; **John Spencer Emtage**, Marlow, all of United Kingdom

[73] Assignee: **Celltech Limited**, Berkshire, United Kingdom

[21] Appl. No.: **303,569**

[22] Filed: **Sep. 7, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 743,329, Sep. 17, 1991, abandoned.

[30] **Foreign Application Priority Data**

Dec. 21, 1989 [GB] United Kingdom 8928874

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

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

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

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Primary Examiner—Donald E. Adams

Attorney, Agent, or Firm—Woodcock Washburn Kurtz Mackiewicz & Norris

[57] **ABSTRACT**

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (**6**, **23**) and/or (**24**, **48**) and/or (**49**, **71**) and/or (**73**, **75**) and/or (**76**) and/or (**78**) and (**88**) and/or (**91**). The CDR-grafted light chains comprise donor residues at at least one of positions (**1**) and/or (**3**) and (**46**) and/or (**47**) or at at least one of positions (**46**, **48**, **58**) and (**71**). The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for in vivo therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

8 Claims, 18 Drawing Sheets

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

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

FIG. 1a

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

FIG. 1b

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

FIG. 2a

OKT 3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

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

FIG. 2b

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


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

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

FIG. 3

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

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


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

FIG. 4

□KT 3 HEAVY CHAIN CDR GRAFTS

1. gh341 and derivatives

```
          1                              26        35  39  43
□kt3vh    QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQ
gH341     QVQLVESGGGVVQ□GRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA178
gH341A    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA185

gH341E    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA198
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA2□7
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA209
gH341D    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA197
gH341*    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA199
gH341C    QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK   JA184

gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA203
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA205
gH341B    QVQLVESGGGVVQPGRSLRLSCSSSGYTFTRYTMHWVRQAPGK   JA183
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA204
gH341*    QVQLVESGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA206
gH341*    QVQLVQSGGGVVQPGRSLRLSCSASGYTFTRYTMHWVRQAPGK   JA208
K□L       QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK
```

FIG. 5a

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

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

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

FIG. 5b

	84 95 102 113		SEQ ID NO:
□kt3vh	SEDSAVYYCARYYDDHY.....CLDYWGQGTTLTVSS		
gH341	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA178	30
gH341A	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA185	12
gH341E	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA198	13
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA207	14
gH341D	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA197	15
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA209	16
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA199	17
gH341C	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA184	18
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA203	19
gH341*	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA205	20
gH341B	PEDTAVYYCARYYDDHY.....CLDYWGQGTTLTVSS	JA183	21
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA204	22
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA206	23
gH341*	PEDTGVYFCARYYDDHY.....CLDYWGQGTTLTVSS	JA208	24
K□L	PEDTGVYFCARDGGHGFCSSASCFGPDYWGQGTPVTVSS		10

FIG. 5c

OKT3 LIGHT CHAIN CDR GRAFTING

1. gL221 and derivatives

```
          1                         24        34      42
Okt3vl    QIVLTQSPAOMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
gL221     DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221A    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221B    QIVMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
gL221C    DIQMTQSPSSLSASVGDRVTITCSASS.SVSYMNWYQQTPGK
REI       DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK

          43     50    56                            85
Okt3vl    SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
gL221     APKLLIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221A    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221B    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
gL221C    APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIAT
REI       APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT (SEQ ID NO:8)


          86   91   96         108
Okt3vl    YYCQQWSSNPFTFGSGTKLEINR      (SEQ ID NO:29)
gL221     YYCQQWSSNPETFGQGTKLQITR      (SEQ ID NO:25)
gL221A    YYCQQWSSNPETFGQGTKLQITR      (SEQ ID NO:26)
gL221B    YYCQQWSSNPETFGQGTKLQITR      (SEQ ID NO:27)
gL221C    YYCQQWSSNPETFGQGTKLQITR      (SEQ ID NO:28)
REI       YYCQQYQSLPYTFGQGTKLQITR      (SEQ ID NO:9)
```

CDR'S ARE UNDERLINED

FRAMEWORK RESIDUES INCLUDED IN THE GENE ARE DOUBLE UNDERLINED

FIG. 6

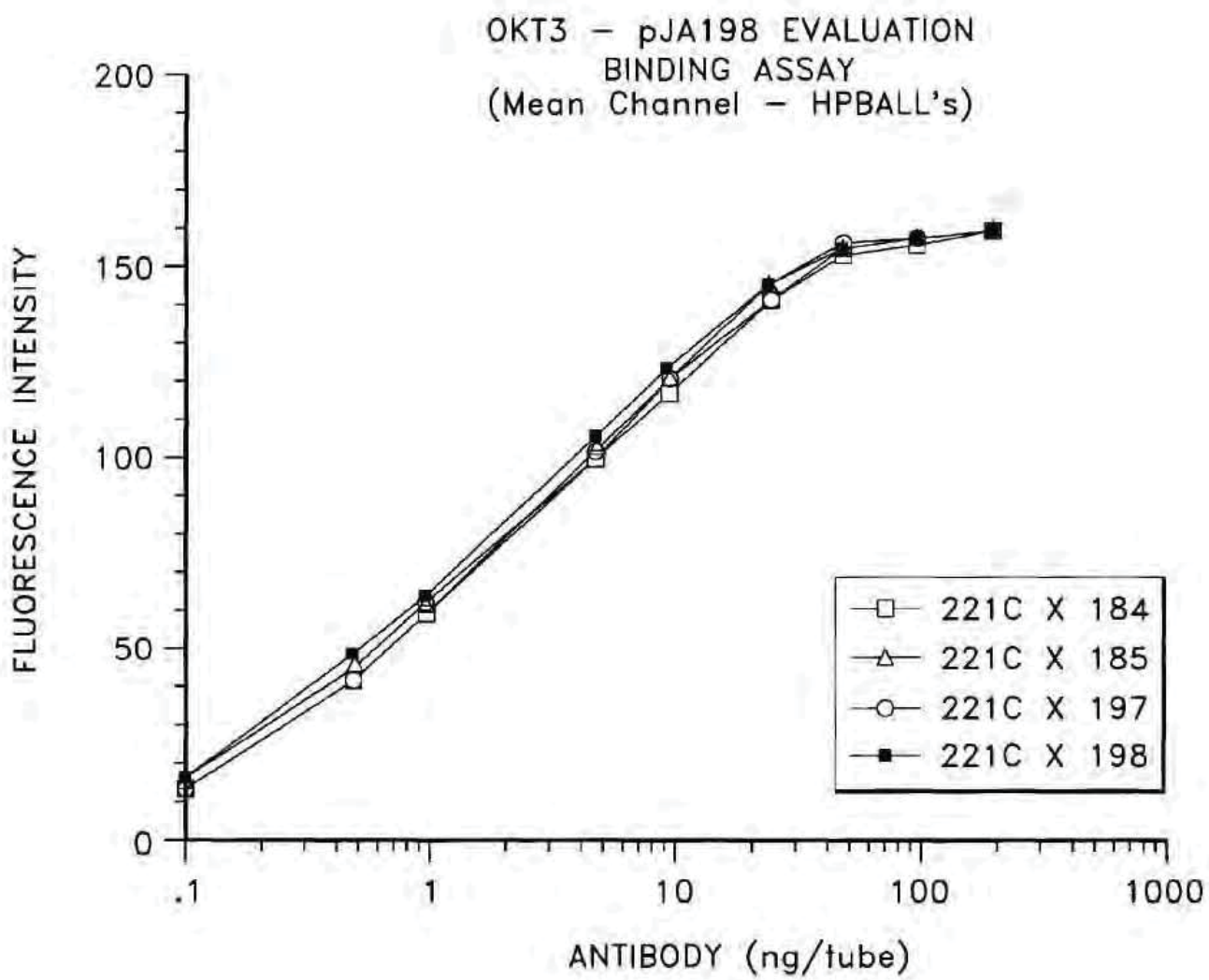
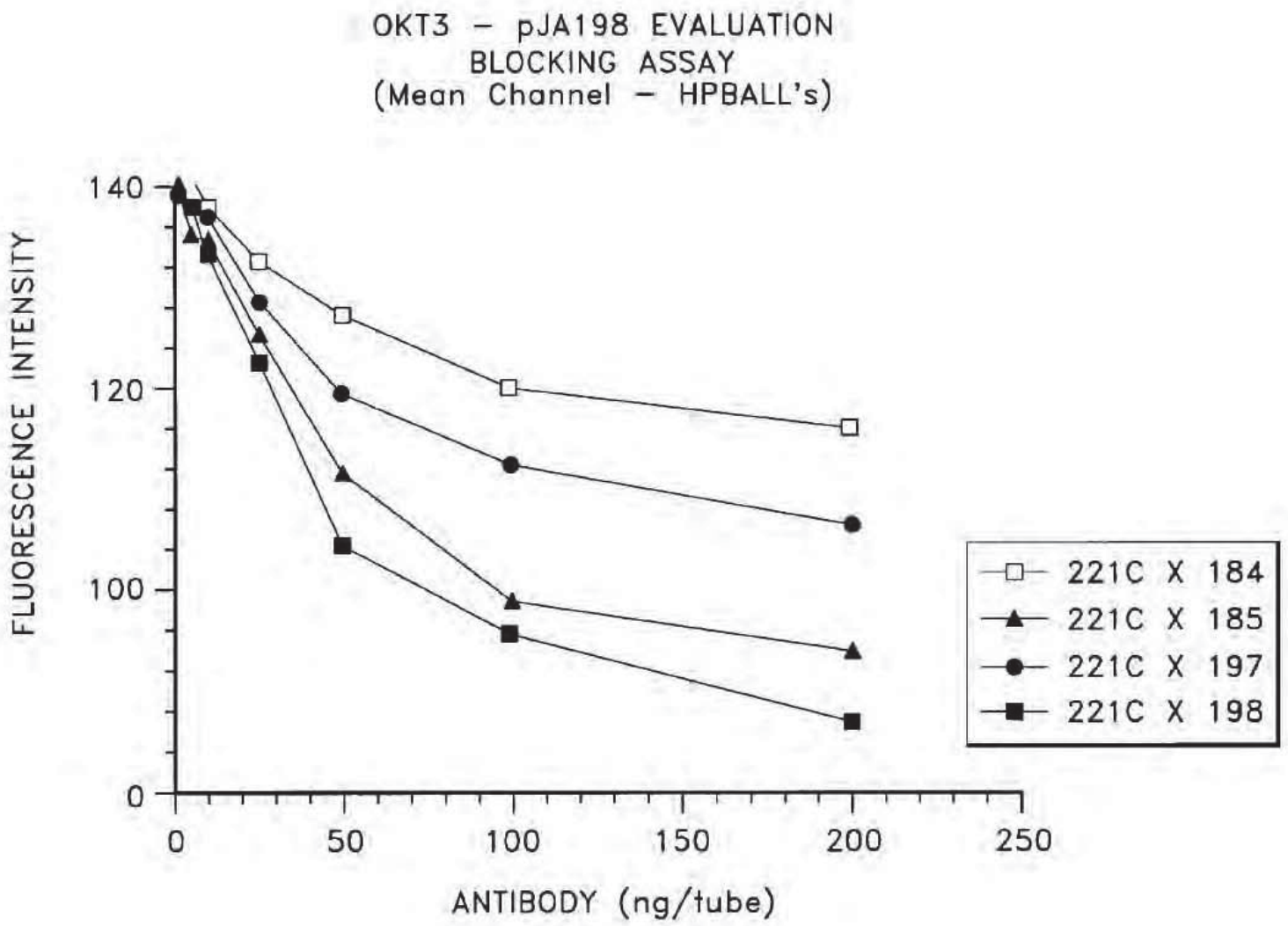

OKT3 – pJA198 EVALUATION
BINDING ASSAY
(Mean Channel – HPBALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
.1
1
10
100
1000
ANTIBODY (ng/tube)
221C X 184
221C X 185
221C X 197
221C X 198

FIG. 7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BLOCKING ASSAY
(Mean Channel – HPBALL's)
FLUORESCENCE INTENSITY
140
120
100
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
221C X 184
221C X 185
221C X 197
221C X 198

FIG. 8

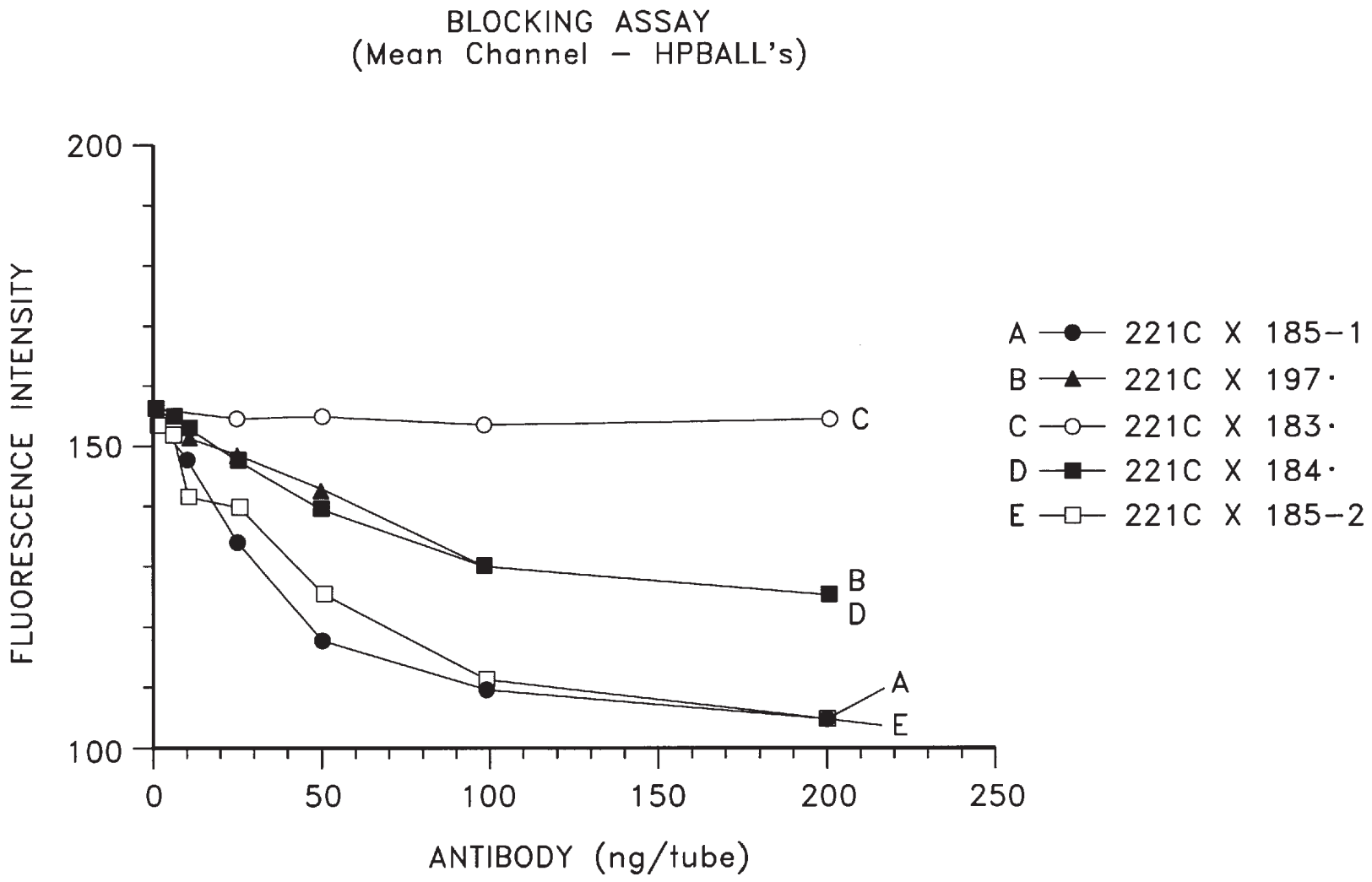
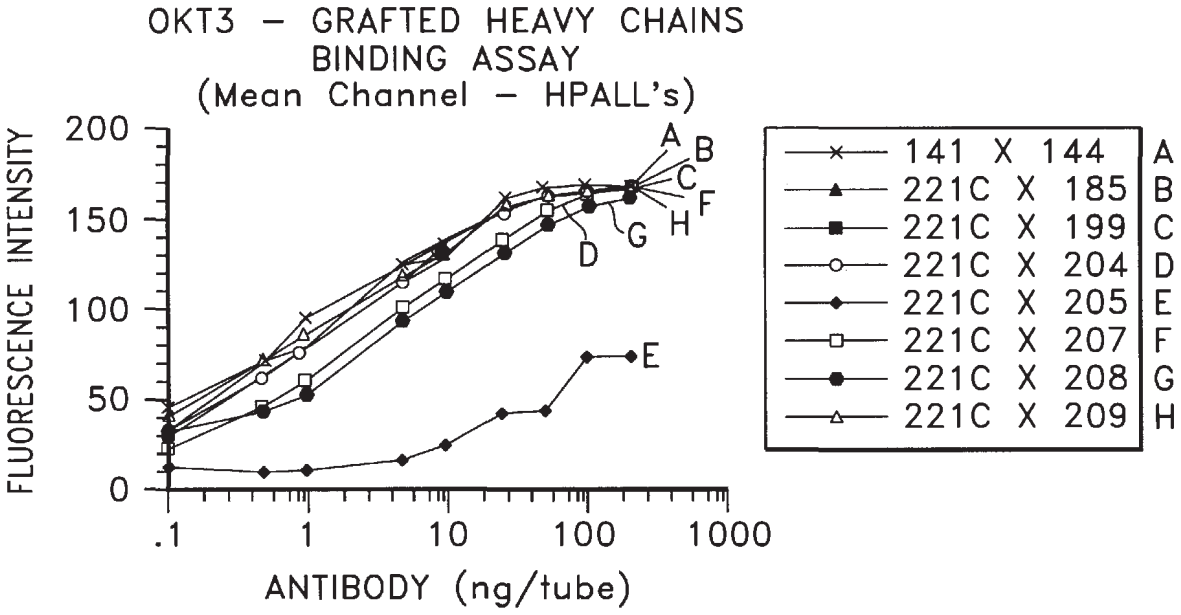
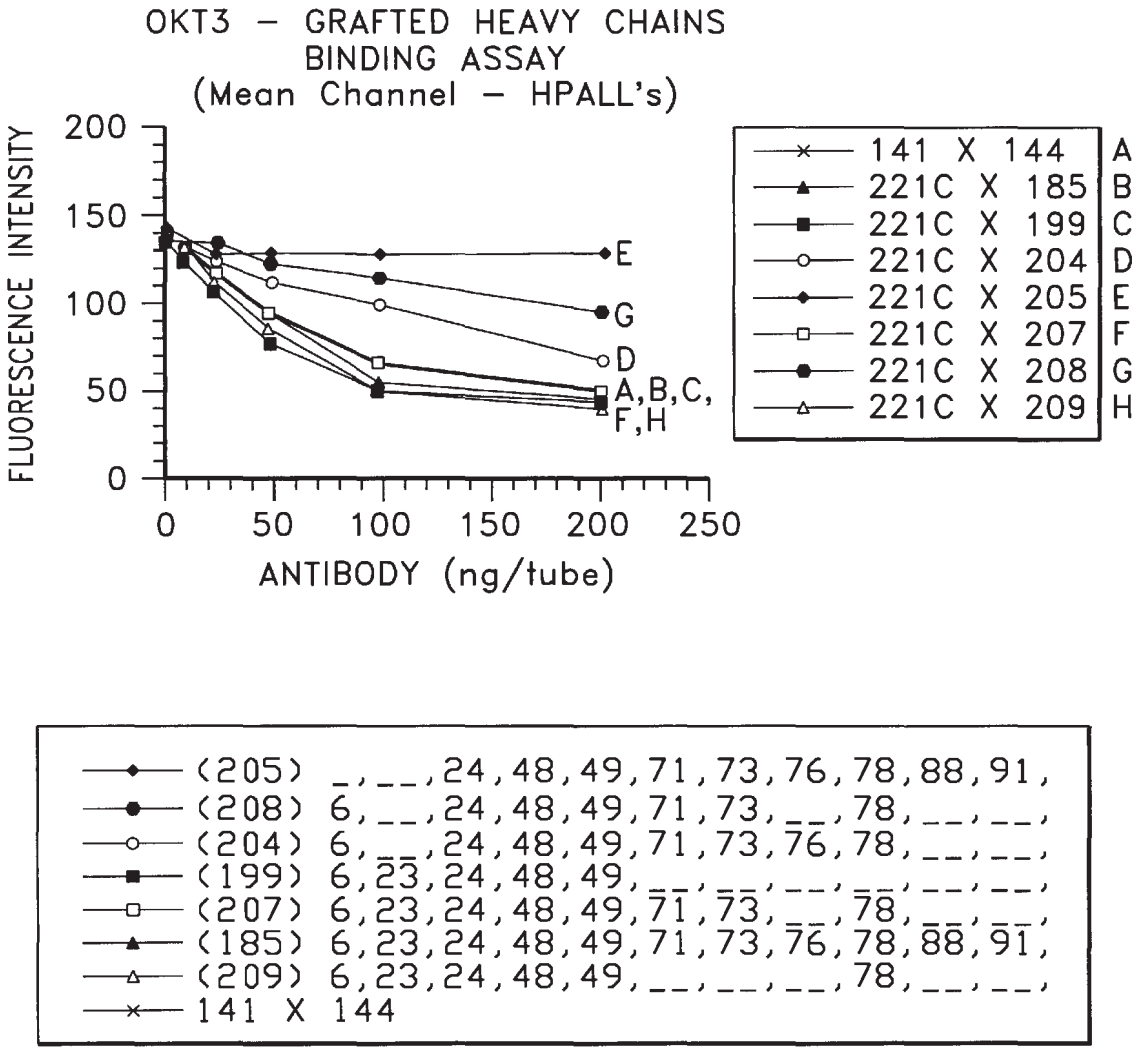

BLOCKING ASSAY
(Mean Channel – HPBALL's)
FLUORESCENCE INTENSITY
200
150
100
0
50
100
150
200
250
ANTIBODY (ng/tube)
A 221C X 185-1
B 221C X 197·
C 221C X 183·
D 221C X 184·
E 221C X 185-2
C
B
D
A
E

FIG. 9



(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

FIG. 10a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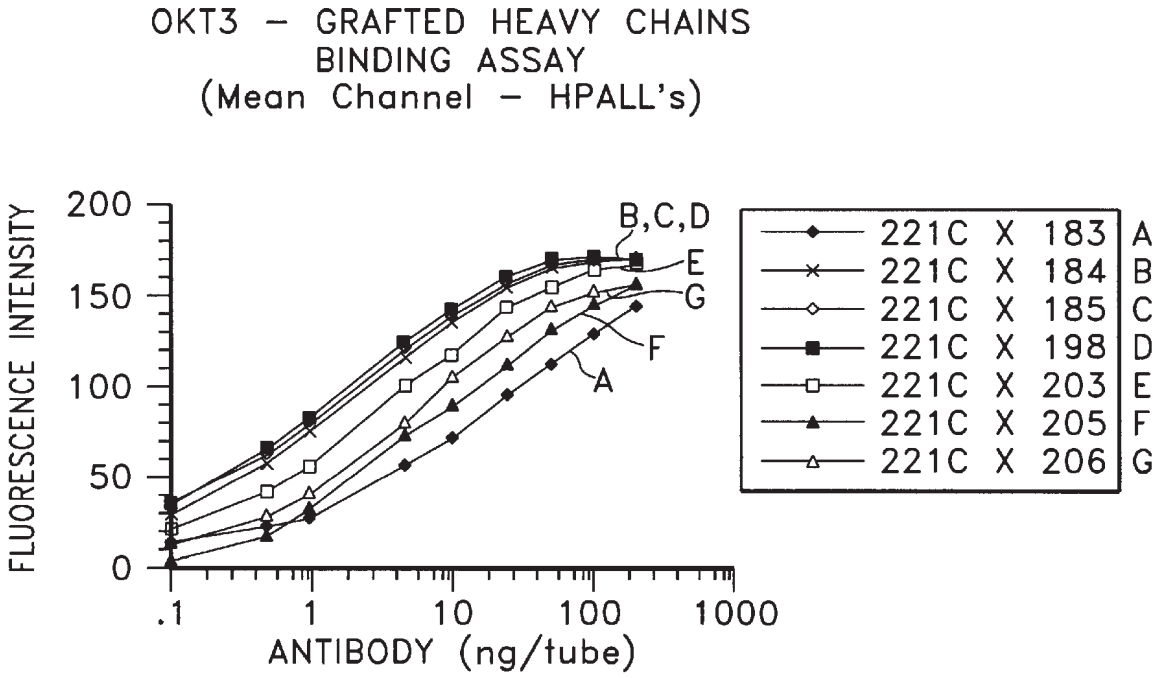
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
200
150
100
50
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
E
G
D
A,B,C,
F,H
141 X 144 A
221C X 185 B
221C X 199 C
221C X 204 D
221C X 205 E
221C X 207 F
221C X 208 G
221C X 209 H
(205) _,__,24,48,49,71,73,76,78,88,91,
(208) 6,__,24,48,49,71,73,__,78,__,__,
(204) 6,__,24,48,49,71,73,76,78,__,__,
(199) 6,23,24,48,49,__,__,__,__,__,__,
(207) 6,23,24,48,49,71,73,__,78,__,__,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(209) 6,23,24,48,49,__,__,__,78,__,__,
141 X 144

FIG. 10b



(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,
(184) 6,23,24,__,__,__,__,__,__,__,__,
(206) _,__,24,48,49,71,73,76,78,__,__,
(203) 6,__,24,48,49,71,73,76,78,88,91,
(185) 6,23,24,48,49,71,73,76,78,88,91,
(198) 6,23,24,48,49,71,73,76,78,__,__,

FIG. 11a

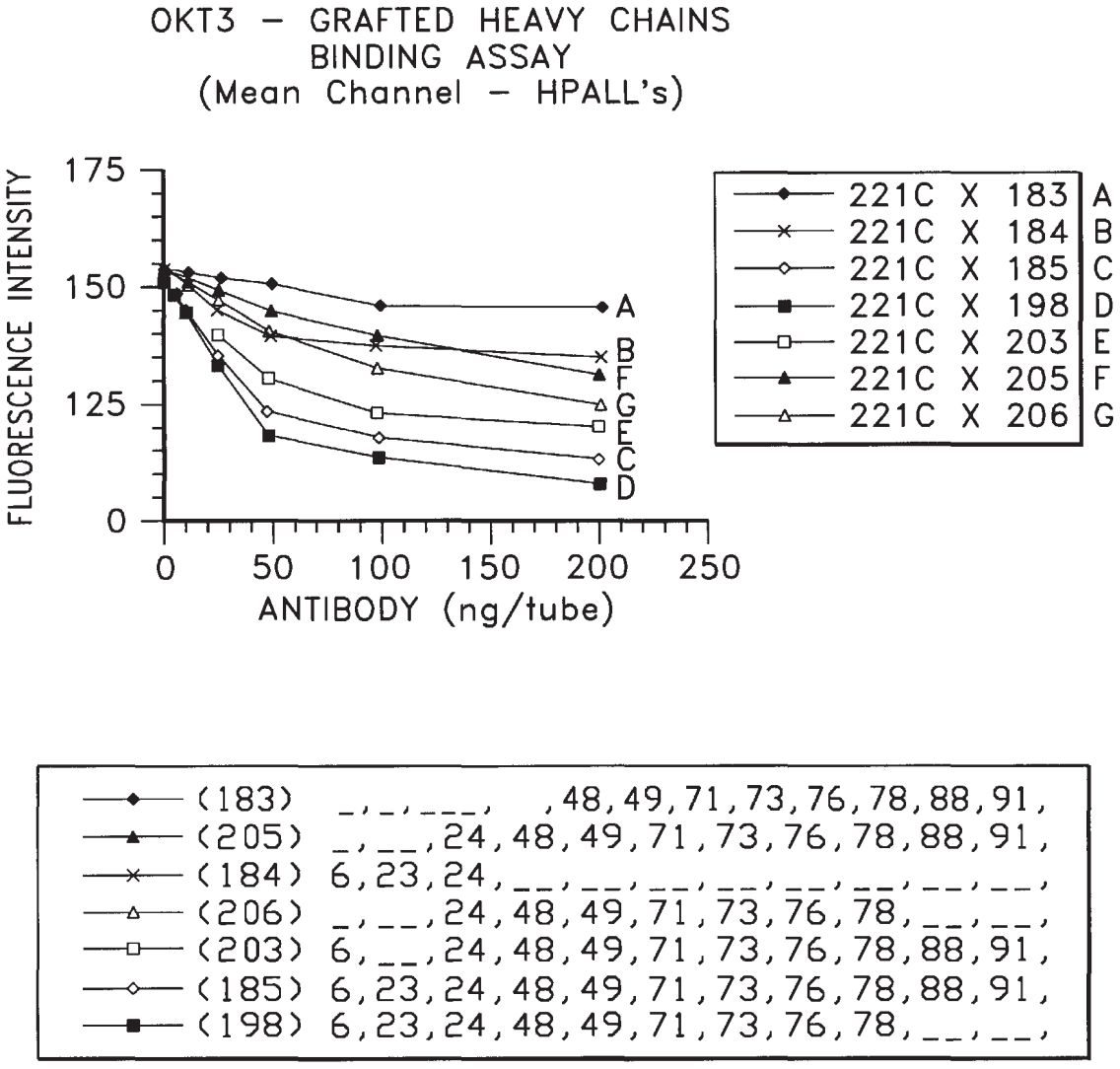

OKT3 – GRAFTED HEAVY CHAINS
BINDING ASSAY
(Mean Channel – HPALL's)
FLUORESCENCE INTENSITY
175
150
125
0
0
50
100
150
200
250
ANTIBODY (ng/tube)
A
B
F
G
E
C
D
221C X 183 A
221C X 184 B
221C X 185 C
221C X 198 D
221C X 203 E
221C X 205 F
221C X 206 G
(183) _,_,___, ,48,49,71,73,76,78,88,91,
(205) _,__,24,48,49,71,73,76,78,88,91,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

FIG. 11b

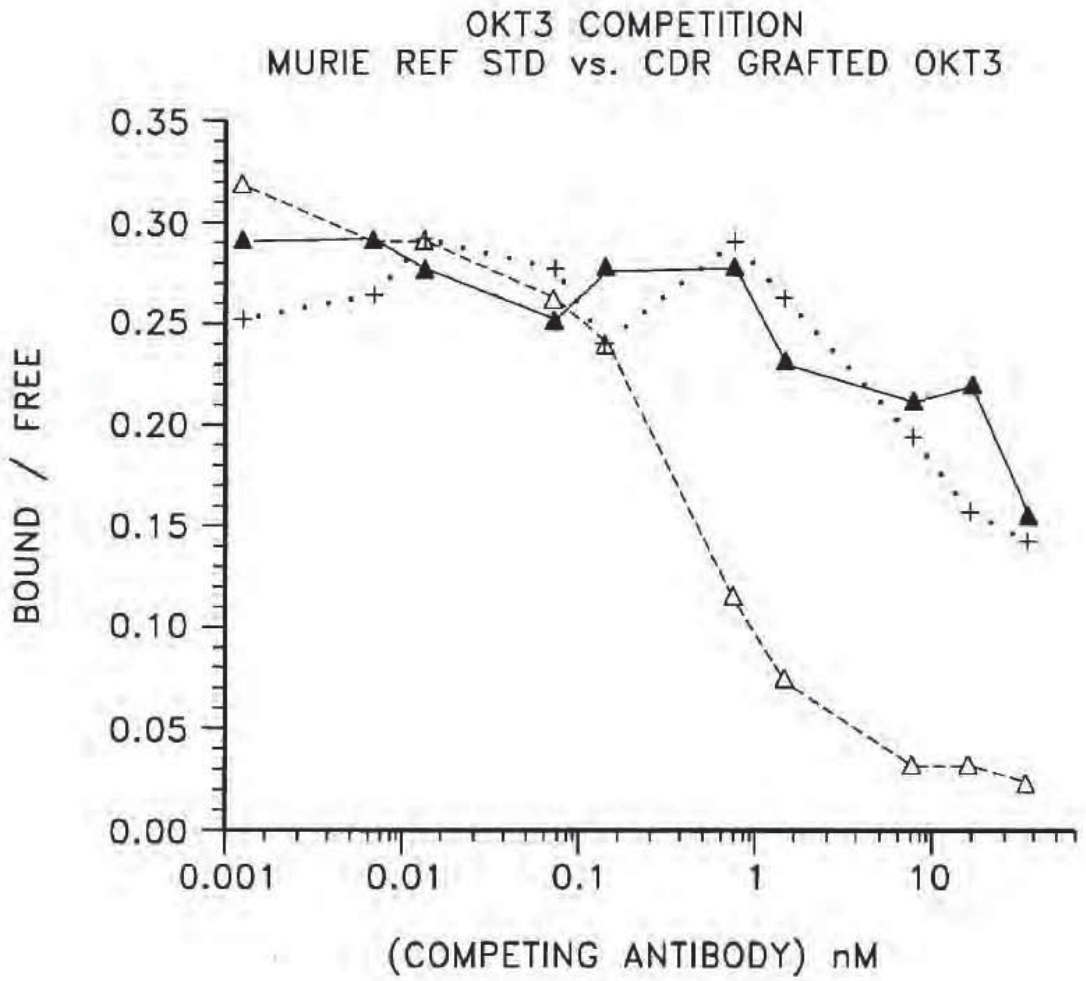

OKT3 COMPETITION
MURIE REF STD vs. CDR GRAFTED OKT3
BOUND / FREE
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.001
0.01
0.1
1
10
(COMPETING ANTIBODY) nM
MEDIA CNTRL
REF IN MEDIA
CDR 221 x 178 * #1
CDR 221 x 178 * #2
* PROTEIN CONCENTRATIONS
APPROXIMATE [ELISA]

FIG. 12

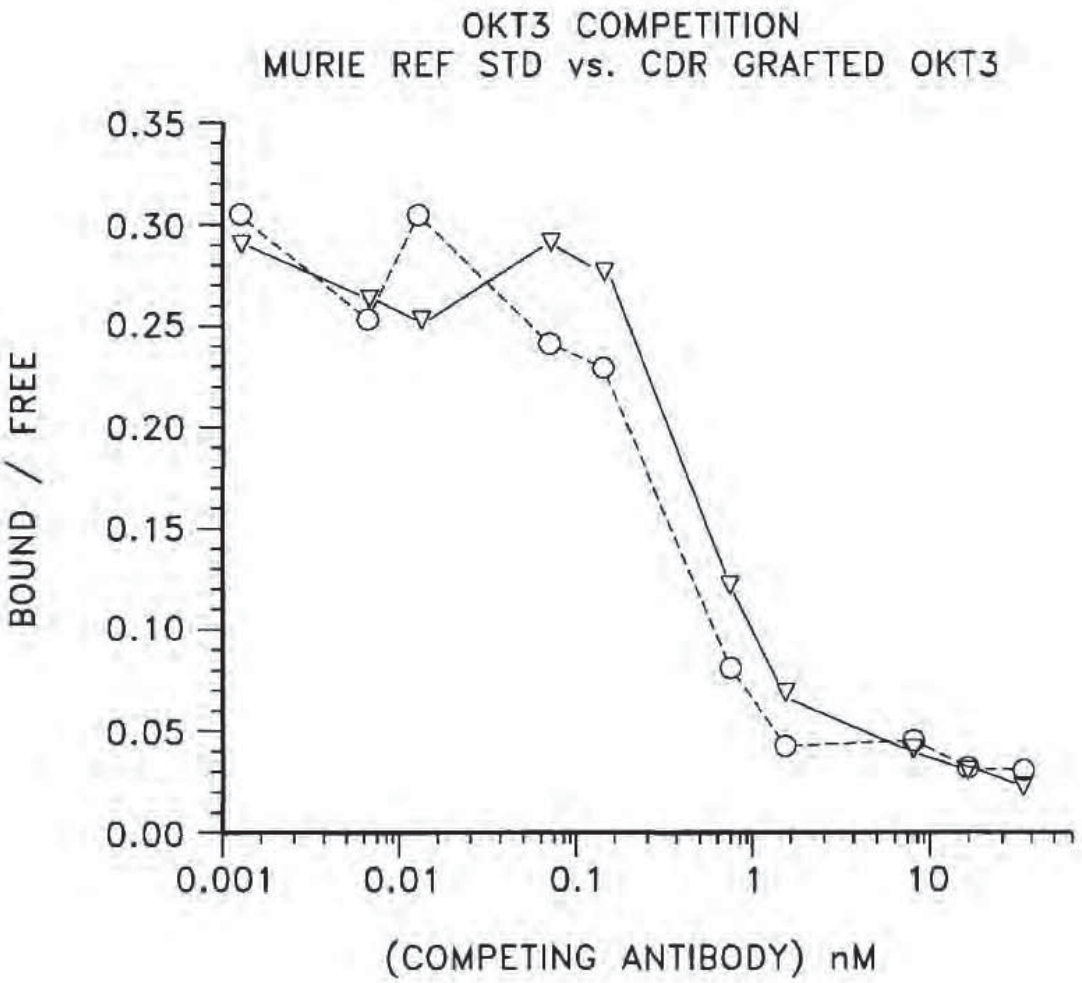

OKT3 COMPETITION
MURIE REF STD vs. CDR GRAFTED OKT3
BOUND / FREE
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.001
0.01
0.1
1
10
(COMPETING ANTIBODY) nM
REF STD
CDR 221C x 185
(PROTEIN CONCENTRATION APPROXIMATE)
* PROTEIN CONCENTRATIONS APPROXIMATE [ELISA]

FIG. 13

HUMANISED ANTIBODIES

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

FIELD OF THE INVENTION

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

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

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

BACKGROUND OF THE INVENTION

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

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

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

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

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

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

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

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

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

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

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

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

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

SUMMARY OF THE INVENTION

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

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

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

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

1 and 3,

72 and 76,

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

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

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

67,

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

91,

88, and

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

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

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

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

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

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

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

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

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

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

1 and 3,

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

and/or

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

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

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

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

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

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

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

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

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

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

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

Protocol

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

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

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

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

2. Heavy Chain

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

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

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

i. 1, 3

ii. 72, 76

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

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

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

vi. 67

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

viii. 91

ix. 88

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

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

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

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

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

i. 1, 3

ii. 63

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

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

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

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

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

Rationale

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

1. The extent of the CDRs

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

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

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

2. Non-CDR residues which contribute to antigen binding

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

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

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

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

2.2 Packing residues near the CDRs.

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

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

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

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

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

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

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

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

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

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

BRIEF DESCRIPTION OF THE FIGURES

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

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

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

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

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

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

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

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

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

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

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

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

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

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Example 1

CDR-grafting of OKT3

Material and Methods

1. Incoming Cells

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

2. Molecular Biology Procedures

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

3. Research Assays

3.1. Assembly Assays

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

3.1.1. COS Cells Transfected With Mouse OKT3 Genes

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

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

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

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

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

3.2. Assay for Antigen Binding Activity

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

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

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

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

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

3.3 Determination of Relative Binding Affinity

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

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

4. cDNA Library Construction

4.1. mRNA Preparation and cDNA Synthesis

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

4.2. Library Construction

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

5. Screening

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

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

6. DNA Sequencing

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

7. Construction of cDNA Expression Vectors

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

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

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

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

8. Expression of cDNAS in COS Cells

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

9. Construction of Chimeric Genes

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

9.1. Light Chain Gene Construction

The mouse light chain cDNA sequence contains an Aval site near the 3' end of the variable region [FIG. **1**(*a*)(SEQ ID NO:4)]. The majority of the sequence of the variable region was isolated as a 396 bp. EcoR1-Aval fragment. An oligonucleotide adapter was designed to replace the remainder of the 3' region of the variable region from the Aval site and to include the 5' residues of the human constant region up to and including a unique Nar1 site which had been previously engineered into the constant region.

A Hind111 site was introduced to act as a marker for insertion of the linker.

The linker was ligated to the V_L fragment and the 413 bp EcoR1-Nar1 adapted fragment was purified from the ligation mixture.

The constant region was isolated as an Nar1-BamH1 fragment from an M13 clone NW361 and was ligated with the variable region DNA into an EcoR1/BamH1/C1P pSP65 treated vector in a three way reaction to yield plasmid JA143. Clones were isolated after transformation into *E.coli* and the linker and junction sequences were confirmed by the presence of the Hind111 site and by DNA sequencing.

9.2 Light Chain Gene Construction—Version 2

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

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

VARIABLE CONSTANT

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

An internal Hind111 site was not included in this adapter, to differentiate the two chimeric light chain genes.

The variable region fragment was isolated as a 376 bp EcoR1-Aval fragment. The oligonucleotide linker was ligated to Nar1 cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into EcoR1/C1P treated pEE6hCMVneo to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing

9.3. Heavy Chain Gene Construction

9.3.1. Choice of Heavy Chain Gene Isotype

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

9.3.2. Gene Construction

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

The linker was ligated to the V_H fragment and the EcoR1-Hind111 adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting pJA91 with EcoR1 and Hind111 removing the intron fragment and replacing it with the V_H to yield pJA142. Clones were isolated after transformation into *E.coli* JM101 and the linker and junction sequences were confirmed by DNA sequencing. (N.B. The Hind111 site is lost on cloning).

10. Construction of Chimeric Expression Vectors

10.1. neo and gpt Vectors

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

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

The chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp EcoR1/BamH1 fragment and cloned into the EcoR1/Bcl1/C1P treated vector fragment of a derivative of pEE6hCMVgpt to yield plasmid pJA144.

10.2. GS Separate Vectors

GS versions of pJA141 and pJA144 were constructed by replacing the neo and gpt cassettes by a BamH1/Sa11/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 to yield the light chain vector pJA179 and the heavy chain vector pJA180.

10.3. GS Single Vector Construction

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

11. Expression of Chimeric Genes

11.1. Expression in COS Cells

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

11.2 Expression in Chinese Hamster Ovary (CHO) Cells

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

12. CDR-grafting

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

12.1. Variable Region Analysis

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

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

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

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

12.1.1. Light Chain

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

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

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

12.1.2. Heavy Chain

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

12.2. Design of Variable Genes

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

12.3. Gene Construction

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

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

13. Construction of Expression Vectors

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

TABLE 1

CDR-GRAFTED GENE CONSTRUCTS

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

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

14. Expression of CDR-grafted Genes

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

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

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) (SEQ ID NO:25) demonstrated some weak binding in association with mH or cH. However, when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 12.1 antigen binding was demonstrated when both of the new constructs, which were termed 121A and 221A were co-expressed with cH. When the effects of these residues were examined in more detail, it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene (SEQ ID NO:27) shows little detectable binding activity in association with cH. The light chain product of gL221C(SEQ ID NO:28), in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH.

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

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

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

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

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

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

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

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

14.3 Production of Fully CDR-grafted Antibody

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

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

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

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

An analysis of the above results is given below.

15. Discussion of CDR-grafting Results

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

15.1. Light Chian

15.1.1. Extent of the CDRs

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

15.1.2. Framework Resides

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

15.2. Heavy Chain

15.2.1. Extent of the CDRs

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

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

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

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

15.2.2. Framework Residues

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

15.3 Interim Conclusions

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

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

16. Further CDR-grafting Experiments

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

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

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

TABLE 2-continued

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3v1	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q	L	L	

(SEQ ID NO:29, 8, 9 and 25–28)

MURINE RESIDUES ARE UNDERLINED

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

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

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

The binding and blocking assay results indicate the following:

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

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

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

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

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

Example 2

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

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

The Light Chain

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

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

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

The Heavy Chain

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

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

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

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

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

Example 3

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

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

(a) B72.3 Light Chain

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

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

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

B72.3 cH/B72.3 cL

and B72.3 cH/B72.3 gL

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

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

(b) B72.3 heavy chain

i. Choice of framework

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

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

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

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

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

ii. Results with grafted heavy chain genes

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

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

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

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

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

iii. Framework changes in B72.3 gH gene

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

iv. Other framework changes

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

v. Other

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

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

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

Example 4

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

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

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

Light Chain

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

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

Heavy Chain

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

Example 5

CDR-Grafting of Murine Anti-TNFa Antibodies

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

61E71

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

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

hTNF1

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

Heavy Chain

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

Light Chain

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

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

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

hTNF3

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

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

101.4

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

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

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

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 31

(2) INFORMATION FOR SEQ ID NO:1:

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

(i i) MOLECULE TYPE: cDNA

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

T C C A G A T G T T A A C T G C T C A C 2 0

(2) INFORMATION FOR SEQ ID NO:2:

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

(i i) MOLECULE TYPE: cDNA

-continued

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

CAGGGGCCAG TGGATGGATA GAC 23

(2) INFORMATION FOR SEQ ID NO:3:

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

(ii) MOLECULE TYPE: peptide

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

Leu Glu Ile Asn Arg Thr Val Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO:4:

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

(ii) MOLECULE TYPE: cDNA

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

TAGAGACAAA GGTCCTGAGA CGCCACCACC AGCTCCCAGC TCCATCCTAT CTTCCCTTCT        782

AAGGTCTTGG AGGCTTCCCC ACAAGCGCTT ACCACTGTTG CGGTGCTCTA AACCTCCTCC        842

CACCTCCTTC TCCTCCTCCT CCCTTTCCTT GGCTTTTATC ATGCTAATAT TTGCAGAAAA        902

TATTCAATAA AGTGAGTCTT TGCCTTGAAA AAAAAAAAAA A                            943
```

(2) INFORMATION FOR SEQ ID NO:5:

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

(i i) MOLECULE TYPE: protein

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

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

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

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

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

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

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

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

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

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

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

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

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

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

-continued

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

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

(2) INFORMATION FOR SEQ ID NO:6:

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

(i i) MOLECULE TYPE: cDNA

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

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

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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

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

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

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

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

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

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

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

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

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

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

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

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

GAC CTC TAC ACC CTC AGC AGC TCA GTG ACT GTA ACC TCG AGC ACC TGG      679
```

-continued

```
Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp
    180                 185                 190

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

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

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

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

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

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

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

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

TGC AAG GTC AAC AAC AAA GAC CTC CCA GCG CCC ATC GAG AGA ACC ATC  1111
Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile
        325                 330                 335

TCA AAA CCC AAA GGG TCA GTA AGA GCT CCA CAG GTA TAT GTC TTG CCT  1159
Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro
    340                 345                 350

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

GTC ACA GAC TTC ATG CCT GAA GAC ATT TAC GTG GAG TGG ACC AAC AAC  1255
Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn
                375                 380                 385

GGG AAA ACA GAG CTA AAC TAC AAG AAC ACT GAA CCA GTC CTG GAC TCT  1303
Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser
            390                 395                 400

GAT GGT TCT TAC TTC ATG TAC AGC AAG CTG AGA GTG GAA AAG AAG AAC  1351
Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn
        405                 410                 415

TGG GTG GAA AGA AAT AGC TAC TCC TGT TCA GTG GTC CAC GAG GGT CTG  1399
Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu
    420                 425                 430

CAC AAT CAC CAC ACG ACT AAG AGC TTC TCC CGG ACT CCG GGT AAA      1444
His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
435                 440                 445

TGAGCTCAGC ACCCACAAAA CTCTCAGGTC CAAAGAGACA CCCACACTCA TCTCCATGCT  1504

TCCCTTGTAT AAATAAAGCA CCCAGCAATG CCTGGGACCA TGTAAAAAAA AAAAAAAAAG  1564

GAATTC                                                          1570
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

-continued

(ii) MOLECULE TYPE: protein

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

```
Met Glu Arg His Trp Ile Phe Leu Leu Leu Leu Ser Val Thr Ala Gly
-19                 -15                 -10                  -5

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

-continued

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

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

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

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

Thr Pro Gly Lys
```

(2) INFORMATION FOR SEQ ID NO:8:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

Glu Asp Ile Ala Thr
                85
```

(2) INFORMATION FOR SEQ ID NO:9:

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

(ii) MOLECULE TYPE: peptide

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

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

Thr Lys Leu Gln Ile Thr Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:10:

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

(ii) MOLECULE TYPE: peptide

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

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

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

-continued

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

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

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

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

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

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

(2) INFORMATION FOR SEQ ID NO: 11:

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

(i i) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:12:

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

(i i) MOLECULE TYPE: peptide

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

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

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

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

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

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

-continued

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

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

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:13:

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

(i i) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:14:

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

(i i) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
```

-continued

115

(2) INFORMATION FOR SEQ ID NO:15:

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

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:16:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO: 17:

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

-continued

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:18:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:19:

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

(ii) MOLECULE TYPE: peptide

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

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

-continued

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:20:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:21:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

-continued

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

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

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

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:22:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:23:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

-continued

100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:24:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:25:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

(2) INFORMATION FOR SEQ ID NO:26:

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

-continued

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

(2) INFORMATION FOR SEQ ID NO:27:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

(2) INFORMATION FOR SEQ ID NO:28:

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

(ii) MOLECULE TYPE: peptide

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

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

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

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

-continued

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

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

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

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

(2) INFORMATION FOR SEQ ID NO:29:

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

(i i) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

Arg
```

(2) INFORMATION FOR SEQ ID NO:30:

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

(i i) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

-continued

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

(2) INFORMATION FOR SEQ ID NO:31:

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

(i i) MOLECULE TYPE: peptide

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

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

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

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

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

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

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

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

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

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

We claim:

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

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

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

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

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

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

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

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

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

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

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

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

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

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

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

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

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

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

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

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

CERTIFICATE OF CORRECTION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CERTIFICATE OF CORRECTION

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

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

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

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

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

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

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

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

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

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

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

CERTIFICATE OF CORRECTION

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

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

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

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

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

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

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

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

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

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

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

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

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

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

Signed and Sealed this

Twelfth Day of November, 2002

Attest:

Attesting Officer

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

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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

(ii) TITLE OF INVENTION: Humanised Antibodies

(iii) NUMBER OF SEQUENCES: 30

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

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

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

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

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

(2) INFORMATION FOR SEQ ID NO:1:

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

(ii) MOLECULE TYPE: cDNA

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

TCCAGATGTT AACTGCTCAC 20

(2) INFORMATION FOR SEQ ID NO:2:

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

(ii) MOLECULE TYPE: cDNA

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

CAGGGGCCAG TGGATGGATA GAC 23

(2) INFORMATION FOR SEQ ID NO:3:

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

(ii) MOLECULE TYPE: peptide

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

Leu Glu Ile Asn Arg Thr Val Ala Ala
1 5

(2) INFORMATION FOR SEQ ID NO:4:

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

(ii) MOLECULE TYPE: cDNA

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

TAGAGACAAA GGTCCTGAGA CGCCACCACC AGCTCCCAGC TCCATCCTAT CTTCCCTTCT        782

AAGGTCTTGG AGGCTTCCCC ACAAGCGCTT ACCACTGTTG CGGTGCTCTA AACCTCCTCC        842

CACCTCCTTC TCCTCCTCCT CCCTTTCCTT GGCTTTTATC ATGCTAATAT TTGCAGAAAA        902

TATTCAATAA AGTGAGTCTT TGCCTTGAAA AAAAAAAAAA A                            943
```

(2) INFORMATION FOR SEQ ID NO:5:

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

(ii) MOLECULE TYPE: protein

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(2) INFORMATION FOR SEQ ID NO:6:

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

(ii) MOLECULE TYPE: cDNA

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

ACA CAA ACC CAT AGA GAG GAT TAC AAC AGT ACT CTC CGG GTG GTC AGT         1015
Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser
                295                 300                 305

GCC CTC CCC ATC CAG CAC CAG GAC TGG ATG AGT GGC AAG GAG TTC AAA         1063
Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys
            310                 315                 320

TGC AAG GTC AAC AAC AAA GAC CTC CCA GCG CCC ATC GAG AGA ACC ATC         1111
Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile
        325                 330                 335
```

```
TCA AAA CCC AAA GGG TCA GTA AGA GCT CCA CAG GTA TAT GTC TTG CCT        1159
Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro
    340                 345                 350

CCA CCA GAA GAA GAG ATG ACT AAG AAA CAG GTC ACT CTG ACC TGC ATG        1207
Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met
355                 360                 365                 370

GTC ACA GAC TTC ATG CCT GAA GAC ATT TAC GTG GAG TGG ACC AAC AAC        1255
Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn
                375                 380                 385

GGG AAA ACA GAG CTA AAC TAC AAG AAC ACT GAA CCA GTC CTG GAC TCT        1303
Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser
            390                 395                 400

GAT GGT TCT TAC TTC ATG TAC AGC AAG CTG AGA GTG GAA AAG AAG AAC        1351
Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn
        405                 410                 415


TGG GTG GAA AGA AAT AGC TAC TCC TGT TCA GTG GTC CAC GAG GGT CTG        1399
Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu
    420                 425                 430


CAC AAT CAC CAC ACG ACT AAG AGC TTC TCC CGG ACT CCG GGT AAA            1444
His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
435                 440                 445

TGAGCTCAGC ACCCACAAAA CTCTCAGGTC CAAAGAGACA CCCACACTCA TCTCCATGCT      1504

TCCCTTGTAT AAATAAAGCA CCCAGCAATG CCTGGGACCA TGTAAAAAAA AAAAAAAAAG      1564

GAATTC                                                                 1570
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 468 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Glu Arg His Trp Ile Phe Leu Leu Leu Leu Ser Val Thr Ala Gly
-19             -15                 -10                 -5
```

```
Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
                1               5                  10

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
    15                  20                  25

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
                50                  55                  60

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
            65                  70                  75

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
        80                  85                  90

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
    95                  100                 105

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro
110                 115                 120                 125

Ser Val Tyr Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser
                130                 135                 140

Val Thr Leu Gly Cys Leu Val Lys Gly Thr Phe Pro Glu Pro Val Thr
            145                 150                 155

Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro
        160                 165                 170

Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val
    175                 180                 185

Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His
190                 195                 200                 205

Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro
                210                 215                 220

Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu
            225                 230                 235

Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu
        240                 245                 250

Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser
    255                 260                 265
```

```
Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu
270                 275                 280                 285

Val His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr
                290                 295                 300

Leu Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser
            305                 310                 315

Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro
        320                 325                 310

Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln
    335                 340                 345

Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val
350                 355                 360                 365

Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val
                370                 375                 380

Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu
            385                 390                 395

Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg
        400                 405                 410

Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val
    415                 420                 425

Val His Glu Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg
430                 435                 440                 445

Thr Pro Gly Lys
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1                 5                  10                  15
```

```
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ile Lys Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Thr Glu Ala Ser Asn Leu Gln Ala Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr
                85
```

2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Tyr Tyr Cys Gln Gln Tyr Gln Ser Leu Pro Tyr Thr Phe Gly Gln Gly
1               5                   10                  15

Thr Lys Leu Gln Ile Thr Arg
            20
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15
```

```
Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Phe Ile Phe Ser Ser Tyr
            20                  25                  30

Ala Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Ile Ile Trp Asp Asp Gly Ser Asp Gln His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Thy Phe Cys
                85                  90                  95

Ala Arg Asp Gly Gly His Gly Phe Cys Ser Ser Ala Ser Cys Phe Gly
            100                 105                 110

Pro Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1                   5                   10                  15

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Try Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95
```

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60
```

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15
```

```
Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
             20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95
```

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ser Ser Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60
```

```
Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Ash Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110
```

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
50 55 60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Thr Met His Trp Val Arg Gln Ala Pro Cys Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Thr Asp Lys Ser Lys Ser Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CIARACTERISTICS:
(A) LENGTI: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLDGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gly Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60
```

```
Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTI: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Gln Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Gln Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTI: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30

```
            Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
            1               5                  10

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
     15                  20                  25

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
 30                  35                  40                  45

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
                 50                  55                  60

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
             65                  70                  75

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
         80                  85                  90

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
     95                  100                 105

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110                 115
```

WF 4-19-99 Amdt 17/H

DOCKET NO.: CARP-0057 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Reeves**

For: **Humanised Antibodies**

I, Doreen Yatko Trujillo, Registration No. 35,179 certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On April 9, 1999

Doreen Yatko Trujillo

Doreen Yatko Trujillo Reg. No. 35,179

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

Pursuant to 37 C.F.R. § 1.115, please amend the above-identified application as a follows.

In the specification:

Page 1, after "September 7, 1994," please insert -- now U.S. Patent No. 5.859,205, --.

Page 1, after "September 17, 1991," please insert -- abandoned, --.

In the claims:

24. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least

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10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence or contributes to antigen binding as determined by X-ray crystallography.

28. (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence or contributes to antigen binding as determined by X-ray crystallography.

29. (Amended) A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity [equivalent to that of a chimeric antibody formed from] similar to that of said donor immunoglobulin.

REMARKS

This paper is being filed in response to the Office Action dated November 16, 1998. A petition for a two-month extension of time and the appropriate fee accompany this response.

Claims 24-31 are pending. In the Office Action, all pending claims were rejected. In view of the foregoing amendments and the arguments that follow, Applicants

respectfully submit that allowable subject matter has been identified and request that the interference be declared.

Preliminarily, as requested by the Examiner, the specification has been amended to update the status of parent applications.

Additionally, the Examiner stated that the Information Disclosure Statements filed in the parent cases will not be considered unless they are filed with the present case and the references have been submitted. This appears to be contrary to MPEP § 609, page 600-103, specifically. As stated therein, information that has been considered by the Office in a parent application of a FWC filed prior to December 1, 1997 will be part of the file and need not be resubmitted to have the information considered. Likewise, an Examiner will consider information that has been considered by the Office in a parent application when examining a continuation under 36 C.F.R. § 1.60. The present application is a continuation under 37 C.F.R. § 1.60 of prior Application Serial No. 08/303,569, filed September 7, 1994, which is a continuation under 37 C.F.R.§ 1.62 (i.e., FWC) of Application Serial No. 07/7443,329, filed September 17, 1991. According to MPEP § 609, then, information considered in both parent applications is to be considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 24-31 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection for the reasons that follow. For the Examiner's convenience, the paragraphs are designated to correspond to the Examiner's paragraphs under this section.

a. The Examiner rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 have been amended herein to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. Support for these amendments can be found, *inter alia*, on page 38, lines 1-

12, and lines 23 through 38, of the application as filed. As is clear therefrom, the contribution to antigen binding can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing, i.e., the residues are spatially near a CDR. On page 17, lines 9-11, of the application as filed, the extents of the heavy chain CDRs are taught. On page 6, lines 25-35, it is indicated that the heavy chain "framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49...." Residue 49 is clearly adjacent a CDR. As evident from Figure 4, residues 6, 23, 24, and 48 contribute to antigen binding, as determined by X-ray crystallography. Applicants respectfully request that this rejection be withdrawn.

b. The Examiner rejected claims 27, 30, and 31, seeking evidence that CD3 is the same as "OKT3" and that CD4 is the same as "OKT4." Actually, one term refers to the antibody, while the other refers to the antigen bound. Specifically, OKT3 refers to a monoclonal antibody that recognizes the CD3 antigen and OKT4 refers to a monoclonal antibody that recognizes the CD4 antigen. Consistent therewith, on page 28, lines 19-22, of the application as filed, the testing of the ability of CDR-grafted OKT3 light chain to bind to CD3 positive cells is disclosed, and on page 52, line 29, of the application as filed, the reference "CD4 (OKT4)" is made. Applicants respectfully request that this rejection be withdrawn.

Claim 29 was rejected under 35 U.S.C. § 112, first paragraph, in view of the phrase "which specifically binds to an antigen with a binding affinity equivalent to that of a chimeric antibody formed from said donor immunoglobulin." The Examiner requested that Applicants point to support in the specification for the phrase. Claim 29 has been amended herein to recite that the binding affinity is "similar to that of" the donor. Support for this amendment can be found, *inter alia*, on page 48, lines 24-27 and page 51, lines 27-31 of the application as filed. Applicants respectfully request that this rejection be withdrawn.

Rejection Under 35 U.S.C. § 102(e)

Claims 24-31 were rejected under 35 U.S.C. § 102(e) in view of U.S. Patent No. 5,585,089 ("Queen et al."). Applicants respectfully traverse this rejection.

The Examiner observed that Queen et al. is entitled to priority back to "at least

12/28/90." (It is assumed that the Examiner meant 12/19/90, the filing date of the latest application designated as a continuation-in-part in the series of Queen et al. applications.) Although seeming to recognize that Queen et al. may not be entitled to a priority date earlier than 12/19/90, the Examiner, nonetheless, proceeded to argue that limitations recited in claims 24-31 are found in the earlier Queen et al. applications. The relevant inquiry for Queen et al. to be an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that the limitation "outside the Kabat and Chothia CDRs" is not found in, nor supported by, the priority documents.

This limitation requires that the framework residues to be replaced be outside both the Kabat and Chothia CDRs. As submitted in the Preliminary Amendment filed concurrently with the present application, however, the earliest Queen et al. applications do not teach, either explicitly or implicitly, that the framework residues to be replaced by donor **must be** outside **both** the Kabat and Chothia CDRs. Indeed, in the only example found in these early applications, and even in the specification of the Queen et al. patent as issued, changes were made to residues inside what Queen et al. denotes as CDRH1 of Chothia, i.e., inside a Chothia CDR. Considering that this limitation was required for patentability, Queen et al. cannot be entitled to a priority date earlier than the filing date of the application in which this limitation was first introduced, i.e., 12/19/90. Queen et al., thus, fails as a reference under 102(e) because, as also submitted in the Preliminary Amendment filed concurrently with the present application, Applicants are entitled to their GB priority date of 12/21/89.

Applicants respectfully request that this rejection be withdrawn.

Presentation of a Revised Proposed Count

Applicants present in Appendix A attached hereto a revised "Proposed Count." In compliance with 37 CFR §1.606, the revised proposed Count 1 is broader than any of claims 1-4, the broadest claims in the Queen patent, and as broad as any one of claims 24-31 being entered into the instant application.

The proposed count contains disjunctive or alternative language to cover the claim terminology of the two parties. Such counts were expressly approved by the Board in *Hsing v. Myers*, 2 USPQ2d 1861 (Bd, Pat,. App. & Int. 1987). It is clear, however, that both alternatives are directed to the same invention as that claimed in the Queen patent.

(c) Identification of Claims Corresponding to the Count

Applicants identify all of the Queen patent claims 1-11 and applicant's claims 24-31 as corresponding to the Count and as being directed to the same patentable invention.

(d) Application of the Terms of Applicants' Disclosure to the Copied Claims

In attached Appendix B, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24 substantially copied from Queen claims 1, 5, 9 and 10. There is, of course, additional support in applicants' application omitted for the sake of brevity.

(e) Applicants' Effective Filing Date

Applicants' present application, being a Rule 60 continuation, has the identical specification and drawings as that originally filed in U.S. application Serial No. 08/303.569, filed September 7, 1994, which is a U.S. national phase application stemming from PCT/GB-90/02017, filed December 21, 1990. The latter PCT application claimed priority benefit of GB national application Serial No. 89/28874.0, filed December 21, 1989.

In attached Appendix C is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claims 28 and 29 which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner advise them as soon as possible whether the Examiner intends to declare an interference between the present application and

Queen et al. Specifically, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo
Registration No. 35,719

Date: April 9, 1999

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

APPENDIX A

PROPOSED COUNT FOR INTERFERENCE

Count 1:

A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with:

(i) an effective antigen binding activity, or

(ii) an affinity constant of at least 10^7 M^{-1} and no greater than about four-fold that of the donor immunoglobulin,

wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside:

(a) the Kabat and Chothia CDRs, or

(b) both the Kabat CDRs and the structural loop CDRs of the variable regions,

wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) (a) contains an atom within a distance of 4 of or (b) is spatially close to a CDR in said humanized immunoglobulin .

APPENDIX B

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1},	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.
and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence	See page 11, lines 16-20, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At page 6, lines 25-35, it is indicated that the heavy chain "framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49...." In the heavy chain, Kabat CDR2 together with [Chothia] structural loop H2 extends from residues 50 to 65. Thus, residue 49 is immediately adjacent the beginning of this CDR2/H2 region.

or contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 6, 23,24, and 48 are identified in Figure 4.

APPENDIX C

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity·	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.
and each of said donor amino acids is adjacent to a CDR in the donor immunoglobulin sequence	See page 7, lines 11-14, showing that homology is maximized between donor and acceptor sequences adjacent CDRs within acceptor framework. At p.5, l. 9-16, reference is made to heavy chain "framework comprises donor at at least one of residues 6, 23 and/or 24, 48 and/or 49...." Residue 49 is immediately adjacent CDR2/H2 loop region.
or contributes to antigen binding as determined by X-ray crystallography.	Page 18, lines 11-17, and lines 33-37, and Figs. 20-21 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 6, 23,24, and 48 are identified in Figure 21.

29. A humanized immunoglobulin according to claim 28 which specifically binds to an antigen with a binding affinity similar to that of said donor immunoglobulin.	Page 23, lines 1-10.

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WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS
A PARTNERSHIP INCLUDING PROFESSIONAL CORPORATIONS
ONE LIBERTY PLACE - 46TH FLOOR
PHILADELPHIA, PA 19103-7301

(215) 568-3100

Facsimile: (215) 568-3439
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75962

DATE: November 4, 1999

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DOCKET NO.: CARP-0037 PATENT

RESPONSE UNDER 37 CFR 1.116
EXPEDITED PROCEDURE
EXAMINING GROUP NO. 1642

21/II
D.G.J.
11/9/99
(NE)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Burke**

For: **Humanised Antibodies**

I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being transmitted by facsimile to Examiner Burke of the U.S. Patent and Trademark Office, Washington, D.C. 20231.

On November 3, 1999

Doreen Yatko Trujillo

Doreen Yatko Trujillo Reg. No. 35,719

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17Nov99

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

11/09/1999 LPENDER 00000003 23 [illegible]
01 FC:117 [illegible]

Pursuant to 37 C.F.R. § 1.116, please amend the above-identified application as a follows.

In the claims:

24. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the

variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

28. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

Please add the following claim:

32. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks at residues 48, 49, 71, 73, 76, 78, 88, and 91.

REMARKS

This paper is being filed in response to the Final Rejection dated May 28, 1999. No extension of time is believed necessary for responding to the Final Rejection. To the extent this belief is in error, Applicants hereby request the necessary extension and the undersigned authorizes charging any such fee to Deposit Account 23-3050.

Claims 24-31 were pending. In the Final Rejection, all pending claims were rejected. Claim 32 has been added herein. In view of the foregoing amendments and the arguments that follow, Applicants respectfully submit that allowable subject matter has been identified and request that the interference be declared.

The Examiner stated that the Information Disclosure Statements filed in the parent cases will be considered once the references are submitted. To the extent the Examiner is requiring that Applicants resubmit references already submitted, this appears to be contrary to MPEP § 609, page 600-103. Applicants are not required to resubmit references to get them considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 24-31 were again rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection for the reasons that follow. For the Examiner's convenience, the paragraphs are designated to correspond to the Examiner's paragraphs for the rejection remaining under this section.

a. and b. The Examiner again rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 were previously amended to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. During a telephone conference between the Examiner and the undersigned, the Examiner indicated that removal of the "adjacent to a CDR" language would

obviate this rejection. Although Applicants disagree with the Examiner's reasoning, the claims have been amended herein to remove the recitation "adjacent to a CDR in the donor immunoglobulin sequence." As Applicants made clear in the previous response, the contribution to antigen binding need not be direct and, indeed, can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing. Naturally, even for an indirect effect, the residues must be spatially near the CDR.

Applicants respectfully request that this rejection be withdrawn.

As the Examiner is aware, Applicants desire to provoke an interference between the present application and the Queen patent (U.S. Patent No. 5,585,089). Although Applicants are confident that the present claims are directed to the same invention as the Queen patent, new claim 32 is submitted herewith. New claim 32 recites the residues changed in example g341B disclosed in Applicants' specification as filed and, indeed, in GB8928874. Of the residues recited, all are either adjacent a CDR (49), or contribute to antigen binding as determined by X-ray crystallography (48, 71, 73, 76, 78, 88, and 91). Claim 32 is clearly allowable and clearly directed to the same invention as claim 1 of the Queen patent.

> If at least one of the presented claims is not rejectable on any [ ] ground and is claiming the same invention as at least one claim of the patent, the examiner should proceed to initiate an interference.

MPEP 2307.02.

Applicants respectfully request that an interference between the present application and the Queen patent be declared.

Rejection Under 35 U.S.C. § 102(e)

Claims 24-31 were again rejected under 35 U.S.C. § 102(e) in view of the Queen patent. Applicants respectfully traverse this rejection. Again, the relevant inquiry as to whether the Queen patent is an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that the limitation "outside the

Kabat and Chothia CDRs" is not found in, nor supported by, the priority documents.

This limitation requires that framework residues be changed outside both the Kabat and Chothia CDRs. This limitation is significant because the "CDRs" as defined by Kabat and Chothia differ.[1] Kabat defines CDR1 of the heavy chain as amino acids 31-35. Chothia defines the first hypervariable loop of the heavy chain as residues 26-32. As submitted in the Preliminary Amendment filed concurrently with the present application, the earliest Queen patent applications do not teach, either explicitly or implicitly, that the framework residues to be replaced by donor **must be outside both** the Kabat and Chothia CDRs. Indeed, in the specification of the Queen patent as issued, changes were made to residues inside what the Queen patent denotes as CDRH1 of Chothia, i.e., inside a Chothia CDR. Considering that the "outside the Kabat and Chothia CDRs" limitation was required for patentability, the Queen patent cannot be entitled to a priority date earlier than the filing date of the application in which this limitation was first introduced, i.e., 12/19/90.

The Examiner argued in the Final Rejection that the limitation is taught, for example, on page 9, lines 1-5 of Queen priority Application Serial No. 07/290,975 ("Queen '975") and page 13, lines 1-8 of Queen priority Application Serial No. 08/310,252 ("Queen '252"). The passages cited by the Examiner, however, do not support the Examiner's position.

The passage on page 9, lines 1-5, of Queen '975, contains a background discussion of the hypervariable regions, which it is therein stated are also called the CDRs. References by Kabat and Chothia are cited, and incorporated by reference. This is the only in passage in Queen '975 linking the Chothia reference to the term "CDRs." Other passages specifically referring to the CDRs as encompassed by the invention of Queen '975 make it clear that the CDRs are as defined by Kabat. For example, on page 10, line 2, the framework regions are defined in terms of Kabat. If the framework regions are defined in terms of Kabat,

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[1]Notably, the Chothia reference refers to loops and carefully distinguishes these loops from the Kabat CDRs.

the CDRs must be as well. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 19-22, residues which fall in positions within a CDR "as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106" are to be donor. In lines 28-30, amino acid 30 is listed as a position immediately adjacent to a CDR to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but within the heavy chain Chothia "CDR" as that term is used in Queen '975. The description of Figure 1 of Queen '975 indicates that it refers to the heavy chains and that the three CDRs are underlined (page 6, lines 1-6). In Figure 1, amino acids 31-35 are underlined for CDR1. Clearly, all specific references to CDRs were to Kabat CDRs only.

Further, in Figure 1, framework amino acids changed to donor are indicated by asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '975 teaches changing only one or two amino acids, and that both can be within the Chothia CDR. There is no support in Queen '975 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Neither is there support for the limitation in Queen '252. In this instance, the passage relied upon by the Examiner for referring to Chothia is in the context of computer programs for computer models. There is no reference to CDRs. Contrastingly, the specific references to CDRs make it clear that the CDRs are as defined by Kabat. On page 8, lines 22-26, Queen '252 reports that the extents of the framework region and CDRs have been "precisely defined" by Kabat. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 20-22, residues which fall in positions within a CDR "as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106" are to be donor. In lines 27-29, amino acid 30 is listed as a position immediately adjacent to a CDR to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but within the heavy chain Chothia "CDR" as that term is used in Queen '975.

Again, in Figure 1, framework amino acids changed to donor are indicated by

asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '252 teaches changing only one or two amino acids to donor, and that both can be within the Chothia CDR. There is no support in Queen '252 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Applicants respectfully request that this rejection be withdrawn.

The Proposed Count is the same as that submitted with the Amendment filed April 9, 1999. Applicants again identify all of the Queen patent claims 1-11 and Applicants' claims 24-32 as corresponding to the Proposed Count.

In attached Appendix A, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24 and new claim 32. There is, of course, additional support in applicants' application omitted for the sake of brevity.

In attached Appendix B is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claim 28 and new claim 38 which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner declare an interference between the

present application and the Queen patent. Specifically, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo
Registration No. 35,719

Date: November 3, 1999

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

APPENDIX A

Claim Limitation	Support in Adair Application
24. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.
which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 6, line 12, to page 7, line 5.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 38, lines 1-12, and lines 23-38, and Figs. 3-4 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are so identified in Figure 4.
32. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 41.

APPENDIX B

Claim Limitation	Support in 1989 GB Application
28. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 1-2 and 10-20; page 5, lines 8, to page 6, line 4; , and page 8.
which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity	See page 5, lines 1-7; page 22, lines 27-35, page 23, lines 5-9, page 24, lines 1-4; page 25, lines 27-33; page 26 last paragraph.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 5, lines 1-7; page 26, last paragraph, to page 27, top paragraph.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks,	See page 5, line 8, to page 6, line 4: page 7, lines 5-20.
and each of said donor amino acids contributes to antigen binding as determined by X-ray crystallography.	Page 18, lines 11-17, and lines 33-37, and Figs. 20-21 of the application as filed reference residues that may "contribute to antigen binding" as determined using X-ray crystallography. Residues 48, 49, 71, 73, 76, 78, 88, and 91 are identified in Figure 21.
32. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains	See page 1, lines 5-16, and page 7, line 32, through page 8, line 21.

which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}.	See page 11, lines 27-30.
wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions,	See page 6, lines 14-23, page 8, lines 13-16, and page 19, line 16, to page 20, line 15.
wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks	See page 6, line 12, to page 7, line 5.
at residues 48, 49, 71, 73, 76, 78, 88, and 91.	See Light chain 341B of Table 1, page 20.

DOCKET NO.: CARP-0057 **PATENT**

~~RESPONSE UNDER 37 CFR 1.116~~
~~EXPEDITED PROCEDURE~~
~~EXAMINING GROUP NO. 1642~~

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: **Adair et al.**

Serial No.: **08/846,658** Group No.: **1642**

Filed: **May 1, 1997** Examiner: **J. Burke**

For: **Humanised Antibodies**

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I, Doreen Yatko Trujillo, Registration No. 35,719 certify that this correspondence is being transmitted by facsimile to Examiner Burke of the U.S. Patent and Trademark Office, Washington, D.C. 20231.

On January 19, 2000
Doreen Yatko Trujillo
Doreen Yatko Trujillo Reg No. 35,719

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Please do not Enter 2/3/00 OK

Dear Sir:

AMENDMENT AND REQUEST FOR RECONSIDERATION

Pursuant to 37 C.F.R. § 1.116, please amend the above-identified application as a follows.

In the claims:

24. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the

variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

28. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an effective antigen binding affinity, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of said donor amino acids [is adjacent to a CDR in the donor immunoglobulin sequence or] contributes to antigen binding as determined by X-ray crystallography.

Please add the following claim:

49. A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1}, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside both the Kabat CDRs and the structural loop CDRs of the variable regions, wherein the donor amino acids replace corresponding amino acids in the acceptor immunoglobulin heavy chain framework at residues 48, 49, 71, 73, 76, 78, 88, and 91.

REMARKS

This paper is being filed following the Advisory Action dated December 2, 1999. A Notice of Appeal was filed November 29, 1999. Accordingly, it is Applicants' belief that no extension of time or accompanying fee is required. If Applicants' belief is erroneous, this serves to request the requisite extension of time and authorizes the charging of any fee to Deposit Account 23-3050.

Claims 24-31 were pending. In the Final Rejection, all pending claims were rejected. An Amendment and Request for Reconsideration ("Amendment") was filed November 3, 1999 in response to the Final Rejection. The Amendment was not entered in view of what the Examiner considered new matter in a newly submitted claim. The Advisory Action, however, indicated that the Amendment would have overcome the then outstanding rejections under 112 and for new matter of claims 24 and 28. The previous amendments to claims 24 and 28 are resubmitted herein. Their entry is earnestly requested.

New claim 49 has been added herein. New claim 49 refers to specific replacements in the heavy chain. In that regard, the Examiner is directed to Table 1 of the application as filed, specifically to the "Heavy Chain" designated as 341 b. Applicants respectfully submit that new claim 49 does not contain new matter and does not raise new 35 U.S.C. § 112, first and second paragraph issues, nor does it raise new 102/103 issues. Claim 49 is submitted herein in an abundance of caution in view of the removal of the phrase "adjacent to a CDR in the donor immunoglobulin sequence" from claims 24 and 28 as suggested by the Examiner in the Final Rejection. Claim 49 recites a specific residue that is adjacent a CDR, i.e., residue 49. If, however, Applicants' submission of claim 49 is all that stands between the application being in condition for interference , Applicants respectfully request that the Examiner so advise the undersigned at (215) 564-8352.

In view of the foregoing amendments and the arguments that follow, Applicants respectfully submit that allowable subject matter has been identified and request that the interference be declared.

The Examiner stated that the Information Disclosure Statements filed in the

parent cases will be considered once the references are submitted. To the extent the Examiner is requiring that Applicants resubmit references already submitted, this appears to be contrary to MPEP § 609, page 600-103. Applicants are not required to resubmit references to get them considered by the Examiner.

Rejections Under 35 U.S.C. § 112, first paragraph

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a. and b. The Examiner again rejected claims 24 and 28 alleging that the specification does not provide support for the concept that only substitutions adjacent CDRs are envisaged. Claims 24 and 28 were previously amended to recite that each of the donor amino acids to be replaced is adjacent a CDR or contributes to antigen binding as determined by X-ray crystallography. During a telephone conference between the Examiner and the undersigned, the Examiner indicated that removal of the "adjacent to a CDR" language would obviate this rejection. Although Applicants disagree with the Examiner's reasoning, the claims have been amended herein to remove the recitation "adjacent to a CDR in the donor immunoglobulin sequence." As Applicants made clear in the previous response, the contribution to antigen binding need not be direct and, indeed, can be indirect, e.g., by affecting antigen binding site topology or inducing stable packing. Naturally, even for an indirect effect, the residues must be spatially near the CDR.

Applicants respectfully request that this rejection be withdrawn.

As the Examiner is aware, Applicants desire to provoke an interference between the present application and the Queen patent (U.S. Patent No. 5,585,089). Although Applicants are confident that the present claims are directed to the same invention as the

Queen patent, new claim 49 is submitted herewith. New claim 49 recites the residues changed in example g341B disclosed in Applicants' specification as filed and, indeed, in GB8928874. Of the residues recited, all are either adjacent a CDR (49), or contribute to antigen binding as determined by X-ray crystallography (48, 71, 73, 76, 78, 88, and 91). Claim 49 is clearly allowable and clearly directed to the same invention as claim 1 of the Queen patent.

> If at least one of the presented claims is not rejectable on any [ ] ground and is claiming the same invention as at least one claim of the patent, the examiner should proceed to initiate an interference.

MPEP 2307.02.

Applicants respectfully request that an interference between the present application and the Queen patent be declared.

Rejection Under 35 U.S.C. § 102(e)

Claims 24-31 were again rejected under 35 U.S.C. § 102(e) in view of the Queen patent. Applicants respectfully traverse this rejection. Again, the relevant inquiry as to whether the Queen patent is an appropriate reference under 102(e) is whether there is support for the claims *as allowed* in the priority applications, see MPEP 2136.03, p. 2100-85, citing *In re Wertheim*, 209 USPQ 554 (CCPA 1981), not simply whether the limitations can be found in the priority document. Regardless, Applicants maintain that the limitation "outside the Kabat and Chothia CDRs" is not found in, nor supported by, the priority documents.

This limitation requires that framework residues be changed outside both the Kabat and Chothia CDRs. This limitation is significant because the "CDRs" as defined by Kabat and Chothia differ.[1] Kabat defines CDR1 of the heavy chain as amino acids 31-35. Chothia defines the first hypervariable loop of the heavy chain as residues 26-32. As submitted in the Preliminary Amendment filed concurrently with the present application, the earliest Queen patent applications do not teach, either explicitly or implicitly, that the

[1]Notably, the Chothia reference refers to loops and carefully distinguishes these loops from the Kabat CDRs.

framework residues to be replaced by donor **must be outside both** the Kabat and Chothia CDRs. Indeed, in the specification of the Queen patent as issued, changes were made to residues inside what the Queen patent denotes as CDRH1 of Chothia, i.e., inside a Chothia CDR. Considering that the "outside the Kabat and Chothia CDRs" limitation was required for patentability, the Queen patent cannot be entitled to a priority date earlier than the filing date of the application in which this limitation was first introduced, i.e., 12/19/90.

The Examiner argued in the Final Rejection that the limitation is taught, for example, on page 9, lines 1-5 of Queen priority Application Serial No. 07/290,975 ("Queen '975") and page 13, lines 1-8 of Queen priority Application Serial No. 08/310,252 ("Queen '252"). The passages cited by the Examiner, however, do not support the Examiner's position.

The passage on page 9, lines 1-5, of Queen '975, contains a background discussion of the hypervariable regions, which it is therein stated are also called the CDRs. References by Kabat and Chothia are cited, and incorporated by reference. This is the only in passage in Queen '975 linking the Chothia reference to the term "CDRs." Other passages specifically referring to the CDRs as encompassed by the invention of Queen '975 make it clear that the CDRs are as defined by Kabat. For example, on page 10, line 2, the framework regions are defined in terms of Kabat. If the framework regions are defined in terms of Kabat, the CDRs must be as well. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 19-22, residues which fall in positions within a CDR "**as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106**" are to be donor. In lines 28-30, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but **within** the heavy chain Chothia "CDR" as that term is used in Queen '975. The description of Figure 1 of Queen '975 indicates that it refers to the heavy chains and that the three CDRs are underlined (page 6, lines 1-6). In Figure 1, amino acids 31-35 are underlined for CDR1. Clearly, all specific references to CDRs were to Kabat CDRs only.

Further, in Figure 1, framework amino acids changed to donor are indicated by

asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '975 teaches changing only one or two amino acids, and that both can be within the Chothia CDR. There is no support in Queen '975 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Neither is there support for the limitation in Queen '252. In this instance, the passage relied upon by the Examiner for referring to Chothia is in the context of computer programs for computer models. There is no reference to CDRs. Contrastingly, the specific references to CDRs make it clear that the CDRs are as defined by Kabat. On page 8, lines 22-26, Queen '252 reports that the extents of the framework region and CDRs have been "precisely defined" by Kabat. On page 21, the protocol for selecting which residues in the heavy chain are to be donor is set out. In lines 20-22, residues which fall in positions within a CDR "**as defined by Kabat, [i.e.,] amino acids 31-35, 50-66, and 99-106**" are to be donor. In lines 27-29, amino acid 30 is listed as a position **immediately adjacent to a CDR** to be changed to donor. Amino acid 30 is adjacent the heavy chain Kabat CDR, but **within** the heavy chain Chothia "CDR" as that term is used in Queen '975.

Again, in Figure 1, framework amino acids changed to donor are indicated by asterisks. Amino acids 27 and 30 are so designated. These residues are clearly within the Chothia "CDR." Neither the specification nor the claims require that more than one amino acid be changed to donor. Thus, Queen '252 teaches changing only one or two amino acids to donor, and that both can be within the Chothia CDR. There is no support in Queen '252 for the limitation that the residues changed to donor must be outside both the Kabat and Chothia "CDRs."

Applicants respectfully request that this rejection be withdrawn.

The Proposed Count is the same as that submitted with the Amendment filed April 9, 1999. Applicants again identify all of the Queen patent claims 1-11 and Applicants' claims 24-31 and 49 as corresponding to the Proposed Count.

In attached Appendix A, applicants illustrate the representative support in their present application disclosure for the limitations of their amended claim 24 and new claim 49. There is, of course, additional support in applicants' application omitted for the sake of brevity.

In attached Appendix B is a diagram of support in applicants' 1989 GB application for each limitation of applicants' amended claim 28 and new claim 49 which are also drawn to the same invention as proposed Count 1. Accordingly, applicants' effective filing date for their invention of Count 1 is 12/21/89, the filing date of their GB national application.

In view of the foregoing, Applicants respectfully submit that allowable subject matter has been identified and request that the Examiner declare an interference between the present application and the Queen patent. Specifically, the Examiner is requested to contact the undersigned at (215) 564-8352.

Respectfully submitted,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. 35,719

Date: January 19, 2000

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

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.

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.

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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BENNETT, L

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FRANCIS A. PAINTIN
WOODCOCK, WASHBURN, KURTZ, MACKIEWICZ & NORRIS
ONE LIBERTY PLACE-46TH FLOOR
PHILADELPHIA, PA 19103

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, ~~4 pages~~
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☑ Notice of Art Cited by Applicant, PTO-1449, ~~4~~ 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. 9-89)

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

Doreen Yatko Trujillo, Reg. No. 35,719

#23H 09

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,

Doreen Yatko Trujillo
Registration No. **35,719**

Date: 29 August 2000

Woodcock Washburn Kurtz
Mackiewicz and Norris LLP
One Liberty Place - 46th Floor,
Philadelphia, PA 19103,
(215) 568-3100

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Cable: WOODCOCK

00035

DATE: September 14, 2000

Please deliver this and the following pages to:

Name:	**Examiner Julie E. Burke, née Reeves, Ph.D.**
Company/Firm:	**U.S. Patent and Trademark Office, Group 1642**
Telecopier No.:	**(703) 305-7401 or (703) 308-4242**
Client/Matter No.:	**CARP-0057; Serial No. 08/846,658**
SENDER'S NAME:	**Doreen Y. Trujillo**
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DOCKET NO.: CARP-0057

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re parent application of: **Adair et al.**

Serial No.: **08/846,658**

Group No.: **1642**

Filed: **May 1, 1997**

Examiner: **J. Burke**

For: **Humanised Antibodies**

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

requested to contact the undersigned at (215) 564-8352 if she feels a telephonic discussion will be helpful.

Respectfully submitted,

Doreen Yatko Trujillo
Registration No. 35,719

Date: September 14, 2000

WOODCOCK WASHBURN KURTZ
MACKIEWICZ & NORRIS LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
(215) 568-3100

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
KO
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.

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**

WOODCOCK WASHBURN LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
Telephone: (215) 568-3100
Facsimile: (215) 568-3439

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.

WOODCOCK WASHBURN
INTELLECTUAL PROPERTY LAW

PHILADELPHIA
One Liberty Place, 46th floor
Philadelphia, PA 19103
Phone: 215-568-3100
Fax: 215-568-3439

SEATTLE
999 Third Avenue, Suite 1606
Seattle, WA 98104
Phone: 206-332-1380
Fax: 206-624-7317

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DATE: March 18, 2002 JOB CODE: 27136.16

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 residue mean 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

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,

Doreen Yatko Trujillo

Doreen Yatko Trujillo
Registration No. **35,719**

Date: August 9, 2002

WOODCOCK WASHBURN LLP
One Liberty Place - 46th Floor
Philadelphia, PA 19103
Telephone: (215) 568-3100
Facsimile: (215) 568-3439

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.

P. E. Crawley
Celltech Limited
216 Bath Road,
Slough,
SL1 4EN Berks.

Ms. L. Summersby. 4/1
Signature & Date

Agent's Reference

PA 259

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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_dIII 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 HindIII 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).

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

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 gL121A (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
```

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.

```
   51  ACTGGATCTT TCTACTGCTG 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

8928874·0

```
  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)

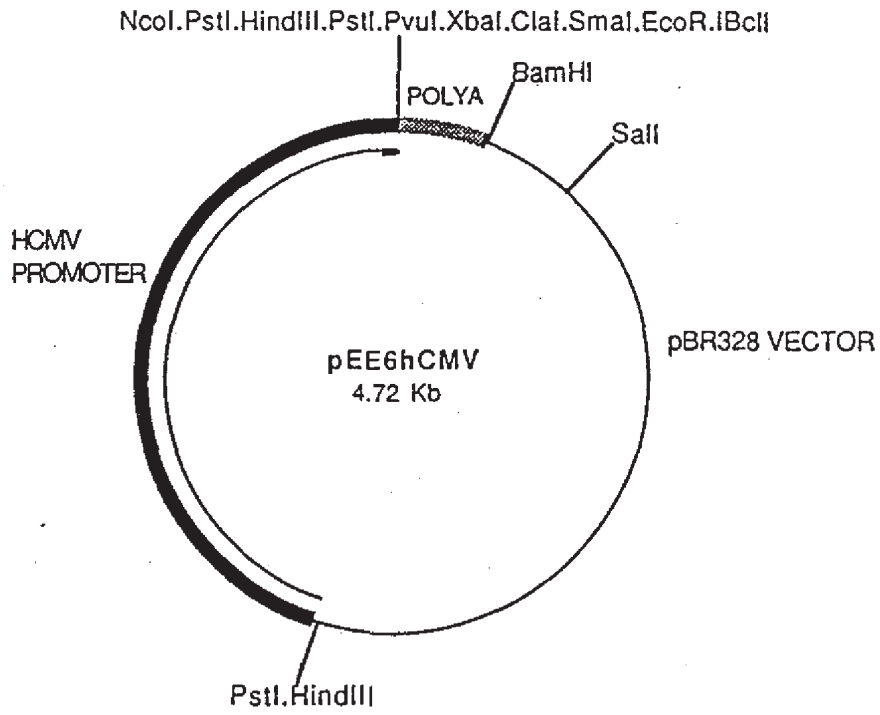


FIGURE 5

A map for the EE6hCMV expression vector used in this study
Only necessary sites are shown.

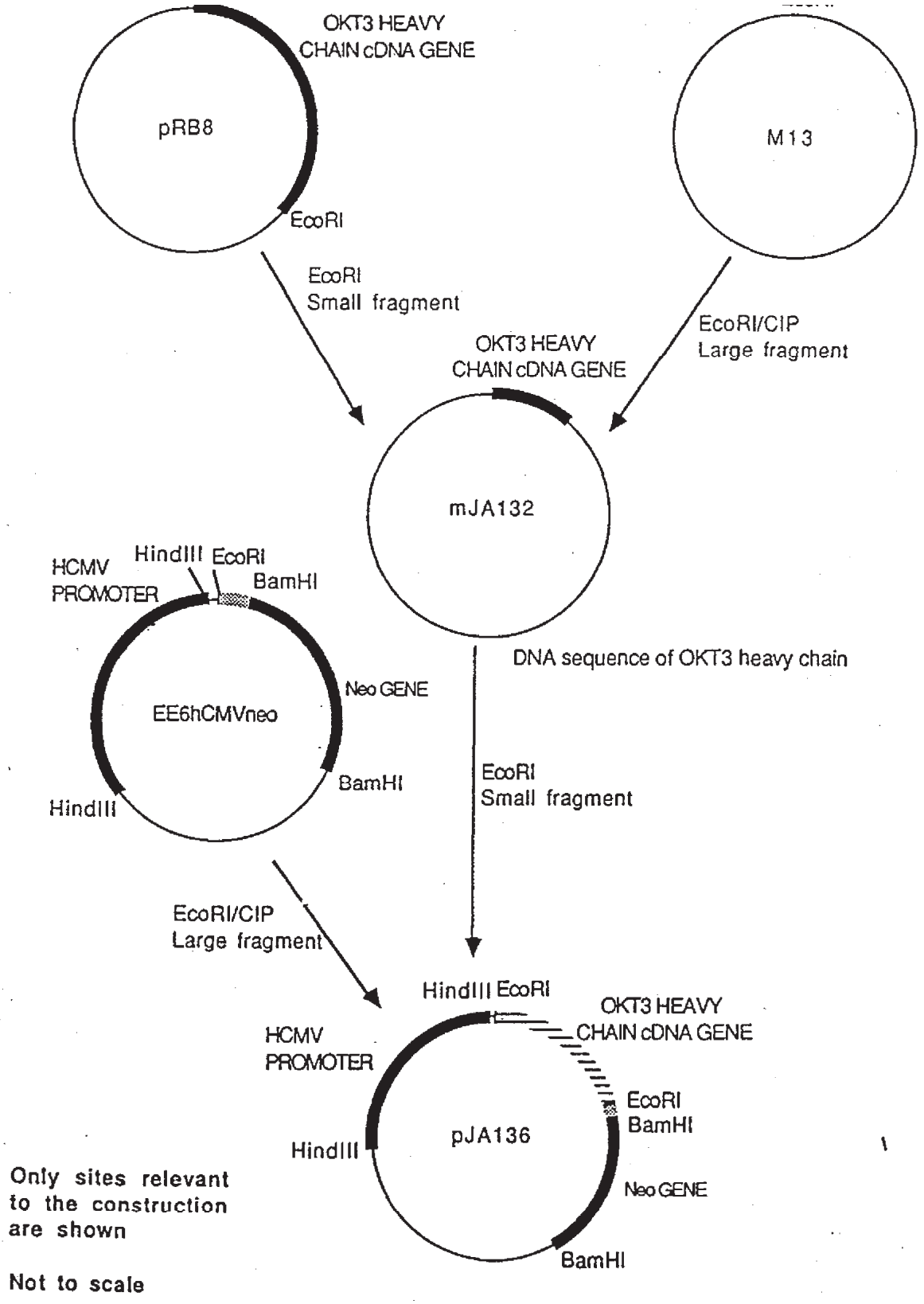


FIGURE 6
An outline schematic of the procedures involved in the construction of pJA136, a vector for the expression in eukaryotic cells of the OKT3 heavy chain cDNA gene.

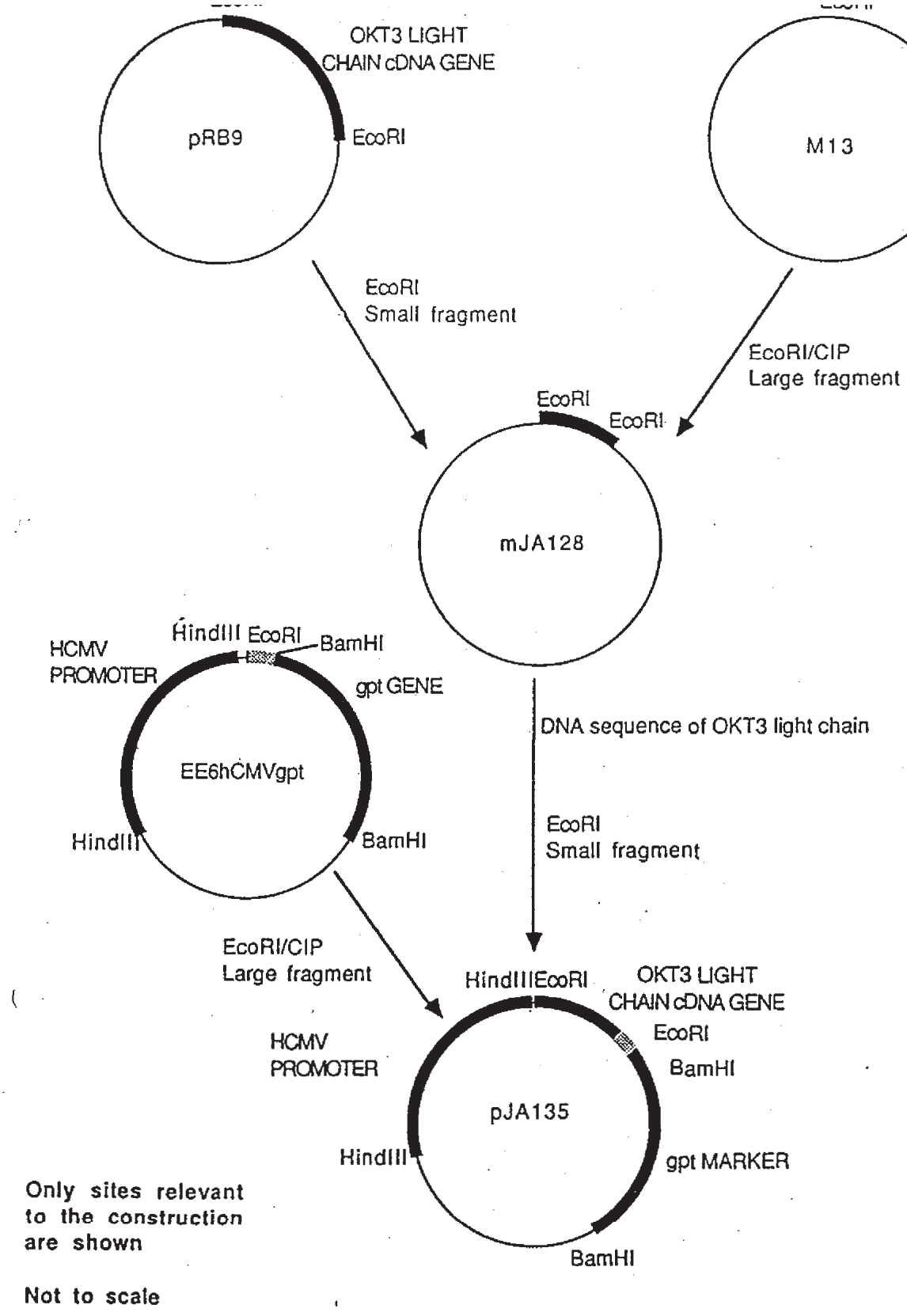


FIGURE 7
An outline schematic of the procedures involved in the construction of pJA135, a vector for the expression in eukaryotic cells of the OKT3 light chain cDNA gene.

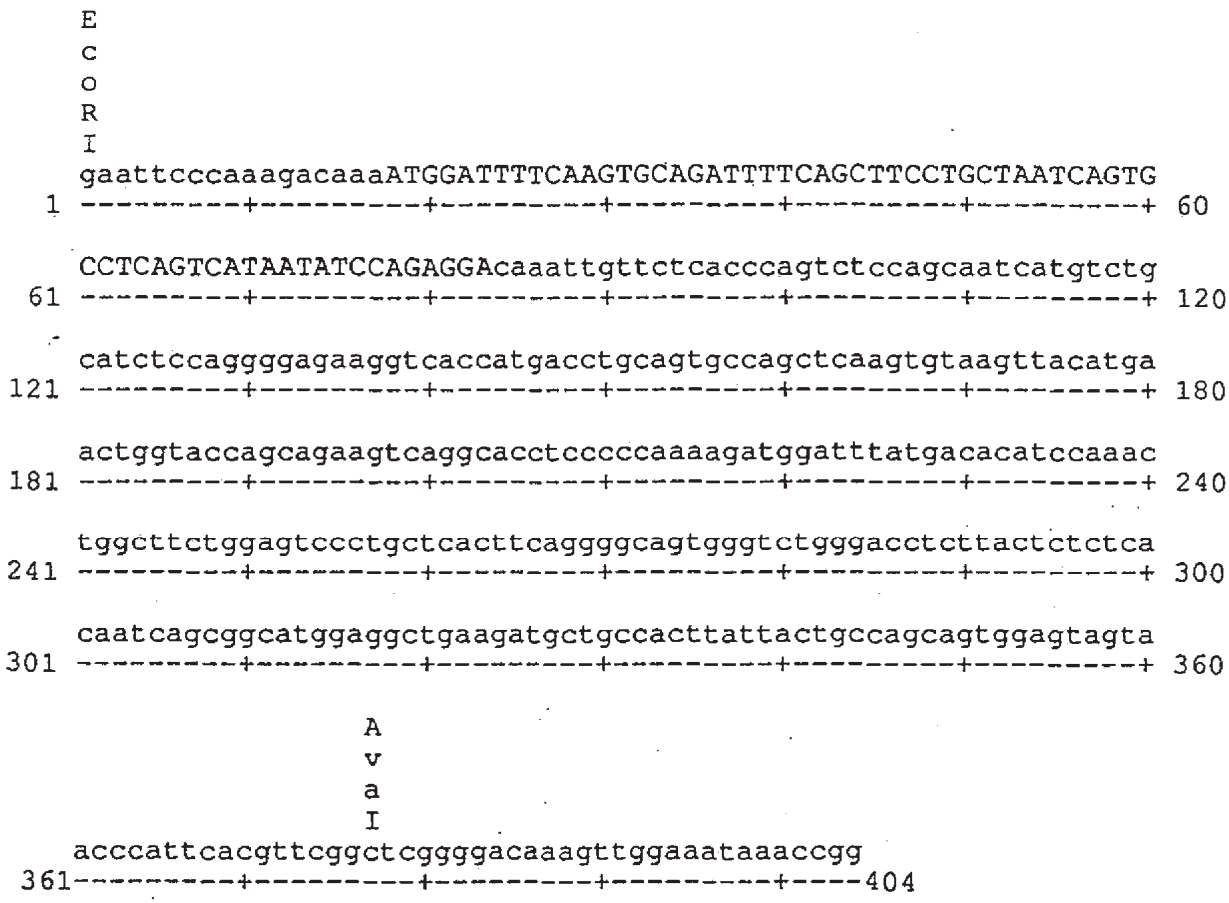
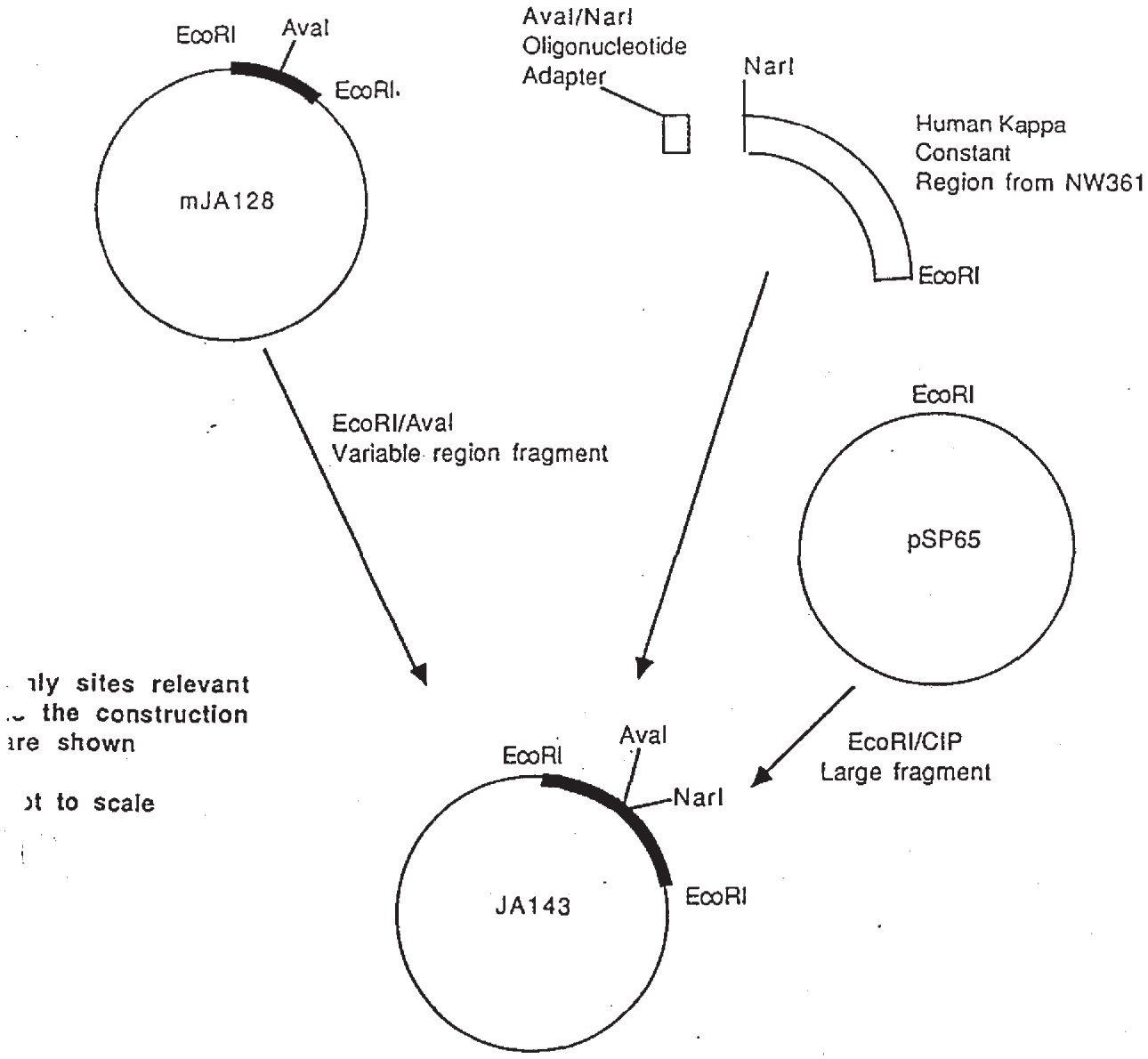


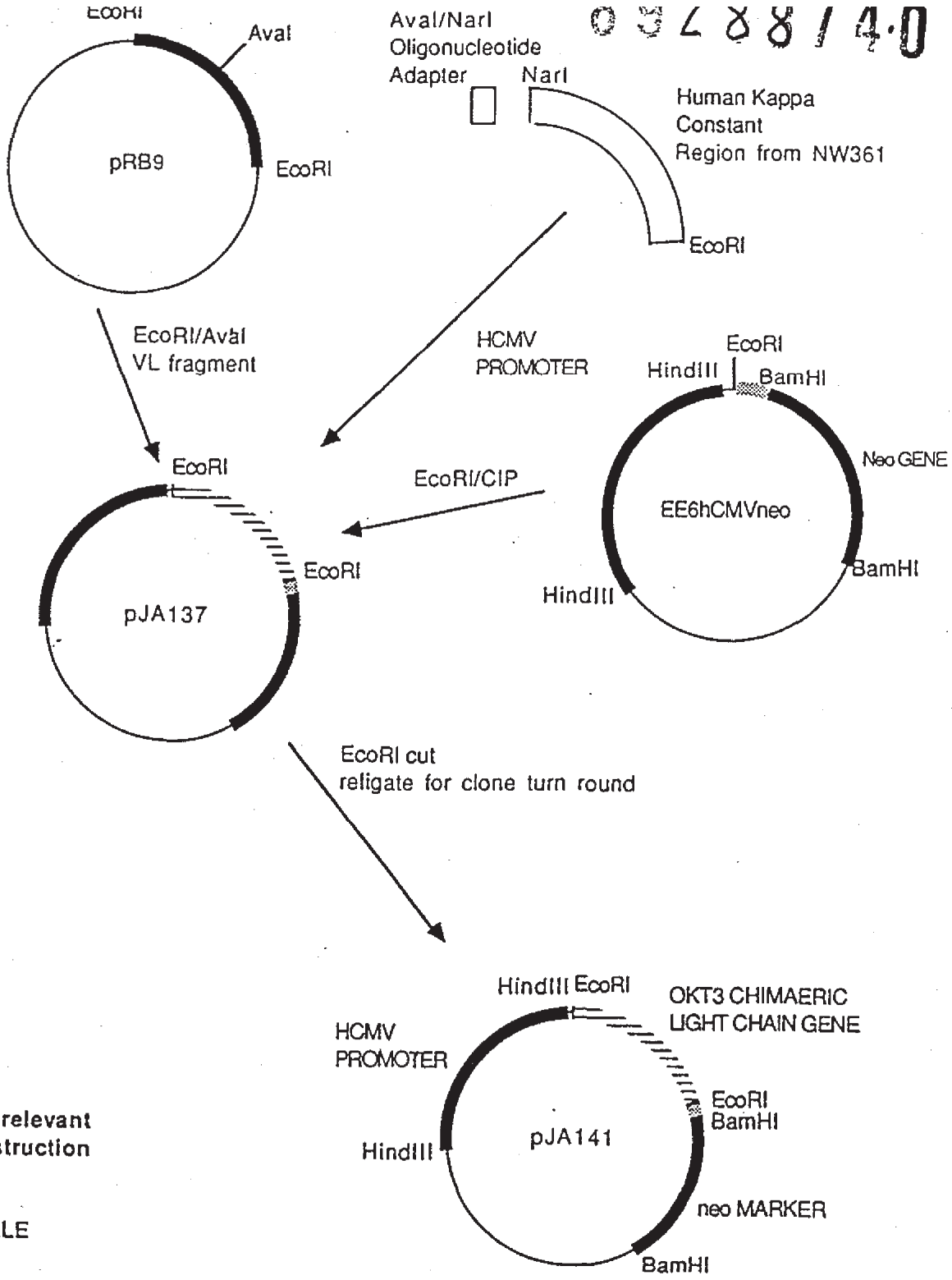
FIGURE 8
The nucleotide sequence for the OKT3 light chain variable region and the location of the EcoRI and AvaI sites used in the construction of the chimaeric OKT3 light chain gene.



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

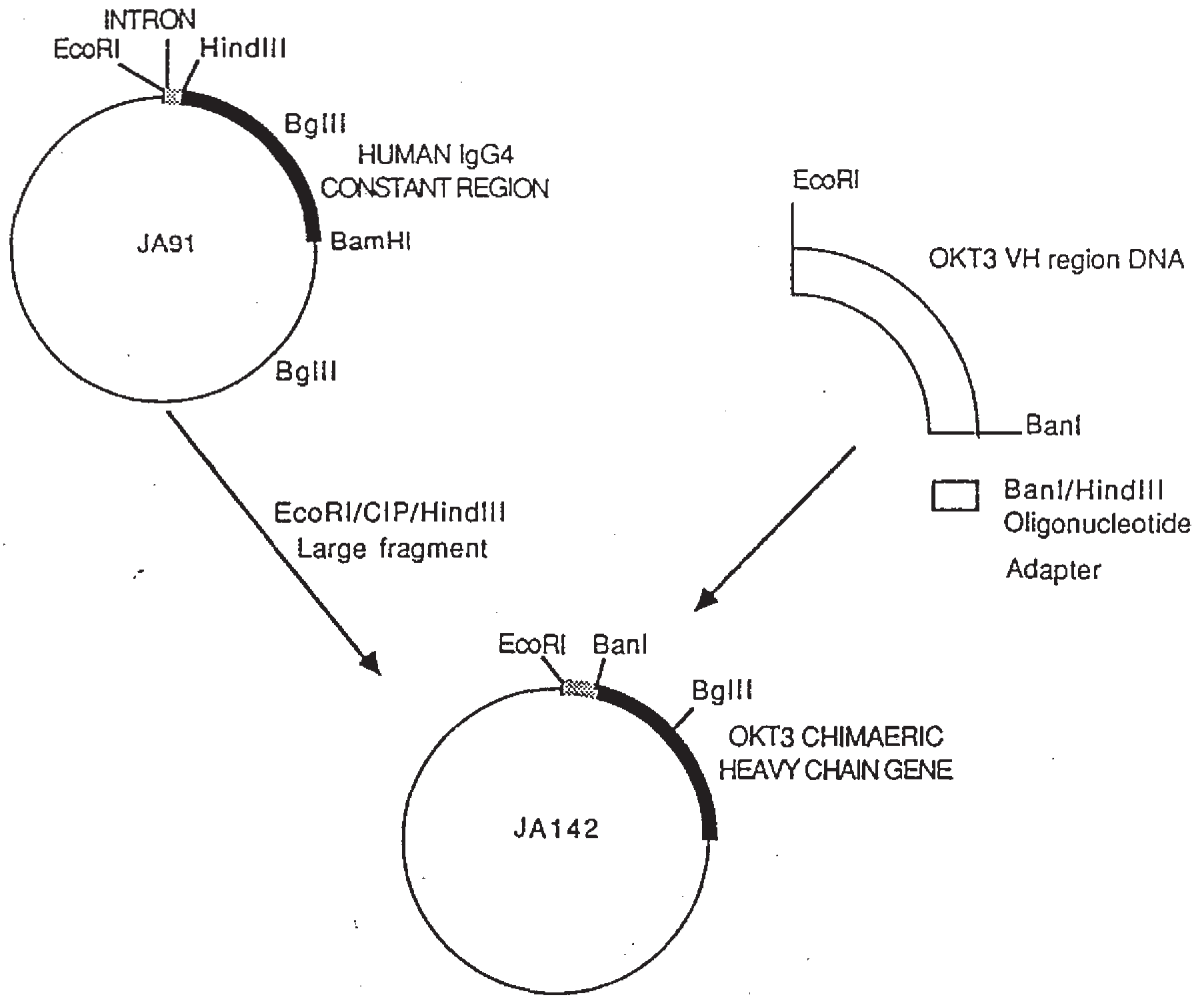
FIGURE 10

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
e 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.

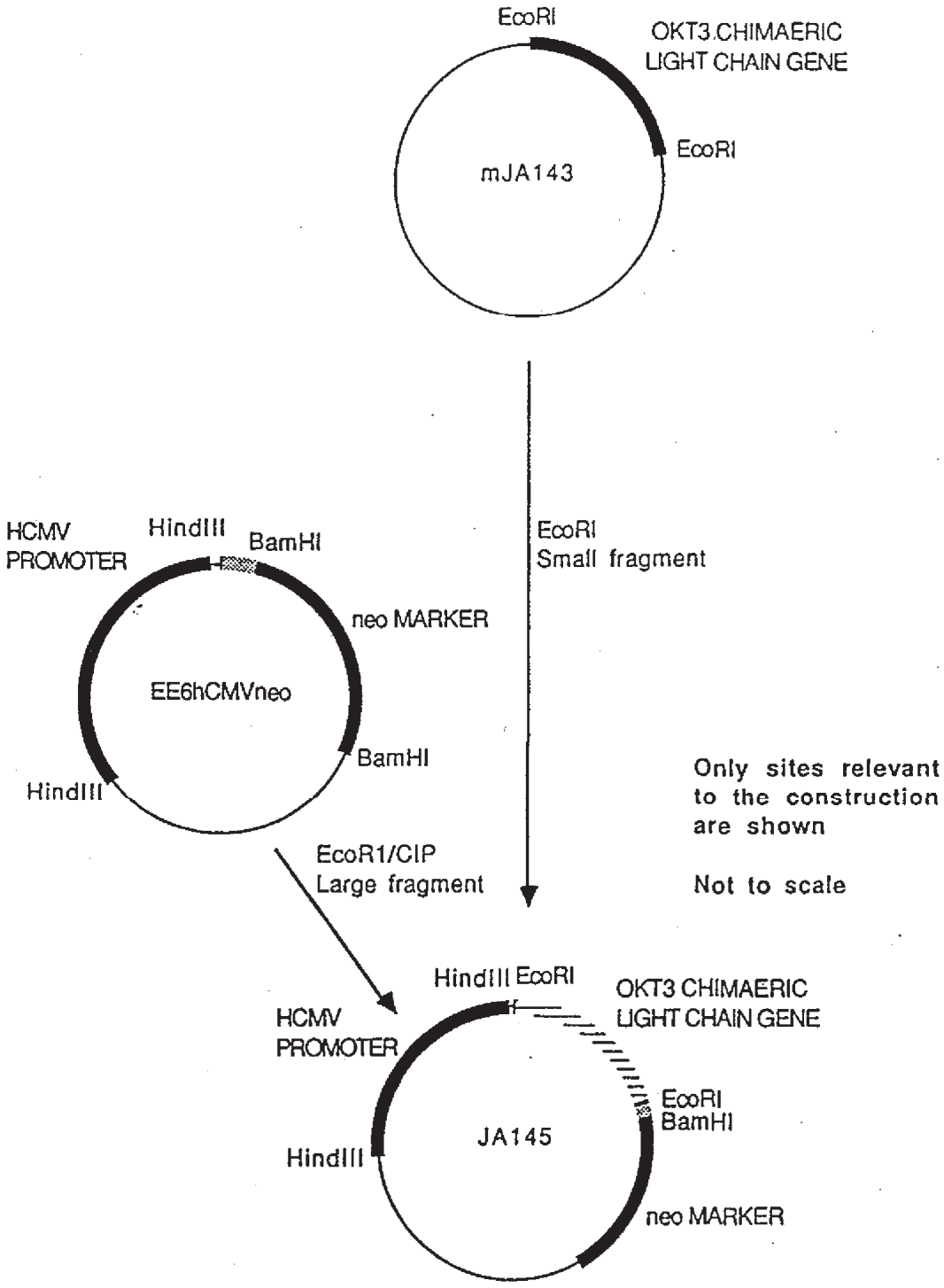


FIGURE 13
An outline schematic of the procedures involved in theconstruction of pJA145, a vector for the expression in eukaryotic cells of the OKT3 chimaeric light chain gene (Version 1).

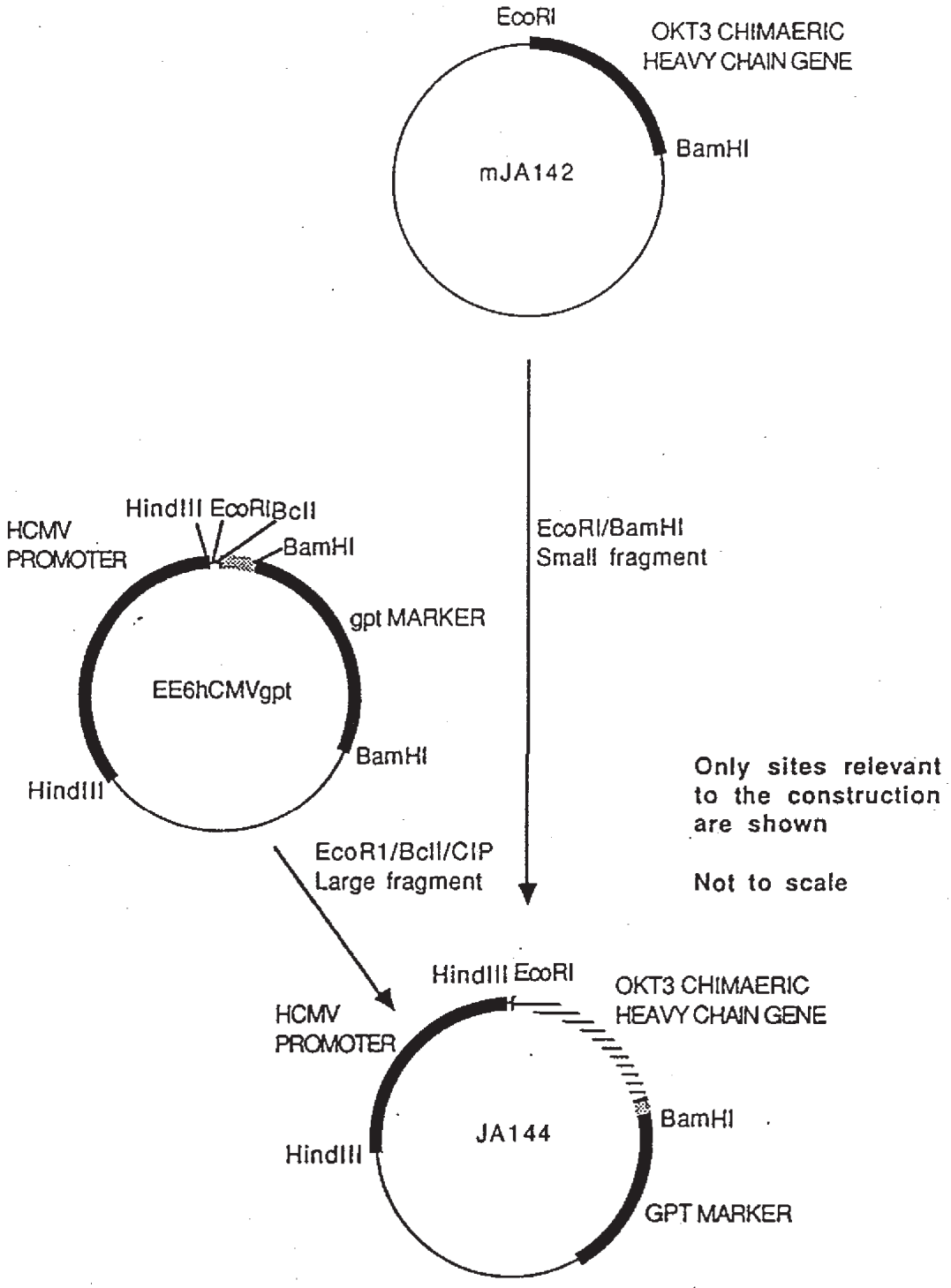


FIGURE 14
An outline schematic of the procedures involved in the construction of pJA144, a vector for the expression in eukaryotic cells of the OKT3 chimaeric heavy chain gene.

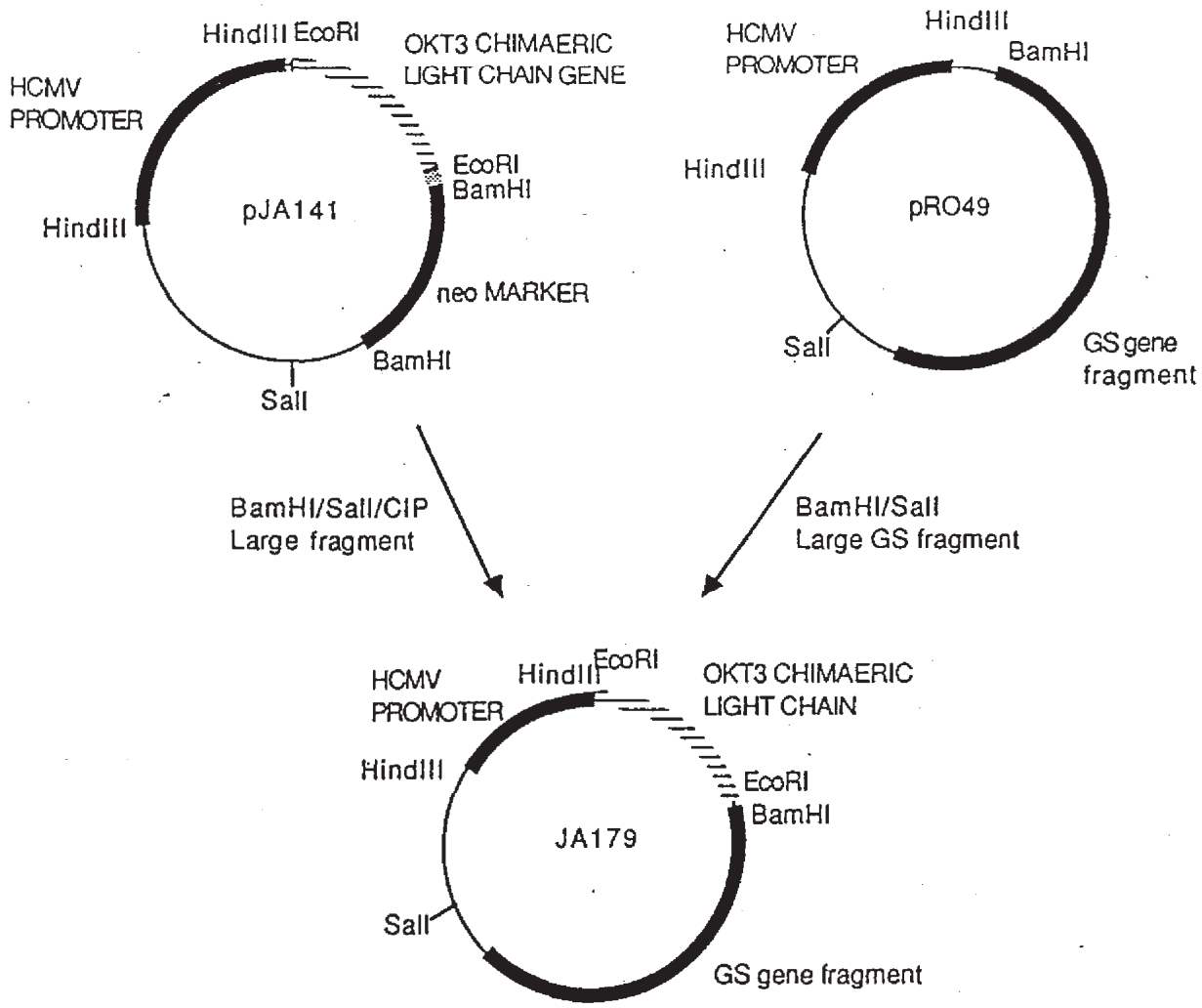


FIGURE 15
An outline schematic of the procedures involved in the construction of pJA179, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric light chain gene (Version 2).

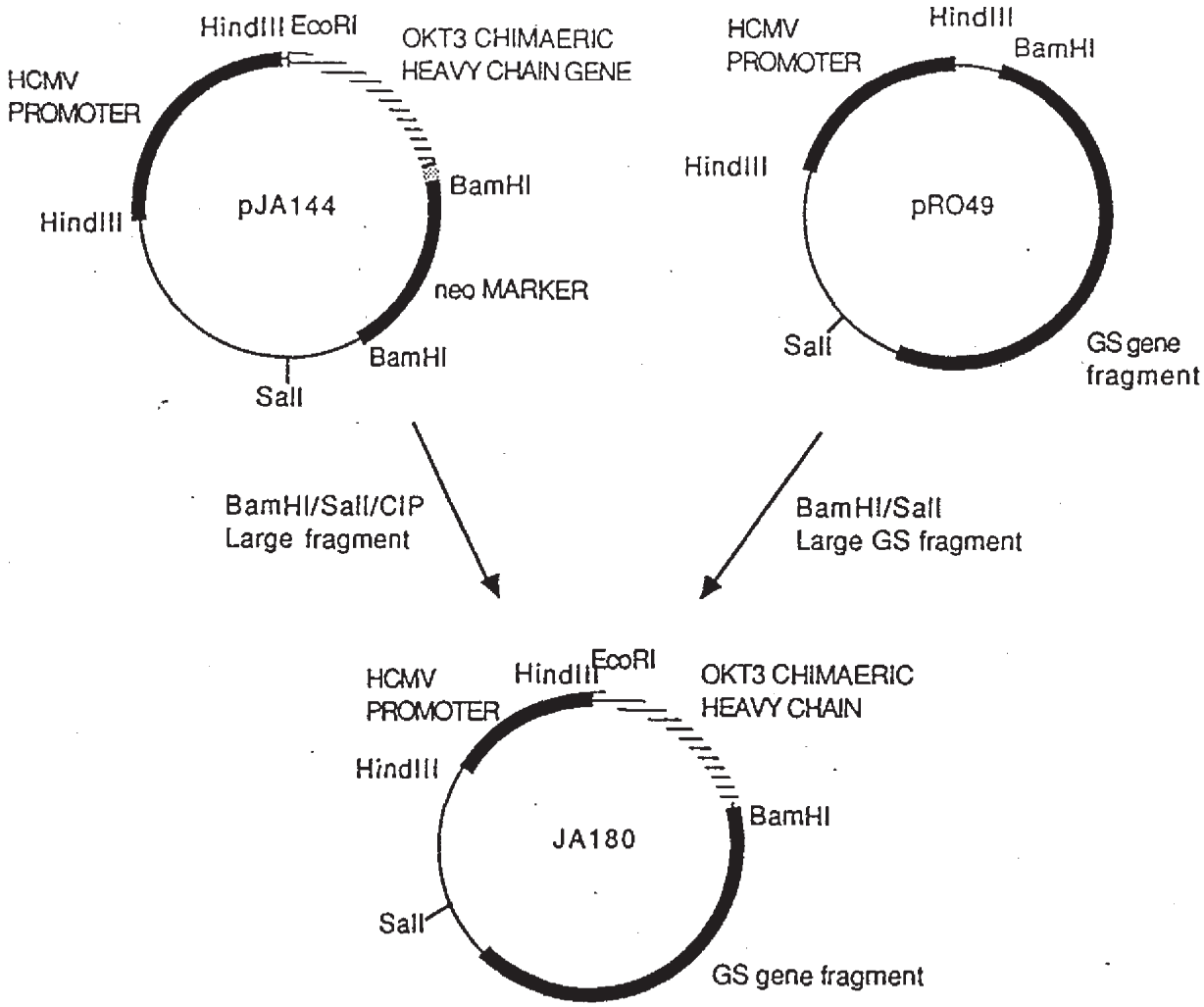


FIGURE 16
An outline schematic of the procedures involved in the construction of pJA179, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric heavy chain gene.

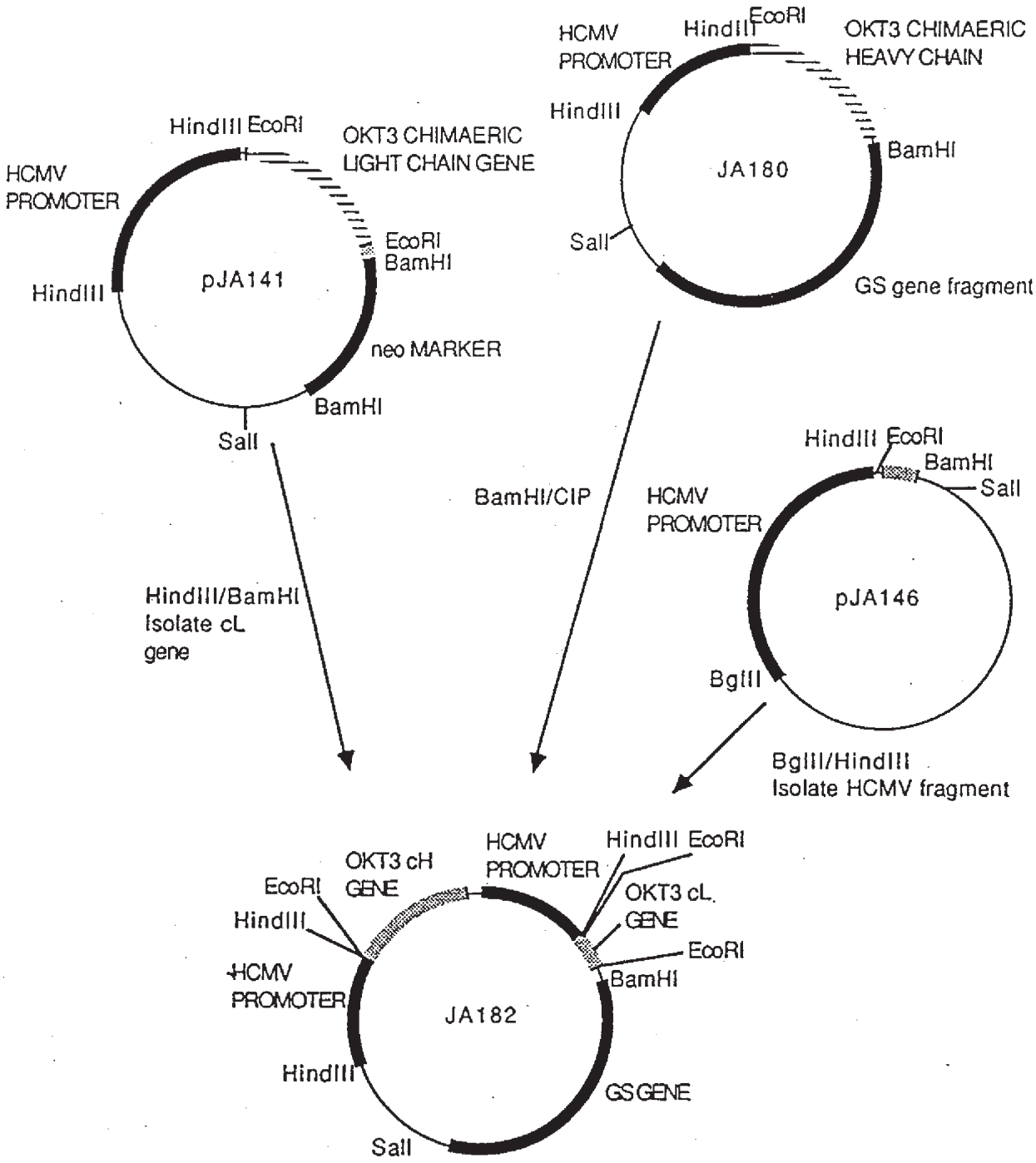


FIGURE 17
An outline schematic of the procedures involved in the construction of pJA182, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric heavy chain gene and chimaeric light chain gene (Version 2) in the transcription order cH>cL>GS.

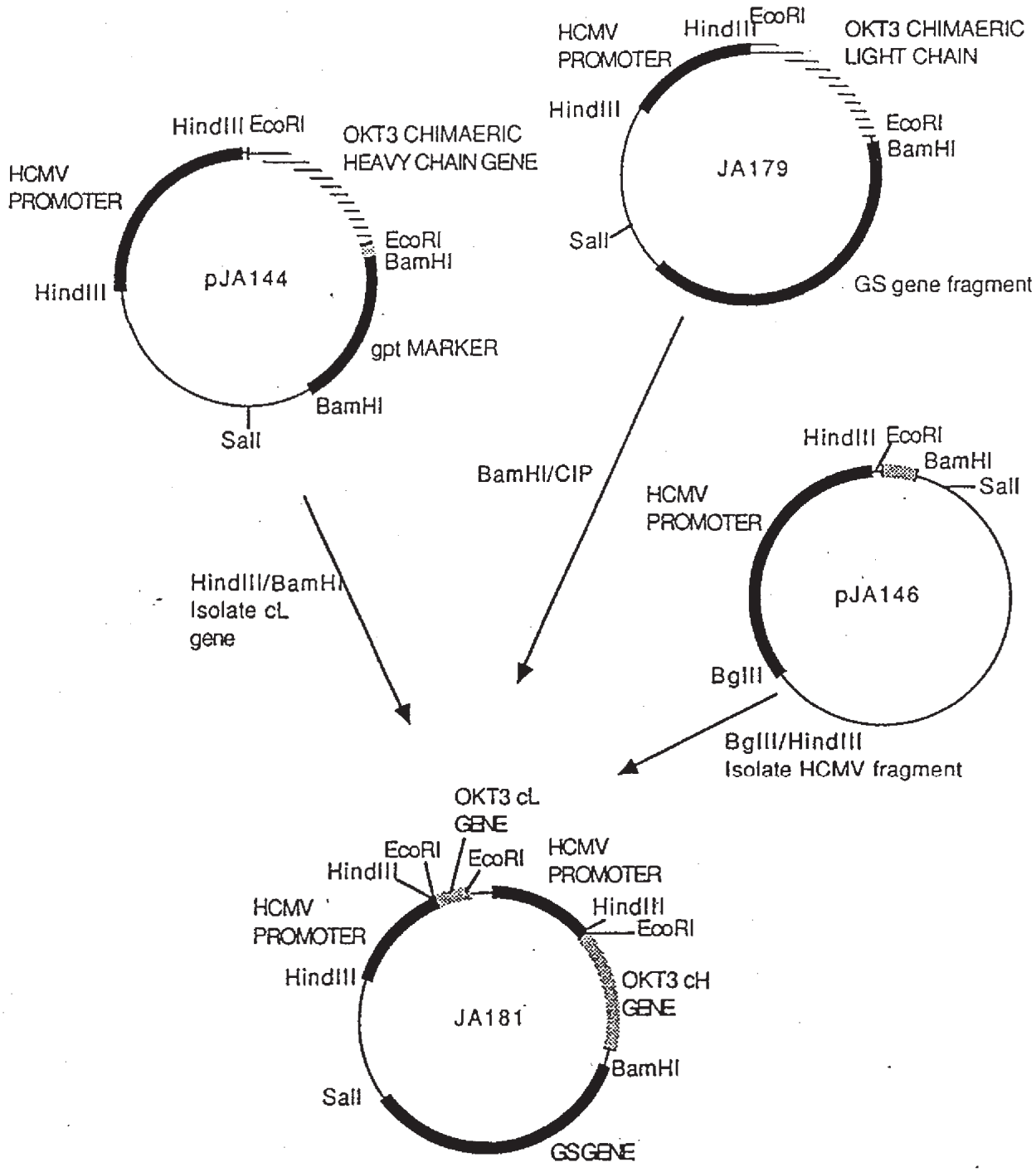


FIGURE 18
An outline schematic of the procedures involved in the construction of pJA181, a vector for the expression in eukaryotic cells using the GS amplification system, of the OKT3 chimaeric light chain gene (Version 2) and chimaeric heavy chain gene in the transcription order cL>cH>GS.

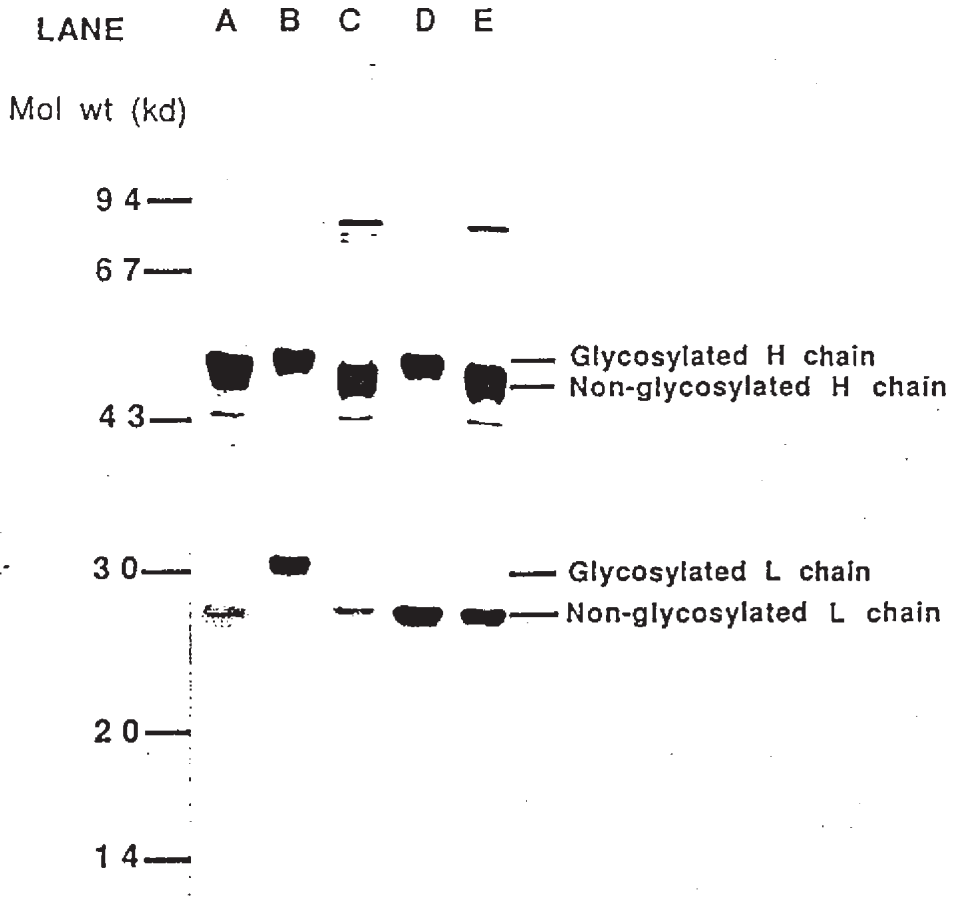


Fig 19. Effect on glycosylation of the presence of tunicamycin during cell growth

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

```
                NN      N                N        N      N
 RES TYPE   SBspSPESssBSbSsSssPSPSPsPSsse*s*p*Pi^ISsSe
Okt3vl      QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYMNWYQQKSGT
REI         DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGK
            ? ?
               CDR1   (LOOP)            *******
               CDR1   (KABAT)         ***********

                          56                              85
            N  NN
 RES TYPE   *IsiPpIeesesssSBEsePsPSBSSEsPspsPsseesSPePb
Okt3vl      SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI         APKLLIYEASNLQAGVPSRFSGSGSGTDYTFTISSLQPEDIAT
            ?  ??                          ?  ?
                    ******* CDR2   (LOOP/KABAT)

                          102   108
RES TYPE    PiPIPies**iPIIsPPSPSPSS
Okt3vl      YYCQQWSSNPFTFGSGTKLEINR
REIvl       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.

```
 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.

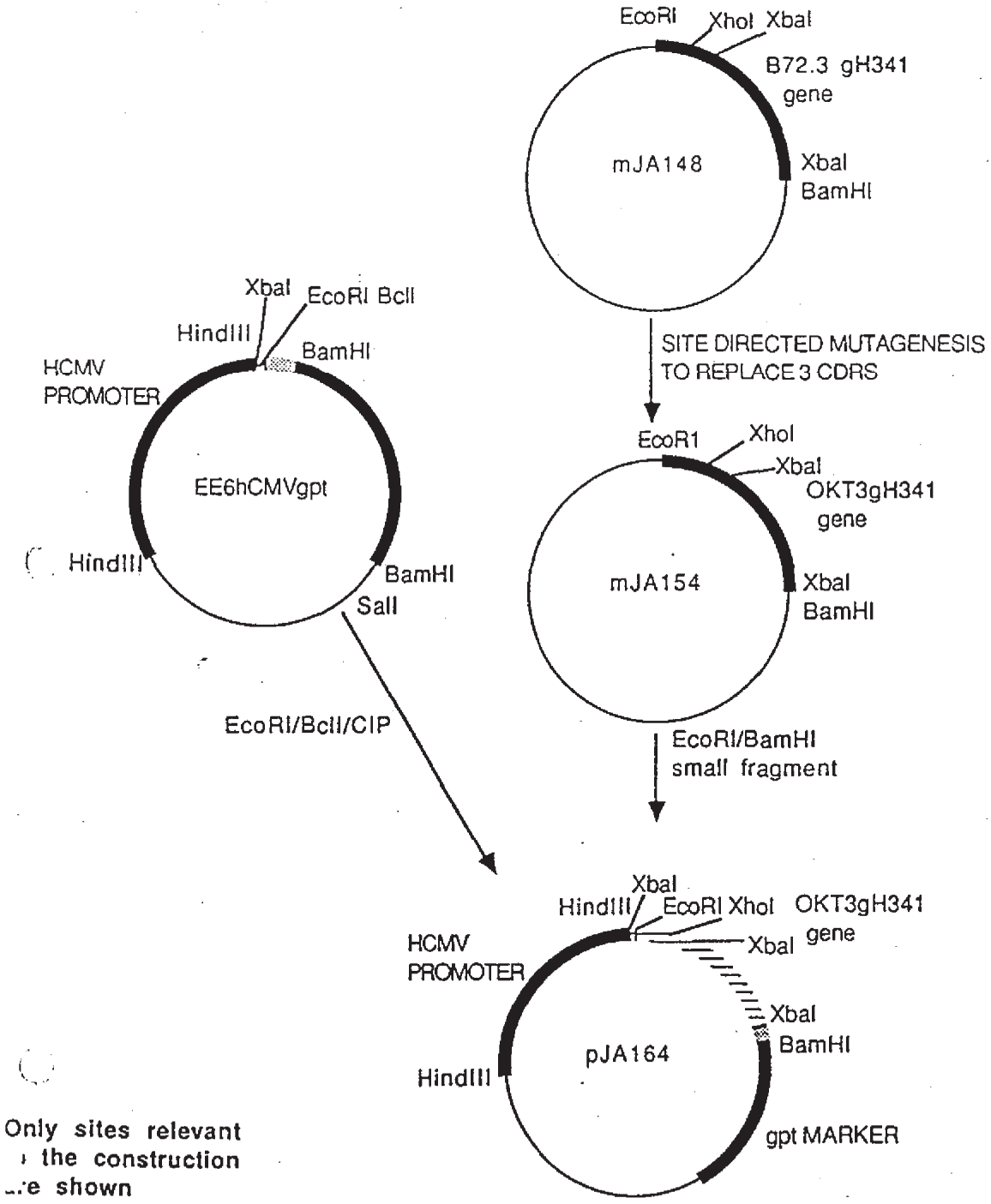


FIGURE 22B
An outline schematic of the procedures involved in the construction of pJA164, a vector for the expression in eukaryotic cells of the OKT3 gH341 CDR grafted heavy chain gene.

```
                                    M  E  W  S  W  V  F  L  F  F  L  S  V
R1387    1  AATTCGCCGCCACCATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTA      51
R1388    2     GCGGCGGTGGTACCTTACCTCGACCCAGAAAGAGAAGAAGGAC            43

                                        MATURE V_H
                                 T  T  G  V  H  S  Q  V  Q  L  V
R1090    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).

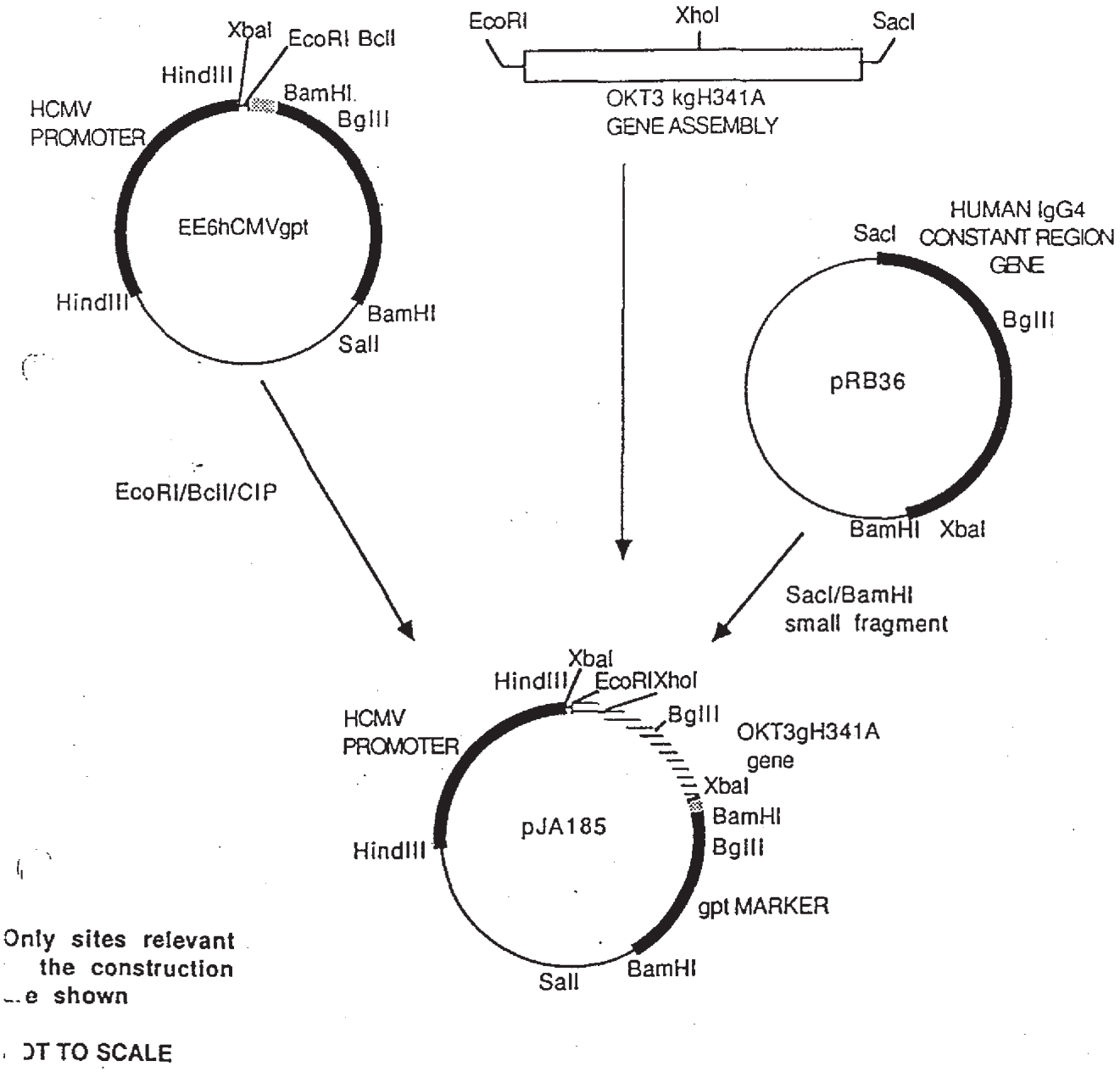


FIGURE 23B
An outline schematic of the procedures involved in theconstruction of pJA185, a vector for the expression in eukaryotic cells of the OKT3 gH341 CDR grafted heavy chain gene.

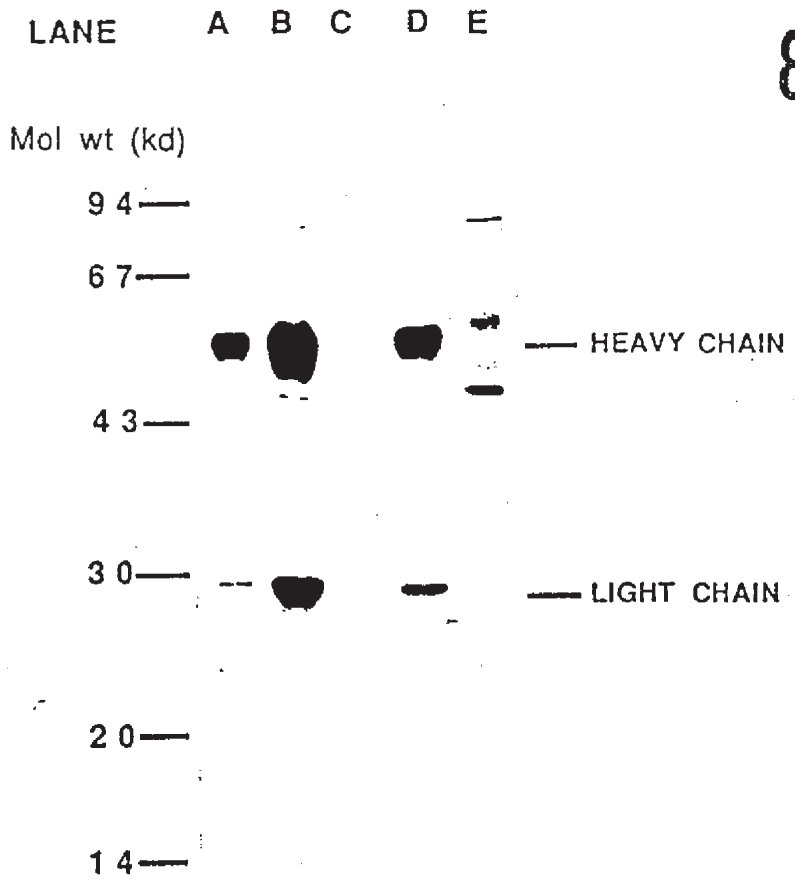


Fig 24a. Effect on antibody expression of the presence of a "Kozak consensus sequence" immediately preceding the gL gene.

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

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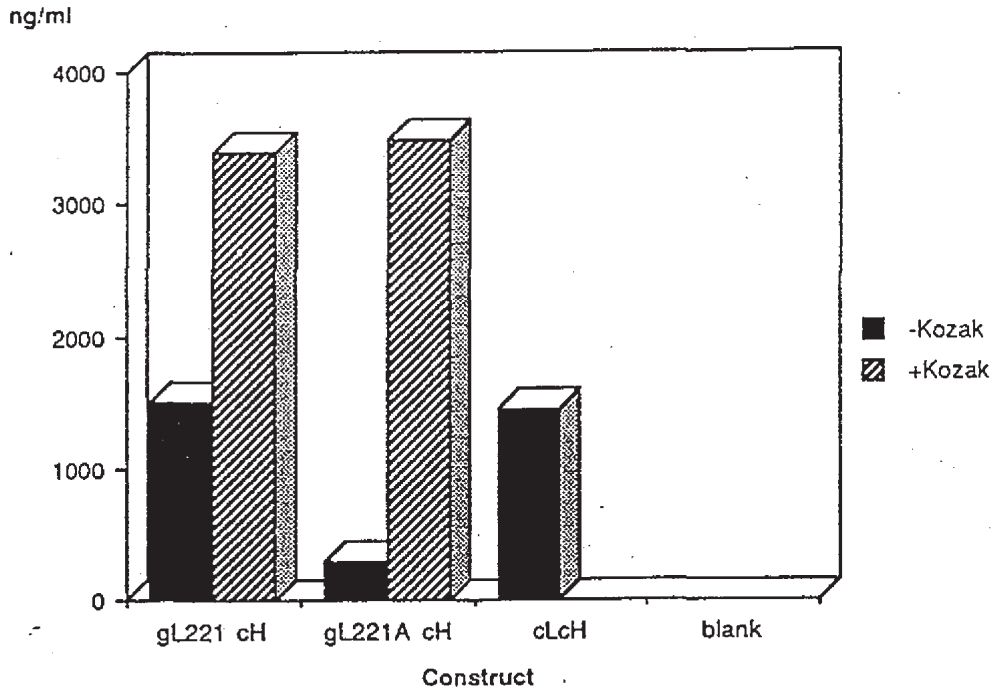

Fig 24b. Effect on antibody expression in the presence of the Kozak consensus sequence immediately preceding the gL gene.

Yield of antibody (ng/ml) from COS cell transient expression experiment.

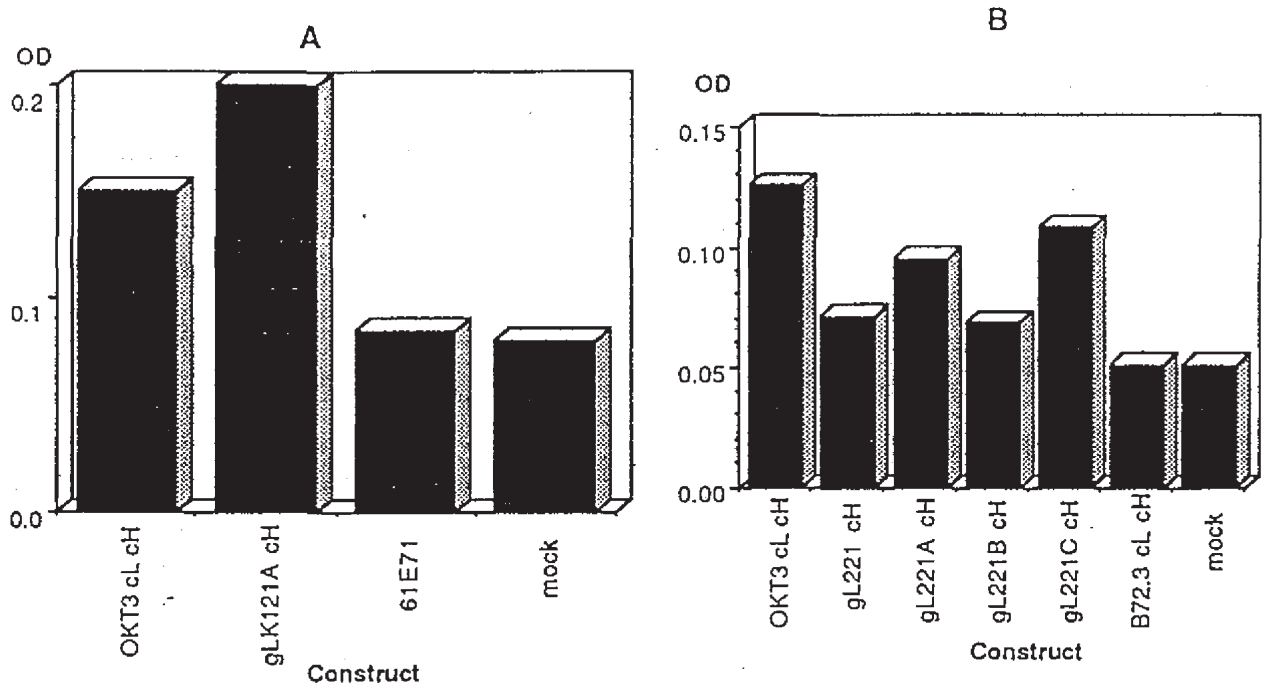

Fig 25. Antigen binding data for gL series genes

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.

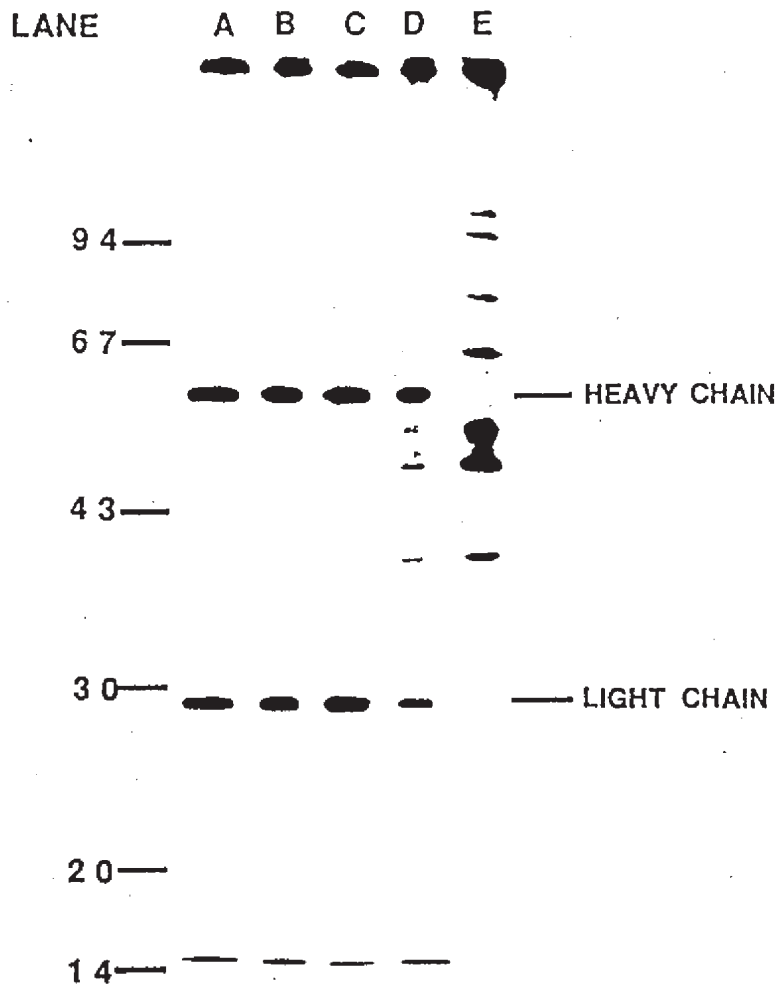

Fig 26. Effect on antibody expression of the presence of a "Kozak consensus sequence" immediatly preceding the gH gene.

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

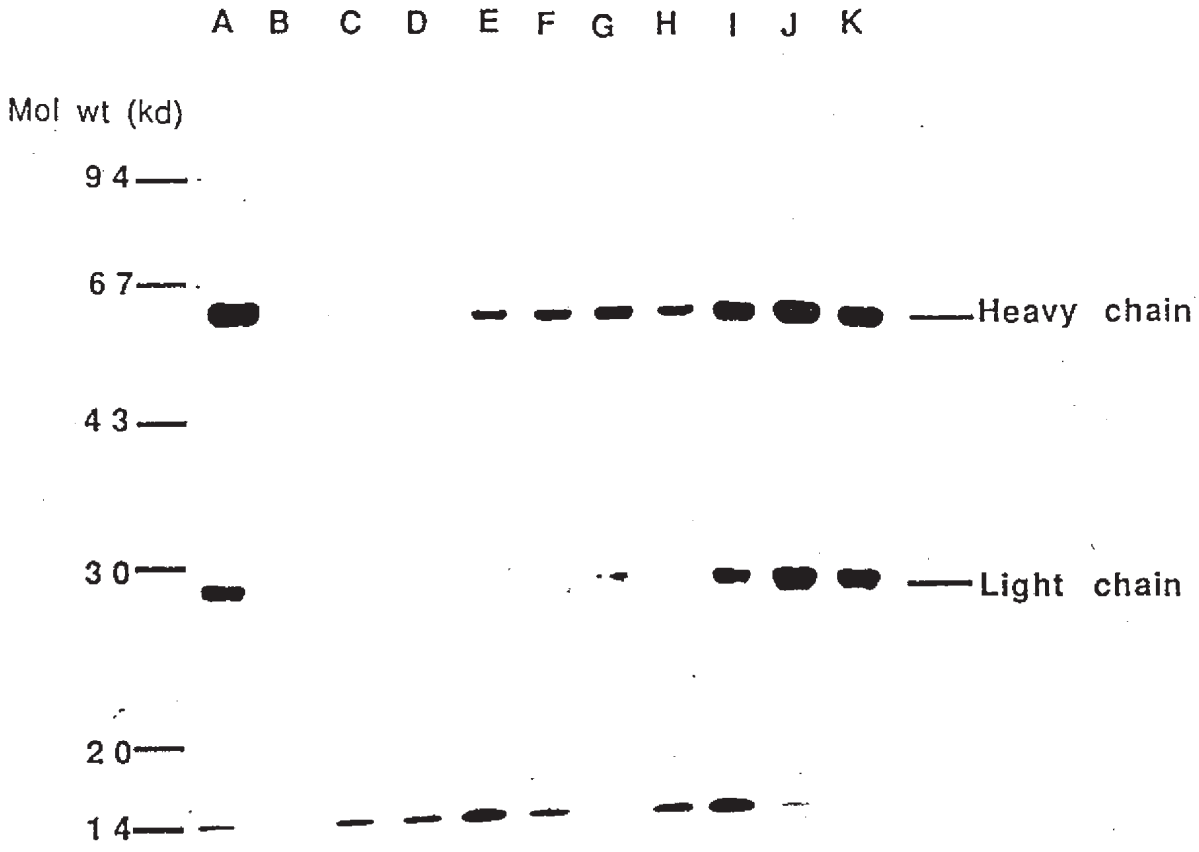

Fig 27. Expression of gH chain genes with cL chain

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

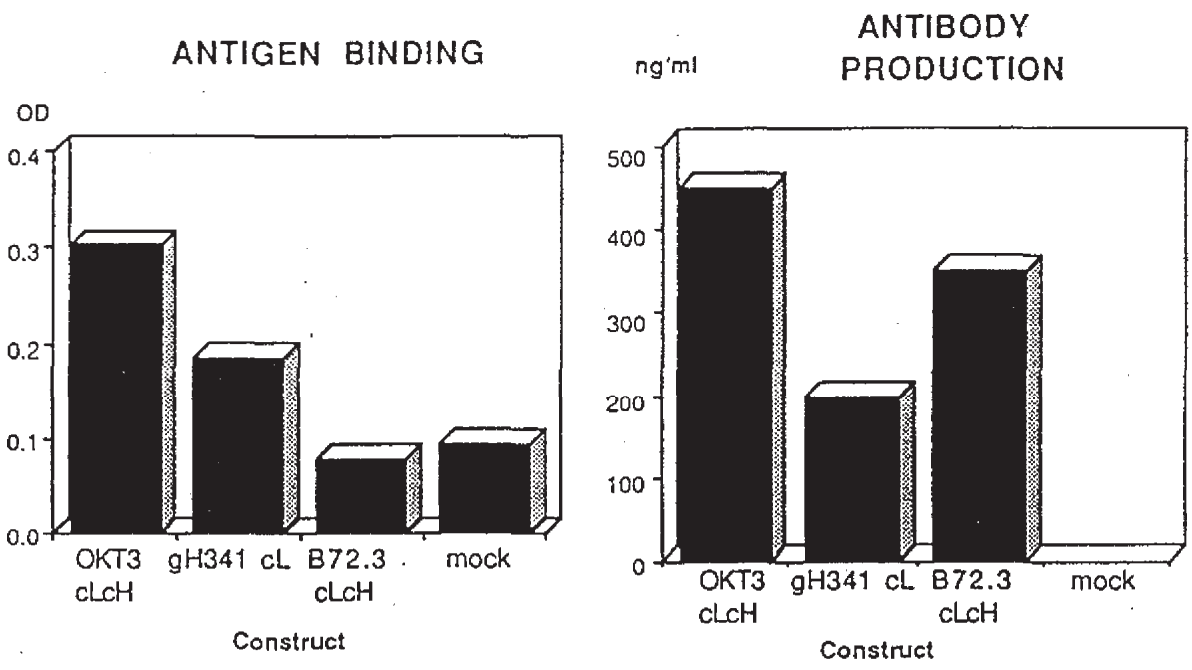

Fig 28 Antigen binding assay for gH341 cL gene combination.

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.

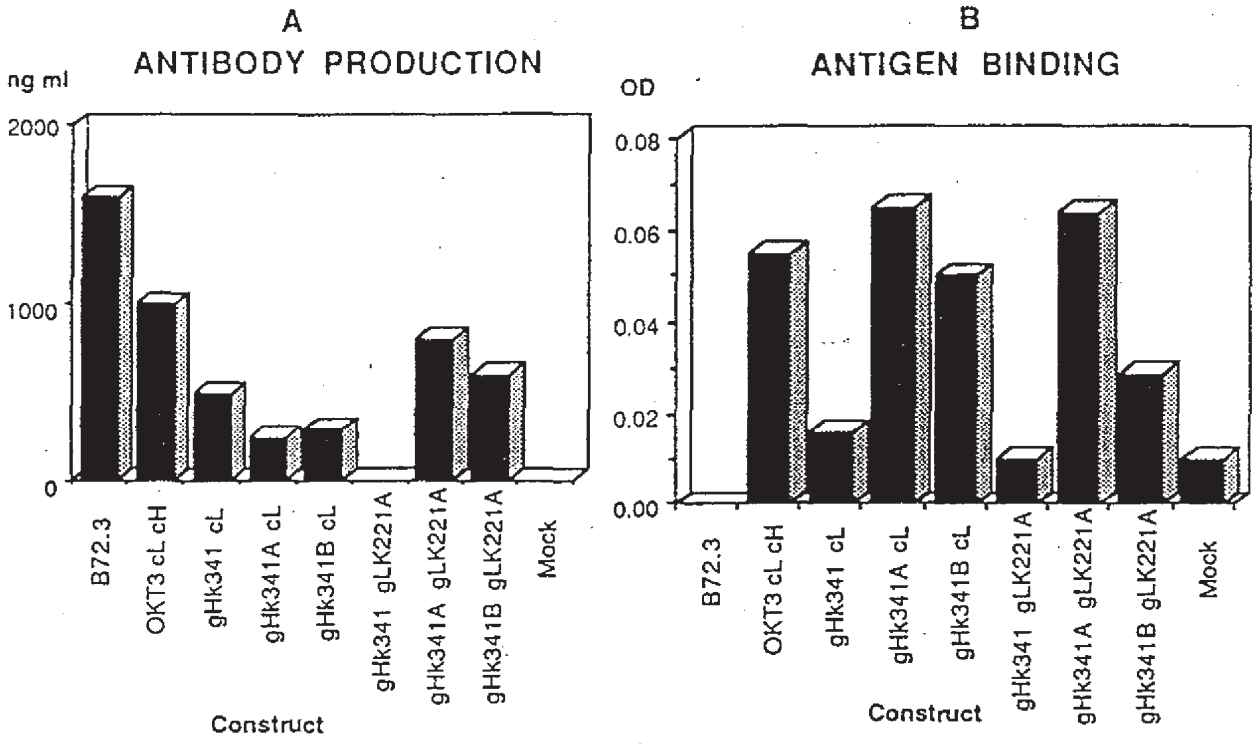

Fig 29 Antigen Binding assay for grafted OKT3 combinations

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.

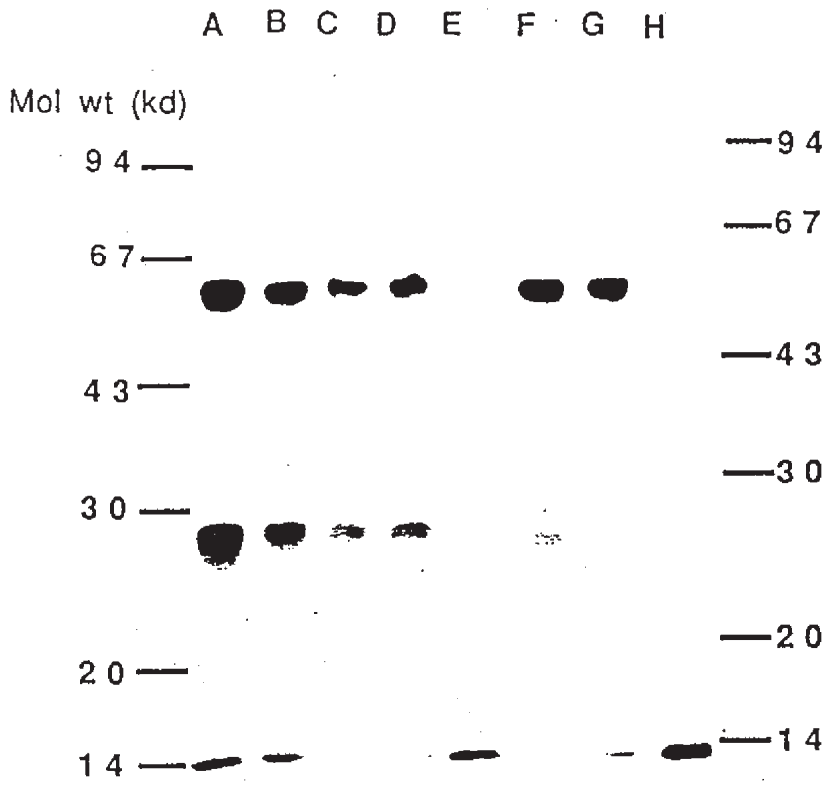

Fig 29c. gH341 series cotransfected with cL and gLK221A

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

HUMANISED ANTIBODIES

Field of the Invention

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, and 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~~typically comprises complementarity determining regions ~~grafted onto~~(CDRs) which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains. ~~The abbreviation "MAb" is used to indicate a monoclonal antibody~~ There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Background of the Invention

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, $(Fab')_2$ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, ~~have been~~were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of ~~techniques~~procedures for the ~~preparation~~production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. ~~Thus, in~~In practice,

MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud et al (2) and Jeffers et al (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotype component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable KAMA response and thus enlarge the areas of use of these very useful antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques ~~generally~~typically 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~~Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP~~-A-0 171 496~~0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), ~~EP-A-0 194 276~~and WO 86/01533 (Celltech Limited)~~ and WO-A-8 702 671 (Int. Gen. Eng. Inc.). The~~. This latter Celltech application (WO 86/01533) discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. ~~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~~Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered over a prolonged period [Begent et al (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides~~. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable regions~~. The present invention relates to ~~HAMs~~humanised antibody molecules prepared according to this alternative approach, i.e. CDR-grafted ~~HAMs~~humanised antibody molecules. Such
CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells~~ respectively~~ were humanised by CDR-grafting ~~are shown~~have been described by Verhoeyen et al (~~2~~5) and

Riechmann et al (~~3~~6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In ~~the latter case (~~Riechmann et al~~)~~/Medical Research Council it was found that transfer of the CDR regions alone ~~(~~[as defined by Kabat refs. ~~4~~(7) and ~~5~~(8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having ~~satisfactory~~improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to
CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Very recently Queen et al (9) have described the preparation of a humanised antibody that binds to the interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanized antibody.

In WO 90/07861 Queen et al propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that criteria two, three or four may be applied in addition or alternatively to criterion one, and may be applied singly or in any combination.

~~In recent years a number of rodent MAbs have been developed for therapeutic applications. For instance, OKT3 a mouse IgG2a/k MAb which recognizes 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.~~
WO 90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanized antibody having specificity for the p55 Tac protein of the IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody Eu (7) being used as acceptor. In the resultant humanized antibody the donor CDRs were as defined by Kabat et al (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanized anti-Tac antibody obtained is reported to have an affinity for p55 of 3×10^9 M^{-1}, about one-third of that of the murine MAb.

We have further ~~investgated~~investigated the preparation of CDR-grafted ~~HAMs~~humanised antibody molecules and have identified ~~residues~~a hierarchy of positions within the framework of the variable ~~region~~regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of ~~which~~the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

Summary of the Invention

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising ~~human~~acceptor framework and ~~non-human (rodent)~~donor antigen binding regions wherein the ~~human~~ framework comprises ~~non-human (rodent)~~donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues.

In particularly preferred embodiments the heavy chain framework additionally comprises donor residues at one, some or all of positions 6, 37, 48 and 94. Also it is particularly preferred that residues at positions of the heavy chain framework which are commonly conserved across species, i.e. positions 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the heavy chain framework additionally comprises donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

In addition the heavy chain framework optionally comprises donor residues at one, some or all of positions:
1 and 3,

72 and 76,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
80 and 20 (if 69 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91,
88, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

In the first and other aspects of the present invention reference is made to CDR-grafted antibody products comprising acceptor framework and donor antigen binding regions. It will be appreciated that the invention is widely applicable to the CDR-grafting of antibodies in general. Thus, the donor and acceptor antibodies may be derived from animals of the same species and even same antibody class or sub-class. More usually, however, the donor and acceptor antibodies are derived from animals of different species. Typically the donor antibody is a non-human antibody, such as a rodent MAb, and the acceptor antibody is a human antibody.

In the first and other aspects of the present invention, the donor antigen binding region typically comprises at least one CDR from the donor antibody. Usually the donor antigen binding region comprises at least two and preferably all three CDRs of each of the heavy chain and/or light chain variable regions. The CDRs may comprise the Kabat CDRs, the structural loop CDRs or a composite of the Kabat and structural loop CDRs and any combination of any of these.

Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

~~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 residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen et al (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising ~~human~~acceptor framework and ~~non-human (rodent)~~donor

antigen binding regions wherein the ~~human~~ framework comprises ~~non-human (rodent)~~donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Preferably the CDR grafted light chain of the second aspect comprises donor residues at positions 46 and/or 47.

The invention also provides in a third aspect a CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

In a preferred embodiment of the third aspect, the framework comprises donor residues at all of positions 46, 48, 58 and 71.

In particularly preferred embodiments of the second and third aspects, the framework additionally comprises donor residues at positions 36, 44, 47, 85 and 87. Similarly positions of the light chain framework which are commonly conserved across species, i.e. positions 2, 4, 6, 35, 49, 62, 64-69, 98, 99, 101 and 102, if not conserved between donor and acceptor, additionally comprise donor residues. Most preferably the light chain framework additionally comprises donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

In addition the framework of the second or third aspects optionally comprises donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form at potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and 21 (if 47 is different between donor and acceptor),
37 and 45 (if 47 is different between donor and acceptor),
and
any one or more of 10, 12, 40, 80, 103 and 105.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain 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~~89-~~96~~97).

The invention further provides in a fourth aspect a CDR-grafted ~~HAM~~antibody molecule comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second or first and third aspects of the invention.

The ~~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~~humanised antibody ~~heavy~~molecules 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~~a Fab ~~or~~, $(Fab')_2$ or FV fragment; a light chain or heavy chain monomer or dimer; or ~~any other~~a single chain antibody, e.g. a single chain FV in which heavy and light chain variable regions are joined by a peptide linker; or any other CDR-grafted molecule with the same specificity as the original ~~non-human (rodent)~~donor antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

~~Alternatively,~~Also the heavy or light chains or ~~HAM~~humanised antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, ~~they~~it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

~~For CDR-grafted products of the invention,~~Any appropriate acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of ~~human~~acceptor framework used is of the same/similar class/type as the donor antibody. ~~Advantageously~~Conveniently, the framework ~~is~~may be 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~~However, a high level of homology between donor and acceptor sequences is not important for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10^5 M^{-1}, preferably at least about 10^8 M^{-1}, or especially in the range 10^8-10^{12} M^{-1}. In principle, the present invention is applicable to any combination of donor and acceptor antibodies irrespective of the level of homology between their sequences. A protocol for applying the invention to any particular donor-acceptor antibody pair is given hereinafter. Examples of human frameworks which may be used are KOL, NEWM, RE1, EU, LAY and POM (refs. 4 and 5) and the like; for instance KOL and NEWM for the heavy chain and RE1 for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also ~~human~~the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM ~~domain~~domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the ~~HAM~~humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of lymphokine activity.

However, the remainder of the ~~HAM~~antibody molecules need not comprise only protein sequences from ~~the human immunoglobulin~~immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably the CDR-grafted antibody heavy and light chain and antibody molecule products are produced by recombinant DNA technology.

Thus in further aspects the invention also includes DNA sequences coding for the CDR-grafted heavy and light chains, cloning and expression vectors containing the DNA sequences, host cells transformed with the DNA sequences and processes for producing the CDR-grafted chains and antibody molecules comprising expressing the DNA sequences in the transformed host cells.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 10 and 11.

The DNA sequences which encode the donor amino acid sequence may be obtained by methods well known in the art. For example the donor coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, RE1, EU and NEWM, are widely available to workers in the art.

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the CDR-grafted products. Desired DNA sequences may be synthesized completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 20) may be used. Also oligonucleotide directed mutagenesis of a pre-exising variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped oligonucleotides using T_4 DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the CDR-grafted heavy and light chains. Bacterial e.g. E. coli, and other microbial systems may be used, in particular for expression of antibody fragments such as FAb and (Fab')2 fragments, and especially FV fragments and single chain antibody fragments e.g. single chain FVs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

Thus, ~~according to~~in a further aspect the present invention provides a process for producing ~~an anti-CD3 HAM which process comprises~~a CDR-grafted antibody product comprising:

(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; and/or

(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~~third aspect of the invention;

(c) transfecting a host cell with the or each vector; and

(d) culturing the transfected cell line to produce the ~~HAM~~CDR-grafted antibody product.

The CDR-grafted product may comprise only heavy or light chain derived polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence is used to transfect the host cells.

For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, the first vector ~~containing~~may contain an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical, except in so far as the coding sequences and selectable markers are concerned, so as to ensure as far as possible that each polypeptide chain is equally expressed. Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both. However, it is preferred that the DNA sequence encoding the heavy or light chain comprises at least partially, genomic DNA, preferably a fusion of cDNA and genomic DNA.

The present invention ~~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.~~is applicable to antibodies of any appropriate specificity. Advantageously, however, the invention may be applied to the humanisation of non-human antibodies which are used for in vivo therapy or diagnosis. Thus the antibodies may be site-specific antibodies such as tumour-specific or cell surface-specific antibodies, suitable for use in in vivo therapy or diagnosis, e.g. tumour imaging. Examples of cell surface-specific antibodies are anti-T cell antibodies, such as anti-CD3, and CD4 and adhesion molecules, such as CR3, ICAM and ELAM. The antibodies may have specificity for interleukins (including lymphokines, growth factors and stimulating factors), hormones and other biologically active compounds, and receptors for any of these. For example, the antibodies may have specificity for any of the following: Interferons α, β, γ, or δ, IL1, IL2, IL3, or IL4, etc., TNF, GCSF, GMCSF, EPO, hGH, or insulin, etc.

The the present invention also includes therapeutic and diagnostic compositions comprising the CDR-grafted products of the invention and uses of such compositions in therapy and diagnosis.

Accordingly in a further aspect the invention provides a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

Accordingly also the invention provides a method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted antibody heavy or light chain or molecule according to previous aspects of the invention to a human or animal subject.

A preferred protocol for obtaining CDR-grafted antibody heavy and light chains in accordance with the present invention is set out below together with the rationale by which we have derived this protocol. This protocol and rationale are given without prejudice to the generality of the invention as hereinbefore described and defined.

Protocol

It is first of all necessary to sequence the DNA coding for the heavy and light chain variable regions of the donor antibody, to determine their amino acid sequences. It is also necessary to choose appropriate acceptor heavy and light chain variable regions, of known amino acid sequence. The CDR-grafted chain is then designed starting from the basis of the acceptor sequence. It will be appreciated that in some cases the donor and acceptor amino acid residues may be identical at a particular position and thus no change of acceptor framework residue is required.

1. As a first step donor residues acceptor residues in the CDRs. For this purpose the CDRs are preferably defined as follows:

Heavy chain	- CDR1:	residues 26-35
	- CDR2:	residues 50-65
	- CDR3:	residues 95-102
Light chain	- CDR1:	residues 24-34
	- CDR2:	residues 50-56
	- CDR3:	residues 89-97

The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows, first of all with respect to the heavy chain and subsequently with respect to the light chain.

2. Heavy Chain

2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor).

2.2 Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

2.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 72, 76
iii. If 48 is different between donor and acceptor sequences, consider 69
iv. If at 48 the donor residue is chosen, consider 38 and 46
v. If at 69 the donor residue is chosen, consider 80 and then 20
vi. 67
vii. If at 67 the donor residue is chosen, consider 82 and then 18
viii. 91
ix. 88
x. 9, 11, 41, 87, 108, 110, 112

3. Light Chain

3.1 Choose donor at 46, 48, 58 and 71

3.2 Check that the following have the same amino acid in donor and acceptor sequences, if not preferably choose donor:

2, 4, 6, 35, 38, 44, 47, 49, 62, 64-69 inclusive, 85, 87, 98, 99, 101 and 102

3.3 To further optimise affinity consider choosing donor residues at one, some or any of:

i. 1, 3
ii. 63
iii. 60, if 60 and 54 are able to form potential saltbridge
iv. 70, if 70 and 24 are able to form potential saltbridge
v. 73, and 21 if 47 is different between donor and acceptor
vi. 37, and 45 if 47 is different between donor and acceptor
vii. 10, 12, 40, 80, 103, 105

Rationale

In order to transfer the binding site of an antibody into a different acceptor framework, a number of factors need to be considered.

1. The extent of the CDRs
The CDRs (Complementary Determining Regions) were defined by Wu and Kabat (refs. 4 and 5) on the basis of an analysis of the variability of different regions of antibody variable regions. Three regions per domain were recognised. In the light chain the sequences are 24-34, 50-56, 89-97 (numbering according to Kabat (ref. 4), Eu Index) inclusive and in the heavy chain the sequences are 31-35, 50-65 and 95-102 inclusive.

When antibody structures became available it became apparent that these CDR regions corresponded in the main to loop regions which extended from the β barrel framework of the light and heavy variable domains. For H1 there was a discrepancy in that the loop was from 26 to 32 inclusive and for H2 the loop was 52 to 56 and for L2 from 50 to 53. However, with the exception of H1 the CDR regions encompassed the loop regions and extended into the β strand frameworks. In H1 residue 26 tends to be a serine and 27 a phenylalanine or tyrosine, residue 29 is a phenylalanine in most cases. Residues 28 and 30 which are surface residues exposed to solvent might be involved in antigen-binding. A prudent definition of the H1 CDR therefore would include residues 26-35 to include both the loop region and the hypervariable residues 33-35.

It is of interest to note the example of Riechmann et al (ref. 3), who used the residue 31-35 choice for CDR-H1. In order to produce efficient antigen binding, residue 27 also needed to be recruited from the donor (rat) antibody.

2. Non-CDR residues which contribute to antigen binding

By examination of available X-ray structures we have identified a number of residues which may have an effect on net antigen binding and which can be demonstrated by experiment. These residues can be sub-divided into a number of groups.

2.1 Surface residues near CDR [all numbering as in Kabat et al (ref. 7)].

2.1.1. Heavy Chain - Key residues are 23, 71 and 73. Other residues which may contribute to a lesser extent are 1, 3 and 76. Finally 25 is usually conserved but the murine residue should be used if there is a difference.

2.1.2 Light Chain - Many residues close to the CDRs, e.g. 63, 65, 67 and 69 are conserved. If conserved none of the surface residues in the light chain are likely to have a major effect. However, if the murine residue at these positions is unusual, then it would be of benefit to analyse the likely contribution more closely. Other residues which may also contribute to binding are 1 and 3, and also 60 and 70 if the residues at these positions and at 54 and 24 respectively are potentially able to form a salt bridge i.e. 60 + 54; 70 + 24.

2.2 Packing residues near the CDRs.

2.2.1 Heavy Chain - Key residues are 24, 49 and 78. Other key residues would be 36 if not a tryptophan, 94 if not an arginine, 104 and 106 if not glycines and 107 if not a threonine. Residues which may make a further contribution to stable packing of the heavy chain and hence improved affinity are 2, 4, 6, 38, 46, 67 and 69. 67 packs against the COR residue 63 and this pair could be either both mouse or both human. Finally, residues which contribute to packing in this region but from a longer range are 18, 20, 80, 82 and 86. 82 packs against 67 and in turn 18 packs against 82. 80 packs against 69 and in turn 20 packs against 80. 86 forms an H bond network with 38 and 46. Many of the mouse-human differences appear minor e.g. Leu-Ile, but could have an minor impact on correct packing which could translate into altered positioning of the CDRs.

2.2.2 Light Chain - Key residues are 48, 58 and 71. Other key residues would be 6 if not glutamine, 35 if not tryptophan, 62 if not phenylalanine or tryosine, 64, 66, 68, 99 and 101 if not glycines and 102 if not a threonine. Residues which make a further contribution are 2, 4, 37, 45 and- 47. Finally residues 73 and 21 and 19 may make long distance packing contributions of a minor nature.

2.3 Residues at the variable domain interface between heavy and light chains - In both the light and heavy chains most of the non-CDR interface residues are conserved. If a conserved residue is replaced by a residue of different character, e.g. size or charge, it should be considered for retention as the murine residue.

2.3.1 Heavy Chain - Residues which need to be considered are 37 if the residue is not a valine but is of larger side chain volume or has a charge or polarity. Other residues are 39 if not a glutamine, 45 if not a leucine, 47 if not a tryptophan, 91 if not a phenylalanine or tyrosine, 93 if not an alanine and 103 if not a tryptophan. Residue 89 is also at the interface but is not in a position where the side chain could be of great impact.

2.3.2 Light Chain - Residues which need to be considered are 36, if not a tyrosine, 38 if not a glutamine, 44 if not a proline, 46, 49 if not a tyrosine, residue 85, residue 87 if not a tyrosine and 98 if not a phenylalanine.

2.4 Variable-Constant region interface - The elbow angle between variable and constant regions may be affected by alterations in packing of key residues in the variable region against the constant region which may affect the position of V_L and V_H with respect to one another. Therefore it is worth noting the residues likely to be in contact with the constant region. In the heavy chain the surface residues potentially in contact with the variable region are conserved between mouse and human antibodies therefore the variable region contact residues may influence the V-C interaction. In the light chain the amino acids found at a number of the constant region contact points vary, and the V & C regions are not in such close proximity as the heavy chain. Therefore the influences of the light chain V-C interface may be minor.

2.4.1 Heavy Chain - Contact residues are 7, 11, 41, 87, 108, 110, 112.

2.4.2 Light Chain - In the light chain potentially contacting residues are 10, 12, 40, 80, 83, 103 and 105.

~~The 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 above analysis coupled with our considerable practical experimental experience in the CDR-grafting of a number of different antibodies have lead us to the protocol given above.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - ~~29.~~13.

Brief Description of the Figures

Figure 1 shows DNA and amino acid sequences of the OKT3 light chain;
Figure 2 shows DNA and amino acid sequences of the OKT3 heavy chain;
Figure 3 shows the alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody RE1;
Figure 4 shows the alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL;
Figure 5 shows the heavy variable region amino acid sequences of OKT3, KOL and various corresponding CDR grafts;
Figure 6 shows the light variable region amino acid sequences of OKT3, RE1 and various corresponding CDR grafts;
Figure 7 shows a graph of binding assay results for various grafted OKT3 antibodies
Figure 8 shows a graph of blocking assay results for various grafted OKT3 antibodies;
Figure 9 shows a similar graph of blocking assay results;
Figure 10 shows similar graphs for both binding assay and blocking assay results;
Figure 11 shows further similar graphs for both binding assay and blocking assay results;
Figure 12 shows a graph of competition assay results for a minimally grafted OKT3 antibody compared with the OKT3 murine reference standard, and
Figure 13 shows a similar graph of competition assay results comparing a fully grafted OKT3 antibody with the murine reference standard.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

EXAMPLE 1

CDR-GRAFTING OF OKT3

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot ~~4882-1~~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~~assayed to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as described in Maniatis et al~~.~~ (ref. ~~6~~9) with, in some cases~~,~~ minor modifications. DNA sequencing was performed as described in Sanger et al~~.~~ (ref. ~~7~~11) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al~~.~~ (ref. ~~8~~12) and the Anglian Biotechnology Ltd~~.~~ handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al~~.~~ (ref. ~~9~~13)

3. RESEARCH ASSAYS

~~3.1~~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~~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~~3.1.2. COS AND CHO CELLS TRANSFECTED WITH ~~CHIMAERIC~~CHIMERIC OR CDR -GRAFTED OKT3 GENES

The assembly assay for ~~intact humanised OKT3~~chimeric or CDR-grafted antibody in COS cell supernatants was an ELISA with the following format:-

96 well microtitre plates were coated with F(ab')2 goat anti-human IgG Fc. The plates were washed and samples added and incubated for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')2 goat anti -mouse IgG Fc (HRPO conjugated) was added. ~~Substrate~~Enzyme substrate was added to reveal the reaction.~~Chimaeric~~ Chimeric B72.3 (IgG4) (ref. 13) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the ~~chimaeric~~chimeric standard.

~~3,2~~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 chimeric B72.3. The positive control was mouse Orthomune OKT3 or

chimeric OKT3, when available. This cell-based assay was difficult to perform, and an alternative assay was developed for CDR-grafted OKT3 which was more sensitive and easier to carry out.

In this system CDR-grafted OKT3 produced by COS cells was tested for its ability to bind to the CD3-positive HPB-ALL (human peripheral blood acute lymphocytic leukemia) cell line. It was also tested for its ability to block the binding of murine OKT3 to these cells. Binding was measured by the following procedure: HPB-ALL cells were harvested from tissue culture. Cells were incubated at 4°C for 1 hour with various dilutions of test antibody, positive control antibody; or negative control antibody. The cells were washed once and incubated at 4°C for 1 hour with an FITC-labelled goat anti-human IgG (Fc-specific, mouse absorbed). The cells were washed twice and analysed by cytofluorography. Chimeric OKT3 was used as a positive control for direct binding. Cells incubated with mock- transfected COS cell supernatant, followed by the FITC-labelled goat anti-human IgG, provided the negative control. To test the ability of CDR-grafted OKT3 to block murine OKT3 binding, the HPB-ALL cells were incubated at 4°C for 1 hour with various dilutions of test antibody or control antibody. A fixed saturating amount of FITC OKT3 was added. The samples were incubated for 1 hour at 4°C, washed twice and analysed by cytofluorography. FITC-labelled OKT3 was used as a positive control to determine maximum binding. Unlabelled murine OKT3 served as a reference standard for blocking. Negative controls were unstained cells with or without mock-transfected cell supernatant. The ability of the CDR-grafted OKT3 light chain to bind CD3-positive cells and block the binding of murine OKT3 was initially tested in combination with the chimeric OKT3 heavy chain. The chimeric OKT3 heavy chain is composed of the murine OKT3 variable region and the human IgG4 constant region. The chimeric heavy chain gene is expressed in the same expression vector used for the CDR-grafted genes. The CDR-grafted light chain expression vector and the chimeric heavy chain expression vector were co-transfected into COS cells. The fully chimeric OKT3 antibody (chimeric light chain and chimeric heavy chain) was found to be fully capable of binding to CD3 positive cells and blocking the binding of murine OKT3 to these cells.

3.3 DETERMINATION OF RELATIVE BINDING AFFINITY

The relative binding affinities of CDR-grafted anti-CD3 monoclonal antibodies were determined by competition binding (ref. 6) using the HPB-ALL human T cell line as a source of CD3 antigen, and fluorescein-conjugated murine OKT3 (FI-OKT3) of known binding affinity as a tracer antibody. The binding affinity of FI-OKT3 tracer antibody was determined by a direct binding assay in which increasing amounts of FI-OKT3 were incubated with HPB-ALL ($5x10^5$) in PBS with 5% foetal calf serum for 60 min. at 4°C. Cells were washed, and the fluorescence intensity was determined on a FACScan flow cytometer calibrated with quantitative microbead standards (Flow Cytometry Standards, Research Triangle Park, NC). Fluorescence intensity per antibody molecule (F/P ratio) was determined by using microbeads which have a predetermined number of mouse IgG antibody binding sites (Simply Cellular beads, Flow Cytometry Standards). F/P equals the fluorescence intensity of beads saturated with FI-OKT3 divided by the number of binding sites per bead. The amount of bound and free FI-OKT3 was calculated from the mean fluorescence intensity per cell, and the ratio of bound/free was plotted against the number of moles of antibody bound. A linear fit was used to determine the affinity of binding (absolute value of the slope).

~~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.~~For competitive binding, increasing amounts of competitor antibody were added to a sub-saturating dose of FI-OKT3 and incubated with $5xl0^5$ HPB-ALL in 200 ml of PBS with 5% foetal calf serum, for 60 min at 4^oC. The fluorescence intensities of the cells were measured on a FACScan flow cytometer calibrated with quantitative microbead standards. The concentrations of bound and free FI-OKT3 were calculated. The affinities of competing antibodies were calculated from the equation [X] - [OKT3] = (l/Kx) - (l/Ka), where Ka is the affinity of murine OKT3, Kx is the affinity of competitor X, [ ] is the concentration of competitor antibody at which bound/free binding is R/2, and R is the maximum bound/free binding.

4. cDNA LIBRARY ~~CONSTRUTION~~CONSTRUCTION

~~4.1~~4.1. mRNA PREPARATION AND cDNA SYNTHESIS

OKT3 producing cells were grown as described above and 1.2×10^9 cells harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

~~4.2~~4.2. LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5' phosphate groups removed by calf intestinal phosphatase (EcoR1/CIP). The ligation was used to transform high transformation efficiency Escherichia coli (E.coli) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides:
5' TCCAGATGTTAACTGCTCAC 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 cDNAs [Figures l(a) and 2(a)] were obtained ~~(Figs 1 and 2).~~and the

corresponding amino acid sequences predicted [(Figures l(b) and 2(b)]. In Figure l(a) the untranslated DNA regions are shown in uppercase, and in both Figures 1 and 2 the signal sequences are underlined.

~~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 l4.6b.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. l0) 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.~~7. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV ~~(Fig. 5)~~ (ref. ~~12~~14) . A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as ~~BamHI~~BamH1 cassettes in the unique ~~BamHI~~BamH1 site of pEE6 hCMV; for instance, the neo marker to provide pEE6 hCMV neo. It is usual practice to insert the neo and gpt markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal ~~EcoRI~~EcoR1 sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.

The mouse sequences were excised ~~as EcoRI~~from the M13 based vectors described above as EcoR1 fragments and cloned into either ~~EE~~pEE6-hCMV-neo for the heavy chain ~~(Fig 6)~~ and into EE6-hCMV-gpt for the light chain ~~(Fig. 7)~~to yield vectors pJA136 and pJA135 respectively.

~~9.~~8. EXPRESSION OF ~~cDNA'S~~cDNAS 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.~~9. CONSTRUCTION OF ~~CHIMAERIC~~CHIMERIC GENES

Construction of ~~chimaeric~~chimeric genes followed a previously described strategy ~~(~~[Whittle et al (ref. ~~9~~ 13)~~)~~]. A restriction site near the 3' end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

~~10.1~~9.1. LIGHT CHAIN GENE CONSTRUCTION ~~- VERSION 1~~

The mouse light chain cDNA sequence ~~showed~~contains an Aval site near the 3' end of the variable region ~~(~~[Fig ~~8.~~ l(a)]. The majority of the sequence of the variable region was isolated as a ~~376~~396 bp. ~~EcoRI~~EcoR1-Aval fragment. An oligonucleotide adapter was designed to replace the remainder of the 3' region of the variable region from the Aval site and to include the 5' residues of the human constant region up to and including a unique Narl site which had been previously engineered into the constant region.

~~TOP STRAND 5TCGGGGACAAAGCTTGAAATAAACAGAACTGTGGCGG 3'~~
~~BOTTOM STRAND 3' CCTGTTTCGAACTTTATTTGTCTTGACACCGCCGC 5'~~

A Hind ~~III site, shown in bold type within the oligonucleotide sequence above,~~111 site was introduced to act as a marker for insertion of the linker.

The linker was ligated to the V_L fragment and the 413 bp ~~EcoRI~~EcoR1-Narl adapted fragment was purified from the ligation mixture.

The constant region was isolated as an Narl-~~BamHI~~BamH1 fragment from an M13 clone NW361 and was ligated with the variable region DNA into an ~~EcoRI/BamHI/ClP~~EcoR1/BamH1/C1P pSP65 treated vector in a three way reaction ~~-~~ to yield plasmid JA143. Clones were isolated after transformation into E.coli and the linker and junction sequences were confirmed by the presence of the ~~HinDIII~~Hind111 site and by DNA sequencing ~~(Fig 9)~~.

~~10.2.~~9.2 LIGHT CHAIN GENE CONSTRUCTION - VERSION 2

The construction of the first ~~chimaeric~~chimeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable-constant region junction. In the case of the OKT3 light chain the amino acids at the ~~chimaera~~chimera junction are:

……. Leu-Glu-~~Ile~~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~~chimeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).
~~TOP STRAND 5' TCGGGGACAMGTIGGAMTAMCAG IIGCTGTGGCGG 3'~~
~~BOTIOM STRAND 3' CCTGTTTCAACCTTTATTTGTCTCGACACCGCCGC 5'~~

~~The~~An internal ~~HinDIII~~Hind111 site ~~present in the version 1 adapter~~ was not included in this adapter, to differentiate the two ~~chimaeric~~chimeric light chain genes.

The variable region fragment was isolated as a 376 bp~~. EcoRI~~ EcoR1-AvaI fragment. The oligonucleotide linker was ligated to NarI cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with ~~EcoRI.~~EcoR1. The variable region fragment and the modified constant region fragment were ligated directly into ~~EcoRI/CIP~~EcoR1/C1P treated pEE6hCMVneo~~.~~ to yield pJA137. Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round~~.~~ and yield plasmid pJA141. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing ~~(Fig10).~~

~~10.3~~9.3. HEADY CHAIN GENE CONSTRUCTION
~~10.3.1~~9.3.1. CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

~~10.3.2~~9.3.2. GENE CONSTRUCTION
The heavy chain cDNA sequence showed a ~~BanI~~Ban1 site near the 3' end of the variable region ~~(~~[Fig ~~11~~. 2(a)). The majority of the sequence of the variable region was isolated as a 426bp. ~~EcoRI/CIP/BanI~~EcoR1/C1P/Ban1 fragment. An oligonucleotide adapter was ~~designed~~designated to replace the remainder of the 3' region of the variable region from the ~~BanI~~Ban1 site up to and including a unique ~~HinDIII~~HindIII site which had been previously engineered into the first two amino acids of the constant region.
~~TOP STRAND 5' GCACCACTCTCACCGTGtlGCTC3'~~
~~BOTTOM STRAND 3'GTGAGAGTGGCACTCGAGTCGA 5'~~

The linker was ligated to the V_H fragment and the ~~EcoRI HinDIII~~EcoR1-Hind111 adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting ~~mJA~~pJA91 with ~~EcoRI~~EcoR1 and Hind~~III~~111 removing the intron fragment and replacing it with the V_H ~~(Fig 12).~~to yield pJA142. Clones were isolated after transformation into E.coli JM101 and the linker and junction sequences were confirmed by DNA sequencing. (~~NB~~N. B. The ~~HindIII~~Hind111 site is lost on cloning).

~~11.~~10. CONSTRUCTION OF ~~CHIMAERIC~~CHIMERIC EXPRESSION VECTORS
~~11.1~~10.1. neo AND gpt VECTORS

The ~~chimaeric~~chimeric light chain (version 1) was removed from pJA143 ~~(Fig 9)~~ as an ~~EcoRI~~EcoR1 fragment and cloned into ~~EcoRI/CIP~~EcoR1/C1P treated pEE6hCMVneo expression vector~~.~~ to yield pJA145. Clones with the insert in the correct orientation were identified by restriction mapping ~~(Fig 13)~~.

The ~~chimaeric~~chimeric light chain (version 2) was constructed as described above ~~(see Fig 10)~~.

The ~~chimaeric~~chimeric heavy chain gene was isolated from pJA142 as a 2.5Kbp ~~EcoRI/BamHI~~EcoR1/BamH1 fragment and cloned into the ~~EcoRI/BclI/CIP~~EcoR1/Bc11/C1P treated vector fragment of ~~pJA97,~~ a derivative of pEE6hCMVgpt ~~(Fig 14).~~to yield plasmid pJA144.

~~11.2~~10.2. GS SEPARATE VECTORS

GS versions of pJA141 ~~(Fig 10)~~ and pJA144 ~~(Fig 14)~~ were constructed by replacing the neo and gpt cassettes by ~~BamHI/SaII/CIP~~a BamH1/Sa11/C1P treatment of the plasmids, isolation of the vector fragment and ligation to a
GS-containing fragment from the plasmid pRO49 ~~(Figs 15 and 16)~~to yield the light chain vector pJA179 and the heavy chain vector pJA180.

~~11.3~~ 10.3. GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL (chimeric light), cH (chimeric heavy) and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS and with transcription of the genes being head to tail ~~ege~~. g. cL>cH>GS were constructed. These plasmids were made by treating pJA179 ~~(Fig 15)~~ or pJA180 ~~(Fig 16)~~ with ~~BamHI/CIP~~BamH1/C1P and ligating in a ~~BgIII/HinDIII~~Bg111/Hind111 hCMV promoter cassette ~~from pJA146~~ along with either the ~~HinDIII/BamHI~~Hind111/BamH1 fragment from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 ~~(Fig17),~~ or the ~~HinDIII/BamHI~~Hind111/BamH1 fragment from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA~~181 (Fig 18).~~181.

~~12.~~11. EXPRESSION OF ~~CHIMAERIC~~CHIMERIC GENES

~~12.1~~11.1. EXPRESSION IN COS CELLS

The ~~chimaeric~~chimeric antibody ~~plasmids~~plasmid pJA145 (cL) and pJA144 (cH) were ~~cotransfected~~co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels ~~(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~~chimeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin ~~(Fig 19)~~. This second version of the ~~chimaeric~~chimeric light chain, when expressed in association with

~~chimaeric~~chimeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

~~12.2~~11.2. EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines ~~are being~~have been prepared from plasmids ~~pJA~~PJA141/pJA144 and from pJA179/pJA180, pJA~~181,~~181 and pJA182 by transfection into CHO cells.

~~13.~~12. CDR ~~-~~GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and ~~chimaeric~~chimeric antibodies.

~~13.1~~12.1. VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

~~A.~~(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.~~ (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.~~ (c) Residues not identified by ~~A~~(a) and ~~B above~~(b) may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

~~13.1.1~~12.1.1. LIGHT CHAIN

Figure ~~20~~3 shows an alignment of sequences for the human framework region ~~REI~~RE1 and the OKT3 light variable region. The structural loops (LOOP) and CDRs (KABAT) believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1~~C. REI~~(c).

Above the sequence in Figure 3 the residue type indicates the spatial location of each residue side chain, derived by examination of resolved structures from X-ray crystallography analysis. The key to this residue type designation is as follows:

N - near to CDR (From X-ray Structures)

P - Packing B - Buried Non-Packing

S - Surface E - Exposed

I - Interface * - Interface

- Packing/Part Exposed

? - Non-CDR Residues which may require to be left as Mouse sequence.

Residues underlined in Figure 3 are amino acids. RE1 was chosen as the human framework because the light chain is a ~~Kappa~~kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region ~~eg~~, e.g. KOL (see below). ~~REI~~RE1 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~~12.1.2. HEAVY CHAIN

Similarly Figure ~~21~~4 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.~~12.1(c). The residue type key and other indicators used in Figure 4 are the same as those used in Figure 3. 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~~12.2. DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage ~~([~~[Grantham and Perrin~~,~~ (ref.~~13~~ 15)~~)~~] and used the B72.3 signal sequences ~~([~~[Whittle et al~~.~~ (ref. ~~9~~13)~~)~~]. The sequences were designed to be attached to the constant region in the same way as for the ~~chimaeric~~chimeric genes described above. Some constructs contained the "Kozak consensus sequence" ~~([~~[Kozak~~,~~ (ref. ~~14~~16)~~)~~] directly linked to the 5' of the signal sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

~~13.3~~ 12.3. GENE CONSTRUCTION

To build the variable regions ~~two~~, various strategies are available. ~~Either to assemble the~~The sequence may be assembled by using oligonucleotides in a manner similar to Jones et al~~.~~ (ref. ~~15~~17) or ~~to~~by simultaneously ~~replace~~replacing all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al~~.~~ (ref. 2) . Both strategies were used and a list of constructions is set out in ~~Table 1.~~Tables 1 and 2 and Figures 4 and 5. 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~~13. CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the ~~chimaeric~~chimeric 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 experiment.~~

TABLE 1 CDR GRAFTED GENE CONSTRUCTIONS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE +
LIGHT CHAIN	ALL HUMAN FRAMEWORK ~~REI~~RE1		
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+ n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d. +
121B	26-32, 50-56, 91-96 inclusive +46, 47	Partial gene assembly	n.d. +
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+ +
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+ +
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+ +
221C	24-34, 50-56, 91-96 inclusive +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.

~~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.~~

14. EXPRESSION OF CDR-GRAFTED GENES

~~15.1~~14.1. PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR ~~CHIMAERIC~~CHIMERIC HEAVY (cH) CHAINS~~.~~

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression ~~(Fig 24a and b)~~. Over an extended series of experiments expression levels were raised from ~~approx~~approximately 200ng/~~mL~~ml to ~~approx.~~approximately 500 ng/~~mL~~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~~12.1 antigen binding ~~can be~~was demonstrated

when both of the new constructs, which were termed 121A and 221A~~, are coexpressed~~ were co-expressed with cH ~~(Fig 25A and B)~~. When the effects of these residues ~~are~~were 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~~ 14.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR ~~CHIMAERIC~~CHIMERIC LIGHT (cL) CHAINS~~.~~

Expression of the gH genes ~~has proven~~proved to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence ~~appears~~appeared to have ~~had~~ no marked effect on expression of gH genes ~~(Fig. 26)~~. Expression ~~may~~appears to be slightly improved but not to the same degree as seen for the grafted light chain.

~~Second~~Also, it ~~has proven~~proved difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used ~~eg~~, e.g. 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~~Moreover, co-expression 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~~were expressed in association with cL, antibody ~~is~~was produced but antibody binding activity ~~has~~was not ~~been~~ detected ~~(Table 2)~~. When the more conservative gH341 gene ~~is~~was used antigen binding ~~can~~could be detected in association with cL or mL, but the activity ~~is~~was only marginally above the background level ~~(Fig 28)~~.

When further mouse residues ~~are~~were substituted based on the arguments in ~~13.1~~12.1, antigen binding ~~can~~could be clearly demonstrated for the antibody produced when kgH341A and kgH341B ~~are~~were expressed in association with cL ~~(Fig 29)~~.

~~15.3~~14.3 PRODUCTION OF FULLY CDR ~~-~~GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A~~,~~ or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression ~~experiment (Fig 29A aned C)~~. For the 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~~chimeric antibody ~~(Fig 29B)~~.

~~DISCUSSION~~

~~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 chimaerlc material was equivalent in binding to the mouse OKT3 (see Fig 19). These observations have been confirmed by Ortho staff (L Jolliffe pers. comm.).~~
An analysis of the above results is given below.

~~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).~~

15. DISCUSSION OF CDR-GRAFTING RESULTS

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

15.1. LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs

For the light chain the regions defining the loops known from structural studies of other antibodies to contain the antigen contacting residues, and those hypervariable sequences defined by Kabat et al (refs. ~~as Cornplementarity~~4 and 5) as Complementarity Determining Regions (CDRs) are equivalent for CDR2. 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~~RE1 has glutamine. For CDR3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from residues 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and ~~REI~~RE1 (Fig~~-20~~. 3). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and ~~coexpressed~~co-expressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

15.1.2. FRAMEWORK RESIDUES

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 ~~gl~~gL221A (gL221 + D1Q, Q3V, L46R, L47W, see ~~Fig 20~~Figure 3 and Table 1) was made, cloned in EE6hCMVneo and ~~coexpressed~~co-expressed 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~~co-expressed with cH, only the gL221C/cH combination showed good antigen binding ~~(Fig 25)~~. When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and ~~coexpressed~~co-expressed with cH, antibody was produced which also bound to antigen ~~(Fig 25)~~.

15.2. HEAVY CHAIN

15.2.1. EXTENT OF THE CDRs

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 ~~incusive~~inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were ~~coexpressed~~co-expressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 ~~ege.g~~. 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~~residues to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when ~~coexpressed~~co-expressed 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~~co-expressed 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)~~.

15.2.2. FRAMEWORK RESIDUES

As in the case of the light chain the heavy chain frameworks were ~~reexamined~~re-examined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes~~,~~ kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to improve domain packing. Both showed ~~good levels of expression with cL or kgL221A (Fig 29A) and both showed~~ antigen binding when combined with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice ~~(Fig 29B)~~.

15.3 INTERIM CONCLUSIONS

It has been demonstrated ~~here~~, therefore, for OKT3 that to transfer antigen binding ability to the humanised antibody, mouse residues outside the CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer

extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human ~~Kappa~~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~~generated only weak binding activity ~~without~~. Therefore the presence of the ~~6 and 23 changes. It would be of interest to determine by~~6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody. It was important, therefore, to further study the individual contribution of ~~the~~other other 8 mouse residues of the kgH341A gene compared to kgH~~341 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.~~341.

16. FURTHER CDR-GRAFTING EXPERIMENTS

Additional CDR-grafted heavy chain genes were prepared substantially as described above. With reference to Table 2 the further heavy chain genes were based upon the gh341 (plasmid pJA178) and gH341A (plasmid pJA185) with either mouse OKT3 or human KOL residues at 6, 23, 24, 48, 49, 63, 71, 73, 76, 78, 88 and 91, as indicated. The CDR-grafted light -chain genes used in these further experiments were gL221, gL221A, gL221B and gL221C as described above.

TABLE 2

OKT3 HEAVY CHAIN CDR GRAFTS

1. gH341 and derivatives

RES NUM	6	23	24	48	49	63	71	73	76	78	88	91	
OKT3vh	Q	K	A	I	G	F	T	K	S	A	A	Y	
gH341	E	S	S	V	A	F	R	N	N	L	G	F	JA178
gH341A	Q	K	A	I	G	V	T	K	S	A	A	Y	JA185
gH341E	Q	K	A	I	G	V	T	K	S	A	G	G	JA198
gH341*	Q	K	A	I	G	V	T	K	N	A	G	F	JA207
gH341*	Q	K	A	I	G	V	R	N	N	A	G	F	JA209
gH341d	Q	K	A	I	G	V	T	K	N	L	G	F	JA197
gH341*	Q	K	A	I	G	V	R	N	N	L	G	F	JA199

gH341C	Q	K	A	V	A	F	R	N	N	L	G	F	JA184
gH341*	Q	S	A	I	G	V	T	K	S	A	A	Y	JA203
gH341*	E	S	A	I	G	V	T	K	S	A	A	Y	JA205
gH341B	E	S	S	I	G	V	T	K	S	A	A	Y	JA183
gH341*	Q	S	A	I	G	V	T	K	S	A	G	F	JA204
gH341*	E	S	A	I	G	V	T	K	S	A	G	F	JA206
gH341*	Q	S	A	I	G	V	T	K	N	A	G	F	JA208
KOL	E	S	S	V	A		R	N	N	L	G	F	

OKT3 LIGHT CHAIN CDR GRAFTS

2. gL221 and derivatives

RES NUM	1	3	46	47	
OKT3vl	Q	V	R	W	
GL221	D	Q	L	L	DA221
gL221A	Q	V	R	W	DA221A
gL221B	Q	V	L	L	DA221B
GL221C	D	Q	R	W	DA221C
RE1	D	Q	L	L	

MURINE RESIDUES ARE UNDERLINED

The CDR-grafted heavy and light chain genes were co-expressed in COS cells either with one another in various combinations but also with the corresponding murine and chimeric heavy and light chain genes substantially as described above. The resultant antibody products were then assayed in binding and blocking assays with BPS-ALL cells as described above.

The results of the assays for various grafted heavy chains co-expressed with the gL221C light chain are given in Figures 7 and 8 (for the JA184, JA185, JA197 and JA198 constructs - see Table 2), in Figure 9 (for the JA183, JA184 , JA185 and JA197 constructs) in Figure 10 (for the chimeric, JA185, JA199, JA204, JA205, JA207, JA208 and JA209 constructs) and in Figure 11 (for the JA183, JA184, JA185, JA198, JA203, JA205 and JA206 constructs).

The basic grafted product without any human to murine changes in the variable frameworks, i.e. gL221 co-expressed with gh341 (JAI78), and also the "fully grafted" product, having most human to murine changes in the grafted heavy chain framework, i.e. gL221C co-expressed with gh341A (JA185), were assayed for relative binding affinity in a competition assay against murine OKT3 reference standard, using HPB-ALL cells. The assay used was as described above in section 3.3. The results obtained are given in Figure 12 for the basic grafted product and in Figure 13 for the fully grafted product. These results indicate that the basic grafted product has neglibible binding ability as compared with the OKT3 murine reference standard; whereas the "fully grafted" product has a binding ability very similar to that of the OKT3 murine reference standard.

The binding and blocking assay results indicate the following:

The JA198 and JA207 constructs appear to have the best binding characteristics and similar binding abilities, both substantially the same as the chimeric and fully grafted gH341A products. This indicates that positions 88 and 91 and position 76 are not highly critical for maintaining the OKT3 binding ability; whereas at least some of positions 6, 23, 24, 48, 49, 71, 73 and 78 are more important.

This is borne out by the finding that the JA209 and JA199, although of similar binding ability to one another, are of lower binding ability than the JA198 and JA207 constructs. This indicates the importance of having mouse residues at positions 71, 73 and 78, which are either completely or partially human in the JA199 and JA209 constructs respectively.

Moreover, on comparing the results obtained for the JA205 and JA183 constructs it is seen that there is a decrease in binding going from the JA205 to the JA183 constructs. This indicates the importance of retaining a mouse residue at position 23, the only position changed between JA205 and JA183.

These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

Similar Experiments were carried out to CDR-graft a number of the rodent antibodies including antibodies having specificity for CD4 (OKT4), ICAM-l (R6-5), TAG72 (B72.3), and TNF α(61E71, 101.4, hTNF1, hTNF2 and hTNF3).

EXAMPLE 2

CDR-GRAFTING OF A MURINE ANTI-CD4 T CELL RECEPTOR ANTIBODY, OKT4A

Anti OKT4A CDR-grafted heavy and light chain genes were prepared, expressed and tested substantially as described above in Example 1 for CDR-grafted OKT3. The CDR grafting of OKT4A is described in detail in Ortho patent application PCT/GB 90 ……. of even date herewith entitled "Humanised Antibodies". The disclosure of this Ortho patent application PCT/GB 90 ……. is incorporated herein by reference. A number of CDR-grafted OKT4

antibodies have been prepared. Presently the CDR-grafted OKT4A of choice is the combination of the grafted light chain LCDR2 and the grafted heavy chain HCDR10.

THE LIGHT CHAIN

The human acceptor framework used for the grafted light chains was RE1. The preferred LCDR2 light chain has human to mouse changes at positions 33, 34, 38, 49 and 89 in addition to the structural loop CDRs. Of these changed positions, positions 33, 34 and 89 fall within the preferred extended CDRs of the present invention (positions 33 and 34 in CDR1 and position 89 in CDR3). The human to murine changes at positions 38 and 49 corresponds to positions at which the amino acid residues are preferably donor murine amino acid residues in accordance with the present invention.

A comparison of the amino acid sequences of the donor murine light chain variable domain and the RE1 human acceptor light chain variable further reveals that the murine and human residues are identical at all of positions 46, 48 and 71 and at all of positions 2, 4, 6, 35, 36, 44, 47, 62, 64 - 69, 85, 87, 98, 99 and 101 and 102. However the amino acid residue at position 58 in LCDR2 is the human RE1 framework residue not the mouse OKT4 residue as would be preferred in accordance with the present invention.

THE HEAVY CHAIN

The human acceptor framework used for the grafted heavy chains was KOL.

The preferred CDR graft HCDR10 heavy chain has human to mouse changes at positions 24, 35, 57, 58, 60, 88 and 91 in addition to the structural loop CDRs.

Of these positions, positions 35 (CDR1) and positions 57, 58 and 60 (CDR2) fall within the preferred extended CDRs of the present invention. Also the human to mouse change at position 24 corresponds to a position at which the amino acid residue is a donor murine residue in accordance with the present invention. Moreover, the human to mouse changes at positions 88 and 91 correspond to positions at which the amino acid residues are optionally donor murine residues.

Moreover, a comparison of the murine OKT4A and human KOL heavy chain variable amino acid sequences reveals that the murine and human residues are identical at all of positions 23, 49, 71, 73 and 78 and at all of positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

Thus the OKT4A CDR-grafted heavy chain HCDR10 corresponds to a particularly preferred embodiment according to the present invention.

EXAMPLE 3

CDR-GRAFTING OF AN ANTI-MUCIN SPECIFIC MURINE ANTIBODY, B72.3

The cloning of the genes coding for the anti-mucin specific murine monoclonal antibody B72.3 and the preparation of B72.3 mouse-human chimeric antibodies has been described previously (ref. 13 and WO 89/01783). CDR-grafted versions of B72.3 were prepared as follows.

(a) B72.3 Light Chain

CDR-grafting of this light chain was accomplished by direct transfer of the murine CDRs into the framework of the human light chain RE1.

The regions transferred were:

CDR Number	Residues
1	24-34
2	50-56
3	90-96

The activity of the resulting grafted light chain was assessed by co-expression in COS cells, of genes for the combinations:

B72.3 cH/B72.3 cL
and B72.3 cH/B72.3 gL

Supernatants were assayed for antibody concentration and for the ability to bind to microtitre plates coated with mucin. The results obtained indicated that, in combination with the B72.3 cH chain, B72.3 cL and B72.3 gL had similar binding properties.

Comparison of the murine B72.3 and RE1 light chain amino acid sequences reveals that the residues are identical at positions 46, 58 and 71 but are different at position 48.

Thus changing the human residue to the donor mouse residue at position 48 may further improve the binding characteristics of the CDR-grafted light chain, (B72.3 gL) in accordance with the present invention.

(b) B72.3 heavy chain

i. Choice of framework

At the outset it was necessary to make a choice of human framework. Simply put, the question was as follows: Was it necessary to use the framework regions from an antibody whose crystal structure was known or could the choice be made on some other criteria?

For B72.3 heavy chain, it was reasoned that, while knowledge of structure was important, transfer of the CDRs from mouse to human frameworks might be facilitated if the overall homology between the donor and receptor frameworks was maximised. Comparison of the B72.3 heavy chain sequence with those in Kabat (ref. 4) for human heavy chains showed clearly that B72.3 had

poor homology for KOL and NEWM (for which crystal structures are available) but was very homologous to the heavy chain for EU.

On this basis, EU was chosen for the CDR-grafting and the following residues transferred as CDRs.

CDR Number	Residues
1	27-36
2	50-63
3	93-102

Also it was noticed that the FR4 region of EU was unlike that of any other human (or mouse) antibody. Consequently, in the grafted heavy chain genes this was also changed to produce a "consensus" human sequence. (Preliminary experiments showed that grafted heavy chain genes containing the EU FR4 sequence expressed very poorly in transient expression systems.)

ii. Results with grafted heavy chain genes

Expression of grafted heavy chain genes containing all human framework regions with either gL or cL genes produced a grafted antibody with little ability to bind to mucin. The grafted antibody had about 1% the activity of the chimeric antibody.

In these experiments, however, it was noted that the activity of the grafted antibody could be increased to ~10% of B72.3 by exposure to pHs of 2-3.5.

This observation provided a clue as to how the activity of the grafted antibody could be improved without acid treatment. It was postulated that acid exposure brought about the protonation of an acidic residue (pKa of aspartic acid = 3.86 and of glutamine acid = 4.25) which in turn caused a change in structure of the CDR loops, or allowed better access of antigen.

From comparison of the sequences of B72.3 (ref. 13) and EU (refs. 4 and 5), it was clear that, in going from the mouse to human frameworks, only two positions had been changed in such a way that acidic residues had been introduced. These positions are at residues 73 and 81, where K to E and Q to E changes had been made, respectively.

Which of these positions might be important was determined by examining the crystal structure of the KOL antibody. In KOL heavy chain, position 81 is far removed from either of the CDR loops.

Position 73, however, is close to both CDRs 1 and 3 of the heavy chain and, in this position it was possible to envisage that a K to E change in this region could have a detrimental effect on antigen binding.

iii. Framework changes in B72.3 gH gene

On the basis of the above analysis, E73 was mutated to a lysine (K). It was found that this change had a dramatic effect on the ability of the grafted Ab to bind to mucin. Further the ability of the

grafted B72.3 produced by the mutated gH/gL combination to bind to mucin was similar to that of the B72.3 chimeric antibody.

iv. Other framework changes

In the course of the above experiments, other changes were made in the heavy chain framework regions. Within the accuracy of the assays used, none of the changes, either alone or together, appeared beneficial.

v. Other

All assays used measured the ability of the grafted Ab to bind to mucin and, as a whole, indicated that the single framework change at position 73 is sufficient to generate an antibody with similar binding properties to B72.3.

Comparison of the B72.3 murine and EU heavy chain sequences reveals that the mouse and human residues are identical at positions 23, 24, 71 and 78.
Thus the mutated CDR-grafted B72.3 heavy chain corresponds to a preferred embodiment of the present invention.

CDR-GRAFTING OF A MURINE ANTI-ICAM-1 MONOCLONAL ANTIBODY

A murine antibody, R6-5-D6 (EP 0314863) having specificity for Intercellular Adhesion Molecule 1 (ICAM-l) was CDR-grafted substantially as described above in previous examples. This work is described in greater detail in co-pending application, British Patent Application No. 9009549.8, the disclosure of which is incorporated herein by reference.

The human EU framework was used as the acceptor framework for both heavy and light chains. The CDR-grafted antibody currently of choice is provided by co-expression of grafted light chain gL221A and grafted heavy chain gH341D which has a binding affinity for ICAM 1 of about 75% of that of the corresponding mouse-human chimeric antibody.

LIGHT CHAIN

gL221A has murine CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition several framework residues are also the murine amino acid. These residues were chosen after consideration of the possible contribution of these residues to domain packing and stability of the conformation of the antigen binding region. The residues which have been retained as mouse are at positions 2, 3, 48 (?), 60, 84, 85 and 87. Comparison of the murine anti-ICAM 1 and human EU light chain amino acid sequences reveals that the murine and human residues are identical at positions 46, 58 and 71.

HEAVY CHAIN

gH341D has murine CDRs at positions 26-35 (CDR1), 50-56 (CDR2) and 94-100B (CDR3). In addition murine residues were used in gH341D at positions 24, 48, 69, 71, 73, 80, 88 and 91.

Comparison of the murine anti-ICAM 1 and human EU heavy chain amino acid sequences are identical at positions 23, 49 and 78.

EXAMPLE 5

CDR-Grafting of murine anti-TNFa antibodies

A number of murine anti-TNFa monoclonal antibodies were CDR-grafted substantially as described above in previous examples. These antibodies include the murine monoclonal antibodies designated 61 E71, hTNFl, hTNF3 and 101.4. A brief summary of the CDR-grafting of each of these antibodies is given below.

61E71

A similar analysis as described above (Example 1, Section 12.1.) was done for 61E71 and for the heavy chain 10 residues were identified at 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain. Three genes were built, the first of which contained 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The second gene also had 75 and 88 as murine residues [gH341(8)] while the third gene additionally had 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only). The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.

Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine 61E71.

hTNF1

hTNF1 is a monoclonal antibody which recognises an epitope on human TNF. The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

Heavy Chain

In the CDR-grafted heavy chain (ghTNF1) mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

Light Chain

In the CDR-grafted light chain (gLhTNF1) mouse CDRs wre used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at

positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted hTNF1 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product.

hTNF3

hTNF3 recognises an epitope on human TNF-α. The sequence of hTNF3 shows only 21 differences compared to 61E71 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chains of the 61E71 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However 61E71 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF-a compared to hTNF3. Based on the 61E71 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF-a, but competes very poorly in the L929 assay. It is possible that in this case also the framework residues identified for OKT3 programme may improve the competitive binding ability of this antibody.

101. 4

101.4 is a further murine monoclonal antibody able to recognise human TNF-a. The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with cL or gL221. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

A number of other antibodies including antibodies having specificity for interleukins e.g. IL1 and cancer markers such as carcinoembryonic antigen (CEA) e.g. the monoclonal antibody A5B7 (ref. 21), have been successfully CDR-grafted according to the present invention.

It will be appreciated that the foregoing examples are given by way of illustration only and are not intended to limit the scope of the claimed invention. Changes and modifications may be made to the methods described whilst still falling within the spirit and scope of the invention.

ABSTRACT

CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91). The CDR-grafted light chains comprise donor residues at at least one of positions (1) and/or (3) and (46) and/or (47) or at at least one of positions (46, 48, 58) and (71).

The CDR-grafted antibodies are preferably humanised antibodies, having non human, e.g. rodent, donor and human acceptor frameworks, and may be used for in vivo therapy and diagnosis. A generally applicable protocol is disclosed for obtaining CDR-grafted antibodies.

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CLAIMS

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to Claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to Claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to Claim 2 or 3, comprising donor residues at one, some or all of positions:
1 and 3,
69 (if 48 is different between donor and acceptor),
38 and 46 (if 48 is the donor residue),
67,
82 and 18 (if 67 is the donor residue),
91, and
any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to Claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to Claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to Claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to Claim 9 or 10, comprising donor residues at one, some or all of positions:
1 and 3,
63,
60 (if 60 and 54 are able to form a potential saltbridge),
70 (if 70 and 24 are able to form a potential saltbridge),
73 and o 21 (if 47 is different between donor and acceptor) ,
37 and 45 (if 47 if different between donor and acceptor), and
any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to anyone of Claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to anyone of Claims 1-5 and at least one CDR-grafted light chain according to anyone of Claims 6-12.

14. A CDR-grafted antibody molecule according to Claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to Claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to anyone of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to Claim 1 or a CDR-grafted light chain according to Claim 6 or Claim 8.

18. A cloning or expression vector containing a DNA sequence according to Claim 17.

19. A host cell transformed with a DNA sequence according to Claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to Claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to Claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to Claim 6 or Claim 8;

(c) transfecting a host cell with the or each vector;

and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to Claim 1, or a CDR-grafted light chain according to Claim 6 or Claim 8, or a CDR-grafted antibody molecule according to Claim 13 to a human or animal subject.

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Statistics:	
	Count
Insertions	743
Deletions	424
Moved from	11
Moved to	11
Style change	0
Format changed	0
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FRANCIS A. PAINTIN
WOODCOCK, WASHBURN, KURTZ, MACKIEWICZ & NORRIS
ONE LIBERTY PLACE-46TH FLOOR
PHILADELPHIA, PA 19103

This is a communication from the examiner in charge of your application.
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☒ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), 0 days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☒ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 1-23 are pending in the application.
   Of the above, claims 0 are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 1-23 are rejected.
5. ☒ Claims 1-23 are objected to.
6. ☒ Claims 0 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. 9-89)

15. The disclosure is objected to because the specification is replete with misspellings including "humanised" and "humanisation" instead of "humanized" and "humanization", respectively; "recognizes" on page 2, line 22, and page 4, lines 2 and 4 instead of "recognizes"; "chimerisation" on page 3, line 8 instead of "chimerization"; "maximise/optimise" on page 11, lines 18 and 19 instead of "maximize/optimize". The specification should be reviewed and amended to identify and correct other such misspellings. Appropriate correction is required.

16. Claims 5, 11-16, 22 and 23 are objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim cannot depend from any other multiple dependent claim. See MPEP 608.01(n).

17. Claims 1-23 are objected to over the recitation of "CDR-grafted" because abbreviations and acronyms are not appropriate in claim language since the same abbreviation can represent multiple terms. This objection by be overcome by amending the claims to recite instead "Complementarity determining region- grafted".

18. 35 U.S.C. 101 reads as follows:

> "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title".

A) Claims 1-12 are rejected under 35 U.S.C. 101 because the claimed invention is inoperative and therefore lacks utility. Claims 1-5 are drawn to CDR-grafted antibody heavy chains having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises comprises donor residues at particular positions. Claims 6-12 are drawn to CDR-grafted antibody light chains having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at

particular positions. The specification discloses that a few particular CDR-grafted heavy chains when co-expressed with chimeric light chains, unmodified light chains or in one case a CDR-grafted light chain in COS cells retain similar binding affinity for the specific antigen as the chimeric antibody or the unmodified donor antibody. The specification also presents a few examples of co-expression of CDR-grafted light chains with chimeric or unmodified heavy chains where the resulting antibody possesses antigen binding affinity similar to the chimeric antibody. However, the specification does not show CDR-grafted antibody heavy or light chains alone which have the ability to bind antigen. Since antigen binding and therapeutic application are the only utility disclosed in the present application and since the prior art does not generally teach that isolated heavy and light antibody chains can bind antigen, a showing that isolated heavy and light chains have useful antigen binding utility would be required to support the operability. The single chains will not form a complete receptor structure for the antigen epitope for which the complete antibody is specific.

B) Claim 17 is rejected under 35 U.S.C. 101 because the claimed invention is drawn to non-statutory subject matter. Claim 17 is drawn to a DNA sequence coding for a CDR-grafted heavy or light chain. However, "DNA sequences" are not patentable because they are algorithms. M.P.E.P. 608.01 (P). This rejection may be overcome by amending the claims to recite "DNA molecule" instead of "DNA sequence".

C) Claims 22-23 are rejected under 35 U.S.C. 101 because the invention is inoperative and therefore lacks patentable utility. Claim 22 is drawn to a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain or a CDR grafted light chain or a CDR-grafted antibody molecule in combination with a pharmaceutical carrier. Claim 23 is drawn to a method of therapy or diagnosis comprising administering an effective amount of a CDR grafted heavy chain or a CDR grafted light chain or a CDR-grafted antibody to a human or animal subject. The specification fails to establish the utility of the claimed method and pharmaceutical composition using a CDR-grafted antibody in humans or any other animal. The specification does not present any **in vivo** or **in vitro** data to support the

utility of any CDR-grafted heavy chain, light chain or complete antibody molecule. Pharmaceutical therapy reshaped monoclonal antibodies is unpredictable in the absence of **in vivo** clinical data for the following reasons: (1) The antibody or the heavy and light chains may be inactivated before producing an effect such as by proteolytic degradation or due to an inherently short half-life of the modified antibody; (2) The humanized antibody or antibody chain may otherwise not reach the target area because the reshaped antibody may be absorbed by fluid cells or tissues where the antibody has no effect or the humanized antibody; (3) The reshaped antibody and antibody chains may have reduced affinity for the antigen, may be incapable of effector functions or both and as a consequence the antibody may not function as the original donor antibody; (4) The reshaped antibody may still be treated by the human or animal subject as a foreign antigen and result in a rejection reaction. See M.P.E.P. 608.01(P).

19. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> "The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention."

The specification is objected to under 35 U.S.C. 112, first paragraph, as failing to adequately teach how to make and use the claimed invention. Claims 1-5 are drawn to CDR-grafted antibody heavy chains having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises comprises donor residues at particular positions. Claims 6-12 are drawn to CDR-grafted antibody light chains having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at particular positions. The specification does not enable the skilled artisan to make and use the claimed CDR-grafted antibody heavy and light chains. The specification does not provide guidance and working examples

showing how to make and use antibody heavy and light chains to bind antigen. Since this is the basis of the utility disclosed in the present application for the claimed antibodies and antibody chains, the specification must demonstrate the operability of isolated antibody heavy and light chains for binding antigen. The specification only demonstrates the ability of CDR-grafted antibodies containing both a heavy and a light chain. The prior art also does not teach that isolated antibody heavy and light chains have the ability to generally bind antigen similar to the complete double chain antibody. Therefore, the isolated heavy and light chains appear to be inoperable for binding antigen as discussed above in the rejection made under 35 U.S.C. 101, and as a consequence undue experimentation would be required of the skilled artisan in order to practice the claimed invention.

Claims 1-12 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

20. The specification is objected to under 35 U.S.C. 112, first paragraph, as failing to adequately teach how to make and use the claimed invention. Claim 22 is drawn to a therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain or a CDR grafted light chain or a CDR-grafted antibody molecule in combination with a pharmaceutical carrier. Claim 23 is drawn to a method of therapy or diagnosis comprising administering an effective amount of a CDR grafted heavy chain or a CDR grafted light chain or a CDR-grafted antibody to a human or animal subject. The present specification does not enable the skilled artisan to make and use CDR-grafted antibody heavy, light antibody chains or complete antibodies as therapeutic or diagnostics. The specification provides no guidance or working examples to support the operability of the claimed therapeutic/diagnostic composition and method of treatment. The prior art also does not teach that CDR-grafted antibodies have general operability in treating disease in human or non-human subjects. The claimed invention appears to be inoperable without supporting **in vivo** data for the reasons discussed in the rejection made under 35 U.S.C. 101. Therefore undue

experimentation would be required of the skilled artisan in order to practice the claimed invention.

Claims 22 and 23 are rejected under 35 U.S.C. 112, first paragraph, for the reasons set forth in the objection to the specification.

21. Claims 13-16 are rejected 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 antibody, i.e. which are similar to the non-humanized donor antibodies. 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 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 for OKT3, OKT4 and Anti-ICAM. However the specification does not clearly establish that every time the recited amino acid positions are the same between donor and acceptor, "good" binding to antigen is observed. The specification does not provide actual binding 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. and Queen et al. 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 in 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 reasonable predict which amino acids will always be effect in increasing of retaining antigen binding ability. Therefore, this analysis shows that undue experimentation would be

required of the skilled artisan in order to practice the claimed invention. See MPEP 706.03(n) and 706.03(z).

22. Claims 1-23 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) Claims 1-5 are indefinite over the recitation of "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". because the claims is unclear as to whether the antibody heavy chain has at least one of 6,23,24,48,49,71,73,75,76,78,88, or 91 or alternatively has at least one (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91) or alternatively has at least one (6,23) and/or (24,48) and/or (49,71) and/or (73,75) and (76) and/or (78 and 88) and/or (91). The claims are further indefinite because these position numbers are arbitrary unless they are identified as positions in a Figure or relative to an identified' numbered sequence, for example. These position numbers are not identified as positions in a amino acid sequence or in a nucleotide sequence.

B) Claims 6-12 are indefinite over the recitation of "at least one of positions 1 and/or 3 and 46 and/or 47". The claims are unclear with regard to whether the light chain contains donor residues at at least one of positions 1,3, 46 and/or 47 or alternatively at at least one of (1 and/or 3) and (46 and/or 47) or alternatively (1) and/or (3 and 26) and/or (47). The claims are further indefinite because these position numbers are arbitrary unless they are identified as positions in a Figure or relative to an identified numbered sequence, for example. These position numbers are not identified as positions in a amino acid sequence or in a nucleotide sequence.

C) Claims 4 and 11 are indefinite and confusing because they are in an improper Markush listing. Correction is required.

D) Claim 21 is indefinite over the recitation of "a complementary antibody light chain" in step (b) because the method is unclear as to what "complementary" refers. Claim 21 is further indefinite over the recitation of "with the or each vector" in step (c) because the method is unclear as to which of the two expression vectors is "the vector" or if "the vector" refers to a different vector which lacks antecedent basis. Additionally step (c) is unclear with regard to whether the same host cell is transfected with both vectors or whether the vectors are transfected into different host cells. Finally, claim 21 is unclear because the recitation of "the transfected cell line" lacks antecedent basis.

23. The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

"A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States."

A) Claims 1, 5, 6-8, 12-22 are rejected under 35 U.S.C. § 102 (b) as being anticipated by Riechmann et al. Claim 1 is drawn to a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6,23, and/or 24,48 and/or 49,71 and/or 73,75 and/or 76 and/or 78 and 88 and/or 91. Claim 5 is further limited wherein the donor CDRs are at positions 26-35, 50-65 and 95-100. Claim 6 is drawn to a CDR-grafted antibody light chain having a variable domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Claim 7 is further limited wherein the donor residues are at positions 46 and 47. Claim 8 is drawn to a CDR-

grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48,, 58 and 71. Claim 13 is drawn to a CDR-grafted antibody molecule comprising at least one CDR-grafted antibody heavy chain of claims 1-5 and at least one CDR-grafted antibody light chain of claims 6-12. Claims 14 and 15 are further limited wherein the antibody is a site-specific antibody molecule (claim 14) and wherein the antibody has specificity for an interleukin, hormone, other biologically active compound. Claim 16 is drawn to a CDR-grafted antibody heavy or light chain or molecule comprising human acceptor residues and non-human donor residues. Claim 17 is drawn to DNA encoding any of the above antibody chains. Claim 18 is drawn to an expression vector and claim 19 is drawn to a host cell transformed with the expression vector. Claim 20 is drawn to a method of making the antibody from the transformed cell and claim 21 is drawn to a process comprising producing an expression vector encoding the heavy chain and another expression vector encoding the light, transfecting a host cell with the vectors and culturing the transfected cell to make the CDR-grafted antibody product.

Riechmann et al. teach a CDR-grafted antibody heavy chains (Figure 1(a)), a CDR-grafted antibody light chain (Figure 1(b)) and a CDR-grafted antibody molecule containing a CDR-grafted heavy chain and a CDR grafted light chain (Figure 2) having a variable region domain comprising human acceptor framework (human NEW for the heavy chain and RE1 for the light chain) and rat donor antigen binding regions (heavy chain CDRs: CDR1 at 31-35, CDR2 at 50-65, and CDR3 at 95-102; light chain CDRs: CDR1 at 24-34, CDR2 at 50-56, CDR3 at 89-97), wherein the framework comprises rat donor residues at the following positions. For the heavy chain rat donor residues are present at amino acid positions 6, 49, 76, 88, 91, and for the light chain rat donor residues are at positions 1, 46 and 47, 48 and 71. Riechmann et al. also teach the DNA encoding these humanized antibodies in Figure 1, expression vectors containing the DNA encoding the reshaped antibodies (see description of Figure 2), a host cell transfected with these expression vectors, i.e lymphoid cells lines (see description of Figure 2) and a process for the production of the humanized antibodies comprising making an expression

vector for the reshaped heavy chain and another expression vector for the light chain, transfecting a host cell with both of these vectors and culturing the host cells so that the heavy and light chains are co-expressed. (description of Figure 2). Applicant should note that claim 1 and claim 6 are being interpreted to mean that the framework has donor residues at at least one of any of positions 6,23,24,48,49,71,73,75,76,78,88 or 91 in the heavy chain and (1,3,46 or 47) or (46,48,58 or 71) in the light chain. Therefore, the teachings of Riechmann et al. anticipate the invention as claimed.

B) Claims 1-6 and 12-22 are rejected under 35 U.S.C. § 102 (b) as being anticipated by Queen et al. Claim 1 is drawn to a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6,23, and/or 24,48 and/or 49,71 and/or 73,75 and/or 76 and/or 78 and 88 and/or 91. Claims 2-5 are further limited wherein donor residues are at positions 23,24,49,71,73,78 or 23,24 and 49 (claim 2), wherein donor residues are additionally present at positions 2,4,6,25,36,37,39,47,48,93,94,103,104,106 and 107 (claim 3), wherein donor residues are additionally present at position at at least on of positions 1 and 3, 69, 38 and 46, 67 82 and 18, 91 and any of 9,11,41,87,108,110 and 112 (claim 4) and wherein the donor CDRs are at positions 26-35, 50-65 and 95-100. Claim 6 is drawn to a CDR-grafted antibody light chain having a variable domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47. Claim 12 further limited wherein the donor CDRs are at positions 24-34, 50-56 and 89-97. Claim 13 is drawn to a CDR-grafted antibody molecule comprising at least one CDR-grafted antibody heavy chain of claims 1-5 and at least one CDR-grafted antibody light chain of claims 6-12. Claims 14 and 15 are further limited wherein the antibody is a site-specific antibody molecule (claim 14) and wherein the antibody has specificity for an interleukin, hormone, other biologically active compound. Claim 16 is drawn to a CDR-grafted antibody heavy or light chain or molecule comprising

human acceptor residues and non-human donor residues. Claim 17 is drawn to DNA encoding any of the above antibody chains. Claim 18 is drawn to an expression vector and claim 19 is drawn to a host cell transformed with the expression vector. Claim 20 is drawn to a method of making the antibody from the transformed cell and claim 21 is drawn to a process comprising producing an expression vector encoding the heavy chain and another expression vector encoding the light, transfecting a host cell with the vectors and culturing the transfected cell to make the CDR-grafted antibody product.

Queen et al. teach a CDR-grafted anti-Tac antibody using a human antibody framework (EU) as acceptor and murine anti-Tac antibody as donor. The antibody heavy chain of Queen et al. as murine amino acids at positions 2,4,6,23,24,25,36,37,39,47,48,49,71,73,78,93,94,103,104,106 and 107 and additionally at positions 1,3,69,67,9,41,108 and 110. (see Figure 2). The humanized antibody of Queen et al. contains murine CDRs at positions 26-35, 50-56 and 95-100. Queen et al. also teach a humanized antibody light chain having a donor amino acid at position 47 and CDRs at positions 24-34, 50-56 and 89-97. Queen et al. further teach the cDNA encoding the the humanized heavy and light chains (page 10030, col. 1 and 10031), the insertion of these cDNAs in expression vectors (10030 col 1-col 2), the transfection of host cells with these expression vectors(10031 col. 2) and the co-expression of the cDNAs to produce a CDR-grafted antibody molecule (10031, col. 2 and 10032). Therefore, since every recited claim limitation is taught by Queen et al., the invention, as claimed, is anticipated by Queen et al.

24. The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary

skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) and (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103.

Claims 1-21 are rejected under 35 U.S.C. 103 as being unpatentable over Riechmann et al. and Queen et al. Both Riechmann 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 Riechmann 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 to the murine antibody the less likely would combining the anti-Tac CDRs with the human framework be to introduce distortions into the CDRs. Therefore, 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. further teach making a molecular model of the donor variable domain (in this case the anti-Tac V domain) based on homology to other antibody V domains whose crystal structure is known. By doing so Queen et al. teach that amino acids

outside the CDRs which are close enough to the CDRs to influence the CDR conformation or to directly interact with the antigen. When the residues where 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 recommends changing these amino acids to the consensus amino acid (page 10032, col. 1). Riechmann et al. and Queen et al. further teach that different changes will be necessary depending of the specific donor and acceptor antibodies which are used. Both references teach 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 into 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 Riechmann et al. and Queen et al. to reshape any given antibody to "humanize" that antibody by making changes in the framework regions of the human acceptor to the donor residue when those residues are close to the CDRS and when those amino acids 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, paragraph 3 through col. 2) and Riechmann et al. (Figure 4).

25. No claims are allowable.

26. 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) 308-4227. 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).

27. Any inquiry concerning this communication or earlier communications from the examiner should be directed to 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.

LTB
Lisa T. Bennett
November 14, 1992

MARGARET MOSKOWITZ
SUPERVISORY PATENT EXAMINER
GROUP 180

FORM PTO-892 (REV. 3-78) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

NOTICE OF REFERENCES CITED

SERIAL NO.	GROUP ART UNIT	ATTACHMENT TO PAPER NUMBER
~~07/743,329~~	1807	11

APPLICANT(S): ADAIR ET AL.

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE
	A						
	B						
	C						
	D						
	E						
	F						
	G						
	H						
	I						
	J						
	K						

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PERTINENT PP. SPEC.
	L								
	M								
	N								
	O								
	P								
	Q								

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)

R CHOTHIA, CYRUS ET AL (Dec. 1989) NATURE, "CONFORMATIONS OF IMMUNOGLOBULIN HYPERVARIABLE REGIONS", VOLUME 342, pp. 877-883.

S QUEEN, C. ET AL (DEC. 1989) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, "A HUMANIZED ANTIBODY THAT BINDS TO INTERLEUKIN 2 RECEPTOR"

T ~~RIECHM~~ VOLUME 86, pp. 10029-10033.

U RIECHMANN ET AL (MARCH 1988) NATURE, "RESHAPING HUMAN ANTIBODIES FOR THERAPY," VOLUME 332, pp. 323-327.

EXAMINER: Lisa L. Bennett

DATE: 11-14-92

* A copy of this reference is not being furnished with this office action.
(See Manual of Patent Examining Procedure, section 707.05 (a).)

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
743,329	8/16/91	Adair et al.	CARP-0009

EXAMINER	
Lisa Bennett	
ART UNIT	PAPER NUMBER
1807	18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lisa Bennett (3) LIZA HOHENSCHUTZ

(2) Scott Chambers (4) FRANCIS A. PAINTIN
GALE MATTHEWS PHILLIP ANSELL
CHRIS MERCER
JOHN ADAIR

Date of interview 1/27/93

Type: ☐ Telephonic ☑ Personal (copy is given to ☐ applicant ☑ applicant's representative).

Exhibit shown or demonstration conducted: ☑ Yes ☐ No. If yes, brief description: Proposed amended claims.

Agreement ☐ was reached with respect to some or all of the claims in question. ☑ was not reached.

Claims discussed: All

Identification of prior art discussed: Queen et al and Reichmann et al

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: proposed amend w/ overcome 112 2nd & spec obj: leaving 101, utility enablement & art. Expln requested narrowing to use of human/mouse or human/rat: at present, specific residues for any antibodies were w/in genus of the claim. Thera claims will be put into a continuat. & applic will properly focus

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1–7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lisa L. Bennett
Examiner's Signature

PTOL-413 (REV. 1-84)

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Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
743,329	8/16/91	Adair et al.	CARP-0009

EXAMINER	
Lisa Bennett	
ART UNIT	PAPER NUMBER
1807	#18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lisa Bennett, Scott Chambers, (3)

(2) Adair. (4)

Date of interview 1/27/93

Type: ☐ Telephonic ☑ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☑ Yes ☐ No. If yes, brief description: Proposed amended claims.

Agreement ☐ was reached with respect to some or all of the claims in question. ☑ was not reached.

Claims discussed: & All

Identification of prior art discussed: Queen et al., Riechmann et al.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: on just binding regions; Can one of art make Ab from any source? Applicant indicates that great conservation of Ab would suggest that acceptor & donor can come from any species. Prior art rejections: Riechmann & Queen. Applic. have found specific residues which they find are important for the invent.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1–7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lisa L. Bennett
Examiner's Signature

PTOL-413 (REV. 1-84)

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Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
743,329	8/16/91	Adair et al.	CARP-0009

EXAMINER	
Lisa Bennett	
ART UNIT	PAPER NUMBER
1807	#18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lisa Bennett, Scott Chambers (3) _____

(2) _____ (4) _____

Date of interview 1/27/93

Type: ☐ Telephonic ☑ Personal (copy is given to ☐ applicant ☑ applicant's representative).

Exhibit shown or demonstration conducted: ☑ Yes ☐ No. If yes, brief description: Proposed Claims.

Agreement ☐ was reached with respect to some or all of the claims in question. ☑ was not reached.

Claims discussed: All

Identification of prior art discussed: Queen et al., Riechmann et al.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Queen starts from point of identifying areas of high homology & then uses computer modeling to determine. It is possible that Queen would come up w/ same sequence but Queen sometimes fails, applicant indicates they have ~~none~~ not had a failure & average is 50-60%. Queen doesn't suggest some of the changes

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1-7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lisa L. Bennett
Examiner's Signature

PTOL-413 (REV. 1-84)

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Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
743,529			

EXAMINER	
ART UNIT	PAPER NUMBER
	18

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lisa Bennett, Scott Chambers

(2) _____ (4) See page 1

Date of interview 1/27/92

Type: ☐ Telephonic ☒ Personal (copy is given to ☒ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description: _____

Agreement ☐ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed: _____

Identification of prior art discussed: _____

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: the changes; applicant suggests that the "comprising" in eg clm 24 is not to be taken as "comprising" more residues than those in clm, i.e. claimed residues are not to be considered open ended. Applic indicated they would clarify the latter issue. Queen does not teach charging residue: 73 HC; 38 HC; #1 on LC #1 on LC + #4 on LC, 36 on LC 46 on LC

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1–7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lisa B. Bennett

Examiner's Signature

PTOL-413 (REV. 1-84)

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Paper No. _____

Filed on behalf of: Party Carter

Filed: May 28, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER'S LIST OF EXHIBITS
(As of May 28, 2010)

CARTER'S LIST OF EXHIBITS

In accordance with ¶ 154.4 of the Standing Order (Paper No. 2) and Part E of the Order - Motion Times - Bd.R. 104(c) (Paper No. 23, p. 8), Carter herein provides a list of exhibits reflecting evidence filed and served in connection with Carter Substantive Motions 1 and 2 (*i.e.*, Ex. 2001-2029, 2031 and 2033-2039).

Designated Exhibit	Description of the Document
Ex. 2001	U.S. Patent No. 6,407,213 to Carter *et al.*, issued June 18, 2002.
Ex. 2002	U.S. Patent Application No. 11/284,261 to Adair *et al.*, filed November 21, 2005.
Ex. 2003	Preliminary Amendment and Request for Interference Under 37 C.F.R. § 42.202 [sic], filed November 21, 2005, in U.S. Patent Application No. 11/284,261 to Adair *et al.*
Ex. 2004	Request for Reconsideration, filed September 9, 2009, in U.S. Patent Application No. 11/284,261 to Adair *et al.*
Ex. 2005	PCT Application No. PCT/GB90/02017 to Adair *et al.*, filed December 21, 1990, published as WO 91/09967 on July 11, 1991 ("the PCT Application").
Ex. 2006	U.S. Patent Application No. 07/743,329 to Adair *et al.*, filed September 17, 1991.
Ex. 2007	Response to Office Action filed January 19, 1993, in U.S. Patent

	Application No. 07/743,329 to Adair *et al*.
Ex. 2008	Amendment filed April 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.
Ex. 2009	Letter regarding amendments filed September 9, 1993, in European Patent Application No. 91901433.2 to Adair *et al*.
Ex. 2010	Amendment filed February 7, 1994, in U.S. Patent Application No. 07/743,329 to Adair *et al*.
Ex. 2011	Riechmann *et al.*, *Nature*, Vol. 332, pp. 323-327 (March 1988).
Ex. 2012	Response to Advisory Action filed May 9, 1994, in U.S. Patent Application No. 07/743,329 to Adair *et al*.
Ex. 2013	Preliminary Amendment filed September 7, 1994, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2014	Amendment filed September 18, 1995, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2015	Preliminary Amendment filed August 23, 1996, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2016	Preliminary Amendment and Request for Interference Under 37 C.F.R. § 1.607 filed May 1, 1997, in U.S. Patent Application No.

	08/846,658 to Adair *et al*.
Ex. 2017	Response and Amendment filed August 20, 1997, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2018	Proposed claims transmitted January 28, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2019	Amendment Pursuant to 37 C.F.R. § 1.312 filed February 23, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2020	Amendment Pursuant to 37 C.F.R. § 1.312 filed July 13, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.
Ex. 2021	Allowed claims filed September 29, 1998, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2022	Fourth Preliminary Amendment filed November 5, 1998, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2023	Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 10029-10033 (December 1989).
Ex. 2024	U.S. Patent No. 5,859,205 to Adair *et al.*, issued January 12, 1999.
Ex. 2025	Amendment and Request for Reconsideration filed April 9, 1999, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2026	Amendment and Request for Reconsideration filed November 3, 1999, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2027	Amendment and Request for Reconsideration filed January 19, 2000, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2028	Office Action mailed September 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al.*
Ex. 2029	Request for Reconsideration filed August 29, 2000, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2030	Not used.
Ex. 2031	Supplemental Amendment and Request for Reconsideration filed September 14, 2000, in U.S. Patent Application No. 08/846,658 to Adair *et al*.
Ex. 2032	Not used.
Ex. 2033	Amendment and Request for Reconsideration filed November 12, 2001, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2034	Proposed amendments to claims transmitted on March 18, 2002, in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2035	Amendment and Request for Reconsideration filed August 9, 2002,

	in U.S. Patent Application No. 08/485,686 to Adair *et al*.
Ex. 2036	Great Britain Application No. 8928874.0 to Adair *et al*., filed December 21, 1989 ("the UK Application").
Ex. 2037	Computer generated comparison (using Workshare™ Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application.
Ex. 2038	Office Action mailed November 18, 1992, in U.S. Patent Application No. 07/743,329 to Adair *et al*.
Ex. 2039	Examiner Interview Summary Record dated January 27, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Respectfully submitted,

May 28, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

ASHE, P.C.
11440 Isaac Newton Square North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 1"** and Exhibits 2001-2029, 2031, and 2033-2039 were filed this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 1"** and Exhibits 2001-2029, 2031, and 2033-2039 were served this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

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Filed on behalf of: Party Carter

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By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

_____

CARTER SUBSTANTIVE MOTION 1
(Adair Claim 24 Is Barred Under 35 U.S.C. § 135(b)(1))

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CARTER SUBSTANTIVE MOTION 1

I. PRECISE RELIEF REQUESTED

Carter moves under 37 C.F.R. § 41.121(a)(1)(iii) for judgment that Adair's involved claim 24 is not patentable to Adair under 35 U.S.C. § 135(b)(1).

II. THE EVIDENCE AND STATEMENT OF MATERIAL FACTS

A list of exhibits, papers, and appendices relied upon in support of this motion is set forth in Appendix 1. A statement of material facts relied upon in support of this motion is set forth in Appendix 2.

III. ARGUMENT

A. Introduction and Overview of Argument

Carter's involved U.S. Patent No. 6,407,213 ("the '213 patent") issued on June 18, 2002. (MF 36). The "critical date" for compliance with 35 U.S.C. § 135(b) is June 18, 2003. (MF 37). More than six years after the critical date, on September 9, 2009, Adair presented its involved claim 24. (MF 40).

To comply with the requirements of § 135(b), Adair must satisfy at least three conditions. First, Adair must have presented a "pre-critical date" claim that is patentable to Adair. Second, Adair must have presented a "pre-critical date" claim that defines the same or substantially the same subject matter as a Carter '213 patent claim.[1] Third, Adair's post-critical date claim (*i.e.*, Adair's involved claim 24) cannot differ in any "material limitation" from Adair's pre-critical date claim(s) that is patentable to Adair and defines substantially the same subject matter as a

[1] None of Adair's pre-critical date claims is identical to (*i.e.*, "the same as") a Carter '213 patent claim. (MF 41).

Carter '213 patent claim.[2] *In re Berger*, 279 F.3d 975, 981-82 (Fed. Cir. 2002), citing *Corbett v. Chisum*, 568 F.2d 759, 196 USPQ 337 (CCPA 1977). If Adair fails to satisfy any one of these three conditions, then Adair's claim 24 is not patentable to Adair and Adair loses its standing to participate in the interference. (37 C.F.R. § 41.201, defining "threshold issue.")

On November 21, 2005, Adair filed its involved application 11/284,261 ("the '261 application"), presented new claim 24, and filed a "Preliminary Amendment and Request for Interference under 37 C.F.R. § 42.202 [sic]."[3] (MF 38-39 and 43). In requesting an interference with the Carter '213 patent, Adair acknowledged the hurdle it faced to comply with the requirements of § 135(b). (MF 43-44). Specifically, Adair argued that its newly presented claim 24 was not barred by § 135(b) because claims 8 and 16 in its PCT application, when considered together, define subject matter that is the same or substantially the same as the subject matter defined by claim 1 of Carter's '213 patent.[4] (MF 44). Adair's position does not withstand scrutiny.

First, Adair's § 135(b) analysis entirely failed to address whether Adair's PCT claims are patentable to Adair, a prerequisite for compliance with § 135(b) of any post-critical date claim

[2] Adair claim 24 is not identical to (*i.e.*, "the same as") any pre-critical date Adair claim. MF 42.

[3] Adair thereafter amended claim 24. Adair's involved claim 24 was presented on September 9, 2009. A comparison of the 2005 version of claim 24 and the 2009 version of involved claim 24 is provided in Appendix 3. (MF 31).

[4] Carter '213 patent claim 1 is not designated as corresponding to Count 1, nor has Adair moved for such relief. (Declaration, Paper No. 1, p. 4; and Adair Motions List, Paper 20, p. 2, item 5).

seeking benefit of a pre-critical date claim.[5] As discussed below, Adair's original PCT claims were also presented as the original claims in Adair's '329 application and were immediately and soundly rejected under 35 U.S.C. §§ 101, 102, 103, and 112 first and second paragraph. (MF 5-6 and 11-20). In response, Adair promptly cancelled the original claims in its PCT and U.S. applications and presented new claims that Adair characterized as more clearly describing Adair's invention and distinguishing the claims over a number of prior art references. (MF 21-26).

Second, Adair's arguments regarding compliance with § 135(b) failed to address the critical issue of whether Adair claim 24 is materially different from Adair's pre-critical date claims. A comparison of Adair's PCT claim 16/8 (*i.e.*, the pre-critical claims identified by Adair for compliance with § 135(b) in its request for interference) and Adair's involved claim 24 reveals that the claims are directed to different subject matter. (MF 32 and Appendix 4). For example, the PCT claim 16/8 recites a "CDR-grafted light chain…" whereas involved claim 24 recites a "humanized antibody comprising a heavy chain…" (MF 32 and Appendix 4). The heavy and light chains of an antibody are different polypeptides and, therefore, a residue substitution in a light chain is physically different from a residue substitution in a heavy chain and *vice-versa*.

In summary, the § 135(b) argument that Adair advanced to provoke an interference with the Carter '213 patent 1) failed to address the unpatentability to Adair of the original PCT/U.S.

[5] As a matter of law, Adair cannot rely on a PCT application to satisfy the requirements of 35 U.S.C. § 135(b). However, Adair's original PCT and original U.S. claims are identical and, therefore, Adair's improper reliance on PCT claims rather than on U.S. claims does not appear to be of immediate significance to the relief requested in this motion.

claims upon which Adair relied for § 135(b) benefit; 2) failed to address the fact that Adair's pre-critical claims do not define the same or substantially the same subject matter as an involved Carter patent claim; and 3) failed to address the differences in material limitations between Adair's original PCT/U.S. claims and Adair post-critical date claim 24.

In this motion, Carter explains that Adair's involved claim 24 is statutorily barred under § 135(b) for any one of the following reasons:

1) none of Adair's original PCT/U.S. claims are patentable to Adair and, therefore, Adair involved claim 24 (or any other post-critical date claim) is not entitled to the benefit of these claims for compliance with § 135(b);

2) none of Adair's pre-critical date claims are directed to the same or substantially same subject matter as any one of Carter's involved '213 patent claims and, therefore, cannot be relied upon for compliance with § 135(b);

3) Adair involved claim 24 differs in material limitations from Adair's pre-critical date claims and, therefore, Adair is not entitled to the benefit of these claims for compliance with § 135(b).

In Carter Substantive Motion 2, Carter moves for judgment that Adair involved claim 24 is unpatentable to Adair for failure to comply with the written description requirement of 35 U.S.C. § 112, first paragraph. Regardless of whether the Board finds Adair claim 24 to satisfy the written description requirement, Adair claim 24 is barred under § 135(b) and adverse judgment should be entered against Adair.

B. <u>Adair Is Not Statutorily Entitled to Any of Its Original U.S. Claims</u>

On December 21, 1990, Adair filed PCT/GB90/02017 ("the PCT application"), containing claims 1-23 (*i.e.*, the "original PCT claims"). (MF 2). On September 17, 1991, Adair filed U.S. Patent Application No. 07/743,329 ("the '329 application"), which claims benefit of

the Adair PCT application. (MF 5). The original claims filed in the '329 application are identical to Adair's original PCT claims. (MF 6).

On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-23 on a variety of statutory grounds (MF 11), including the following:

1. Claims 1-12, 17 and 22-23 were rejected under 35 U.S.C. § 101 as lacking utility (MF 12);

2. Claims 1-16 and 22-23 were rejected under 35 U.S.C. § 112, first paragraph, as failing to adequately teach how to make and use the claimed invention (MF 13);

3. Claims 1-23 were rejected under 35 U.S.C. § 112, second paragraph, as failing to particularly point out and distinctly claim the subject matter which Adair regarded as its invention (MF 14);

4. Claims 1, 5, 6-8, and 12-22 were rejected under 35 U.S.C. § 102(b) as being anticipated by Riechmann *et al.* (MF 15);

5. Claims 1-6 and 12-22 were rejected under 35 U.S.C. § 102(b) as being anticipated by Queen *et al.* (MF 16); and

6. Claims 1-21 were rejected under 35 U.S.C. § 103 as being obvious over Riechmann *et al.* and Queen *et al.* (MF 17).

In rejecting claims 1-16 and 22-23 for lack of enablement under § 112, first paragraph, the Examiner maintained, *inter alia*, that the Adair specification did not support making the range of residue changes recited in the claims and observed that the effects of the residue changes as described in Adair's original claims could not readily be predicted. (MF 18).

In rejecting claims 1-23 under 35 U.S.C. § 112, second paragraph, the Examiner maintained that the claims were indefinite in their recitation of "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" because it was unclear to the Examiner whether the heavy chain framework had donor residues at:

a. at least one of 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91, or alternatively,

b. at least one of (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91), or alternatively,

c. at least one of (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and 76 and/or (78 and 88) and/or (91). (MF 19).

In rejecting the claims 1, 5, 6-8, and 12-22 under 102(b) as being anticipated by Riechmann *et al.*, the Examiner noted that the claims were being interpreted to mean that the framework has donor residues at at least one of any of positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain. (MF 20).

On January 19, 1993, Adair responded to the November 1992 Office action by cancelling original claims 1-20, 22 and 23, amending original claim 21, and adding new claims 24-66. (MF 21).

Adair further responded to the § 112, first paragraph, rejections by relying on the disclosure added to its PCT application that described a "hierarchy of residues" protocol for preparing humanized antibodies according to Adair's invention. (MF 3-4). Adair argued that, in view of the specific rules set forth in Adair's "hierarchy of residues" protocol, it would not require undue experimentation to make and use the subject matter defined by Adair's newly added claims. (MF 3-4, 22).

In its January 1993 response, Adair did not contest the rejections under § 112, second paragraph but, rather, cancelled the rejected claims. (MF 21 and 24). Likewise, Adair did not contest the rejection of the claims under § 102(b) as being anticipated by Riechmann *et al.* and

Queen *et al.* but, rather, relied upon limitations in the newly presented claims to distinguish over these prior art references. (MF 25-26). Adair followed the filing of its response in the '329 application with an in-person Examiner interview on January 27, 1993, wherein Adair asserted that its claims were not open-ended with respect to unrecited residue substitutions. (MF 27).

On September 9, 1993, in the Adair PCT/EP Patent Application No. 91901433.2, Adair filed an amendment deleting original claims 1-23, replacing them with new claims 1-20, and acknowledging grounds of unpatentability of its original claims. (MF 28).

On February 7, 1994, Adair filed an amendment in the '329 application responding to the Office action mailed on September 7, 1993. Although substantial in length, the following quote captures several important points regarding Adair's own characterization of its invention, the scope of its claims, and the interpretation of its specification (MF 10 and 29):

> At a very helpful interview held at the beginning of 1993, there was some discussion of the word "comprising" as used in the claims under consideration at that time. In those claims, it was only specified that certain residues should be donor residues. It was considered that it was not clear whether these were the only residues which could be donor residues. The alternative view was that these were only the minimum number of residues which must be donor but that any of the other residues could also be donor. [Emphasis by Adair].
>
> If the second line of interpretation were taken, the claims could be read to cover a situation in which all except one of the residues in the variable domain were donor residues. In this case, the claims could then be interpreted to cover a structure similar to a "chimeric" antibody comprising a donor variable domain and a human constant region. Such chimeric antibodies were already well known at the priority date. [Emphasis by Adair].
>
> It plainly is not the intention of the Applicants to claim chimeric antibodies or any similar structures. As can be seen from the description, the superhumanised antibodies of the present invention are compared to the prior art chimeric antibodies. Moreover, the present invention was intended to deal with the problem of chimeric antibodies in that chimeric antibodies were believed to be too "foreign" because of the presence of the complete donor variable domain.
>
> For the above reasons, it is clear that the wording of the claims needed to be changed so that the Applicants' intention of excluding chimeric antibodies was

made effective. The language now present in the claims puts this intention clearly into effect.

As to support for this wording, the Examiner is referred firstly to page 16, under the heading "Protocol". It can be seen from this paragraph that the first step in the process involves the choice of an appropriate acceptor chain variable domain. This acceptor domain must be of known sequence. Thus, the protocol starts with a variable domain in which all the residues are acceptor residues. In the sentence bridging pages 16 and 17, it is stated that:

> "The CDR-grafted chain is then designed starting from the basis of the acceptor sequence".

On page 17, in the middle paragraph, it is stated that:

> "The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows ...."

This again shows that, unless a residue is chosen for substitution, it will remain as in the acceptor sequence. [Emphasis by Adair].

It must also be borne in mind that the purpose of the invention is to obviate some of the disadvantages of prior art proposals. The proposal of using chimeric antibodies had the disadvantage that they were more "foreign" than desirable. The problem of making CDR-grafted antibodies was that they generally did not provide good recovery of affinity. Thus, the aim of the present invention was to minimise as far as possible the "foreign" nature of the antibody while maximising as far as possible its affinity.

Bearing the passages referred to above and the aim of the invention in mind, it would have been abundantly clear to the skilled person reading the application that as many residues as possible should remain as acceptor residues. If this were not the case, it could hardly be said that the composite chain is based on the acceptor sequence.

The skilled person reading the application can plainly see that certain residues have been considered for changing from acceptor to donor. These are clearly set out in the description. It would be plain to the skilled person that all other residues should not be considered for changing at all. It would therefore be obvious that any residue which is not specified as being under consideration for changing must remain as in the acceptor chain.

It may be that there is no explicit statement in the description that the specified residues should remain as in the acceptor chain. However, the disclosure in a specification is not limited to the explicit disclosure but also includes that which is implicit. It is implicit, in the recitation that the chain is based on the acceptor and that only certain residues are considered for changing, that all non-specified residues must remain as acceptor residues. Subject matter which might

be fairly deduced from the disclosure is not new matter. *Acme Highway Products Corp. v. D.S. Brown Co.*, 431 F.2d 1074, 1080, 167 U.S.P.Q. 129, 132-133(6th Cir. 1970), *cert denied*, 401 U.S. 956 (1971).

In summary, Adair has acknowledged that it is not statutorily entitled to its original PCT/U.S. claims, as these claims were ambiguous and could be reasonably interpreted as reading on the disclosures in Queen *et al.* and Reichmann *et al.* of humanized antibody polypeptides meeting the requirements of these original Adair claims, as well as on known chimeric antibody structures. The express teachings of the Adair specification and Adair's representations regarding the same provide compelling evidence that Adair is not statutorily entitled to its original PCT/U.S. claims and, therefore cannot rely upon those claims for purposes of satisfying the statutory requirements of § 135(b). *Adang v. Umbeck*, 2007 U.S. App. LEXIS 25198 (Fed. Cir. 2007); *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 927 (Fed. Cir. 2004); *PIN/NIP, Inc. v. Platte Chem. Co.*, 304 F.3d 1235, 1247-48 (Fed. Cir. 2002); *In re Curtis*, 354 F.3d 1347, 1353-54 (Fed. Cir. 2004).

C. None of Adair's Pre-Critical Date Claims Defines the Same or Substantially the Same Subject Matter As Any One of Carter's Involved Patent Claims

None of Adair's pre-critical date claims define the same or substantially the same subject matter as an involved Carter '213 patent claim because each of Adair's pre-critical date claims differ in one or more material limitations relative to Carter's '213 patent claims. (MF 41). As a consequence, none of Adair's pre-critical date claims can serve as a basis for overcoming the barrier of § 135(b).

As discussed above, Adair's original PCT/U.S. claims were found not to comply with numerous statutory provisions for patentability, including that the claims were indefinite under 35 U.S.C. § 112, second paragraph. (MF 11-20). By contrast, Adair does not contest that Carter's involved patent claims are definite and thus satisfy 35 U.S.C. § 112, second paragraph.

Logically, Adair's original claims must differ in ways having patentable significance (*i.e.*, in material limitations) from Carter's '213 claims because the former were found to be indefinite by the Examiner, while the latter are conceded by Adair to not be indefinite. Adair's original PCT/U.S. claims are thus not directed to the same or substantially the same subject matter as any Carter's '213 patent claim.

After Adair cancelled its original PCT/U.S. claims in 1992, Adair presented a plethora of other claims prior to June 18, 2003 ("Adair's non-original pre-critical date claims"). (MF 30). None of Adair's non-original pre-critical date claims can be relied upon to satisfy the requirements of § 135(b).

First, many of Adair's non-original pre-critical date claims were determined to be not patentable to Adair and, therefore, cannot be relied upon to satisfy the requirements of § 135(b).

Second, Adair characterized these non-original pre-critical date claims as incorporating essential features of its "hierarchy of residues" protocol. Adair repeatedly emphasized that this protocol was the hallmark of its invention, and was the reason why its newly added claims were patentable over prior art. Adair also asserted that the newly presented claims, because they incorporated limitations adhering to its "protocol" of multiple required substitutions, cured a variety of maladies that plagued the patentability of Adair's original claims.

At page 6 of its involved specification, Adair stated (MF 7):

> We have further investigated the preparation of CDR-grafted humanized antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e., outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9).

In the amendment filed on January 19, 1993, responding to the enablement rejections, Adair again emphasized specific rules governing the "hierarchy of residues" set forth in the protocol in Adair's specification. Adair stated (MF 22):

> In contrast, the teaching in the present application can be applied without any undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9.
>
> …There is then no need to carryout computer modeling to determine which donor residues to substitute into the acceptor sequence. The protocol in the present application provides the teaching directly. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.
>
> …Thus, producing recombinant chains and testing them for affinity merely involves routine experimentation following a protocol which is clearly defined in the application. [Emphasis added].

After cancelling its original claims and presenting new claims in the January 1993 amendment, Adair made the following argument (MF 23):

> It is submitted that this identifies where the present invention makes a significant departure from the prior art. The prior art indicates that each antibody has to be treated individually. In contrast, the present invention teaches that, by following the protocol set forth in the present application, it is possible to reshape any antibody.

Adair also relied upon the "hierarchy of residues" to overcome prior art rejections. For example, in an amendment filed on April 7, 1993, Adair amended a claim reciting residues 71, 73 and 78 to distinguish over the anti-TAC antibody disclosed by Queen *et al.*. (MF 9 and 33):

> In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

In the April 1993 amendment, Adair again pointed to the substance of its protocol setting forth rules for substitutions to support its newly proposed claims. In particular, Adair explained

that, with respect to residues 23, 24, 49, 71, 73 and 78, there were only two alternatives of humanized antibodies being described in its specification; one involving changes at three residues in the heavy chain, and the other involving six residue changes (MF 9 and 34):

> It is stated on page 7, lines 1 to 5, that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative.

In other words Adair characterized the universe of choices for changes to residues 71, 73 and 78 to consist of two alternatives; all donor or all acceptor.

In responding to the Examiner's repeated enablement rejections, Adair made the following statements in an Amendment filed on February 7, 1994, in the '329 application (MF 35):

> It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann [sic] and Queen, at least. Thus, the skilled person would not rely on Reichmann [sic] and Queen as teachings relevant to whether the present description is enabling.
>
> It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims.

Consistent with these arguments, Adair's non-original pre-critical date claims recite positions that must all be donor residues. In contrast, the Carter '213 patent claims do not require that each recited position have a donor residue but, rather, allow for combinations of donor and acceptor residues at the recited positions. The Adair pre-critical claims are not directed to the same or substantially the same subject matter as the Carter '213 patent claims because the claims differ in material limitations. Accordingly, Adair cannot rely upon any of Adair's non-original pre-critical date claims for purposes of complying with the requirements of 35 U.S.C. § 135(b).

D. Adair Involved Claim 24 Differs In Material Limitations From Adair's Non-Original Pre-Critical Date Claims

Adair involved claim 24 is not entitled to the benefit of any pre-critical date claims because there are differences in material limitations in the pre-critical date claims that are not present in Adair involved claim 24.

As noted above, Adair cannot rely on its original PCT/U.S. claims because the claims are not patentable to Adair. (MF 11-20). Also as explained above, Adair's non-original pre-critical date claims are firmly grounded in the specific rules governing the "hierarchy of residues" to which Adair attributed the patentability of its claims. (MF 30). These claims emphasize the criticality of each recited position having a donor residue. Adair's specification and prosecution history also emphasize the criticality of adhering to these rules to render the claims patentable over the prior art such as the Riechmann *et al.* and Queen *et al.* references.

Adair involved claim 24 does not contain material limitations present in Adair's non-original pre-critical date claims. Rather, claim 24 allows for any possible donor/acceptor residue combination at positions 23, 24, 49, 71, 73 and 78. Claim 24 also allows for donor residues at any unrecited position in the heavy chain variable region. In other words, the very concepts and claim limitations upon which Adair relied to distinguish over prior art and to justify the enablement of its claims are absent in Adair claim 24.

IV. CONCLUSION

For the above-stated reasons, Adair involved claim 24 is barred under § 135(b) and adverse judgment should be entered against Adair.

Respectfully submitted,

May 28, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 1"** was filed this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel.: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

May 28, 2010 /Oliver R. Ashe, Jr./
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Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Appendix 1

EVIDENCE

I. Exhibits Cited

The following exhibits are cited in support of this motion:

Ex. 2001 U.S. Patent No. 6,407,213 to Carter *et al.*, issued June 18, 2002.

Ex. 2002 U.S. Patent Application No. 11/284,261 to Adair *et al*., filed November 21, 2005.

Ex. 2003 Preliminary Amendment and Request for Interference Under 37 C.F.R. § 42.202 [sic], filed November 21, 2005, in U.S. Patent Application No. 11/284,261 to Adair *et al*.

Ex. 2004 Request for Reconsideration, filed September 9, 2009, in U.S. Patent Application No. 11/284,261 to Adair *et al*.

Ex. 2005 PCT Application No. PCT/GB90/02017 to Adair *et al*., filed December 21, 1990, published as WO 91/09967 on July 11, 1991 ("the PCT Application").

Ex. 2006 U.S. Patent Application No. 07/743,329 to Adair *et al*., filed September 17, 1991.

Ex. 2007 Response to Office Action filed January 19, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2008 Amendment filed April 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2009 Letter regarding amendments filed September 9, 1993, in European Patent Application No. 91901433.2 to Adair *et al*.

Ex. 2010 Amendment filed February 7, 1994, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2011 Riechmann *et al.*, *Nature*, Vol. 332, pp. 323-327 (March 1988).

Ex. 2012 Response to Advisory Action filed May 9, 1994, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2013 Preliminary Amendment filed September 7, 1994, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2014 Amendment filed September 18, 1995, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2015 Preliminary Amendment filed August 23, 1996, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2016 Preliminary Amendment and Request for Interference Under 37 C.F.R. § 1.607 filed May 1, 1997, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2017 Response and Amendment filed August 20, 1997, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2018 Proposed claims filed January 28, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2019 Amendment Pursuant to 37 C.F.R. § 1.312 filed February 23, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2020 Amendment Pursuant to 37 C.F.R. § 1.312 filed July 13, 1998, in U.S. Patent Application No. 08/303,569 to Adair *et al*.

Ex. 2021 Allowed claims filed September 29, 1998, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2022 Fourth Preliminary Amendment filed November 5, 1998, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2023 Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 10029-10033 (December 1989).

Ex. 2024 U.S. Patent No. 5,859,205 to Adair *et al.*, issued January 12, 1999.

Ex. 2025 Amendment and Request for Reconsideration filed April 9, 1999, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2026 Amendment and Request for Reconsideration filed November 3, 1999, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2027 Amendment and Request for Reconsideration filed January 19, 2000, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2028 Office Action mailed September 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al.*

Ex. 2029 Request for Reconsideration filed August 29, 2000, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2031 Supplemental Amendment and Request for Reconsideration filed September 14, 2000, in U.S. Patent Application No. 08/846,658 to Adair *et al*.

Ex. 2033 Amendment and Request for Reconsideration filed November 12, 2001, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2034 Pending claims filed March 18, 2002, in U.S. Patent Application No.

08/485,686 to Adair *et al*.

Ex. 2035 Amendment and Request for Reconsideration filed August 9, 2002, in U.S. Patent Application No. 08/485,686 to Adair *et al*.

Ex. 2036 Great Britain Application No. 8928874.0 to Adair *et al*., filed December 21, 1989 ("the UK Application").

Ex. 2037 Computer generated comparison (using Workshare™ Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application.

Ex. 2038 Office Action mailed November 18, 1992, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2039 Examiner Interview Summary Record dated January 27, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

II. Papers Cited

The following papers are cited in support of this motion:

Paper No. 1 Declaration entered February 2, 2010.

Paper No. 5 Adair Clean Copy of Claims filed February 16, 2010.

Paper No. 20 Adair Motions List filed April 9, 2010.

III. Appendices Cited

The following papers are cited in support of this motion:

Appendix 1 Evidence.

Appendix 2 Statement of Material Facts Relied Upon in Motion.

Appendix 3	Claim chart comparing Adair claim 24 presented in 2005 and Adair involved claim 24.
Appendix 4	Claim chart comparing Adair original PCT claims 8 and 16 with Adair involved claim 24.

Appendix 2

STATEMENT OF MATERIAL FACTS RELIED UPON IN MOTION

1. On December 21, 1989, Adair filed Great Britain Application No. 8928874.0 ("Adair UK Application"). (Ex. 2036).

2. On December 21, 1990, Adair filed PCT/GB90/02017("Adair's PCT application"), which contained claims 1-23. (Ex. 2005, pp. 67-70).

3. Exhibit 2037 is a computer generated comparison (using Workshare™ Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application. The last page of Exhibit 2037 contains a color-coded legend for identifying deletions, additions, and movement of text.

4. The PCT Application contains a section titled "Protocol" that is not contained in the UK Application. (Ex. 2005, pp. 16-19; Ex. 2036; and Ex. 2037, pp. 10-11).

5. On September 17, 1991, Adair entered the U.S. national stage by filing U.S. Patent Application No. 07/743,329 ("the '329 application"), claiming benefit to Adair's PCT application. (Ex. 2006).

6. Adair's U.S. '329 application contained claims 1-23, which are identical to claims 1-23 as originally filed with Adair's PCT application. (Ex. 2005, pp. 67-70 and Ex. 2006, pp. 67-70).

7. At page 6 of its involved specification, Adair stated:

> We have further investigated the preparation of CDR-grafted humanized antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e., outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining

satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen et al (9). [Ex. 2002, p. 6, lns. 15-28].

8. At page 6, lines 31-37, the Adair specification reads as follows:

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91. [Ex. 2002, p. 6, lns. 31-37].

9. At page 7, lines 1-5, the Adair specification reads as follows:

> In preferred embodiments, the heavy chain framework comprises donor residues at positions 23, 24, 49, 71, 73 and 78 or at positions 23, 24 and 49. The residues at positions 71, 73 and 78 of the heavy chain framework are preferably either all acceptor or all donor residues. [Ex. 2002, p. 7, lns. 1-5].

10. At page 17, lines 27-30, the involved Adair specification reads as follows under a section titled "Protocol":

> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor). [Ex. 2002, p. 17, lns. 27-30].

11. On November 18, 1992, the U.S. Patent and Trademark Office ("the USPTO") entered a non-final office action rejecting claims Adair's claims 1-23 on several statutory grounds. (Ex. 2038).

12. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-12, 17 and 22-23 under 35 U.S.C. § 101 for lack of utility. (Ex. 2038, pp. 1-3).

13. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-16 and 22-23 under 35 U.S.C. § 112, first paragraph, for failing to adequately teach how to make and use the claimed invention. (Ex. 2038, pp. 3-6).

14. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-23 were rejected under 35 U.S.C. § 112, second paragraph, for failing to particularly point out and distinctly claim the subject matter which Adair regarded as its invention. (Ex. 2038, pp. 6-7).

15. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1, 5, 6-8, and 12-22 under 35 U.S.C. § 102(b) as being anticipated by Riechmann *et al.*, *Nature*, Vol. 332, pp. 323-327 (March 1988). (Ex. 2038, pp. 7-9 and Ex. 2011).

16. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-6 and 12-22 under 35 U.S.C. § 102(b) as being anticipated by Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 10029-10033 (December 1989). (Ex. 2038, pp. 9-10 and Ex. 2023).

17. On November 18, 1992, the USPTO rejected the original Adair '329 claims 1-21 under 35 U.S.C. § 103 as being obvious over Riechmann *et al.* and Queen *et al.*. (Ex. 2038, pp. 10-12).

18. At pages 3-6 of the November 1992 office action, the Examiner rejected claims 1-16 and 22-23 for lack of enablement under § 112, first paragraph, on the grounds that, *inter alia*, the specification did not support making the range of residue changes recited in the claims and that the effects of the residue changes as described in Adair's original claims could not readily be predicted. (Ex. 2038, pp. 3-6).

19. At page 6 of the November 1992 office action, the Examiner rejected claims 1-5 under 35 U.S.C. § 112, second paragraph, as being indefinite in their recitation of "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" because it was unclear to the Examiner whether the heavy chain,

a. had at least one of 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91, or alternatively,

b. had at least one of (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91), or alternatively,

c. had at least one (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and 76 and/or (78 and 88) and/or (91). (Ex. 2038, p. 6).

20. At page 9 of the November 1992 office action, the Examiner rejected Adair's claims in view of Riechmann *et al.*, noting that the Examiner interpreted the claims to mean that the framework has donor residues at at least one of any of positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain. (Ex. 2038, p. 9).

21. In a January 19, 1993 amendment, Adair responded to the November 1992 Office action by cancelling original claims 1-20, 22 and 23, amending original claim 21, and adding new claims 24-66. (Ex. 2007, pp. 1-13).

22. In the January 1993 amendment, Adair stated the following:

> In contrast, the teaching in the present application can be applied without any undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9. …
>
> There is then no need to carryout computer modeling to determine which donor residues to substitute in to the acceptor sequence. <u>The protocol in the present application provides the teaching directly</u>. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.
>
> …Thus, producing recombinant chains and testing them for affinity merely <u>involves routine experimentation following a protocol which is clearly defined in the application</u>. [Ex. 2007, pp. 26-27; Emphasis added].

23. In the January 1993 amendment, Adair stated the following:

> It is submitted that this identifies where the present invention makes a significant departure from the prior art. The prior art indicates that each antibody has to be treated individually. In contrast, the present invention teaches that, by following the protocol set forth in the present application, it is possible to reshape any antibody. [Ex. 2007, p. 28].

24. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 112, second paragraph, by cancelling claims 1-12. (Ex. 2007, pp. 29-32).

25. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Riechmann *et al.* as follows:

> In Part A of this rejection, claims 1, 5, 6-8, and 12-22 were rejected as anticipated by Riechmann et al. The Examiner stated that claim 1 and claim 6 were interpreted to mean that the framework has donor residues in at least one of any of positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 in the heavy chain and (1, 3, 46, or 47) or 46, 48, 58, or 71) in the light chain, and thus, the teachings of Riechmann et al. anticipate the invention as claimed.
>
> The Examiner contends that the original claims lacked novelty over Riechmann et al. Claims 1, 5, 6-8, 12 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.
>
> In present claims 24 and 25, it is specified that residues 23 and 24 in the heavy chain should be donor residues. However, as can be seen from Fig. 1, panel (a) in Riechmann et al., in the recombinant antibody shown there, residues 23 and 24 are acceptor residues. [Ex. 2007, p. 32-33].

26. In the January 1993 amendment, Adair responded to the rejection of claims under 35 U.S.C. § 102(b) in view of Queen *et al.* as follows:

> In Part B of the rejection, the Examiner rejected claims 1-6 and 12-22 as anticipated by Queen et al.
>
> Claims 1-6, 12-20 and 22 have been cancelled without prejudice and submitted as new claims that more distinctly point out certain aspects of the present invention.
>
> In present claims 24 and 25, it is specified that residues 48, 66, 67, 68, 93, 103 to 108 and 110 should all be acceptor residues. However, in Queen et al., as can be seen from Fig. 2B, in these positions Queen et al. uses donor, rather than acceptor, residues. It should again be borne in mind that Queen et al. does <u>not</u> use the Kabat numbering and it is therefore necessary to look carefully at the disclosure in Queen et al. before it is possible to come to any final conclusion. [Emphasis by Adair].

> In present claim 38, it is specified that residue 71 should be a donor residue. However, as can be seen from Fig. 2A of Queen et al., in that position Queen et al. uses an acceptor, rather than a donor residue.
>
> Applicants' claimed antigen-binding molecules are thus not anticipated by Queen et al. Withdrawal of this entire 35 USC § 102 (b) rejection is respectfully requested. [Ex. 2007, pp. 33-34].

27. An Examiner Interview Summary Record dated January 27, 1993, states "applicant suggests that the 'comprising' in eg clm 24 is not to be taken as 'comprising' more residues than those in clm, i.e. claimed residues are not to be considered open ended. Applicant indicated they would clarify the latter issue. Queen does not teach changing residues: 73HC; 38HC; 71 on LC # 1 on LC + #4 on LC, 36 on LC 46 on LC." (Ex. 2039, p. 4; Emphasis by Examiner).

28. On September 9, 1993, in the Adair PCT/EP Patent Application 91901433.2, Adair filed an amendment deleting original claims 1-23 and replacing them with new claims 1-20 and made the following statements:

> 2.10. In new claim 1, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that new claim 1 is novel over the anti-TAC antibody disclosed in PNAS-USA, 86, 10029-10033, 1989 (Queen) (cited in the International Search Report). This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2009, p. 3].

29. On February 7, 1994, Adair filed an amendment in the '329 application responding to an office action mailed on September 7, 1993 **(**Ex. 2028**)**, wherein Adair made the following statements:

> At a very helpful interview held at the beginning of 1993, there was some discussion of the word "comprising" as used in the claims under consideration at that time. In those claims, it was only specified that certain residues should be donor residues. [Emphasis by Adair]. It was considered that it was not clear whether these were the only residues which could be donor residues. The

alternative view was that these were only the minimum number of residues which must be donor but that any of the other residues could also be donor.

If the second line of interpretation were taken, the claims could be read to cover a situation in which all except one of the residues in the variable domain were donor residues. [Emphasis by Adair]. In this case, the claims could then be interpreted to cover a structure similar to a "chimeric" antibody comprising a donor variable domain and a human constant region. Such chimeric antibodies were already well known at the priority date.

It plainly is not the intention of the Applicants to claim chimeric antibodies or any similar structures. As can be seen from the description, the superhumanised antibodies of the present invention are compared to the prior art chimeric antibodies. Moreover, the present invention was intended to deal with the problem of chimeric antibodies in that chimeric antibodies were believed to be too "foreign" because of the presence of the complete donor variable domain.

For the above reasons, it is clear that the wording of the claims needed to be changed so that the Applicants' intention of excluding chimeric antibodies was made effective. The language now present in the claims puts this intention clearly into effect.

As to support for this wording, the Examiner is referred firstly to page 16, under the heading "Protocol". It can be seen from this paragraph that the first step in the process involves the choice of an appropriate acceptor chain variable domain. This acceptor domain must be of known sequence. Thus, the protocol starts with a variable domain in which all the residues are acceptor residues. In the sentence bridging pages 16 and 17, it is stated that:

> "The CDR-grafted chain is then designed starting from the basis of the acceptor sequence". [Emphasis by Adair].

On page 17, in the middle paragraph, it is stated that:

> "The positions at which donor residues are to be substituted for acceptor in the framework are then chosen as follows ...."

This again shows that, unless a residue is chosen for substitution, it will remain as in the acceptor sequence.

It must also be borne in mind that the purpose of the invention is to obviate some of the disadvantages of prior art proposals. The proposal of using chimeric antibodies had the disadvantage that they were more "foreign" than desirable. The problem of making CDR-grafted antibodies was that they generally did not provide good recovery of affinity. Thus, the aim of the present

invention was to minimise as far as possible the "foreign" nature of the antibody while maximising as far as possible its affinity.

Bearing the passages referred to above and the aim of the invention in mind, it would have been abundantly clear to the skilled person reading the application that as many residues as possible should remain as acceptor residues. If this were not the case, it could hardly be said that the composite chain is based on the acceptor sequence.

The skilled person reading the application can plainly see that certain residues have been considered for changing from acceptor to donor. These are clearly set out in the description. It would be plain to the skilled person that all other residues should not be considered for changing at all. It would therefore be obvious that any residue which is not specified as being under consideration for changing must remain as in the acceptor chain.

It may be that there is no explicit statement in the description that the specified residues should remain as in the acceptor chain. However, the disclosure in a specification is not limited to the explicit disclosure but also includes that which is implicit. It is implicit, in the recitation that the chain is based on the acceptor and that only certain residues are considered for changing, that all non-specified residues must remain as acceptor residues. Subject matter which might be fairly deduced from the disclosure is not new matter. *Acme Highway Products Corp. v. D.S. Brown Co.*, 431 F.2d 1074, 1080, 167 U.S.P.Q. 129, 132-133(6th Cir. 1970), *cert denied*, 401 U.S. 956 (1971).

Another way to look at it is to consider a different way in which the claim could be drafted. It could be specified that in the composite chain, at least a certain minimum number of residues are donor residues (as in the present claims) and at most a certain maximum number of residues are donor residues. The maximum number would be derived by listing all the residues which are considered for changing. Such an amendment would have clear explicit basis in the description because all those residues are mentioned as such. However, the effect of such an amendment would be to produce claims of exactly the same scope as the present claims. It can thus be seen that the present claims do not add subject matter but are plainly properly based on the disclosure in the description.

It is therefore submitted that the claims are fully supported by the description, are commensurate in scope with the disclosure in the description, and are properly delimited over the prior art. [Ex. 2010, pp. 3-7].

30. Adair's non-original pre-critical date claims are grounded in the specific rules governing the "hierarchy of residues" to which Adair attributed the patentability of its claims. (Ex. 2007-2010, 2012-2022, 2024-2027, 2029, 2031, and 2031-2035).

31. Appendix 3 is a claim chart comparing Adair claim 24 as originally filed in 2005 and Adair involved claim 24.

32. Appendix 4 is an accurate comparison of Adair original PCT claims 8 and 16 to Adair involved claim 24.

33. In an amendment filed on April 7, 1993, Adair amended a claim reciting residues 71, 73 and 78, stating the following:

> In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor. [Ex. 2008, p. 14].

34. In the April 1993 amendment, Adair stated the following:

> It is stated on page 7, lines 1 to 5, that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative. [Ex. 2008, p. 15].

35. In an Amendment filed on February 7, 1994, in the '329 application, Adair stated the following:

> It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann [sic] and Queen, at least. Thus, the skilled person would not rely on Reichmann [sic] and Queen as teachings relevant to whether the present description is enabling.
>
> It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims. [Ex. 2010, pp. 11-12].

36. Carter's involved U.S. Patent No. 6,407,213 ("the '213 patent") issued on June 18, 2002. (Ex. 2001).

37. One year from the date on which the Carter '213 patent issued is June 18, 2003. (Ex. 2001).

38. On November 21, 2005, Adair filed its involved application 11/284,261 ("the 261 application"). (Ex. 2002).

39. On November 21, 2005, Adair presented new claim 24 as follows:

> Claim 24 (new) A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat. [Ex. 2003, p. 3].

40. On September 9, 2009, Adair presented its involved claim 24 in the '261 application, which reads as follows:

> Claim 24 (currently amended): A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat. [Ex. 2004, p. 2; Adair Clean Copy of Claims, Paper No. 5, p. 4].

41. None of Adair's pre-critical date claims is identical to a Carter '213 patent claim. (Ex.2001, 2005-2010, 2012-2022, 2024-2027, 2029, 2031, and 2031-2035).

42. None of Adair's pre-critical date claims is identical to Adair's involved claim 24. (Ex. 2005-2010, 2012-2022, 2024-2027, 2029, 2031, and 2031-2035 and Adair Clean Copy Of Claims, Paper No. 5, p. 4).

43. Concurrent with the filing of the '261 application, Adair filed a "Preliminary Amendment and Request for Interference under 37 CFR § 42.202 [sic]." (Ex. 2003).

44. On page 4 of the 2005 amendment, Adair stated the following:

> **(b) Compliance with 35 USC § 135(b)**
> Although the present rules do not require a showing of compliance under 35 USC § 135(b), Applicants submit the following to advance the examination of the present application to allowability. […] Claims 1-23 as filed in the PCT application are attached as Appendix A.
>
> Under 35 USC § 135(b)(l), Applicants must show that they had a claim to the same, or substantially the same, subject matter as a claim of the 213 patent within one year of the issuance of the 213 patent, or June 18, 2003. The 213 patent issued on June 18, 2002. The PCT application was filed on December 21, 1990, over 10 years earlier than the 213 patent issued. The time limit of Section 135(b)(l) has been complied with fully. See *Corbett v. Chisholm*, 196 USPQ 337 (CCPA 1977).
>
> To meet the "same or substantially the same invention" requirement of Section 135(b)(l), Applicants must show that their claim contained all material limitations, i.e. limitations necessary to patentability, of the claim of the 213 patent alleged to be to the same, or substantially the same, invention. *Corbett v. Chisholm*, 196 USPQ 337 (C.C.P.A. 1977), *citing Wetmore v. Miller*, 477 F.2d 960, 177 USPQ 699 (C.C.P.A. 19730).
>
> As is evident from Appendix A, Applicants made a claim for the same, or substantially the same, subject matter as a claim of the 213 patent well before the issuance of the 213 patent. Claim 16 of the PCT application, as depending from claim 8, is to substantially the same subject matter as at least claim 1 of the 213 patent. For the Office's convenience, all three claims are duplicated below.
>
> > **Claim 8 of the PCT application**: A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, **58** and 71.
> >
> > **Claim 16 of the PCT application**: A CDR-grafted antibody heavy or **light chain** or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.
> >
> > **Claim 1 of the 213 patent**: A humanized antibody variable domain comprising non-human Complementarity Determining

Region (CDR) amino acid residues which bind an antigen incorporated into a human antibody variable domain, and further comprising a Framework Region (FR) amino acid substitution at a site selected from the group consisting of: 4L, 38L, 43L, 44L, **58L**, 62L, 65L, 66L, 67L, 68L, 69L, 73L, 85L, 98L, 2H, 4H, 36H, 39H, 43H, 45H, 69H, 70H, 74H, and 92H, utilizing the numbering system set forth in Kabat. [Ex. 2003, pp. 4-6; Emphasis by Adair].

Appendix 3

CLAIM CHART COMPARING ADAIR CLAIM 24 PRESENTED IN 2005 AND ADAIR INVOLVED CLAIM 24

Adair Claim 24 Presented in 2005	Adair Involved Claim 24
A humanised antibody heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises an amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.	A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Appendix 4

CLAIM CHART COMPARING
ADAIR ORIGINAL PCT CLAIMS 8 AND 16 WITH ADAIR INVOLVED CLAIM 24

Claims 8 and 16 of Adair PCT/GB90/02017	Involved Claim 24 of Adair 11/284,261
8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58, and 71. 16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.	A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Paper No. _____

Filed on behalf of: Party Carter

Filed: May 28, 2010

By: Oliver R. Ashe, Jr., Esq.
ASHE, P.C.
11440 Isaac Newton Sq. North
Suite 210
Reston, VA 20190
Tel.: (703) 467-9001
Fax: (703) 467-9002
E-mail: oashe@ashepc.com

Jeffrey P. Kushan, Esq.
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
Tel.: (202) 736-8914
Fax: (202) 736-8711
E-mail: jkushan@sidley.com

UNITED STATES PATENT AND TRADEMARK OFFICE

_____

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

_____

PAUL J. **CARTER** AND LEONARD G. PRESTA
Junior Party
(Patent 6,407,213),

v.

JOHN ROBERT **ADAIR**, DILJEET SINGH ATHWAL,
AND JOHN SPENCER EMTAGE
Senior Party
(Application No. 11/284,261),

_____

Patent Interference 105,744 (SGL)
Technology Center 1600

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CARTER SUBSTANTIVE MOTION 2
(Adair Claim 24 Lacks Written Description)

Table of Contents

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CARTER SUBSTANTIVE MOTION 2

I. PRECISE RELIEF REQUESTED

Carter moves under 37 C.F.R. § 41.121(a)(1)(iii) for judgment that involved claim 24 of Adair's U.S. Patent Application No. 11/284,261 ("the '261 application") is not patentable to Adair under 35 U.S.C. § 112, first paragraph, for lack of written description.

II. THE EVIDENCE AND STATEMENT OF MATERIAL FACTS

A list of exhibits, papers, and appendices relied upon in support of this motion is set forth in Appendix 1. A statement of material facts relied upon in support of this motion is set forth in Appendix 2.

III. ARGUMENT

A. Overview

Adair involved claim 24 recites a human heavy chain framework region that "…comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof…." (MF 37). Giving claim 24 its broadest reasonable construction, it encompasses a human heavy chain framework polypeptide having a single non-human amino acid residue at any of positions 23, 24, 49, 71, 73 or 78. Conversely, claim 24 encompasses human heavy chain framework region polypeptides having non-human residue substitutions within the human framework sequence at any number of locations, up to six, and in any combination. There is no description in Adair's specification of a human heavy chain framework region sequence as defined by claim 24.

The Adair specification recites a set of minimum requirements for its modified human heavy chain framework region polypeptides. These requirements are set forth at pages 16-19 of the specification in a detailed "Protocol" for producing humanized heavy chain framework region

polypeptides. (MF. 12; Ex. 2002). Step 1 of the protocol instructs one to insert all of the donor complementarity determining region ("CDR") sequences into the heavy and light human framework regions. See, *Id.* at 17, lines 7-22. Step 2 then sets forth rules for "the positions at which residues are to be substituted for acceptor in the framework." *Id.* at 17. The specification states (MF 13):

> 2. Heavy Chain
> 2.1 Choose donor residues at all of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or all of positions 23, 24 and 49 (71, 73 and 78 are always either all donor or all acceptor). [Emphasis added].

That these substitutions are mandatory is demonstrated by the next rule in the Adair protocol, which uses different language to indicate optional substitutions. Specifically, Rule 2.2 states (MF 14):

> 2.2. Check that the following have the same amino acid in donor and acceptor sequences, and if not preferably choose the donor: 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107. [Emphasis added].

Thus, Adair expressly teaches that positions 23, 24 and 49 in the human heavy chain framework region must always be donor (*e.g.*, non-human) residues, and following the Adair rules yields only two possible sets of changes to the heavy chain framework region:

(i) replacement of residues 23, 24 and 49 of the human sequence with donor residues (*i.e.*, where residues 71, 73 and 78 are "all acceptor"), or

(ii) replacement of residues 23, 24, 49, 71, 73 and 78 of the human sequence with donor residues (*i.e.*, where 71, 73 and 78 are "all donor").

Therefore, the Adair specification does not provide written description for a claim encompassing acceptor/residues at any of positions 23, 24 and 49, as allowed by Adair claim 24.

Adair also does not describe human heavy chain framework substitutions that involve various combinations of substitutions at positions 23, 24, 49, 71, 73 and 78, as recited in Adair

claim 24. As noted above, the Adair specification requires all of residues 23, 24 and 49 to be donor residues and that positions 71, 73 and 78 must be either all donor (*i.e.*, non-human) residues or all acceptor (*i.e.*, human) residues. (MF 13). Therefore, the Adair specification does not provide written description for a claim encompassing acceptor/donor combinations at any of positions 23, 24, 49 71, 73 and 78, as recited in Adair claim 24.

Other portions of the Adair specification reinforce, rather than relax, these rules. For example, at pages 19-23, Adair offers a "rationale" for its protocol. (MF 15). For surface area residues near the CDR regions, the specification at page 20, line 27, states "Heavy Chain - Key residues are 23, 71 and 73." (MF 16). For the "packing residues near the CDRs," the specification at page 21, line 9, states "Heavy Chain - Key residues are 24, 49 and 78." (MF 17). In other words, the "key" residues to which Adair refers are residues 23, 24, 49, 71, 73 and 78 – the same set of residues governed by specific substitution rules set forth in Adair specification.

Adair's rules and rationale are further reinforced by Adair's observations in its working examples. For example, the Adair specification states: "the presence of the 6, 23 and 24 changes are important to maintain a binding affinity similar to that of the murine antibody." (MF 18). Then, at page 52, lines 25-29, the specification states (MF 19):

> These and other results lead us to the conclusion that of the 11 mouse framework residues used in the gH341A (JA185) construct, it is important to retain mouse residues at all of positions 6, 23, 24, 48 and 49, and possibly for maximum binding affinity at 71, 73 and 78.

The fact that some of the express rules governing substitution of residues 23, 24, 49, 71, 73 and 78 are characterized as "preferred" embodiments does not alter the conclusion that involved claim 24 lacks adequate written description under 35 U.S.C. § 112. First, there is no other disclosure in the Adair specification that describes changes to the specific group of residues recited in claim 24 other than the passages that set forth and reinforce the Adair protocol.

Second, Adair has repeatedly emphasized the necessity of following the express rules governing residues 23, 24, 49, 71, 73 and 78 in distinguishing over prior art references and in response to enablement rejections. (MF 20-33). Moreover, each of these express rules was newly added to the Adair disclosure when Adair filed its PCT benefit application PCT/GB90/02017 ("the PCT application"), at which time Adair also added disclosure to the PCT application acknowledging "recent" developments in the prior art. (MF 3 and 7).

Thus, viewing the involved Adair specification as a whole, claim 24 is unpatentable to Adair because it lacks adequate written description under 35 U.S.C. § 112.

In Carter Substantive Motion 1, Carter moves for judgment that Adair's involved claim 24 is unpatentable for failure to comply with the requirements of 35 U.S.C. § 135(b)(1). However, regardless of whether Adair claim 24 satisfies the written description requirement, Adair claim 24 is barred under § 135(b) and adverse judgment should be entered against Adair.

B. <u>Adair Cannot Rely On Involved Claim 24 Itself For Written Description</u>

Adair presented its first version of involved claim 24 in a preliminary amendment at the time of filing the '261 application (*i.e.*, November 21, 2005).[1] (MF 35-36). Thus, while Adair's involved '261 application is described as being a continuation of U.S. Patent Application No. 08/846,658 ("the '658 application"), claim 24 is not part of the disclosure of the '658 application. Thus, claim 24 itself cannot serve as a basis for satisfying the written description requirement. *Turbocare Division of Demag Delaval Turbomachinary Corporation v. General Electric Company*, 264 F.3d 1111, 1120, 60 U.S.P.Q.2d 1017, 1024 (Fed. Cir. 2001). See also 37 C.F.R.

[1] Adair thereafter amended claim 24. Adair's involved claim 24 was presented on September 9, 2009. (MF 37). A comparison of the 2005 version of claim 24 and the 2009 version of involved claim 24 is provided in Appendix 3.

§ 1.63(d)(1)(iii), which requires that "[t]he specification and drawings filed in the continuation or divisional application contain no matter that would have been new matter in the prior application."[2] Accordingly, any written description for claim 24 must be found elsewhere in the involved Adair '261 specification.

C. Adair Involved Claim 24 - Claim Construction

Adair involved claim 24 reads as follows (MF 37):

> A humanised antibody comprising a heavy chain variable domain comprising non-human complementarity determining region amino acid residues which bind an antigen and a human framework region wherein said framework region comprises a non-human amino acid substitution at a residue selected from the group consisting of 23, 24, 49, 71, 73, and 78, and combinations thereof, as numbered according to Kabat.

Given its broadest reasonable interpretation, claim 24 encompasses, *inter alia*, a framework region comprising a non-human amino acid substitution at <u>any one</u> of the human framework residues 23, 24, 49, 71, 73, and 78, and <u>any combination of these recited residues</u>. (MF 39-42). Claim 24 thus encompasses a multitude of different combinations of non-human and human amino acid residues at the recited positions, including:

- <u>non-human</u> (donor) amino acids at positions 71, 73 and 78 and <u>human</u> (acceptor) amino acids at positions 23, 24, and 49 (MF 40);
- <u>non-human</u> (donor) amino acids at positions 23 and 71 and <u>human</u> (acceptor) amino acids at positions 24, 49, 73 and 78 (MF 41); or

[2] Adair did not present a newly executed declaration at the time of filing the '261 application but, rather, relied on the inventor declaration from the parent application to purportedly satisfy the requirements of 37 C.F.R. § 1.63. (MF 34).

- non-human (donor) amino acids at position 23 and human (acceptor) amino acids at positions 24, 49, 71, 73 and 78 (MF 42).

However, as explained below, the express teachings of the involved Adair specification and positions advanced by Adair in *ex parte* prosecution clearly establish that none of the above-identified combinations (as well as many other combinations) within the scope of Adair claim 24 is described, literally or otherwise, by the involved Adair specification. Accordingly, claim 24 is unpatentable to Adair under 35 U.S.C. § 112, first paragraph, for lack of written description.

D. Adair Disclosures Relevant To Recited Residues 23, 24, 49, 71, 73, 78

1. The General Teachings Of The Adair Specification Are Ambiguous

The involved Adair specification contains two passages that, if read alone or in combination without reference to the remainder of the Adair specification, lead to a myriad of possible interpretations. First, the Abstract of Adair's specification reads, in part (MF 9):

> CDR-grafted antibody heavy and light chains comprise acceptor framework and donor antigen binding regions, the heavy chains comprising donor residues at at least one of positions (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and/or (76) and/or (78) and (88) and/or (91).

Second, page 6, lines 31-37, of the Adair specification reads (MF 10):

> Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

While both of these passages contain the same words and residue numbers, there is considerable ambiguity as to the meaning and scope of each passage, alone and in combination.

- In instances in the Abstract where two positions are set forth in parentheses (*e.g.,* "(6, 23)," "(24, 48)," *etc.*), it is unclear whether one or both positions must be donor residues.

- It is not clear whether the passage at page 6, which does not contain parenthetical descriptions, is intended to describe a different concept than what is described in the Abstract.
- With respect to both passages, the use of the term "and/or" appears to suggest that changes at each one of the series may be required (*i.e.*, 6 + (23 and/or 24) + (48 and/or 49), *etc.*). The alternative reading of this passage would render the term "and/or" superfluous (*i.e.*, to mean any of 6, 23, 24, 49, *etc*.). This reading also ignores the plural reference to "residues" in both passages (*i.e.*, "… comprising donor residues at…").

The Examiner struggled with each of these issues when attempting to interpret the same language when it was presented as part of Adair's original U.S. claims. In particular, original claim 1 of Adair's U.S. Patent Application 07/743,329 ("the '329 application") tracked the text in the Summary of Invention section and read as follows (MF 4 and 6):

> 1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

The Examiner rejected claim 1 (among others) under 35 U.S.C. § 112, second paragraph, characterizing as indefinite the recitation "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." (MF 4 and 22). In particular, the Examiner stated it was unclear whether the heavy chain,

a. had at least one of 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91, or alternatively,

b. had at least one of (6) or (23 and/or 24) or (48 and/or 49) or (71 and/or 73) or (75 and/or 76 and/or 78 and 88 and/or 91), or alternatively,

c. had at least one of (6, 23) and/or (24, 48) and/or (49, 71) and/or (73, 75) and 76 and/or (78 and 88) and/or (91).

In response, Adair did not challenge the Examiner's conclusions but, rather, cancelled claim 1 and replaced it with claims reciting specific positions in the framework region that were required to be donor residues. (MF 25).

2. Additional Teachings In The Adair Specification

The broad and ambiguous passages in the Abstract and at page 6 of the Adair specification stand in sharp contrast to the way heavy chain substitutions are described in the remainder of the Adair specification.

The first clear example of Adair's description of its invention appears immediately after the ambiguous language on page 6. Specifically, at page 7, lines 1-5, Adair describes as a "preferred" embodiment a heavy chain framework region which contains donor residues at positions 23, 24, 49, 71, 73 and 78 or alternatively, at positions 23, 24 and 49. (MF 11). Adair explains at this location in the specification that substitutions at positions 71, 73, and 78 must be either all acceptor or all donor residues, while the residues at positions 23, 24, and 49 must always be donor residues. (MF 11).

At page 16, line 30 to page 19, line 9, Adair describes this "preferred protocol" for obtaining CDR-grafted antibodies. (MF 12). For heavy chains, Adair's protocol provides the following instruction: "Choose donor residues at <u>all</u> of positions 23, 24, 49, 71, 73 and 78 of the heavy chain or <u>all</u> of positions 23, 24 and 49 (71, 73 and 78 are always either <u>all donor</u> or <u>all acceptor</u>). (Emphasis added; MF 13)." Again, the Adair disclosure does not describe unrestricted combinations of acceptor/donor residues at any of the positions 23, 24, 49, 71, 73 and 78, as recited in Adair involved claim 24. Rather, Adair teaches two minimum <u>sets</u> of

residue substitutions are required. The first set must consist of donor residues at positions 23, 24 and 49. The second set concerns residues 71, 73 and 78. For this second set, two options are allowed; either 71, 73 and 78 are all from the donor antibody, or 71, 73 and 78 are all from the acceptor (*i.e.*, the human framework region).

Adair's description thus affirmatively rules out modifications to the human framework region that would involve inserting one donor residue at any of positions 23, 24, 49, 71, 73 or 78 where all other residues at these positions are acceptor (*e.g.*, human residues). It also rules out substitutions involving combinations of two, four or five donor residues at these positions. Indeed, it rules out any substitution involving less than 3 residues (*i.e.*, to insert donor residues at positions 23, 24 and 49 where all of the residues at positions 71, 73 and 78 are acceptor), and, in the alternative, permits only one other set of substitutions involving 6 residues (*i.e.*, residues 23, 24, 49 plus the scenario where 71, 73 and 78 are all donor residues).

These additional teachings make clear that Adair did not consider its invention to be any possible combination of acceptor/donor residues at positions 6, 23, 24, 48, 49, 71, 73, 75, 76, 78, 88, or 91 much less unrestricted combinations of acceptor/donor residues at positions 23, 24, 49, 71, 73 and 78, as presently recited in Adair claim 24. Thus, Adair involved claim 24 literally conflicts with Adair's written description, and, therefore, is not patentable to Adair.

E. Prosecution History Regarding Residues 23, 24, 49, 71, 73, 78

As discussed above, the Adair specification contains broad and ambiguous disclosures regarding acceptor/donor residue substitutions, followed by a more detailed description of mandatory rules regarding specific acceptor/donor residue combinations. Although Adair uses the terminology "preferred" in the context of the setting forth these rules, both the Adair specification and the subsequent prosecution history show that Adair believed following these

rules was necessary both to distinguish its invention from prior publications of other scientists and to establish support for its claims.

For example, at pages 4-6 of the specification, Adair provides a discussion of "recent" disclosures by Queen *et al.* relating to CDR-grafted antibodies and the substitution of acceptor framework residues with donor residues. (MF 7). At page 6, lines 22-28, the Adair specification states: "This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance does not coincide with the residues identified by Queen…." (Emphasis added; MF 8).

In an amendment filed on January 19, 1993, in the '329 application, Adair relied upon the recitation of donor residues at positions 23 and 24 as grounds to distinguish over Riechmann *et al*., which disclosed acceptor residues at positions 23 and 24. (MF 26). In the same amendment, Adair argued its claims included limitations that distinguished the claimed antibody chains over the humanized antibody disclosed in Queen *et al*. (MF 27).

In the January 1993 amendment, Adair also relied on its "protocol" to respond to enablement rejections that had been imposed on claims that recited, *inter alia*, changes to residues 71, 73 and 78 of the heavy chain. Adair stated (MF 21 and 28):

> In contrast, the teaching in the present application can be applied without any undue experimentation to any antibody. All that is required is experimentation following a protocol which is clearly set out in the description, in particular at page 16, line 30 to page 19, line 9. …
>
> There is then no need to carryout computer modeling to determine which donor residues to substitute in to the acceptor sequence. The protocol in the present application provides the teaching directly. It instructs the skilled person to compare the two sequences and change certain specified residues in the acceptor sequence to donor residues.

> …Thus, producing recombinant chains and testing them for affinity merely involves routine experimentation following a protocol which is clearly defined in the application. [Emphasis added].

In an amendment filed on April 7, 1993, Adair amended a claim reciting residues 71, 73 and 78, stating the following (MF 30):

> In claim 67, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that claim 67 is novel over the anti-TAC antibody disclosed by Queen. This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

In the April 1993 amendment, Adair again pointed to its protocol setting forth rules for substitutions to support its newly proposed claims. In particular, Adair explained that its rules specified only two alternatives for substitutions within the set of residues consisting of residues 23, 24, 49, 71, 73 and 78 (MF 30):

> It is stated on page 7, lines 1 to 5, that residues 71, 73 and 78 should all be either acceptor or donor. Claims 73, 80, 87, 94 and 101 cover the first alternative and claims 74, 81, 88, 95 and 102 cover the second alternative. [Emphasis added].

As Adair stated, the claims defining Adair's first alternative (*i.e.*, claims 73, 80, 87, 94 and 101) require donor residues to be inserted only at positions 23, 24 and 49, while the claims defining its second alternative require donor residues to be included at positions 23, 24, 49, 71, 73 and 78. Thus, Adair represented to the PTO that its specification defined two alternatives as to changes to the heavy chain: one involving insertion of donor residues at three locations (*i.e.*, where 71, 73 and 78 are all acceptor), and the other involving insertion of donor residues at six locations (*i.e.*, where 71, 73 and 78 are all donor). (MF 30).

It is noted that Adair's arguments often use terms such as "in general" and "can be" and "should be" when drawing substance from the rules set forth in the Adair specification. Such

attorney argument is not consistent with the express teachings of the Adair specification and is not a substitute for written description in an originally filed application.

In an amendment filed on September 9, 1993, (*i.e.*, after the rejection of its original U.S. claims), Adair cancelled its original claims in Adair's PCT benefit application/EP Patent Application No. 91901433.2 and submitted a substitute set of claims, stating (MF 5 and 31):

> 2.10. In new claim 1, it has been specified that residues 71, 73 and 78 are all donor residues in order to ensure that new claim 1 is novel over the anti-TAC antibody disclosed in PNAS-USA, 86, 10029-10033, 1989 (Queen) (cited in the International Search Report). This anti-TAC antibody has an acceptor residue at residue 73. However, as can be seen from page 7, lines 1 to 5, the Applicant considers that in general, residues 71, 73 and 78 can be either all donor or all acceptor.

In an amendment filed on February 7, 1994, Adair again pointed to its "protocol" to respond to the Examiner's repeated enablement rejections, stating (MF 32):

> It is specifically stated in the application that the present protocol represents a departure from the procedures of Reichmann [sic] and Queen, at least. Thus, the skilled person would not rely on Reichmann [sic] and Queen as teachings relevant to whether the present description is enabling.
>
> It is submitted that the skilled person would rely on the clear teaching given in the application and find that it is enabling. The specification plainly sets out what actions need to be taken. It is presumed that the Examiner agrees that the skilled person could have taken those actions. The application also sets out that, contrary to the teachings of Reichmann and Queen, the protocol is generally applicable. The application further shows that it had been successfully implemented. Thus, it is submitted that the skilled person would find that the present application is properly enabled the full extent of the claims.

Thus, throughout examination of its related '329 application, Adair consistently relied upon the "protocol" as providing the basis for distinguishing claims specifying substitutions at positions corresponding to those recited in Adair's involved claim 24 over the prior art and for supporting enablement and description of these claims. (MF 20-33).

The express teachings of the Adair specification and Adair's representations regarding the same provide compelling evidence that Adair did not consider its invention to encompass any

combination of acceptor/donor residues at positions 23, 24, 49, 71, 73 and 78, as involved Adair claim 24 specifies, and, therefore, Adair claim 24 is not patentable under 35 U.S.C. § 112, first paragraph. *Adang v. Umbeck*, 2007 U.S. App. LEXIS 25198 (Fed. Cir. 2007); *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 927 (Fed. Cir. 2004); *PIN/NIP, Inc. v. Platte Chem. Co.*, 304 F.3d 1235, 1247-48 (Fed. Cir. 2002); *In re Curtis*, 354 F.3d 1347, 1353-54 (Fed. Cir. 2004).

F. Case Law Compels The Conclusion That Adair's Involved Claim 24 Lacks Written Description Support

A claim must be supported by an adequate written description of the invention. *Ariad Pharms., Inc. v. Eli Lilly and Co.*, 598 F.3d 1336, 94 U.S.P.Q.2d 1161 (Fed. Cir. 2010)(en banc). "To satisfy the written description requirement, a patent applicant must 'convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed.'" *ICU Med., Inc., v. Alaris Med. Sys., Inc*, 558 F.3d 1368 (Fed. Cir. 2009)(citing *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 U.S.P.Q.2d 1111 (Fed.Cir.1991)). The description of the invention in the disclosure is what must be assessed. See *Carnegie Mellon University v. Hoffman La-Roche, Inc.*, 545 F.3d. 1115, 88 USPQ2d 1233 (Fed. Cir. 2008)("The basic function of a patent specification is to disclose an invention. It has long been the case that a patentee 'can lawfully claim only what he has invented and described, and if he claims more his patent is void.' (citing *O'Reilly v. Morse,* 56 U.S. (15 How.) 62, 121, 14 L.Ed. 601 (1853))."

A broad claim is not patentable when the entirety of the specification clearly indicates that the invention is of a much narrower scope. *Carnegie Mellon,* 545 F.3d at 1127, citing *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473 (Fed.Cir.1998). This is precisely the circumstance presented by Adair's involved claim. Adair's disclosure plainly envisioned a far more restricted invention that what Adair involved claim 24 now defines. First, Adair's

specification requires mandatory changes to the residues at locations 23, 24 and 49 of the human heavy chain framework region to incorporate the donor residue at each of those locations. Second, Adair's specification mandates a rule for a second set of residues at positions 71, 73 and 78, *i.e.*, these residues must all be donor or must all be acceptor. Thus, as Adair itself acknowledged during prosecution of its related '329 application, there are two alternative "minimum" sets of changes to heavy chain residues; one involving changes at three residue positions (*i.e.*, 23, 24 and 49, because residue positions 71, 73 and 78 are all to remain "acceptor" residues), and a second involving six residue positions (*i.e.*, 23, 24, and 49, plus the "donor" residues at all of positions 71, 73 and 78). Despite this description of minimum requirements, Adair's involved claim 24 allows for substitutions ranging from a single donor residue among the six recited positions, donor residues at any number of residue positions up to six, and any permutation of possible acceptor/donor substitutions at these six positions, including substitutions that violate Adair express rules regarding residues at positions 23, 24, 49, 71, 73 and 78. The stark contrast between the scope of the Adair specification and the scope of Adair's involved claim 24 compels a determination that claim 24 is not patentable under 35 U.S.C. § 112, first paragraph.

IV. CONCLUSION

Adair's involved claim 24 lacks written description and, therefore, is not patentable under 35 U.S.C. § 112, first paragraph. Judgment should be entered against Adair.

Respectfully submitted,

May 28, 2010

/Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.
Registration No. 40,491
Counsel for Party Carter

CERTIFICATE OF FILING

The undersigned certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 2"** was filed this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), with:

The Board of Patent Appeals and Interferences
Madison Building East, 9th Floor
600 Dulany Street
Alexandria, VA 22314
Tel: 571-272-4683
Fax: 571-273-0042
E-mail: BoxInterferences@USPTO.GOV

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of the paper entitled **"CARTER SUBSTANTIVE MOTION 2"** was served this 28th day of May, 2010, via Interference Web Portal (https://acts.uspto.gov/ifiling/), on the Attorney of Record for Adair:

Doreen Yatko Trujillo, Esq.
Cozen O'Connor P.C.
1900 Market Street, 7th Floor
Philadelphia, PA 19103
Tel.: 215-665-6593
Fax: 215-701-2005
E-mail: dtrujillo@cozen.com

May 28, 2010 /Oliver R. Ashe, Jr./
Oliver R. Ashe, Jr.

Appendix 1

EVIDENCE

I. Exhibits Cited

The following exhibits are cited in support of this motion:

Ex. 2002 U.S. Patent Application No. 11/284,261 to Adair *et al*., filed November 21, 2005.

Ex. 2003 Preliminary Amendment and Request for Interference Under 37 C.F.R. § 42.202 [sic], filed November 21, 2005, in U.S. Patent Application No. 11/284,261 to Adair *et al*.

Ex. 2004 Request for Reconsideration, filed September 9, 2009, in U.S. Patent Application No. 11/284,261 to Adair *et al*.

Ex. 2005 PCT Application No. PCT/GB90/02017 to Adair *et al*., filed December 21, 1990, published as WO 91/09967 on July 11, 1991 ("the PCT Application").

Ex. 2006 U.S. Patent Application No. 07/743,329 to Adair *et al*., filed September 17, 1991.

Ex. 2007 Response to Office Action filed January 19, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2008 Amendment filed April 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2009 Letter regarding amendments filed September 9, 1993, in European Patent Application No. 91901433.2 to Adair *et al*.

Ex. 2010 Amendment filed February 7, 1994, in U.S. Patent Application No.

07/743,329 to Adair *et al*.

Ex. 2011 Riechmann *et al.*, *Nature*, Vol. 332, pp. 323-327 (March 1988).

Ex. 2023 Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, Vol. 86, pp. 10029-10033 (December 1989).

Ex. 2028 Office Action mailed September 7, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al.*

Ex. 2036 Great Britain Application No. 8928874.0 to Adair *et al*., filed December 21, 1989 ("the UK Application").

Ex. 2037 Computer generated comparison (using Workshare™ Professional 5.2 SR2 software) of the typewritten text of the UK Application to the typewritten text of the PCT Application.

Ex. 2038 Office Action mailed November 18, 1992, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

Ex. 2039 Examiner Interview Summary Record dated January 27, 1993, in U.S. Patent Application No. 07/743,329 to Adair *et al*.

II. Papers Cited

The following papers are cited in support of this motion:

Paper No. 5 Adair Clean Copy of Claims filed February 16, 2010.

III. Appendices Cited

The following papers are cited in support of this motion:

Appendix 1 Evidence.