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106. (TWICE AMENDED) [An] A humanized antibody which lacks immunogenicity compared to a non-human parent antibody upon repeated administration to a human patient in order to treat a chronic disease in that patient[ and] wherein the humanized antibody comprises a consensus human variable domain of a human heavy chain immunoglobulin subgroup, wherein amino acid residues forming Complementarity Determining Regions (CDRs) thereof comprise non-human antibody amino acid residues, and further [comprising] comprises a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) [comprises] introduces a glycosylation site which affects the antigen binding or affinity of the antibody; or (d) participates in the  $V_L$ - $V_H$  interface by affecting the proximity or orientation of the  $V_L$  and  $V_H$  regions with respect to one another.

107. (AMENDED) The humanized antibody of claim 106 comprising a non-human FR residue which noncovalently binds antigen directly.

108. (AMENDED) The humanized antibody of claim 106 comprising a non-human FR residue which interacts with a CDR.

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109. (AMENDED) The humanized antibody of claim 106 comprising a non-human FR residue which [comprises] introduces a glycosylation site which affects the antigen binding or affinity of the antibody.

110. (AMENDED) The humanized antibody of claim 106 comprising a non-human FR residue which participates in the  $V_L$ - $V_H$  interface by affecting the proximity or orientation of the  $V_L$ - $V_H$  regions with respect to one another.

111. (AMENDED) A humanized antibody comprising a consensus human variable domain of human  $V_H$  subgroup III, wherein amino acid residues forming Complementarity Determining Regions (CDRs) thereof comprise non-human antibody amino acid residues, and further comprising a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) [comprises] introduces a glycosylation site which affects the antigen binding or affinity of the antibody; or (d) participates in the  $V_L$ - $V_H$  interface by affecting the proximity or orientation of the  $V_L$  and  $V_H$  regions with respect to one another.

112. (Reiterated) The humanized antibody of claim 111 which lacks immunogenicity compared to a non-human parent antibody upon repeated administration to a human patient in order to treat a chronic disease in that patient.

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113. (AMENDED) A humanized variant of a non-human parent antibody which binds an antigen with better affinity than the parent antibody and comprises a consensus human variable domain of a human heavy chain immunoglobulin subgroup wherein amino acid residues forming Complementarity Determining Regions (CDRs) thereof comprise non-human antibody amino acid residues, and further [comprising] comprises a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) [comprises] introduces a glycosylation site which affects the antigen binding or affinity of the antibody; or (d) participates in the V<sub>L</sub>-V<sub>H</sub> interface by affecting the proximity or orientation of the V<sub>L</sub> and V<sub>H</sub> regions with respect to one another.

114. (AMENDED) The humanized variant of claim 113 which binds the antigen at least about 3-fold more tightly than the parent antibody binds antigen.

Please add the following claims to the above-identified application:

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--115. (NEW) A humanized antibody heavy chain variable domain comprising non-human Complementarity Determining Region (CDR) amino acid residues which bind antigen incorporated into a human antibody variable domain, and further comprising an amino acid substitution at a site selected from the group consisting of: 24H, 73H, 76H, 78H, and 93H, utilizing the numbering system set forth in Kabat.

116. (NEW) The humanized variable domain of claim 115 wherein the substituted residue is the residue found at the corresponding location of the non-human antibody from which the non-human CDR amino acid residues are obtained.

117. (NEW) The humanized variable domain of claim 115 wherein no human Framework Region (FR) residue other than those set forth in the group has been substituted.

118. (NEW) The humanized variable domain of claim 115 wherein the human antibody variable domain is a consensus human variable domain.

119. (NEW) The humanized variable domain of claim 115 wherein the residue at site 24H has been substituted.

120. (NEW) The humanized variable domain of claim 115 wherein the residue at site 73H has been substituted.

121. (NEW) The humanized variable domain of claim 115 wherein the residue at site 76H has been substituted.

122. (NEW) The humanized variable domain of claim 115 wherein the residue at site 78H has been substituted.

123. (NEW) The humanized variable domain of claim 115 wherein the residue at site 93H has been substituted.

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124. (NEW) The humanized variable domain of claim 115 which further comprises an amino acid substitution at site 71H.

125. (NEW) The humanized variable domain of claim 115 which further comprises amino acid substitutions at sites 71H and 73H.

126. (NEW) The humanized variable domain of claim 115 which further comprises amino acid substitutions at sites 71H, 73H and 78H.

127. (NEW) An antibody comprising the humanized variable domain of claim 115.

128. (NEW) A humanized variant of a non-human parent antibody which binds an antigen, wherein the humanized variant comprises Complementarity Determining Region (CDR) amino acid residues of the non-human parent antibody incorporated into a human antibody variable domain, and further comprises a Framework Region (FR) substitution where the substituted FR residue: (a) noncovalently binds antigen directly; (b) interacts with a CDR; or (c) participates in the  $V_L$ - $V_H$  interface by affecting the proximity or orientation of the  $V_L$  and  $V_H$  regions with respect to one another, and wherein the humanized variant binds the antigen more tightly than the parent antibody

binds as.

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Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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<p>In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES</p>	<p>Group Art Unit: 1642 Examiner: J. Reeves</p>	<p>FEB 4 1999 MATRIX CUSTOMER SERVICE CENTER</p>
<p><b>CERTIFICATE OF HAND DELIVERY.</b> I hereby certify that this correspondence is being hand delivered in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on February 1, 1999 <i>R. H. Mitchell</i></p>		

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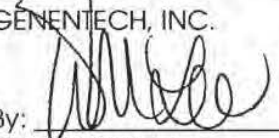
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Further to the Supplemental Amendment fax-filed on January 15, 1999, please find enclosed priority documents USSN 07/290,975 and USSN 07/310,252 for the "PDL Patents" as promised on page 11 of that amendment.

Applicants further submit herewith a Supplemental Information Disclosure Statement. In this respect, Applicants bring to the Examiner's attention a Celltech press release entitled: "Celltech Antibody Technology Platform Further Strengthened Through New Patents in US and Europe." (Exhibit A attached) This press release refers to an allowed US "Adair" patent application. Applicants believe this US Adair patent application corresponds to WO91/09967 (of record) and EP 460,167 B1 (copy attached).

Should the Examiner have questions concerning this communication, she is invited to call the undersigned.

Respectfully submitted,  
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By:   
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Date: January 29, 1999

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## Celltech Antibody Technology Platform Further Strengthened Through New Patents in US and Europe

SLOUGH, U.K., Sept. 26 /PRNewswire/ -- Celltech announced today that the U.S. Patent Office has allowed one of its key patent applications covering engineered human antibodies. The grant of this "Adair" patent will occur in early 1997 and will expire in 2014. This broad product patent covers a key approach to the construction of new human antibodies which is essential in order to achieve full therapeutic activity. It covers all antibodies which have been constructed using this approach. A corresponding patent has already been granted in Europe, although it is anticipated that the financial benefit to Celltech from the U.S. patent will be more significant in the near term because of the numbers of antibodies in late-stage development in the U.S.

The "Adair" patent is an important new element in Celltech's technology platform, and complements previous Celltech patents in the field of antibody engineering. It covers all of Celltech's own antibodies currently in clinical development, thus substantially extending their period of patent protection. In addition the patent covers a range of antibodies under development by other companies. This would result in royalty revenues should these products reach the market.

There are already a number of process patents covering the manufacture of engineered antibodies including those granted to Celltech, Genentech, the Medical Research Council and Protein Design Laboratories. Celltech has agreements in place with Genentech and the Medical Research Council relating to the commercial exploitation of some of these patents. Celltech pursues the strategy of licensing its existing antibody patents to any interested party for products which are not directly competitive with Celltech's own products. This policy will be pursued with the new "Adair" patent and all licensees who have directly licensed pre-existing patents from Celltech (in particular the "Boss" antibody engineering patents) will be offered favorable terms for the "Adair" patent.

Commenting on the news today, Dr. Peter Fellner, CEO, said, "Celltech has built a very valuable platform technology in the field of antibody engineering and the grant of this patent will further strengthen our position. We expect a continued growth in royalty revenues from our licensed patents which will make a significant contribution to the profitability of the company. The potential in this area can be seen from the growing success of ReoPro(TM) (Lilly/Centocor). Royalties on the sales of this product are paid to both Genentech and Celltech." SOURCE Celltech Therapeutics Ltd.

### NOTE TO EDITORS:

1. The Adair product patent covers any antibody in which the antigen binding regions from a donor antibody have been transferred to the framework of a human antibody, and specifies certain requirements in specific amino acid residues within the product which are necessary to recover full antigen binding activity of the newly created antibody.

2. Antibodies are natural proteins which bind tightly and specifically to antigens. This binding property is particularly important in providing a defense mechanism against infectious organisms such as bacteria and viruses. For some time, scientists have been able to produce antibodies in the

laboratory and their availability has had a profound impact on diagnostic medicine. In contrast, they have had little impact on therapeutic medicine. The reason for this is that the first antibodies were derived from animal sources. When these animal antibodies were injected into humans they induced a significant immune response which led to either adverse reactions or a rapid loss of therapeutic efficacy. More recently techniques have been developed to produce engineered human antibodies which are virtually identical to natural human antibodies. The main advantage of these antibodies is that they do not cause a significant immune response in man and they are very well tolerated. Because of their good tolerance, their binding properties are being used in a wide variety of therapeutic applications in areas such as blockade of receptor functions in heart disease, neutralization of cytokine in rheumatoid arthritis and killing of cancer cells./

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CONTINUING DATA VERIFIED

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TITLE

NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS

This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application as originally filed which is identified above.

By authority of the COMMISSIONER OF PATENTS AND TRADEMARKS

*C. A. Lane*  
Certifying Officer



Date 1 JAN 1990

07/290975

PATENT APPLICATION SERIAL NO. \_\_\_\_\_

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NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS

Field of the Invention

5 The present invention relates generally to the combination of recombinant DNA and monoclonal antibody technologies for developing novel therapeutic agents and, more particularly, to the production of non-immunogenic antibodies specific for the human interleukin-2 receptor and their uses.

10 Background of the Invention

15 In mammals, the immune response is mediated by two types of cells that interact specifically with foreign material, i.e., antigens. One of these cell types, B-cells, are responsible for the production of antibodies. The second cell class, T-cells, include a wide variety of cellular subsets controlling the in vivo function of both B-cells and a wide variety of other hematopoietic cells, including T-cells.

20 One way in which T-cells exert this control is through the production of a lymphokine known as interleukin-2 (IL-2), originally named T-cell growth factor. IL-2's prime function appears to be the stimulation and maintenance of T-cells. Indeed, some immunologists believe that IL-2 may be at the center of the entire immune response (see, Farrar, J., et al., Immunol. Rev. 63:129-166 (1982), which is incorporated herein by reference).

25 To exert its biological effects, IL-2 interacts with a specific high-affinity membrane receptor (Greene, W., et al., Progress in Hematology XIV, E. Brown, Ed., Grune and Statton, New York (1986), at pgs. 283 ff). The human IL-2 receptor is a complex multichain glycoprotein, with one chain, known as the Tac peptide, being about 55kD in size (see, Leonard, W., et al., J. Biol. Chem. 260:1872 (1985), which is incorporated herein by reference). A gene encoding this protein has been isolated, and predicts a 272 amino acid peptide, including a 21 amino acid signal peptide (see,

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Leonard, W., et al., Nature 311: 626 (1984)). The 219 NH<sub>2</sub>-terminal amino acids of the p55 Tac protein apparently comprise an extracellular domain (see, Leonard, W., et al., Science, 230:633-639 (1985), which is incorporated herein by reference).

5                    Much of the elucidation of the human IL-2 receptor's structure and function is due to the development of specifically reactive monoclonal antibodies. In particular, one mouse monoclonal antibody, known as anti-Tac (Uchiyama, et al., J. Immunol. 126:1393 (1981)) has shown  
10                    that IL-2 receptors can be detected on T-cells, but also on cells of the monocyte-macrophage family, Kupffer cells of the liver, Langerhans' cells of the skin and, of course, activated T-cells. Importantly, resting T-cells, B-cells or circulating macrophages typically do not display the IL-2  
15                    receptor (Herrmann, et al., J. Exp. Med. 162:1111 (1985)).

                    The anti-Tac monoclonal antibody has also been used to define lymphocyte functions that require IL-2 interaction, and has been shown to inhibit various T-cell functions, including the generation of cytotoxic and suppressor T  
20                    lymphocytes in cell culture. Also, based on studies with anti-Tac and other antibodies, a variety of disorders are now associated with improper IL-2 receptor expression by T-cells, in particular adult T-cell leukemia.

                    More recently, the IL-2 receptor has been shown to  
25                    be an ideal target for novel therapeutic approaches to T-cell mediated diseases. It has been proposed that IL-2 receptor specific antibodies, such as the anti-Tac monoclonal antibody, can be used either alone or as an immunoconjugate (e.g., with Ricin A, isotopes and the like) to effectively  
30                    remove cells bearing the IL-2 receptor. These agents can, for example, theoretically eliminate IL-2 receptor-expressing leukemic cells, certain B-cells, or activated T-cells involved in a disease state, yet allow the retention of mature normal T-cells and their precursors to ensure the  
35                    capability of mounting a normal T-cell immune response as needed. In general, most other T-cell specific agents can destroy essentially all peripheral T-cells, which limits the

agents' therapeutic efficacy. Overall, the use of appropriate monoclonal antibodies specific for the IL-2 receptor may have therapeutic utility in autoimmune diseases, organ transplantation and any unwanted response by activated T-cells. Indeed, clinical trials have been initiated using, 5 e.g., anti-Tac antibodies (see, generally, Waldman, T., et al., Cancer Res. 45:625 (1985) and Waldman, T., Science 232:727-732 (1986), both of which are incorporated herein by reference).

10 Unfortunately, the use of the anti-Tac and other non-human monoclonal antibodies have certain drawbacks, particularly in repeated therapeutic regimens as explained below. Mouse monoclonal antibodies, for example, do not fix human complement well, and lack other important immunoglobulin functional characteristics when used in 15 humans.

Perhaps more importantly, anti-Tac and other non-human monoclonal antibodies contain substantial stretches of amino acid sequences that will be immunogenic when injected into a human patient. Numerous studies have shown that, 20 after injection of a foreign antibody, the immune response elicited by a patient against an antibody can be quite strong, essentially eliminating the antibody's therapeutic utility after an initial treatment. Moreover, as increasing numbers of different mouse or other antigenic (to humans) 25 monoclonal antibodies can be expected to be developed to treat various diseases, after the first and second treatments with any different non-human antibodies, subsequent treatments even for unrelated therapies can be ineffective or even dangerous in themselves.

30 While the production of so-called "chimeric antibodies" (e.g., mouse variable regions joined to human constant regions) has proven somewhat successful, a significant immunogenicity problem remains. In general, the production of human immunoglobulins reactive with the human 35 IL-2 receptor, as with many human antigens, has been extremely difficult using typical human monoclonal antibody production techniques. Similarly, utilizing recombinant DNA

technology to produce so-called "humanized" antibodies (see,  
e.g., EPO Publication No. 0239400), provides uncertain  
results, in part due to unpredictable binding affinities.

Thus, there is a need for improved forms of human-  
like immunoglobulins specific for the human IL-2 receptor  
5 that are substantially non-immunogenic in humans, yet easily  
and economically produced in a manner suitable for  
therapeutic formulation and other uses. The present  
invention fulfills these and other needs.

10 Summary of the Invention

The present invention provides novel compositions  
useful, for example, in the treatment of T-cell mediated  
human disorders, the compositions containing human-like  
immunoglobulins specifically capable of blocking the binding  
15 of human IL-2 to its receptor and/or capable of binding to  
the p55 Tac protein on human IL-2 receptors. The  
immunoglobulins can have two pairs of light chain/heavy chain  
complexes, typically at least one pair having chains  
comprising mouse complementarity determining regions  
20 functionally joined to human framework region segments. For  
example, mouse complementarity determining regions, with or  
without additional naturally-associated mouse amino acid  
residues, can be used to produce human-like antibodies  
capable of binding to the human IL-2 receptor at affinity  
25 levels stronger than about  $10^8 M^{-1}$ .

The immunoglobulins, including binding fragments  
and other derivatives thereof, of the present invention may  
be produced readily by a variety of recombinant DNA  
techniques, with ultimate expression in transfected cells,  
30 preferably immortalized eukaryotic cells, such as myeloma or  
hybridoma cells. Polynucleotides comprising a first sequence  
coding for human-like immunoglobulin framework regions and a  
second sequence set coding for the desired immunoglobulin  
complementarity determining regions can be produced  
35 synthetically or by combining appropriate cDNA and genomic  
DNA segments.

The human-like immunoglobulins may be utilized alone in substantially pure form, or complexed with a cytotoxic agent, such as a radionuclide, a ribosomal inhibiting protein or a cytotoxic agent active at cell surfaces. All of these compounds will be particularly useful in treating T-cell mediated disorders. The human-like immunoglobulins or their complexes can be prepared in a pharmaceutically accepted dosage form, which will vary depending on the mode of administration.

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## BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Comparison of sequences of anti-Tac heavy chain (upper lines) and Eu heavy chain (lower lines). The 1-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDRs are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an \*.

Figure 2. Comparison of sequences of anti-Tac light chain (upper lines) and Eu light chain (lower lines). The single-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDRs are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an \*.

Figure 3. Nucleotide sequence of the gene for the humanized anti-Tac heavy chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature heavy chain sequence begins with amino acid #20 Q.

Figure 4. Nucleotide sequence of the gene for the humanized anti-Tac light chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature light chain sequence begins with amino acid #21 D.

Figure 5. A. Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac heavy chain gene, printed 5' to 3'. B. Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide.

Figure 6. (A) Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac light chain gene, printed 5' to 3'. (B) Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide. The position of a Hind III site in the overlap of JFD2 and JFD3 is shown.

Figure 7. Schematic diagram of the plasmid pHUGTAC1 used to express the humanized anti-Tac heavy chain. Relevant restriction sites are shown, and coding regions of the heavy chain are displayed as boxes. The direction of transcription from the immunoglobulin (Ig) promoter is shown by an arrow. E<sub>H</sub> = heavy chain enhancer, Hyg = hygromycin resistance gene.

Figure 8. Schematic diagram of the plasmid pHULTAC used to express the humanized anti-Tac light chain. Relevant restriction sites are shown, and coding regions of the light chain are displayed as boxes. The direction of transcription from the Ig promoter is shown by an arrow.

Figure 9. Fluorocytometry of HUT-102 and Jurkat cells stained with anti-Tac antibody or humanized anti-Tac antibody followed respectively by fluorescein-conjugated goat anti-mouse Ig antibody or goat anti-human Ig antibody, as labeled. In each panel, the dotted curve shows the results when the first antibody was omitted, and the solid curve the results when first and second (conjugated) antibodies were included as described.

Figure 10. (A) Fluorocytometry of HUT-102 cells stained with 0-40 ng of anti-Tac as indicated, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin. (B) Fluorocytometry of HUT-102 cells stained with the indicated antibody, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin.

## DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, human-like immunoglobulins specifically reactive with the IL-2 receptor on human T-cells are provided. These immunoglobulins, which have binding affinities of at least about  $10^8 M^{-1}$ , and preferably  $10^9 M^{-1}$  to  $10^{10} M^{-1}$  or stronger, are capable of, e.g., blocking the binding of IL-2 to human IL-2 receptors. The human-like immunoglobulins will have a human-like framework and can have complementarity determining regions (CDR's) from an immunoglobulin, typically a mouse immunoglobulin, specifically reactive with an epitope on p55 Tac protein. The immunoglobulins of the present invention, which can be produced economically in large quantities, find use, for example, in the treatment of T-cell mediated disorders in human patients by a variety of techniques.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25kD) and one "heavy" chain (about 50-70kD). The  $NH_2$ -terminus of each chain begins a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The COOH terminus of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as either kappa or lambda. Heavy chains are classified (and subclassified) as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 12 more amino acids. (See, generally, Fundamental Immunology, Paul, W., Ed., Chapter 7, pgs. 131-166, Raven Press, N.Y. (1984), which is incorporated herein by reference.)

The variable regions of each light/heavy chain pair form the antibody binding site. The chains all exhibit the same general structure of relatively conserved framework



regions joined by three hypervariable regions, also called CDR's (see, "Sequences of Proteins of Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987), which are incorporated herein by reference). The CDR's from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope.

As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. The immunoglobulins may exist in a variety of forms besides antibodies: including, for example, Fv, Fab, and F(ab)<sub>2</sub>, as well as in single chains (e.g., Huston, et al., Proc. Nat. Acad. Sci. U.S.A., 85:5879-5883 (1988) and Bird, et al., Science, 242:423-426 (1988), which are incorporated herein by reference). (See, generally, Hood, et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, Nature, 323:15-16 (1986), which are incorporated herein by reference).

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin gene segments belonging to different species. For example, the variable (V) segments of the genes from a mouse monoclonal antibody may be joined to human constant (C) segments, such as  $\gamma_1$  and  $\gamma_3$ . A typical therapeutic chimeric antibody is thus a hybrid protein consisting of the V or antigen-binding domain from a mouse antibody and the C or effector domain from a human antibody (e.g., A.T.C.C. Accession No. CRL 9688 secretes an anti-Tac chimeric antibody), although other mammalian species may be used.

As used herein, the term "framework region" refers to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved (i.e., other

than the CDR's) among different immunoglobulins in a single species, as defined by Kabat, et al., op. cit. As used herein, a "human-like framework region" is a framework region that in each existing chain comprises at least about 70 or more amino acid residues, typically 75 to 85 or more residues, identical to those in human immunoglobulins.

As used herein, the term "human-like immunoglobulin" refers to an immunoglobulin comprising a human-like framework and in which any constant region present is substantially homologous to a human immunoglobulin constant region, i.e., at least about 85-90%, preferably about 95% identical. Hence, all parts of a human-like immunoglobulin, except possibly the CDR's, are substantially homologous to corresponding parts of one or more native human immunoglobulin sequences. For example, a human-like immunoglobulin would not encompass a chimeric mouse variable region/human constant region antibody.

Human-like antibodies have at least three potential advantages over mouse or and in some cases chimeric antibodies for use in human therapy:

- 1) because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).
- 2) The human immune system should not recognize the framework or C region of the human-like antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign mouse antibody or a partially foreign chimeric antibody.
- 3) Injected mouse antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of normal antibodies (Shaw, D., et al., J. Immunol. 138:4534-4538 (1987)). Injected human-like

antibodies will presumably have a half-life essentially identical to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

5 In one aspect, the present invention is directed to recombinant DNA segments encoding the heavy and/or light chain CDR's from an immunoglobulin capable of binding to a desired epitope on the human IL-2 receptor, such as the anti-Tac monoclonal antibody. The DNA segments encoding these regions will typically be joined to DNA segments encoding appropriate human-like framework regions. The preferred DNA sequences, which on expression code for the polypeptide chains comprising the anti-Tac heavy and light chain hypervariable regions (with human-like framework regions), are shown in Figures 1 and 2, respectively. Due to codon degeneracy and non-critical amino-acid substitutions, other DNA sequences can be readily substituted for those sequences, as detailed below.

20 The DNA segments will typically further include an expression control DNA sequence operably linked to the human-like antibody coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow.

30 Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B-cells (see, Kabat op. cit. and WP87/02671). The CDR's for producing the immunoglobulins of the present invention will be similarly derived from monoclonal antibodies capable of binding to the

human IL-2 receptor and produced in any convenient mammalian source, including, mice, rats, rabbits, or other vertebrate capable of producing antibodies by well known methods. Suitable source cells for the DNA sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In addition to the human-like immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary from the sequences in Figures 3 and 4 at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the human-like immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, Gene 8:81-97 (1979) and Roberts, S. et al, Nature 328:731-734 (1987), both of which are incorporated herein by reference).

Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). Also because like many genes, the immunoglobulin-related genes contain separate functional regions, each having one or more distinct biological activities, the genes may be fused to functional regions from other genes (e.g., enzymes, see, commonly assigned U.S.S.N. 132,387, filed Dec. 15, 1987, which is incorporated herein by reference) to produce fusion proteins (e.g., immunotoxins) having novel properties.

The nucleic acid sequences of the present invention capable of ultimately expressing the desired human-like antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic sequences is presently the most common method of production, but cDNA sequences may also be utilized (see, European Patent Publication No. 0239400 and Reichmann, L., et al., Nature 332:323-327 (1988), both of which are incorporated herein by reference).

As stated previously, the DNA sequences will be expressed in hosts after the sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362, which is incorporated herein by reference).

E. coli is one prokaryotic host useful particularly for cloning the DNA sequences of the present invention. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. Saccharomyces is a preferred host, with suitable vectors having expression control sequences, such as promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987), which is incorporated herein by reference). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, etc, but preferably transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen, C., et al., Immunol. Rev. 89:49-68 (1986), which is incorporated herein by reference), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, and the like.

The vectors containing the DNA segments of interest (e.g., the heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts. (See, generally, Maniatis, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, (1982), which is incorporated herein by reference.)

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin

forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982))).

5 Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically (including  
10 extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981))).

15 The antibodies of the present invention will typically find use individually in treating a T-cell mediated disease state. Generally, where the cell linked to a disease has been identified as IL-2 receptor bearing, then the human-like antibodies capable of blocking the binding of IL-2 to  
20 the human IL-2 receptor are suitable (see, U.S.S.N. 085,707, entitled "Treating Human Malignancies and Disorders," which is incorporated herein by reference).

For example, typical disease states suitable for treatment include graft versus host disease and transplant  
25 rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include autoimmune diseases, such as Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

30 The human-like antibodies of the present invention may also be used in combination with other antibodies, particularly human monoclonal antibodies reactive with other markers on cells responsible for the disease. For example, suitable T-cell markers can include those grouped into the  
35 so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop,

Leukocyte Typing, Bernard, et al., Eds., Springer-Verlag, N.Y. (1984), which is incorporated herein by reference.

The antibodies can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

A preferred pharmaceutical composition of the present invention comprises the use of the subject antibodies in immunotoxins. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 163-190 (1982), which is incorporated herein by reference.

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g.,



phospholipase C). (See, generally, commonly assigned U.S.S.N. \_\_\_\_\_ (Townsend and Townsend Docket No. 11823-7-2) filed concurrently herewith, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), all of which are incorporated herein by reference.)

The delivery component of the immunotoxin will include the human-like immunoglobulins of the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The human-like antibodies and pharmaceutical compositions thereof of this invention are particularly useful for parenteral administration, *i.e.*, subcutaneously, intramuscularly or intravenously. The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, *e.g.*, water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, *i.e.*, from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania (1980), which is incorporated herein by reference.

The antibodies of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins and art-known lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g., with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

The compositions containing the present human-like antibodies or a cocktail thereof can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest the disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the infection and the general state of the patient's own immune system, but generally range from about 1 to about 200 mg of antibody per dose, with dosages of from 5 to 25 mg per patient being more commonly used. It must be kept in mind that the materials of this invention may generally be employed in serious disease states, that is life-threatening or potentially life-threatening situations. In such cases,

in view of the minimization of extraneous substances and the lower probability of "foreign substance" rejections which are achieved by the present human-like antibodies of this invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these antibodies.

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In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend upon the patient's state of health and general level of immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per patient. A preferred prophylactic use is for the prevention of kidney transplant rejection.

20  
Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the antibody(ies) of this invention sufficient to effectively treat the patient.

25  
Human-like antibodies of the present invention can further find a wide variety of utilities in vitro. By way of example, the antibodies can be utilized for T-cell typing, for isolating specific IL-2 receptor bearing cells or fragments of the receptor, for vaccine preparation, or the like.

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For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the human-like antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme co-factors, enzyme inhibitors, ligands (particularly haptens),

etc. Numerous types of immunoassays are available and are well known to those skilled in the art.

Kits can also be supplied for use with the subject antibodies in the protection against or detection of a cellular activity or for the presence of a selected antigen. Thus, the subject antibody composition of the present invention may be provided, usually in a lyophilized form in a container, either alone or in conjunction with additional antibodies specific for the desired cell type. The antibodies, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of active antibody, and usually present in total amount of at least about 0.001% wt. based again on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric antibody is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above.

The following examples are offered by way of illustration, not by limitation.

## EXPERIMENTAL

Design of genes for human-like light and heavy chains

The sequence of the human antibody Eu (Sequences of Proteins of Immunological Interest, Kabat, E., et al., U.S. Dept. of Health and Human Services, 1983) was used to provide the framework of the humanized antibody, because the amino acid sequence of the heavy chain of anti-Tac is more homologous to the heavy chain of this antibody than to any other heavy chain sequence in the National Biomedical Foundation Protein Identification Resource.

To select the sequence of the humanized heavy chain, the anti-Tac heavy chain sequence (see, commonly assigned U.S.S.N.'s 186,862 and 223,037, which are incorporated herein by reference) was aligned with the sequence of the Eu heavy chain (Figure 1). At each position, the Eu amino acid was selected for the humanized sequence, unless that position fell in any one of the following categories, in which case the anti-Tac amino acid was selected.

- (1) The position fell within a complementarity determining region (CDR), as defined by Kabat, et al., op. cit. (amino acids 31-35, 50-66, 99-106);
- (2) The Eu amino acid was unusual for human heavy chains at that position, whereas the anti-Tac amino acid was typical for human heavy chains at that position (amino acids 27, 93, 95, 98, 107-109, 111);
- (3) The position was immediately adjacent to a CDR in the amino acid sequence of the anti-Tac heavy chain (amino acids 30 and 67).
- (4) 3-dimensional modeling of the anti-Tac antibody suggested that the amino acid was physically close to the antigen binding region (amino acids 48 and 68).

Some amino acids fell in more than one of these categories but are only listed in one.

To select the sequence of the humanized light chain, the anti-Tac light chain sequence was aligned with the sequence of the Eu light chain (Figure 2). The Eu amino acid was selected at each position, unless the position again fell into one of the categories (1) - (4), (with light chain replacing heavy chain in the category definitions):

- (1) CDRs (amino acids 24-34, 50-56, 89-97).
- (2) Anti-Tac amino acid more typical than Eu (amino acids 48 and 63).
- (3) Adjacent to CDRs (no amino acids; Eu and anti-Tac were already the same at all these positions).
- (4) Possible 3-dimensional proximity to binding region (amino acid 60).

The actual nucleotide sequence of the heavy (Figure 3) and light chain (Figure 4) genes were selected as follows:

- (1) the nucleotide sequences code for the amino acid sequences chosen as described above.
- (2) 5' of these coding sequences, the nucleotide sequences code for a leader (signal) sequence, namely the leader of the light chain of the antibody MOPC 63 and the leader of the heavy chain of the antibody PCH 108A (Kabat et al., op. cit.). These leader sequences were chosen as typical of antibodies.
- (3) 3' of the coding sequences, the nucleotide sequences are the sequences that follow the mouse light chain J5 segment and the mouse heavy chain J2 segment, which are part of the anti-Tac sequences. These sequences are included because they contain splice donor signals.
- (4) At each end of the sequence is an Xba I site to allow cutting at the Xba I sites and cloning into the Xba I site of a vector.

Construction of humanized light and heavy chain genes

To synthesize the heavy chain, four oligonucleotides HES12, HES13, HES14, HES15 (Figure 5A) were synthesized using an Applied Biosystems 380B DNA synthesizer. Two of the oligonucleotides are part of each strand of the heavy chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 5B). Together, the oligonucleotides cover the entire humanized heavy chain (Figure 3) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

Each oligonucleotide was phosphorylated using ATP and T4 polynucleotide kinase by standard procedures (see, Maniatis, op. cit.). To anneal the phosphorylated oligonucleotides, they were suspended together in 40 ul of TA (33 mM Tris acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate) at a concentration of about 3.75 uM each, heated to 95 deg for 4 min. and cooled slowly to 4 deg. To synthesize the complete gene from the oligonucleotides by synthesizing the opposite strand of each oligonucleotide (Figure 5B), the following components were added in a final volume of 100ul:

	10 ul	annealed oligonucleotides
	0.16 mM each	deoxyribonucleotide
25	0.5 mM	ATP
	0.5 mM	DTT
	100 ug/ml	BSA
	3.5 ug/ml	T4 g43 protein (DNA polymerase)
30	25 ug/ml	T4 g44/62 protein (polymerase accessory protein)
	25 ug/ml	45 protein (polymerase accessory protein)

The mixture was incubated at 37 deg for 30 min. Then 10 u of T4 DNA ligase was added and incubation at 37 deg resumed for 30 min. The polymerase and ligase were inactivated by incubation of the reaction at 70 deg for

15 min. To digest the gene with Xba I, to the reaction was added 50 ul of 2x TA containing BSA at 200 ug/ml and DTT at 1 mM, 43 ul of water, and 50 u of Xba I in 5 ul. The reaction was incubated for 3 hr at 37 deg, and run on a gel. The 431 bp Xba I fragment was purified from a gel and cloned into the Xba I site of the plasmid pUC19 by standard methods. Four plasmid isolates were purified and sequenced using the dideoxy method. One of these had the correct sequence (Figure 3).

To synthesize the light chain, four oligonucleotides JFD1, JFD2, JFD3, JFD4 (Figure 6A) were synthesized. Two of the oligonucleotides are part of each strand of the light chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 6B). Together, the oligonucleotides cover the entire humanized light chain (Figure 4) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

The light chain gene was synthesized from these oligonucleotides in two parts. 0.5 ug each of JFD1 and JFD2 were combined in 20 ul sequenase buffer (40 mM Tris-HCl, pH 7.5, 20 mM magnesium chloride, 50 mM sodium chloride), heated at 70 deg for 3 min and allowed to cool slowly to 23 deg in order for the oligonucleotides to anneal. JFD3 and JFD4 were treated in the same way. Each reaction was made 10 mM in DTT and 0.5 mM in each deoxyribonucleotide and 6.5 u of sequenase (US Biochemicals) was added, in a final volume of 24 ul, and incubated for 1 hr at 37 deg to synthesize the opposite strands of the oligonucleotides. Xba I and Hind III were added to each reaction to digest the DNA (there is a Hind III site in the region where JFD2 and JFD3 overlap and therefore in each of the synthesized DNAs; Figure 6B). The reactions were run on polyacrylamide gels, and the Xba I - Hind III fragments were purified and cloned into pUC18 by standard methods. Several plasmid isolates for each fragment were sequenced by the dideoxy method, and correct ones chosen.



Construction of plasmids to express humanized light and heavy chains

5 The heavy chain Xba I fragment was isolated from the pUC19 plasmid in which it had been inserted and then inserted into the Xba I site of the vector pV71 (see, commonly assigned U.S.S.N. 223,037) in the correct orientation by standard methods, to produce the plasmid pHuGTAC1 (Figure 7). This plasmid will express high levels of a complete heavy chain when transfected into an appropriate host cell.

10 The two light chain Xba I - Hind III fragments were isolated from the pUC18 plasmids in which they had been inserted. The vector plasmid pV $\kappa$ 1 (see, commonly assigned U.S.S.N. 223,037) was cut with Xba I, dephosphorylated and ligated with the two fragments by standard methods. The  
15 desired reaction product has the circular form: vector - Xba I - fragment 1 - Hind III - fragment 2 - Xba I - vector. Several plasmid isolates were analyzed by restriction mapping and sequencing, and one with this form chosen. This plasmid, pHuLTAC (Figure 8), therefore contains the complete humanized  
20 light chain (Figure 4) and will express high levels of the light chain when transfected into an appropriate host cell.

Synthesis and affinity of humanized antibody

25 The plasmids pHuGTAC1 and pHuLTAC were transfected into mouse Sp2/0 cells, and cells that integrated the plasmids were selected on the basis of resistance to mycophenolic acid and/or hygromycin B conferred by the gpt and hyg genes on the plasmids (Figures 7,8) by standard methods. To verify that these cells secreted antibody that  
30 binds to the IL-2 receptor, supernatant from the cells was incubated with HUT-102 cells that are known to express the IL-2 receptor. After washing, the cells were incubated with fluorescein-conjugated goat anti-human antibody, washed, and analyzed for fluorescence on a FACSCAN cytofluorometer. The  
35 results (Figure 9A), clearly show that the humanized antibody binds to these cells, but not to Jurkat T-cells that do not express the IL-2 receptor (Figure 9D). As controls, the

original mouse anti-Tac antibody was also used to stain these cells (Figure 9B,C), giving similar results.

For further experiments, cells producing the humanized antibody were injected into mice, and the resultant ascites collected. Humanized antibody was purified to substantial homogeneity from the ascites by passage through an affinity column of goat anti-human immunoglobulin antibody, prepared on an Affigel-10 support (Bio-Rad Laboratories, Inc., Richmond, CA) according to standard techniques. To determine the affinity of the humanized antibody relative to the original anti-Tac antibody, a competitive binding experiment was performed. About  $5 \times 10^5$  HUT-102 cells were incubated with known quantities (10 - 40 ng) of the anti-Tac antibody and the humanized anti-Tac antibody for 10 min at 4 deg. Then 100 ng of biotinylated anti-Tac was added to the cells and incubated for 30 min at 4 deg. This quantity of anti-Tac had previously been determined to be sufficient to saturate the binding sites on the cells, but not to be in large excess. Then the cells were washed twice with 2 ml of phosphate buffered saline (PBS) containing 0.1% sodium azide. The cells were then incubated for 30 min at 4 deg with 250 ng of phycoerythrin-conjugated avidin, which bound to the biotinylated anti-Tac already bound to the cells. The cells were washed again as above, fixed in PBS containing 1% paraformaldehyde, and analyzed for fluorescence on a FACSCAN cytofluorometer.

Use of increasing amounts (10 - 40 ng) of the anti-Tac antibody as competitor in the first step decreased the amount of biotinylated anti-Tac that could bind to the cells in the second step, and therefore the amount of phycoerythrin-conjugated avidin that bound in the last step, thus decreasing fluorescence (Figure 10A). Equivalent amounts (20 ng) of anti-Tac, and humanized anti-Tac used as competitor decreased the fluorescence to approximately the same degree (Figure 10B). This shows that these antibodies have approximately the same affinity, because if one had greater affinity, it would have more effectively competed

with the biotinylated anti-Tac, thus decreasing fluorescence more.

Biological properties of the humanized antibody

5 For optimal use in treatment of human disease, the humanized antibody should be able to destroy T-cells in the body that express the IL-2 receptor. One mechanism by which antibodies may destroy target cells is antibody-dependent cell-mediated cytotoxicity, abbreviated ADCC (Fundamental Immunology, Paul, W., Ed., Raven Press, New York (1984), at 10 pg. 681), in which the antibody forms a bridge between the target cell and an effector cell such as a macrophage that can lyse the target. To determine whether the humanized antibody and the original mouse anti-Tac antibody can mediate ADCC, a chromium release assay was performed by standard 15 methods. Specifically, human leukemia HUT-102 cells, which express the IL-2 receptor, were incubated with <sup>51</sup>Cr to allow them to absorb this radionuclide. The HUT-102 cells were then incubated with an excess of either anti-Tac or humanized anti-Tac antibody. The HUT-102 cells were next incubated for 20 4 hrs with either a 30:1 or 100:1 ratio of effector cells, which were normal purified human peripheral blood mononuclear cells that had been activated by incubation for about 20 hrs with human recombinant IL-2. Release of <sup>51</sup>Cr, which indicated 25 lysis of the target HUT-102 cells, was measured and the background subtracted (Table 1). The results show that at either ratio of effector cells, anti-Tac did not lyse a significant number of the target cells (less than 5%), while the humanized antibody did (more than 20%). Hence, the humanized antibody is likely to be more efficacious than the 30 original mouse antibody in treating T-cell leukemia or other T-cell mediated diseases.

35

TABLE 1

Percent  $^{51}\text{Cr}$  release after ADCC

	<u>Effector: Target ratio</u>	
	30:1	100:1
<u>Antibody</u>		
Anti-Tac	4%	< 1%
Humanized anti-Tac	24%	23%

From the foregoing, it will be appreciated that the human-like immunoglobulins of the present invention offer numerous advantages of other human IL-2 receptor-specific antibodies. In comparison to anti-Tac mouse monoclonal antibodies, the present human-like immunoglobulin can be more economically produced and contain substantially less foreign amino acid sequences. This reduced likelihood of antigenicity after injection into a human patient represents a significant therapeutic improvement.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WE CLAIM:

1. A composition comprising a substantially pure human-like immunoglobulin specifically reactive with p55 Tac protein.

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2. A composition according to Claim 1, wherein the immunoglobulin comprises two pairs of light/heavy chain dimers, wherein each chain comprises a variable region and a constant region.

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3. A composition according to Claim 2, wherein a variable region of at least one chain comprises at least about 75 amino acids from a human immunoglobulin variable region framework.

15

4. A composition comprising a substantially pure human-like immunoglobulin capable of inhibiting binding of human interleukin-2 (IL-2) to a human IL-2 receptor.

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5. A composition according to Claims 1 or 4, wherein the immunoglobulin exhibits a binding affinity to a human IL-2 receptor of about  $10^8 \text{ M}^{-1}$  or stronger.

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6. A composition according to Claims 1 or 4, wherein the immunoglobulin comprises complementarity determining regions from one immunoglobulin and framework regions from at least one different immunoglobulin.

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7. A recombinant immunoglobulin composition comprising a human-like framework and one or more foreign complementarity determining regions not naturally associated with the framework, wherein said immunoglobulin is capable of binding to a human interleukin-2 receptor.

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8. A composition according to Claim 7, wherein one or more of the foreign CDR's are substantially homologous to a CDR from an immunoglobulin reactive with human p55 Tac protein.

5 9. A composition according to Claim 7, wherein all of the foreign CDR's are located on heavy chains of the immunoglobulin.

10 10. A composition according to Claim 7, wherein the immunoglobulin is an IgG, immunoglobulin isotype.

15 11. A composition according to Claim 7, wherein the mature light and heavy variable region protein sequences are substantially homologous to the sequences in Figures 3 and 4.

20 12. A human-like immunoglobulin having two pairs of light chain/heavy chain dimers and capable of specifically reacting with an epitope on a human interleukin-2 receptor with an affinity of at least about  $10^8 \text{ M}^{-1}$ , said light and heavy chains comprising complementarity determining regions (CDR's) and human-like framework regions, wherein the CDR's are from different immunoglobulin molecules than the framework regions.

25 13. An immunoglobulin according to Claim 12, which binds to an epitope located on a p55 Tac protein.

30 14. An immunoglobulin according to Claim 12, which is capable of blocking the binding of interleukin-2 (IL-2) to human IL-2 receptors.

35 15. An immunoglobulin according to Claim 12, wherein the human-like framework regions comprise amino acids sequences from at least two human immunoglobulins.

16. An immunoglobulin according to Claim 12, wherein the CDR's are from a mouse immunoglobulin.

17. A humanized immunoglobulin capable of binding to human interleukin-2 receptors, said immunoglobulin comprising one or more complementarity determining regions (CDR's) from anti-Tac antibody in a human-like framework.

18. A humanized immunoglobulin according to Claim 17, wherein the human framework is substantially homologous to an Eu immunoglobulin framework.

19. A humanized immunoglobulin according to Claim 17, having a mature heavy chain variable sequence as shown in Figure 3, and a mature light chain sequence as shown in Figure 4.

20. A humanized immunoglobulin according to Claim 17 which is capable of blocking the binding of IL-2 to interleukin-2 receptors on human T-cells.

21. A method of treating T-cell mediated disorders in a human patient, said method comprising administering to said patient a therapeutically effective dose of an immunoglobulin according to Claims 1, 5, 12, or 17.

22. An immunoglobulin according to Claims 1, 5, 12, or 17 which was produced in a myeloma or hybridoma cell.

23. A human-like immunoglobulin heavy chain comprising a human-like heavy chain framework region and a hypervariable region which is substantially identical to a monoclonal antibody heavy chain hypervariable region secreted by the cell line designated A.T.C.C. Accession No. CRL 9688.



24. A human-like immunoglobulin light chain comprising a human light chain framework region and a hypervariable region which is substantially identical to a monoclonal antibody light chain hypervariable region secreted by the cell line designated A.T.C.C. Accession No. CRL 9688.

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25. A polynucleotide molecule comprising a first sequence coding for human-like immunoglobulin framework regions and a second sequence coding for one or more mouse immunoglobulin complementarity determining regions, wherein upon expression said polynucleotide encodes an immunoglobulin specifically reactive with p55 Tac protein and capable of blocking the binding of interleukin-2 (IL-2) to the IL-2 receptor on human T-cells.

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26. A cell line transfected with a polynucleotide of Claim 25.

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NOVEL, IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS

## ABSTRACT OF THE DISCLOSURE

Human-like immunoglobulins specifically reactive  
with human IL-2 receptors are prepared employing recombinant  
DNA technology for use in, e.g., treatment of T-cell mediated  
disorders.

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WP50/1DL/1ATAPP-8.PTO

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VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
37 CFR 1.9(f) and 1.27(c) - SMALL BUSINESS CONCERN

Applicant or Patentee: Cary L. Queen and Harold Edwin Selick  
Serial No.: \_\_\_\_\_ Filing Date: December 28, 1988  
Patent No.: \_\_\_\_\_ Issued: \_\_\_\_\_  
For: NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS

I hereby declare that I am

- the owner of the small business concern identified below:  
 an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN Protein Design Labs, Inc., a Delaware Corporation  
ADDRESS OF CONCERN 3181 Porter Drive  
Palo Alto, California 94304

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled NOVEL IL-2 RECEPTOR-SPECIFIC HUMAN IMMUNOGLOBULINS by inventor(s) Cary L. Queen and Harold Edwin Selick described in

- the application filed herewith  
 application serial no. \_\_\_\_\_, filed \_\_\_\_\_  
 patent no. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
 INDIVIDUAL  SMALL BUSINESS CONCERN  NONPROFIT ORGANIZATION

NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
 INDIVIDUAL  SMALL BUSINESS CONCERN  NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.25(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Laurence Jay Korn  
TITLE OF PERSON OTHER THAN OWNER President  
ADDRESS OF PERSON SIGNING 3181 Porter Drive, Palo Alto, California 94304

SIGNATURE *Laurence Jay Korn* DATE 28/12/88

07/290975

FIGURE 1

1	Q	V	Q	L	Q	Q	S	G	A	E	L	A	K	P	G	A	S	V	K	M
1	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V
21	S	C	K	A	S	G	Y	T	F	T	S	Y	R	M	H	W	V	K	Q	R
21	S	C	K	A	S	G	G	T	F	S	R	S	A	I	I	W	V	R	Q	A
						*				*	-----									
41	P	G	Q	G	L	E	W	I	G	Y	I	N	P	S	T	G	Y	T	E	Y
41	P	G	Q	G	L	E	W	M	G	G	I	V	P	M	F	G	P	P	N	Y
							*	-----												
61	N	Q	K	F	K	D	K	A	T	L	T	A	D	K	S	S	S	T	A	Y
61	A	Q	K	F	Q	G	R	V	T	I	T	A	D	E	S	T	N	T	A	Y
	-----						*	*												
81	M	Q	L	S	S	L	T	F	E	D	S	A	V	Y	Y	C	A	R	G	
81	M	E	L	S	S	L	R	S	E	D	T	A	F	Y	F	C	A	G	G	Y
												*	*	*	*	*	*	-----		
100	G	G	V	F	D	Y	W	G	Q	G	T	T	L	T	V	S	S			
101	G	I	Y	S	P	E	E	Y	N	G	G	L	V	T	V	S	S			
	-----								*	*	*	*								

17/290975

FIGURE 2

1	Q	I	V	L	T	Q	S	P	A	I	M	S	A	S	P	G	E	K	V	T
1	D	I	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	T
21	I	T	C	S	A	S	S	S	I		S	Y	M	H	W	F	Q	Q	K	P
21	I	T	C	R	A	S	Q	S	I	N	T	W	L	A	W	Y	Q	Q	K	P
-----																				
40	G	T	S	P	K	L	W	I	Y	T	T	S	N	L	A	S	G	V	P	A
41	G	K	A	P	K	L	L	M	Y	K	A	S	S	L	E	S	G	V	P	S
-----																				
60	R	F	S	G	S	G	S	G	T	S	Y	S	L	T	I	S	R	M	E	A
61	R	F	I	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P
-----																				
80	E	D	A	A	T	Y	Y	C	H	Q	R	S	T	Y	P	L	T	F	G	S
81	D	D	F	A	T	Y	Y	C	Q	Q	Y	N	S	D	S	K	M	F	G	Q
-----																				
100	G	T	K	L	E	L	K													
101	G	T	K	V	E	V	K													

17/290975

FIGURE 3

10 20 30 40 50 60  
TCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTGCCT  
M G W S W I F L F L L S G T A G V H

70 80 90 100 110 120  
CTCAGGYCCAGCTTGTCCAGTCTGGGGCTGAACTCAAGAAACCTGGCTCGAGCGTGAAGG  
S Q V Q L V Q S G A E V K K P G S S V K

130 140 150 160 170 180  
TCTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACAGGATGCACTGGGTAAGGCAGG  
V S C K A S G Y T F T S Y R M H W V R Q

190 200 210 220 230 240  
CCCCTGGACAGGGTCTGGAATGGATTGGATATATTAATCCGTGCACTGGGTATACTGAAT  
A P G Q G L E W I G Y I N P S T G Y T E

250 260 270 280 290 300  
ACAATCAGAAGTTCAAGGACAAGGCAACAATTACTGCAGACGAATCCACCAATACAGCCT  
Y N Q K F K D K A T I T A D E S T N T A

310 320 330 340 350 360  
ACATGGAAGTCTGAGCAGCCTGAGATCTGAGGACACCGCAGTCTATTACTGTGCAAGAGGGG  
Y M E L S S L R S E D T A V Y Y C A R G

370 380 390 400 410 420  
GGGGGCTCTTTGACTACTGGGGCCAAGGAACCCTGGTCACAGTCTCCTCAGGTGAGTCCT  
G G V F D Y W G Q G T L V T V S S

430  
TAAAACCTCTAGA

07/290975

FIGURE 4

10 20 30 40 50 60  
TCTAGATGGAGACCGATACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCACAGGATCAA  
M E T D T L L L W V L L L W V P G S

70 80 90 100 110 120  
CCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGATAGGG  
T G D I Q M T Q S P S T L S A S V G D R

130 140 150 160 170 180  
TCACCATAACCTGCTCTGCCAGCTCAAGTATAAGTTACATGCACTGGTACCAGCAGAAGC  
Y T I T C S A S S S I S Y M H W Y Q Q K

190 200 210 220 230 240  
CAGGCAAAGCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTG  
P G K A P K L L I Y T T S N L A S G V P

250 260 270 280 290 300  
CTCGCTTCAGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGC  
A R F S G S G S G T E F T L T I S S L Q

310 320 330 340 350 360  
CAGATGATTTCCGCACTTATTACTGCCATCAAAGGAGTACTTACCCACTCACGTTCCGGTC  
P D D F A T Y Y C H Q R S T Y P L T F G

370 380 390 400  
AGGGGACCAAGGTGGAGGTCAAACGTAAGTACACTTTTCTAGA  
Q G T K V E V K



17/290975

FIGURE 5

A

HES12 AGCTTCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTG  
CACTCTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTG  
AAGGTC

HES13 CCCAGTCGACGGATTAATATATATCCAATCCATTCCAGACCCTGTCCAGGGGCCTGCCTTAC  
CCAGTGCATCCTGTAGCTAGTAAAGGTGTAGCCAGAAGCCTTGCAGGAGACCTTCACGCT  
CGAGCCAGG

HES14 TATATTAATCCGTCGACTGGGTATACTGAATACAATCAGAAGTTCAAGGACAAGGCAACA  
ATTACTGCAGACGAATCCACCAATACAGCCTACATGGAAGTGAAGCAGCCTGAGATCTGAG  
GACA

HES15 ATATCGTCTAGAGGTTTTAAGGACTCACCTGAGGAGACTGTGACCAGGGTTCCTTGGCCC  
CAGTAGTCAAAGACCCCCCCCCCTCTTGACAGTAATAGACTGCGGTGTCCTCAGATCTC  
AGGCTGCT

B



3 2 2 3  
3 3 3 3  
3 3 3 3  
3 3 3 3

17/290975

FIGURE 6

A

JFD1 CAAATCTAGATGGAGACCGATACCCTCCTGCTATGGGTCTCCTGCTATGGGTCCCAGGA  
TCAACCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGAT

JFD2 ATAAATTAGAAGCTTGGGAGCTTTGCCTGGCTTCTGCTGGTACCAATGTCATGTAAGTTAT  
ACTTGAGCTGGCAGAGCAGGTTATGGTGACCCTATCCCCGACGCTAGCAGAGAG

JFD3 GCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTC  
AGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGCCAGATGAT  
TTC

JFD4 TATATCTAGAAAAGTGTACTTACGTTTGACCTCCACCTTGGTCCCCTGACCGAACGTGAG  
TGGGTAAGTACTCCTTTGATGGCAGTAATAAGTGGCGAAATCATCTGGCTGCAGAGAGCT  
GA

B

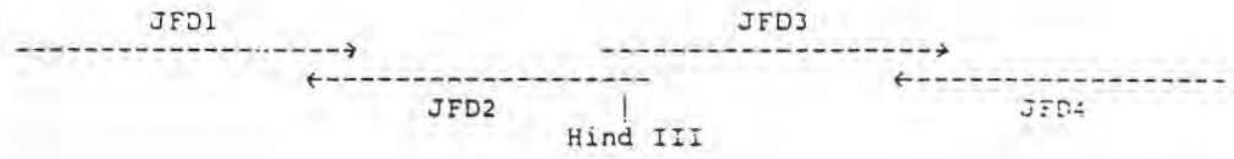


FIGURE 7

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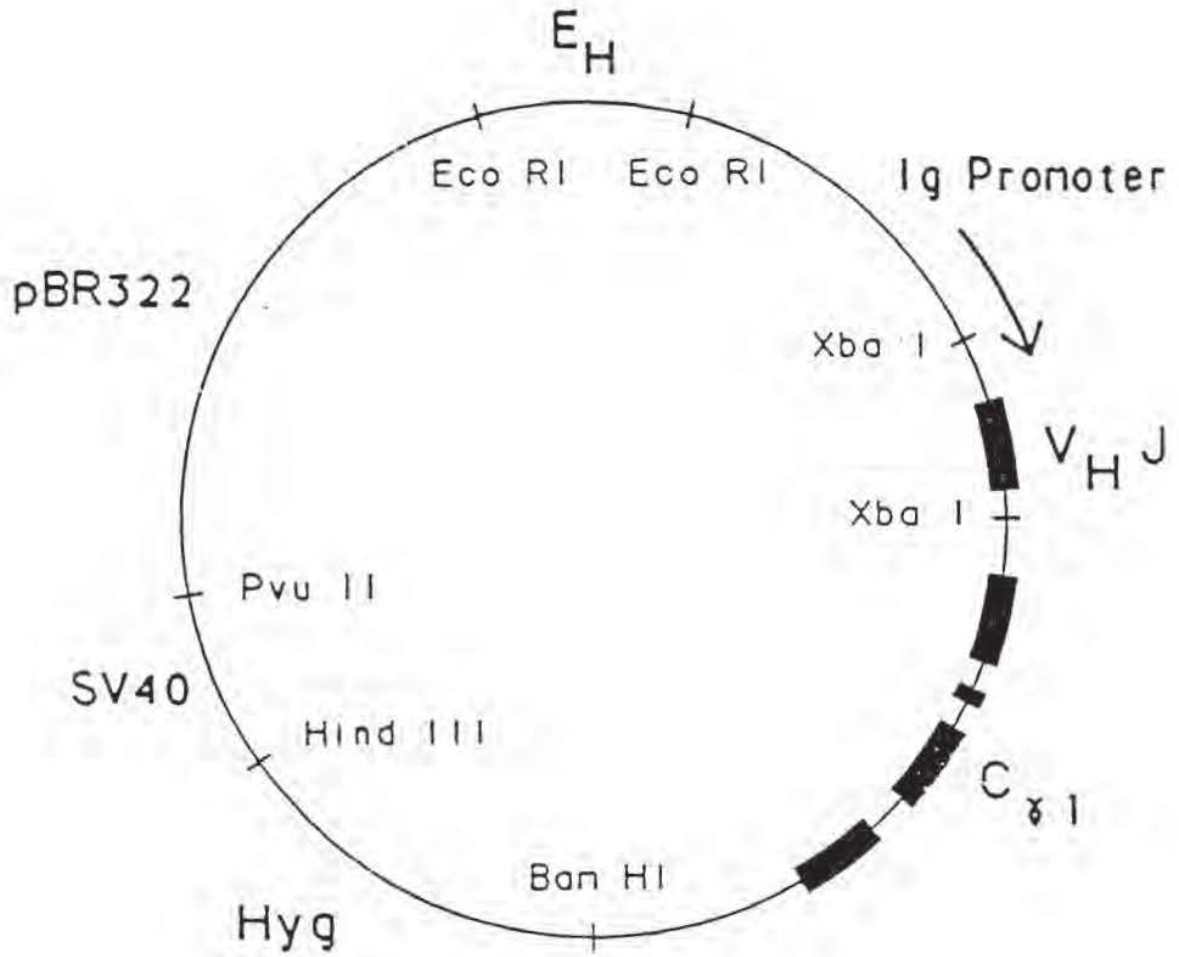
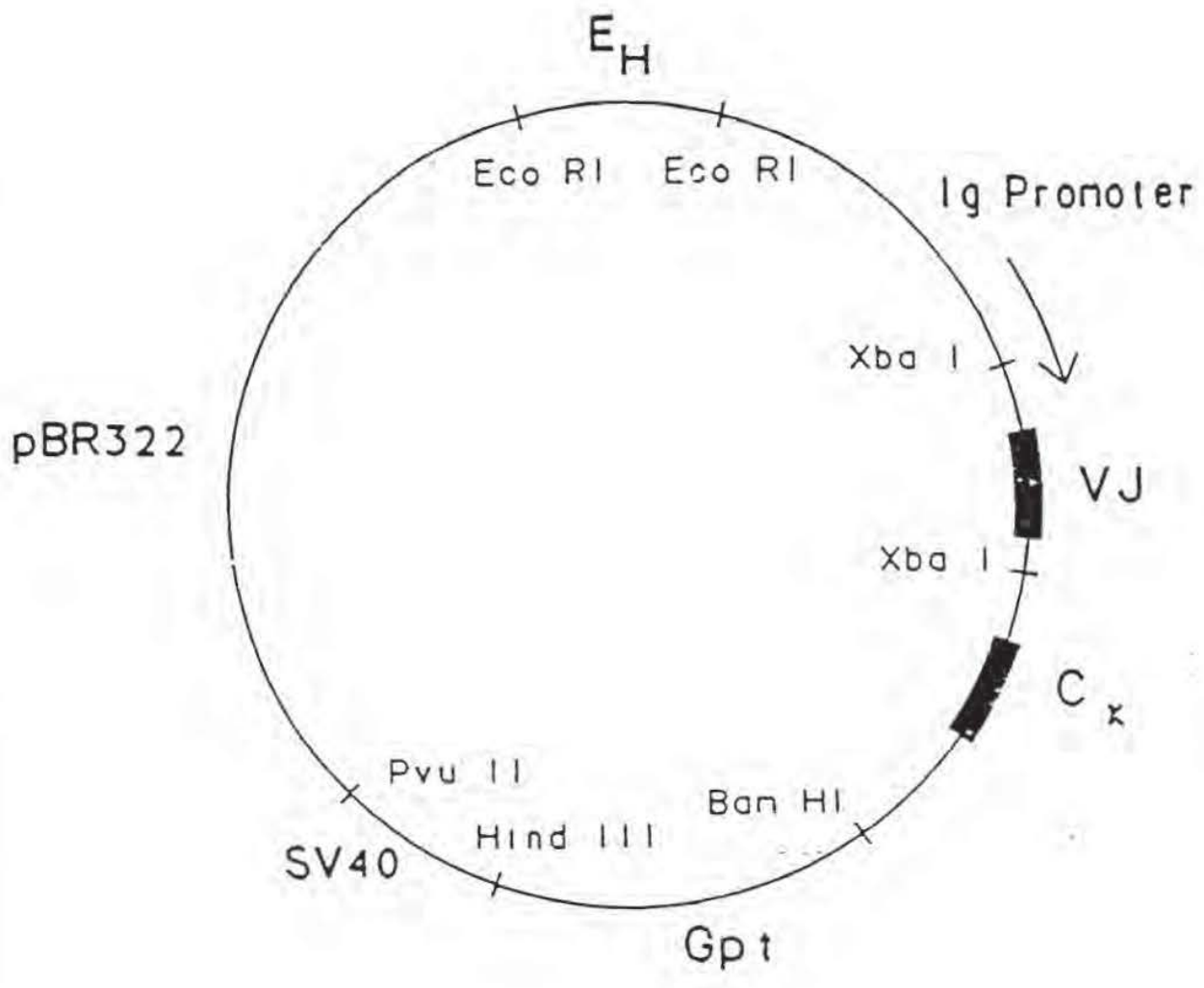
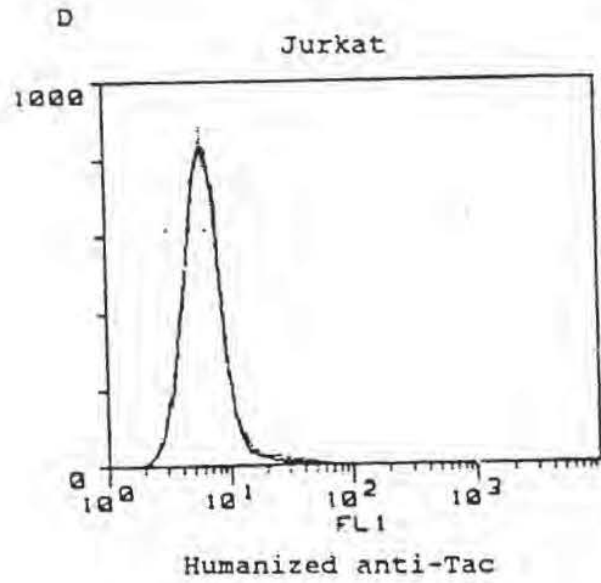
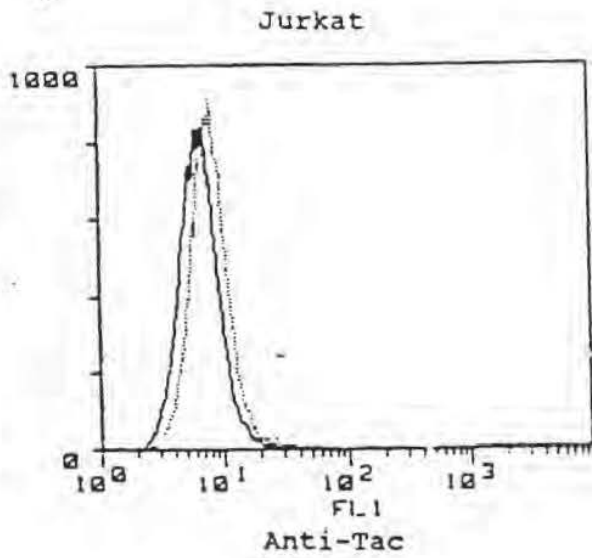
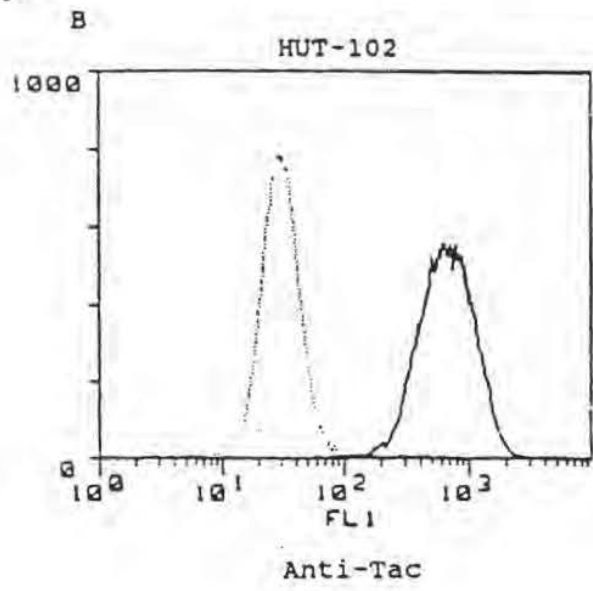
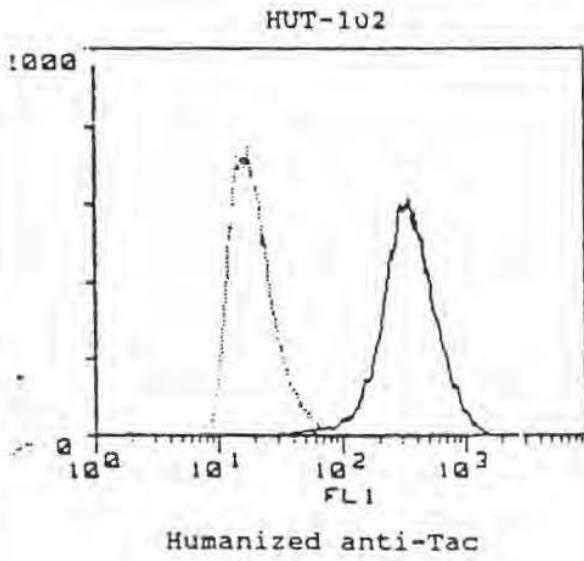


FIGURE 8



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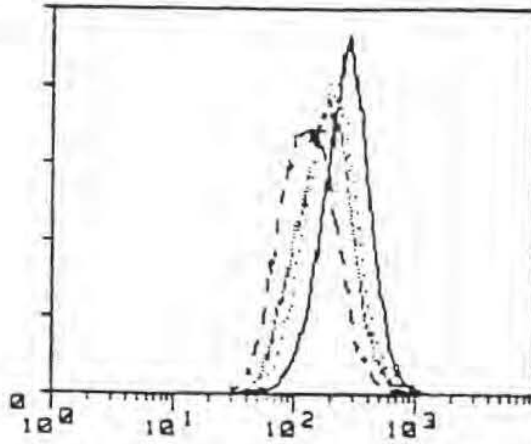
FIGURE 9



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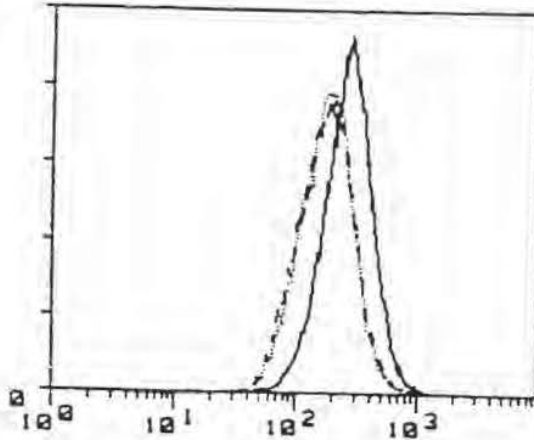
FIGURE 10

A



— 0 ng anti-Tac  
..... 10 ng  
- - - - 20 ng  
- · - · 40 ng

B



— 0 ng anti-Tac  
..... 20 ng anti-Tac  
- - - - 20 ng humanized anti-Tac

SERIAL NUMBER Series of 1987	07/310252	PATENT DATE	PATENT NUMBER
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8

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
07/310252	02/13/94	435		135	

APPLICANTS

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\*\*\*CONTINUING DATA\*\*\*\*\*  
 VERIFIED THIS APPLN IS A CIP OF 07/290,975 12/28/88

REC'D 28 DEC 1989  
 WIPO PCT

\*\*\*FOREIGN/PCT APPLICATIONS\*\*\*\*\*  
 VERIFIED

PRIORITY DOCUMENT

FOREIGN FILING LICENSE GRANTED 03/03/89 \*\*\*\*\* SMALL ENTITY \*\*\*\*\*

Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> yes <input type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS OR WGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S COCKET NO.
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TITLE  
 DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

FORM 1001 (REV. 10-78)



This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application as originally filed which is identified above.

By authority of the  
 COMMISSIONER OF PATENTS AND TRADEMARKS

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 Certifying Officer

PATENT APPLICATION SERIAL NO. 07/310252

U.S. DEPARTMENT OF COMMERCE  
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A-07-31,9252  
11823-9

DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

CROSS-REFERENCE TO RELATED APPLICATION

5 This is a continuation-in-part application of  
commonly assigned patent application U.S.S.N. 290,975, filed  
December 28, 1988, which is incorporated herein by reference.

Field of the Invention

10 The present invention relates generally to the  
combination of recombinant DNA and monoclonal antibody  
technologies for developing novel therapeutic agents and,  
more particularly, to the production of non-immunogenic  
antibodies having strong affinity for a predetermined  
antigen.

15

Background of the Invention

20 The advent of monoclonal antibody technology in the  
mid 1970's heralded a new age of medicine. For the first  
time, researchers and clinicians had access to essentially  
unlimited quantities of uniform antibodies capable of binding  
to a predetermined antigenic site and having various  
immunological effector functions. These proteins, known as  
"monoclonal antibodies" were thought to hold great promise  
in, e.g., the removal of harmful cells *in vivo*. Indeed, the  
25 clinical value of monoclonal antibodies seemed limitless for  
this use alone.

30 Unfortunately, the development of appropriate  
therapeutic products based on these proteins has been  
severely hampered by a number of drawbacks inherent in  
monoclonal antibody production. For example, most monoclonal  
antibodies are mouse derived, and thus do not fix human  
complement well. They also lack other important  
immunoglobulin functional characteristics when used in  
humans.

35 Perhaps most importantly, non-human monoclonal  
antibodies contain substantial stretches of amino acid  
sequences that will be immunogenic when injected into a human

patient. Numerous studies have shown that after injection of a foreign antibody, the immune response mounted by a patient can be quite strong, essentially eliminating the antibody's therapeutic utility after an initial treatment. Moreover, as increasing numbers of different mouse or other antigenic (to humans) monoclonal antibodies can be expected to developed to treat various diseases, after the first or second treatments with any non-human antibodies, subsequent treatments, even for unrelated therapies, can be ineffective or even dangerous in themselves.

While the production of so called "chimeric antibodies" (e.g., mouse variable regions joined to human constant regions) has proven somewhat successful, a significant immunogenicity problem remains. Moreover, efforts to immortalize human B-cells or generate human hybridomas capable of producing human immunoglobulins against a desired antigen have been generally unsuccessful, particularly with many important human antigens. Most recently, recombinant DNA technology has been utilized to produce immunoglobulins which have human framework regions combined with complementarity determining regions (CDR's) from a donor mouse or rat immunoglobulin (see, e.g., EPO Publication No. 0239400, which is incorporated herein by reference). These new proteins are called "humanized immunoglobulins" and the process by which the donor immunoglobulin is converted into a human-like immunoglobulin by combining its CDR's with a human framework is called "humanization". Humanized antibodies are important because they bind to the same antigen as the original antibodies, but are less immunogenic when injected into humans.

However, a major problem with present humanization procedures has been a loss of affinity for the antigen, usually by at least 2 to 3-fold (Jones et al., Nature, 321:522-525 (1986)) and in some instances as much as 10-fold or more, especially when the antigen is a protein (Verhoeven et al., Science, 239:1534-1536 (1988)). Loss of any affinity is, of course, highly undesirable. At the least, it means that more of the humanized antibody will have to be injected

into the patient, at higher cost and greater risk of adverse effects. Even more critically, an antibody with reduced affinity may have poorer biological functions, such as complement lysis, antibody-dependent cellular cytotoxicity, or virus neutralization. For example, the loss of affinity in the partially humanized antibody HuVHCAMP may have caused it to lose all ability to mediate complement lysis (see, Riechmann et al., Nature, 332:323-327 (1988); Table 1).

Thus, there is a need for improved means for producing humanized antibodies specifically reactive with strong affinity to a predetermined antigen. These humanized immunoglobulins should remain substantially non-immunogenic in humans, yet be easily and economically produced in a manner suitable for therapeutic formulation and other uses. The present invention fulfills these and other needs.

#### Summary of the Invention

The present invention provides novel methods for designing humanized immunoglobulin chains having one or more complementarity determining regions (CDR's) from a donor immunoglobulin and a framework region from a human immunoglobulin, the preferred methods comprising first comparing the framework or variable region amino acid sequence of the donor immunoglobulin to corresponding sequences in a collection of human immunoglobulin chains, and selecting as the human immunoglobulin one of the more homologous sequences from the collection. The human immunoglobulin, or acceptor immunoglobulin, sequence is typically selected from a collection of at least 10 to 20 immunoglobulin chain sequences, and usually will have the highest homology to the donor immunoglobulin sequence of any sequence in the collection. The human immunoglobulin framework sequence will typically have about 65 to 70% homology or more to the donor immunoglobulin framework sequences. The donor immunoglobulin may be either a heavy chain or light chain (or both), and the human collection will contain the same kind of chain. A humanized light and heavy chain can be used to form a complete humanized immunoglobulin

or antibody, having two light/heavy chain pairs, with or without partial or full-length human constant regions and other proteins.

In another embodiment of the present invention, either in conjunction with the above comparison step or separately, additional amino acids in an acceptor immunoglobulin chain may be replaced with amino acids from the CDR-donor immunoglobulin chain. More specifically, further optional substitutions of a human framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from a donor immunoglobulin will be made at positions in the immunoglobulins where:

(a) the amino acid in the human framework region of an acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is common for that position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to be within about 3Å of the CDR's in a three-dimensional immunoglobulin model and capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

The humanized immunoglobulin chain will typically comprise at least about 3 amino acids from the donor immunoglobulin in addition to the CDR's, usually at least one of which is immediately adjacent to a CDR in the donor immunoglobulin. The heavy and light chains may each be designed by using any one or all three of the position criteria.

When combined into an intact antibody, the humanized light and heavy chains of the present invention will be substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen (such as a protein or other compound containing an epitope). These affinity levels can vary from about  $10^8 \text{ M}^{-1}$  or higher, and may be within about 4 fold of the donor immunoglobulin's original affinity to the antigen.

Once designed, the immunoglobulins, including binding fragments and other immunoglobulin forms, of the present invention may be produced readily by a variety of recombinant DNA or other techniques. Preferably, polynucleotides encoding the desired amino acid sequences are produced synthetically or by joining appropriate nucleic acid sequences for expression in a suitable host (e.g., cell culture). The humanized immunoglobulins will be particularly useful in treating human disorders susceptible to monoclonal antibody therapy, but find a variety of other uses as well.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Comparison of sequences of anti-Tac heavy chain (upper lines) and Eu heavy chain (lower lines). The 1-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDR's are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an \*.

Figure 2. Comparison of sequences of anti-Tac light chain (upper lines) and Eu light chain (lower lines). The single-letter code for amino acids is used. The first amino acid on each line is numbered at the left. Identical amino acids in the two sequences are connected by lines. The 3 CDR's are underlined. Other amino acid positions for which the anti-Tac amino acid rather than the Eu amino acid was used in the humanized anti-Tac heavy chain are denoted by an \*.

Figure 3. Nucleotide sequence of the gene for the humanized anti-Tac heavy chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature heavy chain sequence begins with amino acid #20 Q.

Figure 4. Nucleotide sequence of the gene for the humanized anti-Tac light chain variable region gene. The translated amino acid sequence for the part of the gene encoding protein is shown underneath the nucleotide sequence. The nucleotides TCTAGA at the beginning and end of the gene are Xba I sites. The mature light chain sequence begins with amino acid #21 D.

Figure 5. A. Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac heavy chain gene, printed 5' to 3'. B. Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide.

Figure 6. (A) Sequences of the four oligonucleotides used to synthesize the humanized anti-Tac light chain gene, printed 5' to 3'. (B) Relative positions of the oligonucleotides. The arrows point in the 3' direction for each oligonucleotide. The position of a Hind III site in the overlap of JFD2 and JFD3 is shown.

Figure 7. Schematic diagram of the plasmid pHuGTAC1 used to express the humanized anti-Tac heavy chain. Relevant restriction sites are shown, and coding regions of the heavy chain are displayed as boxes. The direction of transcription from the immunoglobulin (Ig) promoter is shown by an arrow.  $E_H$  = heavy chain enhancer, Hyg = hygromycin resistance gene.

Figure 8. Schematic diagram of the plasmid pHuLTAC used to express the humanized anti-Tac light chain. Relevant restriction sites are shown, and coding regions of the light chain are displayed as boxes. The direction of transcription from the Ig promoter is shown by an arrow.

Figure 9. Fluorocytometry of HUT-102 and Jurkat cells stained with anti-Tac antibody or humanized anti-Tac antibody followed respectively by fluorescein-conjugated goat anti-mouse Ig antibody or goat anti-human Ig antibody, as labeled. In each panel, the dotted curve shows the results when the first antibody was omitted, and the solid curve the results when first and second (conjugated) antibodies were included as described.

Figure 10. (A) Fluorocytometry of HUT-102 cells stained with 0-40 ng of anti-Tac as indicated, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin. (B) Fluorocytometry of HUT-102 cells stained with the indicated antibody, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin.

#### DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, novel means of designing humanized immunoglobulins capable of specifically binding to a predetermined antigen with strong affinity are provided. These improved methods produce immunoglobulins that are substantially non-immunogenic in humans but have binding affinities of at least about  $10^8 \text{ M}^{-1}$ , preferably  $10^9 \text{ M}^{-1}$  to  $10^{10} \text{ M}^{-1}$ , or stronger. The humanized immunoglobulins will have a human framework and have one or more complementary determining regions (CDR's), plus a limited number of other amino acids, from a donor immunoglobulin specifically reactive with an antigen. The immunoglobulins can be produced economically in large quantities and find use, for example, in the treatment of various human disorders by a variety of techniques.

In order that the invention may be more completely understood, several definitions are set forth. As used herein, the term "immunoglobulin" refers to a protein having one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd, about 214 amino acids) are encoded by a variable region gene at the NH<sub>2</sub>-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH - terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd, about 446 amino acids), are similarly encoded by a variable region gene (encoding about 116 amino acids) and one of the other

aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

One form of immunoglobulin constitutes the basic structural unit of an antibody. This form is a tetramer and consists of two identical pairs of immunoglobulin chains, each pair having one light and one heavy chain. In each pair, the light and heavy chain variable regions are together responsible for binding to an antigen, and the constant regions are responsible for the antibody effector functions. In addition to antibodies, immunoglobulins may exist in a variety of other forms (including less than full-length that retain the desired activities), including, for example, Fv, Fab, and F(ab')<sub>2</sub>, as well as single chain antibodies (e.g., Huston et al., Proc. Nat. Acad. Sci. U.S.A., 85:5879-5883 (1988) and Bird et al., Science, 242:423-426 (1988), which are incorporated herein by reference). (See, generally, Hood et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, Nature, 323:15-16 (1986), which are incorporated herein by reference).

An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, also called CDR's. The extent of the framework region and CDR's have been precisely defined (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, (1983); which is incorporated herein by reference). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDR's. The CDR's are primarily responsible for binding to an epitope of an antigen.

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to human constant segments, such as



gamma 1 and gamma 3. A typical therapeutic chimeric antibody is thus a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody (e.g., A.T.C.C. Accession No. CRL 9688 secretes an anti-Tac chimeric antibody), although other mammalian species may be used.

As used herein, the term "humanized" immunoglobulin refers to an immunoglobulin comprising a substantially human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the "donor" and a human immunoglobulin providing the framework is called the "acceptor". Constant regions need not be present, but if they are, they must be substantially homologous to human immunoglobulin constant regions, i.e., at least about 85-90%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially homologous to corresponding parts of natural human immunoglobulin sequences. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody as defined above, e.g., because the entire variable region of a chimeric antibody is non-human. One says that the donor antibody has been "humanized", by the process of "humanization", because the resultant humanized antibody is expected to bind to the same antigen as the donor antibody that provides the CDR's.

Humanized immunoglobulins, including humanized antibodies, have been constructed by means of genetic engineering. Most humanized immunoglobulins that have been previously described (Jones et al., op. cit.; Verhoeyen et al., op. cit.; Riechmann et al., op. cit.) have comprised a framework that is identical to the framework of a particular human immunoglobulin chain, the acceptor, and three CDR's from a non-human donor immunoglobulin chain. In one case (Riechmann et al., op. cit.), two additional amino acids in the framework were changed to be the same as amino acids in

other human framework regions. The present invention includes criteria by which a limited number of amino acids in the framework of a humanized immunoglobulin chain are chosen to be the same as the amino acids at those positions in the donor rather than in the acceptor, in order to increase the affinity of an antibody comprising the humanized immunoglobulin chain.

The present invention is based in part on the model that two contributing causes of the loss of affinity in prior means of producing humanized antibodies (using as examples mouse antibodies as the source of CDR's) are:

(1) When the mouse CDR's are combined with the human framework, the amino acids in the framework close to the CDR's become human instead of mouse. Without intending to be bound by theory, we believe that these changed amino acids may slightly distort the CDR's, because they create different electrostatic or hydrophobic forces than in the donor mouse antibody, and the distorted CDR's may not make as effective contacts with the antigen as the CDR's did in the donor antibody;

(2) Also, amino acids in the original mouse antibody that are close to, but not part of, the CDR's (*i.e.*, still part of the framework), may make contacts with the antigen that contribute to affinity. These amino acids are lost when the antibody is humanized, because all framework amino acids are made human.

To avoid these problems, and to produce humanized antibodies that have a very strong affinity for a desired antigen, the present invention uses the following four criteria for designing humanized immunoglobulins. These criteria may be used singly, or when necessary in combination, to achieve the desired affinity or other characteristics.

Criterion I: As acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. For example,

comparison of the sequence of a mouse heavy (or light) chain variable region against human heavy (or light) variable regions in a data bank (for example, the National Biomedical Research Foundation Protein Identification Resource) shows that the extent of homology to different human regions varies greatly, typically from about 40% to about 60-70%. By choosing as the acceptor immunoglobulin one of the human heavy (respectively light) chain variable regions that is most homologous to the heavy (respectively light) chain variable region of the donor immunoglobulin, fewer amino acids will be changed in going from the donor immunoglobulin to the humanized immunoglobulin. Hence, and again without intending to be bound by theory, it is believed that there is a smaller chance of changing an amino acid near the CDR's that distorts their conformation. Moreover, the precise overall shape of a humanized antibody comprising the humanized immunoglobulin chain may more closely resemble the shape of the donor antibody, also reducing the chance of distorting the CDR's.

Typically, one of the 3-5 most homologous heavy chain variable region sequences in a representative collection of at least about 10 to 20 distinct human heavy chains will be chosen as acceptor to provide the heavy chain framework, and similarly for the light chain. Preferably, one of the 1-3 most homologous variable regions will be used. The selected acceptor immunoglobulin chain will most preferably have at least about 65% homology in the framework region to the donor immunoglobulin.

Regardless of how the acceptor immunoglobulin is chosen, higher affinity may be achieved by selecting a small number of amino acids in the framework of the humanized immunoglobulin chain to be the same as the amino acids at those positions in the donor rather than in the acceptor. The following criteria define what amino acids may be so selected. Preferably, at most or all amino acid positions satisfying one of these criteria, the donor amino acid will in fact be selected.

Criterion II: If an amino acid in the framework of the human acceptor immunoglobulin is unusual (i.e., "rare", which as used herein indicates an amino acid occurring at that position in no more than about 10% of human heavy (respectively light) chain V region sequences in a representative data bank), and if the donor amino acid at that position is typical for human sequences (i.e., "common", which as used herein indicates an amino acid occurring in at least about 25% of sequences in a representative data bank), then the donor amino acid rather than the acceptor may be selected. This criterion helps ensure that an atypical amino acid in the human framework does not disrupt the antibody structure. Moreover, by replacing an unusual amino acid with an amino acid from the donor antibody that happens to be typical for human antibodies, the humanized antibody may be made less immunogenic.

Criterion III: In the positions immediately adjacent to the 3 CDR's in the humanized immunoglobulin chain, the donor amino acid rather than acceptor amino acid may be selected. These amino acids are particularly likely to interact with the amino acids in the CDR's and, if chosen from the acceptor, distort the donor CDR's and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit et al., Science, 233, 747-753 (1986), which is incorporated herein by reference) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

Criterion IV: A 3-dimensional model, typically of the original donor antibody, shows that certain amino acids outside of the CDR's are close to the CDR's and have a good probability of interacting with amino acids in the CDR's by hydrogen bonding, Van der Waals forces, hydrophobic interactions, etc. At those amino acid positions, the donor amino acid rather than the acceptor immunoglobulin amino acid may be selected. Amino acids according to this criterion will

generally have a side chain atom within about 3 angstrom units of some site in the CDR's and must contain atoms that could interact with the CDR atoms according to established chemical forces, such as those listed above. Computer programs to create models of proteins such as antibodies are generally available and well known to those skilled in the art (see, Loew et al., Int. J. Quant. Chem., Quant. Biol. Symp., 15:55-66 (1988); Bruccoleri et al., Nature, 335, 564-568 (1988); Chothia et al., Science, 233:755-758 (1986), all of which are incorporated herein by reference). These do not form part of the invention. Indeed, because all antibodies have similar structures, the known antibody structures, which are available from the Brookhaven Protein Data Bank, can be used if necessary as rough models of other antibodies. Commercially available computer programs can be used to display these models on a computer monitor, to calculate the distance between atoms, and to estimate the likelihood of different amino acids interacting (see, Ferrin et al., J. Mol. Graphics, 6:13-27 (1988)).

Humanized antibodies generally have at least three potential advantages over mouse or in some cases chimeric antibodies for use in human therapy:

1) Because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).

2) The human immune system should not recognize the framework or constant region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign mouse antibody or a partially foreign chimeric antibody.

3) Injected mouse antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of normal antibodies (D. Shaw et al., J. Immunol., 138:4534-4538 (1987)). Injected humanized antibodies will presumably have a half-life more similar to

naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

In one aspect, the present invention is directed to designing humanized immunoglobulins that are produced by expressing recombinant DNA segments encoding the heavy and light chain CDR's from a donor immunoglobulin capable of binding to a desired antigen, such as the human IL-2 receptor, to DNA segments encoding acceptor human framework regions. Exemplary DNA sequences designed in accordance with the present invention and, which on expression code for the polypeptide chains comprising heavy and light chain CDR's with substantially human framework regions, are shown in Figures 3 and 4, respectively. Due to codon degeneracy and non-critical amino acid substitutions, other DNA sequences can be readily substituted for those sequences, as detailed below. In general, the criteria of the present invention find applicability to designing substantially any humanized immunoglobulin.

The DNA segments will typically further include an expression control DNA sequence operably linked to the humanized immunoglobulin coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the humanized light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow (see, S. Beychok, Cells of Immunoglobulin Synthesis, Academic Press, N.Y., (1979), which is incorporated herein by reference.

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B-cells (see, Kabat

op. cit. and WP87/02671). The CDR's for producing the immunoglobulins of the present invention will be similarly derived from monoclonal antibodies capable of binding to the predetermined antigen, such as the human IL-2 receptor, and produced by well known methods in any convenient mammalian source including, mice, rats, rabbits, or other vertebrate capable of producing antibodies. Suitable source cells for the constant region and framework DNA sequences, and host cells for immunoglobulin expression and secretion, can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference).

In addition to the humanized immunoglobulins specifically described herein, other "substantially homologous" modified immunoglobulins to the native sequences can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the framework regions can vary specifically from the sequences in Figures 3 and 4 at the primary structure level by several amino acid substitutions, terminal and intermediate additions and deletions, and the like. Moreover, a variety of different human framework regions may be used singly or in combination as a basis for the humanized immunoglobulins of the present invention. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, Gene, 8:81-97 (1979) and S. Roberts et al., Nature, 328:731-734 (1987), both of which are incorporated herein by reference).

Alternatively, polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities (e.g., complement fixation activity). Also because like many genes, the immunoglobulin-related genes contain separate functional regions, each having one or more distinct biological activities, the genes may be fused to functional regions from other genes (e.g., enzymes, see,

commonly assigned U.S.S.N. 132,387, filed Dec. 15, 1987, which is incorporated herein by reference) to produce fusion proteins (e.g., immunotoxins) having novel properties. The nucleic acid sequences of the present invention capable of ultimately expressing the desired humanized antibodies can be formed from a variety of different polynucleotides (genomic or cDNA, RNA, synthetic oligonucleotides, etc.) and components (e.g., V, J, D, and C regions), as well as by a variety of different techniques. Joining appropriate genomic sequences is presently the most common method of production, but cDNA sequences may also be utilized (see, European Patent Publication No. 0239400 and L. Reichmann et al., Nature, 312:323-327 (1988), both of which are incorporated herein by reference).

As stated previously, the DNA sequences will be expressed in hosts after the sequences have been operably linked to (i.e., positioned to ensure the functioning of) an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired DNA sequences (see, e.g., U.S. Patent 4,704,362, which is incorporated herein by reference).

*E. coli* is one prokaryotic host useful particularly for cloning the DNA sequences of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (*trp*) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site



sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, may also be used for expression. Saccharomyces is a preferred host, with suitable vectors having expression control sequences, such as  
5 promoters, including 3-phosphoglycerate kinase or other glycolytic enzymes, and an origin of replication, termination sequences and the like as desired.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the  
10 polypeptides of the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987), which is incorporated herein by reference). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins have been  
15 developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, etc, but preferably transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an  
20 enhancer (Queen et al., Immunol. Rev., 89:49-68 (1986), which is incorporated herein by reference), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences  
25 are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, and the like.

The vectors containing the DNA segments of interest (e.g., the heavy and light chain encoding sequences and  
30 expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts. (See, generally, Maniatis et al.,  
35 Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, (1982), which is incorporated herein by reference.)

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see, generally, R. Scopes, "Protein Purification", Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The antibodies of the present invention will typically find use individually in treating substantially any disease susceptible to monoclonal antibody-based therapy. In particular, the immunoglobulins can be used for passive immunization or the removal of unwanted cells or antigens, such as by complement mediated lysis, all without substantial immune reactions (e.g., anaphylactic shock) associated with many prior antibodies. For example, where the cell linked to a disease has been identified as IL-2 receptor bearing, then humanized antibodies that bind to the human IL-2 receptor are suitable (see, U.S.S.N. 085,707, entitled "Treating Human Malignancies and Disorders," which is incorporated herein by reference). For such a humanized immunoglobulin, typical disease states suitable for treatment include graft versus host disease and transplant rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include autoimmune diseases, such as Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

The method of producing humanized antibodies of the present invention can be used to humanize a variety of donor

antibodies, especially monoclonal antibodies reactive with markers on cells responsible for a disease. For example, suitable antibodies bind to antigens on T-cells, such as those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard et al., Eds., Springer-Verlag, N.Y. (1984), which is incorporated herein by reference.

The antibodies of the present invention can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

A preferred pharmaceutical composition of the present invention comprises the use of the subject antibodies in immunotoxins. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference.

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides,

such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase C). (See, generally, commonly assigned U.S.S.N. 290,968 (Townsend and Townsend Docket No. 11823-7-2) filed in U.S.P.T.O. on December 28, 1988, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), all of which are incorporated herein by reference.)

The delivery component of the immunotoxin will include the humanized immunoglobulins of the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

For diagnostic purposes, the antibodies may either be labeled or unlabeled. Unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the humanized antibody, such as antibodies specific for human immunoglobulin constant regions. Alternatively, the antibodies can be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluors, enzymes, enzyme substrates, enzyme co-factors, enzyme inhibitors, ligands (particularly haptens), etc.. Numerous types of immunoassays are available and are well known to those skilled in the art.

The following examples are offered by way of illustration, not by limitation.

## EXPERIMENTAL

Design of genes for humanized light and heavy chains

The sequence of the human antibody Eu (Sequences of Proteins of Immunological Interest, E. Kabat et al., U.S. Dept. of Health and Human Services, 1983) was used to provide the framework of the humanized antibody, because the amino acid sequence of the heavy chain variable region of anti-Tac is more homologous to the heavy chain of this antibody than to any other complete heavy chain variable region sequence in the National Biomedical Foundation Protein Identification Resource.

To select the sequence of the humanized heavy chain, the anti-Tac heavy chain sequence (see, commonly assigned U.S.S.N.'s 186,862 and 223,037, which are incorporated herein by reference) was aligned with the sequence of the Eu heavy chain (Figure 1). At each position, the Eu amino acid was selected for the humanized sequence, unless that position fell in any one of the following categories, in which case the anti-Tac amino acid was selected:

(1) The position fell within a complementarity determining region (CDR), as defined by Kabat, et al., op. cit. (amino acids 31-35, 50-66, 99-106);

(2) The Eu amino acid was rare for human heavy chains at that position, whereas the anti-Tac amino acid was common for human heavy chains at that position (amino acids 27, 93, 95, 98, 107-109, 111);

(3) The position was immediately adjacent to a CDR in the amino acid sequence of the anti-Tac heavy chain (amino acids 30 and 67); or

(4) 3-dimensional modeling of the anti-Tac antibody suggested that the amino acid was physically close to the antigen binding region (amino acids 48 and 68).

Amino acid #27 is listed in category (4) because the acceptor Eu amino acid Gly is rare, and the donor anti-Tac amino acid Tyr is chemically similar to the amino acid Phe, which is common, but the substitution was actually made because #27

also fell in category (4). Although some amino acids fell in more than one of these categories, they are only listed in one. Categories (2) - (4) correspond to criteria (2) - (4) described above.

To select the sequence of the humanized light chain, the anti-Tac light chain sequence was aligned with the sequence of the Eu light chain (Figure 2). The Eu amino acid was selected at each position, unless the position again fell into one of the categories (1) - (4) (with light chain replacing heavy chain in the category definitions):

- (1) CDR's (amino acids 24-34, 50-56, 89-97);
- (2) Anti-Tac amino acid more typical than Eu (amino acids 48 and 63);
- (3) Adjacent to CDR's (no amino acids; Eu and anti-Tac were already the same at all these positions); or
- (4) Possible 3-dimensional proximity to binding region (amino acid 60).

The actual nucleotide sequence of the heavy (Figure 3) and light chain (Figure 4) genes were selected as follows:

- (1) The nucleotide sequences code for the amino acid sequences chosen as described above;
- (2) 5' of these coding sequences, the nucleotide sequences code for a leader (signal) sequence, namely the leader of the light chain of the antibody MOPC 63 and the leader of the heavy chain of the antibody PCH 108A (Kabat *et al.*, *op. cit.*). These leader sequences were chosen as typical of antibodies;
- (3) 3' of the coding sequences, the nucleotide sequences are the sequences that follow the mouse light chain J5 segment and the mouse heavy chain J2 segment, which are part of the anti-Tac sequences. These sequences are included because they contain splice donor signals; and
- (4) At each end of the sequence is an Xba I site to allow cutting at the Xba I sites and cloning into the Xba I site of a vector.

Construction of humanized light and heavy chain genes

To synthesize the heavy chain, four oligonucleotides HES12, HES13, HES14, HES15 (Figure 5A) were synthesized using an Applied Biosystems 380B DNA synthesizer. Two of the oligonucleotides are part of each strand of the heavy chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 5B). Together, the oligonucleotides cover the entire humanized heavy chain variable region (Figure 3) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

Each oligonucleotide was phosphorylated using ATP and T4 polynucleotide kinase by standard procedures (see, Maniatis, *op. cit.*). To anneal the phosphorylated oligonucleotides, they were suspended together in 40 ul of TA (33 mM Tris acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate) at a concentration of about 3.75  $\mu$ M each, heated to 95 deg for 4 min. and cooled slowly to 4 deg. To synthesize the complete gene from the oligonucleotides by synthesizing the opposite strand of each oligonucleotide (Figure 5B), the following components were added in a final volume of 100ul:

	10 ul	annealed oligonucleotides
	0.16 mM each	deoxyribonucleotide
25	0.5 mM	ATP
	0.5 mM	DTT
	100 ug/ml	BSA
	3.5 ug/ml	T4 g43 protein (DNA polymerase)
30	25 ug/ml	T4 g44/62 protein (polymerase accessory protein)
	25 ug/ml	45 protein (polymerase accessory protein)

The mixture was incubated at 37 deg for 30 min. Then 10 u of T4 DNA ligase was added and incubation at 37 deg resumed for 30 min. The polymerase and ligase were inactivated by incubation of the reaction at 70 deg for

15 min. To digest the gene with Xba I, to the reaction was added 50 ul of 2x TA containing BSA at 200 ug/ml and DTT at 1 mM, 43 ul of water, and 50 u of Xba I in 5 ul. The reaction was incubated for 3 hr at 37 deg, and run on a gel. The 431 bp Xba I fragment was purified from a gel and cloned into the Xba I site of the plasmid pUC19 by standard methods. Four plasmid isolates were purified and sequenced using the dideoxy method. One of these had the correct sequence (Figure 3).

To synthesize the light chain, four oligonucleotides JFD1, JFD2, JFD3, JFD4 (Figure 6A) were synthesized. Two of the oligonucleotides are part of each strand of the light chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 6B). Together, the oligonucleotides cover the entire humanized light chain variable region (Figure 4) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

The light chain gene was synthesized from these oligonucleotides in two parts. 0.5 ug each of JFD1 and JFD2 were combined in 20 ul sequenase buffer (40 mM Tris-HCl, pH 7.5, 20 mM magnesium chloride, 50 mM sodium chloride), heated at 70 deg for 3 min and allowed to cool slowly to 23 deg in order for the oligonucleotides to anneal. JFD3 and JFD4 were treated in the same way. Each reaction was made 10 mM in DTT and 0.5 mM in each deoxyribonucleotide and 6.5 u of sequenase (US Biochemicals) was added, in a final volume of 24 ul, and incubated for 1 hr at 37 deg to synthesize the opposite strands of the oligonucleotides. Xba I and Hind III were added to each reaction to digest the DNA (there is a Hind III site in the region where JFD2 and JFD3 overlap and therefore in each of the synthesized DNAs; Figure 6B). The reactions were run on polyacrylamide gels, and the Xba I - Hind III fragments were purified and cloned into pUC18 by standard methods. Several plasmid isolates for each fragment were sequenced by the dideoxy method, and correct ones chosen.



Construction of plasmids to express humanized light and heavy chains

5 The heavy chain Xba I fragment was isolated from the pUC19 plasmid in which it had been inserted and then inserted into the Xba I site of the vector pV71 (see, commonly assigned U.S.S.N. 223,037) in the correct orientation by standard methods, to produce the plasmid pHuGTAC1 (Figure 7). This plasmid will express high levels of a complete heavy chain when transfected into an appropriate host cell.

10 The two light chain Xba I - Hind III fragments were isolated from the pUC18 plasmids in which they had been inserted. The vector plasmid pVκ1 (see, commonly assigned U.S.S.N. 223,037) was cut with Xba I, dephosphorylated and ligated with the two fragments by standard methods. The  
15 desired reaction product has the circular form: vector - Xba I - fragment 1 - Hind III - fragment 2 - Xba I - vector. Several plasmid isolates were analyzed by restriction mapping and sequencing, and one with this form chosen. This plasmid, pHuLTAC (Figure 8), therefore contains the complete humanized  
20 light chain (Figure 4) and will express high levels of the light chain when transfected into an appropriate host cell.

Synthesis and affinity of humanized antibody

25 The plasmids pHuGTAC1 and pHuLTAC were transfected into mouse Sp2/0 cells, and cells that integrated the plasmids were selected on the basis of resistance to mycophenolic acid and/or hygromycin B conferred by the *gpt* and *hyg* genes on the plasmids (Figures 7,8) by standard methods. To verify that these cells secreted antibody that  
30 binds to the IL-2 receptor, supernatant from the cells was incubated with HUT-102 cells that are known to express the IL-2 receptor. After washing, the cells were incubated with fluorescein-conjugated goat anti-human antibody, washed, and analyzed for fluorescence on a FACSCAN cytofluorometer. The  
35 results (Figure 9A), clearly show that the humanized antibody binds to these cells, but not to Jurkat T-cells that do not express the IL-2 receptor (Figure 9D). As controls, the

original mouse anti-Tac antibody was also used to stain these cells (Figure 9B,C), giving similar results.

For further experiments, cells producing the humanized antibody were injected into mice, and the resultant ascites collected. Humanized antibody was purified to  
5 substantial homogeneity from the ascites by passage through an affinity column of goat anti-human immunoglobulin antibody, prepared on an Affigel-10 support (Bio-Rad Laboratories, Inc., Richmond, CA) according to standard techniques. To determine the affinity of the humanized  
10 antibody relative to the original anti-Tac antibody, a competitive binding experiment was performed. About  $5 \times 10^5$  HUT-102 cells were incubated with known quantities (10 - 40 ng) of the anti-Tac antibody and the humanized anti-Tac antibody for 10 min at 4 deg. Then 100 ng of biotinylated  
15 anti-Tac was added to the cells and incubated for 30 min at 4 deg. This quantity of anti-Tac had previously been determined to be sufficient to saturate the binding sites on the cells, but not to be in large excess. Then the cells were washed twice with 2 ml of phosphate buffered saline  
20 (PBS) containing 0.1% sodium azide. The cells were then incubated for 30 min at 4 deg with 250 ng of phycoerythrin-conjugated avidin, which bound to the biotinylated anti-Tac already bound to the cells. The cells were washed again as above, fixed in PBS containing 1%  
25 paraformaldehyde, and analyzed for fluorescence on a FACSCAN cytofluorometer.

Use of increasing amounts (10 - 40 ng) of the anti-Tac antibody as competitor in the first step decreased the amount of biotinylated anti-Tac that could bind to the  
30 cells in the second step, and therefore the amount of phycoerythrin-conjugated avidin that bound in the last step, thus decreasing fluorescence (Figure 10A). Equivalent amounts (20 ng) of anti-Tac, and humanized anti-Tac used as competitor decreased the fluorescence to approximately the  
35 same degree (Figure 10B). This shows that these antibodies have approximately the same affinity, because if one had greater affinity, it would have more effectively competed

with the biotinylated anti-Tac, thus decreasing fluorescence more.

Biological properties of the humanized antibody

5 For optimal use in treatment of human disease, the humanized antibody should be able to destroy T-cells in the body that express the IL-2 receptor. One mechanism by which antibodies may destroy target cells is antibody-dependent cell-mediated cytotoxicity, abbreviated ADCC (Fundamental Immunology, Paul, W., Ed., Raven Press, New York (1984), at 10 pg. 681), in which the antibody forms a bridge between the target cell and an effector cell such as a macrophage that can lyse the target. To determine whether the humanized antibody and the original mouse anti-Tac antibody can mediate ADCC, a chromium release assay was performed by standard 15 methods. Specifically, human leukemia HUT-102 cells, which express the IL-2 receptor, were incubated with <sup>51</sup>Cr to allow them to absorb this radionuclide. The HUT-102 cells were then incubated with an excess of either anti-Tac or humanized anti-Tac antibody. The HUT-102 cells were next incubated for 20 4 hrs with either a 30:1 or 100:1 ratio of effector cells, which were normal purified human peripheral blood mononuclear cells that had been activated by incubation for about 20 hrs with human recombinant IL-2. Release of <sup>51</sup>Cr, which indicated lysis of the target HUT-102 cells, was measured and 25 the background subtracted (Table 1). The results show that at either ratio of effector cells, anti-Tac did not lyse a significant number of the target cells (less than 5%), while the humanized antibody did (more than 20%). Hence, the humanized antibody is likely to be more efficacious than the 30 original mouse antibody in treating T-cell leukemia or other T-cell mediated diseases.

35

TABLE 1

5 Percent <sup>51</sup>Cr release after ADCC

	<u>Effector: Target ratio</u>	
	30:1	100:1
10 <u>Antibody</u>		
Anti-Tac	4%	< 1%
Humanized anti-Tac	24%	23%
15		
20		
25		
30		
35		

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

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## WE CLAIM:

1. A method of designing a humanized immunoglobulin (Ig) chain having one or more complementarity determining regions (CDR's) from a donor Ig and a framework region from a human Ig, said method comprising: comparing  
5 the framework or variable region amino acid sequence of the donor Ig with corresponding sequences in a collection of human Ig chains; and selecting to provide the human Ig framework one of the about three most homologous sequences  
10 from the collection.
2. A method according to Claim 1, wherein the human Ig sequence is selected from a collection of at least about ten to twenty Ig chain sequences.  
15
3. A method according to Claim 1, wherein the human Ig chain sequence selected has the highest homology in the collection to the donor Ig sequence.  
20
4. A method according to Claim 1, wherein the human Ig framework sequence selected is at least about 65% homologous to the donor Ig framework sequence.  
25
5. A method according to Claim 1, wherein the immunoglobulin chain is a heavy chain.  
30
6. A method according to Claim 1, wherein the humanized Ig chain comprises a human constant region.  
35
7. An immunoglobulin comprising two light/heavy chain pairs, wherein at least one chain is designed in accordance with Claim 1.

8. A method of designing a humanized immunoglobulin chain having a framework region from a human acceptor immunoglobulin and complementarity determining regions (CDR's) from a donor immunoglobulin capable of binding to an antigen, said method comprising the steps of substituting at least one human framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from the donor immunoglobulin at a position in the immunoglobulins where:

(a) the amino acid in the human framework region of the acceptor immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to have a side chain atom within about 3Å of the CDR's in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

9. A method according to Claim 8, wherein the humanized immunoglobulin chain comprises in addition to the CDR's at least three amino acids from the donor immunoglobulin chosen by criteria (a), (b) or (c).

10. A method according to Claim 9, wherein at least one of the amino acids substituted from the donor is immediately adjacent a CDR.

11. A method according to Claim 9, wherein said humanized immunoglobulin chain is a heavy chain.

12. An immunoglobulin comprising two light/heavy chain pairs, wherein at least one chain is designed in accordance with Claim 8.

13. An immunoglobulin according to Claim 12, which is specifically reactive with an antigen at an affinity of at least about  $10^8 \text{ M}^{-1}$  or stronger.

5 14. An immunoglobulin according to Claim 12, wherein the designed chain is a light chain comprising about 214 amino acids.

10 15. An immunoglobulin according to Claim 12, wherein the designed chain is a heavy chain comprising about 446 amino acids.

15 16. A DNA sequence which upon expression encodes a humanized immunoglobulin chain according to Claim 1 or Claim 8.

17. A method for improving the affinity of a humanized immunoglobulin (Ig) to an antigen, by replacing amino acids of the human Ig framework with amino acids from the donor Ig framework at positions where:

20 (a) the amino acid in the human framework region of the first immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

25 (b) the amino acid is immediately adjacent to one of the CDR's; or

30 (c) the amino acid is predicted to have a side chain atom within about 3Å of the CDR's in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or the CDR's of the humanized immunoglobulin.

35 18. A method according to Claim 17, wherein the additional amino acids comprise up to three amino acids, each of which is immediately adjacent to one of the CDR's in the second Ig.



19. A method according to Claim 17, wherein the additional amino acids comprise one amino acid immediately adjacent to a CDR.

5 20. A method according to Claim 17, wherein the additional amino acids comprise at least two amino acids from the donor Ig which are predicted by modelling to be capable of interacting with the antigen or the CDR's.

10 21. A method according to Claim 20, wherein said two or more amino acids are predicted to be within about 3Å of the donor Ig CDR's.

15 22. A method according to Claim 17, wherein the humanized Ig has an affinity to the antigen within about 2 to 3 fold of the donor Ig.

23. A method according to Claim 17, wherein the antigen is a protein.

20 24. A method of producing a humanized immunoglobulin containing a heavy chain and a light chain designed in accordance with Claim 17, said method comprising:  
25 culturing a host capable of expressing said heavy chain, said light chain, or both, under conditions suitable for production of said chains; and  
recovering from the culture said humanized immunoglobulin.

30 25. A polynucleotide composition comprising a DNA sequence coding for a humanized immunoglobulin designed in accordance with Claim 17.

35 26. A method of producing an improved humanized immunoglobulin comprising expressing the polynucleotide composition of Claim 25.

27. A cell transformed with a polynucleotide composition according to Claim 25.

28. A composition comprising a humanized immunoglobulin secreted by a cell line according to Claim 24.

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DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

## ABSTRACT OF THE DISCLOSURE

5 Novel methods for designing humanized  
immunoglobulins having one or more complementarity  
determining regions (CDR's) from a donor immunoglobulin and a  
framework region from a human immunoglobulin comprising first  
10 comparing the framework or variable region amino acid  
sequence of the donor immunoglobulin to corresponding  
sequences in a collection of human immunoglobulin chains, and  
selecting as the human immunoglobulin one of the more  
homologous sequences from the collection. Each humanized  
15 immunoglobulin chain may comprise about 3 or more amino acids  
from the donor immunoglobulin in addition to the CDR's,  
usually at least one of which is immediately adjacent to a  
CDR in the donor immunoglobulin. The heavy and light chains  
may each be designed by using any one or all three additional  
20 position criteria. When combined into an intact antibody,  
the humanized immunoglobulins of the present invention will  
be substantially non-immunogenic in humans and retain  
substantially the same affinity as the donor immunoglobulin  
to the antigen, such as a protein or other compound  
25 containing an epitope.

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DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:  
My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

the specification of which  is attached hereto or  was filed on \_\_\_\_\_ as Application Serial No. \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

COUNTRY	APPLICATION NUMBER	DATE OF FILING	PRIORITY CLAIMED UNDER 35 U.S.C. 119	
			Yes	No
			Yes	No
			Yes	No

I claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	DATE OF FILING	STATUS
290,975	December 28, 1988	<input type="checkbox"/> Patented <input checked="" type="checkbox"/> Pending <input type="checkbox"/> Abandoned
		<input type="checkbox"/> Patented <input type="checkbox"/> Pending <input type="checkbox"/> Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) who are partners and associates in the firm of Townsend and Townsend to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:  
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				Zip Code

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201 <i>Cary Queen</i>	Signature of Inventor 202 <i>Harold P. Selick</i>	Signature of Inventor 203
Date 2/11/89	Date 2/10/89	Date

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
(37 CFR 1.91) and 1.27(c) - SMALL BUSINESS CONCERN

Applicant or Patentee: Cary L. Queen and Harold Edwin Selick  
Serial No.: NOT YET ASSIGNED Filing Date: FEBRUARY 13, 1989  
Patent No.: \_\_\_\_\_ Issued: \_\_\_\_\_  
For: DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS

I hereby declare that I am

- the owner of the small business concern identified below;
- an official of the small business concern empowered to act on behalf of the concern identified below

NAME OF CONCERN PROTEIN DESIGN LABS, INC.  
ADDRESS OF CONCERN 3191 Porter Drive  
Palo Alto, California 94304

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-15, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled DESIGNING IMPROVED HUMANIZED IMMUNOGLOBULINS by inventor(s) Cary L. Queen and Harold Edwin Selick described in

- the application filed herewith
- application serial no. \_\_\_\_\_, filed \_\_\_\_\_
- patent no. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
 INDIVIDUAL  SMALL BUSINESS CONCERN  NONPROFIT ORGANIZATION

NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
 INDIVIDUAL  SMALL BUSINESS CONCERN  NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.25(a))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Shirley J. Clayton  
TITLE OF PERSON OTHER THAN OWNER Chief Financial Officer  
ADDRESS OF PERSON SIGNING Protein Design Labs, Inc., 3191 Porter Drive  
Palo Alto, CA 94304

SIGNATURE Shirley J. Clayton DATE 2/13/89

FIGURE 1

1	Q	V	Q	L	Q	Q	S	G	A	E	L	A	K	P	G	A	S	V	K	M
1	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V
21	S	C	K	A	S	G	Y	T	F	T	S	Y	R	M	H	W	V	K	Q	R
21	S	C	K	A	S	G	G	T	F	S	R	S	A	I	I	W	V	R	Q	A
						*				*	-----									
41	P	G	Q	G	L	E	W	I	G	Y	I	N	P	S	T	G	Y	T	E	Y
41	P	G	Q	G	L	E	W	M	G	G	I	V	P	M	F	G	P	P	N	Y
							*				-----									
1	N	Q	K	F	K	D	K	A	T	L	T	A	D	K	S	S	S	T	A	Y
61	A	Q	K	F	Q	G	R	V	T	I	T	A	D	E	S	T	N	T	A	Y
	-----						*	*												
81	M	Q	L	S	S	L	T	F	E	D	S	A	V	Y	Y	C	A	R	G	
81	M	E	L	S	S	L	R	S	E	D	T	A	F	Y	F	C	A	G	G	Y
												*		*			*	-----		
100	G	G	V	F	D	Y	W	G	Q	G	T	T	L	T	V	S	S			
101	G	I	Y	S	P	E	E	Y	N	G	G	L	V	T	V	S	S			
	-----							*	*	*	*									

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FIGURE 2

1	Q	I	V	L	T	Q	S	P	A	I	M	S	A	S	P	G	E	K	V	T
1	D	I	Q	M	T	Q	S	P	S	T	L	S	A	S	V	G	D	R	V	T
21	I	T	C	S	A	S	S	S	I		S	Y	M	H	W	F	Q	Q	K	P
21	I	T	C	R	A	S	Q	S	I	N	T	W	L	A	W	Y	Q	Q	K	P
-----																				
40	G	T	S	P	K	L	W	I	Y	T	T	S	N	L	A	S	G	V	P	A
41	G	K	A	P	K	L	L	M	Y	K	A	S	S	L	E	S	G	V	P	S
-----																				
60	R	F	S	G	S	G	S	G	T	S	Y	S	L	T	I	S	R	M	E	A
61	R	F	T	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P
-----																				
80	E	D	A	A	T	Y	Y	C	H	Q	R	S	T	Y	P	L	T	F	G	S
81	D	D	F	A	T	Y	Y	C	Q	Q	Y	N	S	D	S	K	M	F	G	Q
-----																				
100	G	T	K	L	E	L	K													
101	G	T	K	V	E	V	K													

FIGURE 3

10            20            30            40            50            60  
 TCTAGATGGGATGGAGCTGGATCTTTCTC.TCCTCCTGTCAGGTACCGCGGGCGTGCCT  
   M G W S W I F L F L L S G T A G V H

70            80            90            100           110           120  
 CTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTGAAGG  
   S Q V Q L V Q S G A E V K K P G S S V K

130           140           150           160           170           180  
 TCTCCTGCAAGGCTTCTGGCTACACCTTACTAGCTACAGGATGCACTGGGTAAGGCAGG  
   V S C K A S G Y T F T S Y R M H W V R Q

190           200           210           220           230           240  
 CCCCTGGACAGGGTCTGGAATGGATTGGATATATT. ATCCGTCGACTGGGTATACTGAAT  
   A P G Q G L E W I G Y I N P S T G Y T E

250           260           270           280           290           300  
 ACAATCACAAGTICAAGGACAAGGCAACAATTACTGCAGACGAATCCACCAATACAGCCT  
   Y N Q K F K D K A T I T A D E S T N T A

310           320           330           340           350           360  
 ACAAGGAACTGAGCAGCCTGAGATCTGAGGACACCGCAGTCTATTACTGTGCAAGAGGGG  
   Y M E L S S L R S E D T A V Y Y C A R G

370           380           390           400           410           420  
 GGGGGTCTTTGACTACTGGGGCCAAGGAACCCTGGTCACAGTCTCCTCAGGTGAGTCCT  
   G G V F D Y W G Q G T L V T V S S

430  
 TAAAACCTCTAGA



FIGURE 4

10 20 30 40 50 60  
 TCTAGATGGAGACCGATAACCCTCCTGCTATGGGTCCTCCTGCTATGGGTCCCAGGATCAA  
 M E T D T L L L W V L L L W V P G S  
 70 80 90 100 110 120  
 CCGGACATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGATAGGG  
 T G D I Q M T Q S P S T L S A S V G D R  
 130 140 150 160 170 180  
 TCACCATAACCTGCTCTGCCAGCTCAAGTATAAGTTACATGCACCTGGTACCAGCAGAAGC  
 V T I T C S A S S S I S Y M H W Y Q Q K  
 190 200 210 220 230 240  
 CAGGCAAAGCTCCCAAGCTTCTAATTTATAACCACATCCAACCTGGCTTCTGGAGTCCCTG  
 P G K A P K L L I Y T T S N L A S G V P  
 250 260 270 280 290 300  
 CTCGCTTCAGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGC  
 A R F S G S G S G T E F T L T I S S L Q  
 310 320 330 340 350 360  
 CAGATGATTTCCGCACTTATTACTGCCATCAAAGGAGTACTTACCCACTCACGTTCCGGTC  
 P D D F A T Y Y C H Q R S T Y P L T F G  
 370 380 390 400  
 AGGGGACCAAGGTGGAGGTCAAACGTAAGTACACTTTTCTAGA  
 Q G T K V E V K

FIGURE 5

A

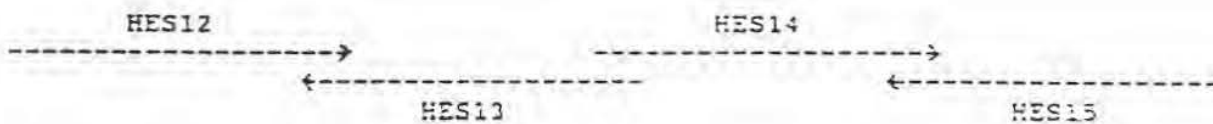
HES12 AGCTTCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTG  
CACTCTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTG  
AAGGTC

HES13 CCCAGTCGACGGATTAATATATATCCAATCCATTCCAGACCCTGTCCAGGGGCCTGCCTTAC  
CCAGTGCATCCTGTAGCTAGTAAAGGTGTAGCCAGAAGCCTTGCAGGAGACCTTCACGCT  
CGAGCCAGG

HES14 TATATTAATCCGTCGACTGGGTATACTGAATACAATCAGAAGTTCAGGACAAGGCAACA  
ATTACTGCAGACGAATCCACCAATACAGCCTACATGGAAGTGAAGCAGCCTGAGATCTGAG  
GACA

HES15 ATATCGTCTAGAGGTTTTAAGGACTCACCTGAGGAGACTGTGACCAGGGTTCCTTGSCCC  
CAGTAGTCAAAGACCCCCCCCCCTCTTGCACAGTAATAGACTGCGGTGTCCTCAGATCTC  
AGGCTGCT

B



07-310252

FIGURE 6

A

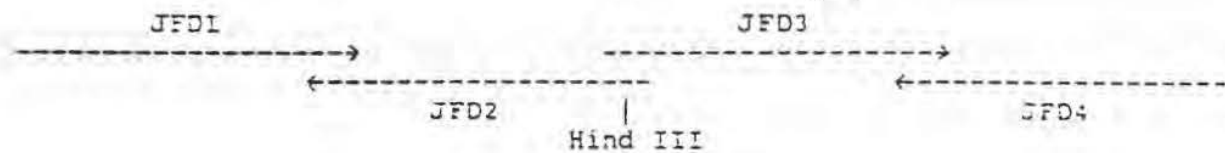
JFD1 CAAATCTAGATGGAGACCGATAACCTCCTGCTATGGGTCCCTCCTGCTATGGGTCCCAGGA  
TCAACCGGAGATATTCAGATGACCCAGTCTCCATCTACCCTCTCTGCTAGCGTCGGGGAT

JFD2 ATAAATTAGAAGCTTGGGAGCTTGCCTGGCTTCTGCTGGTACCAGTGCATGTAACCTTAT  
ACTTGAGCTGGCAGAGCAGGTTATGGTGACCCTATCCCCGACGCTAGCAGAGAG

JFD3 GCTCCCAAGCTTCTAATTTATACCACATCCAACCTGGCTTCTGGAGTCCCTGCTCCCTTC  
AGTGGCAGTGGATCTGGGACCGAGTTCACCCTCACAATCAGCTCTCTGCAGCCAGATGAT  
TTC

JFD4 TATATCTAGAAAAGTGTACTTACGTTTGACCTCCACCTTGGTCCCCTGACCGAACCTGAG  
TGGGTAAGTACTCCTTTGATGGCAGTAATAAGTGGCGAAATCATCTGGCTGCAGAGAGCT  
GA

B



07/310252

FIGURE 7

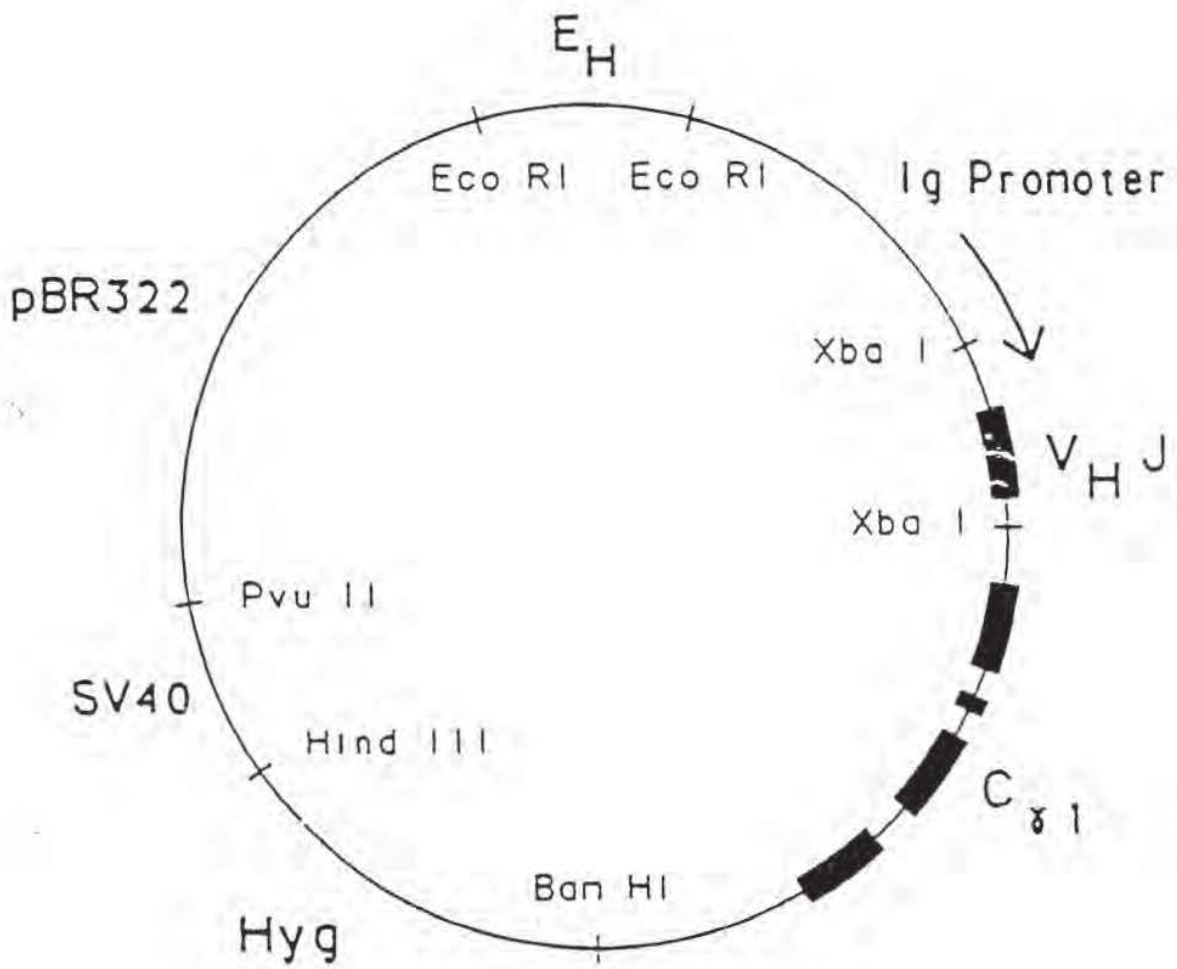
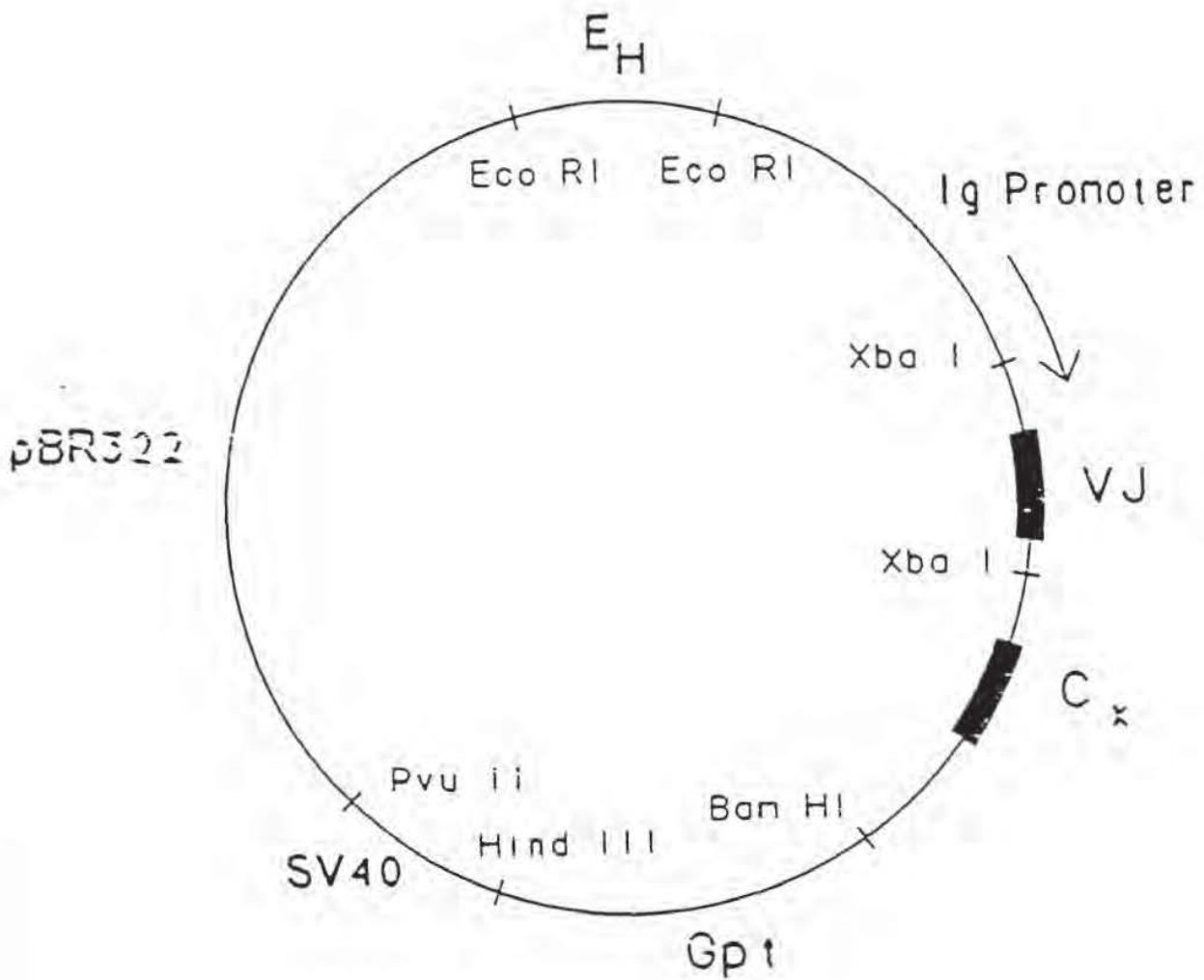


FIGURE 8



17/310252

FIGURE 9

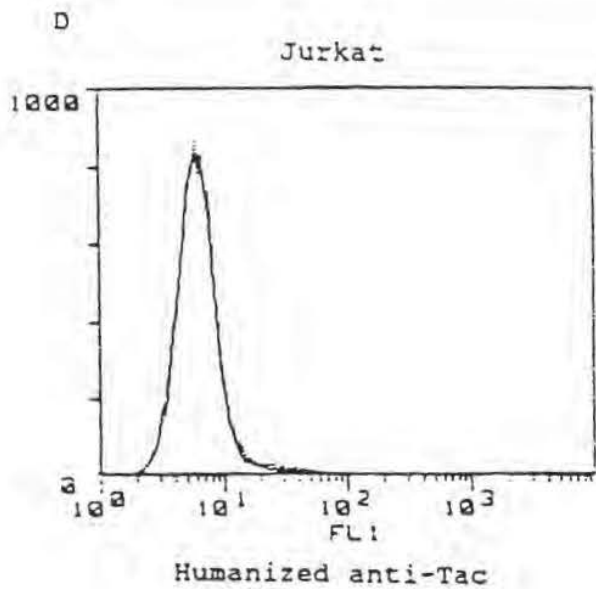
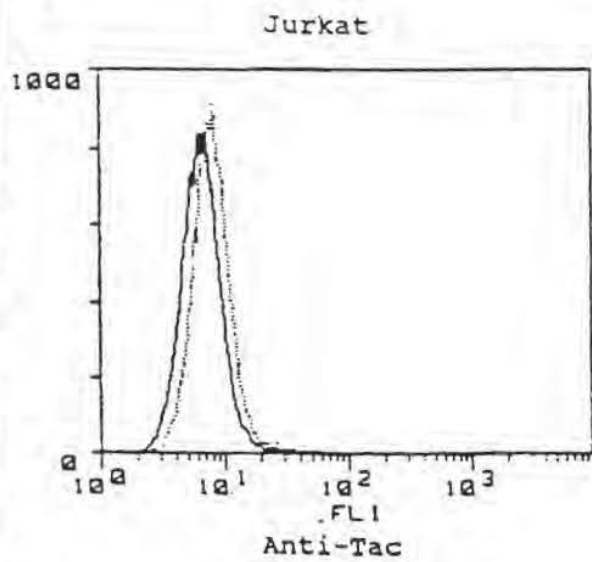
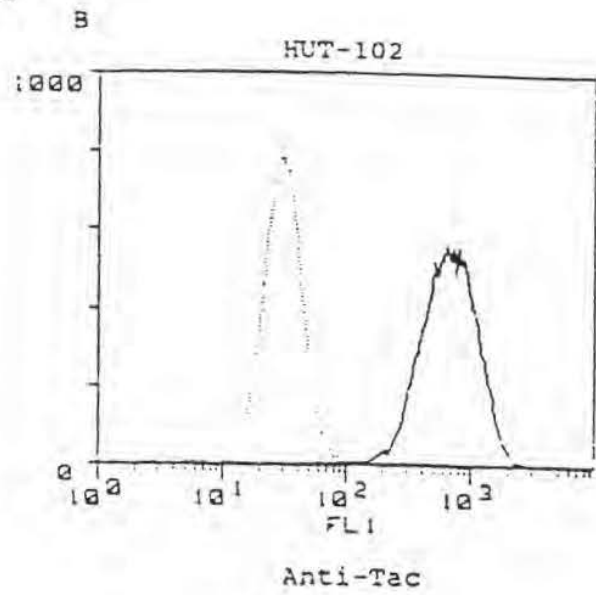
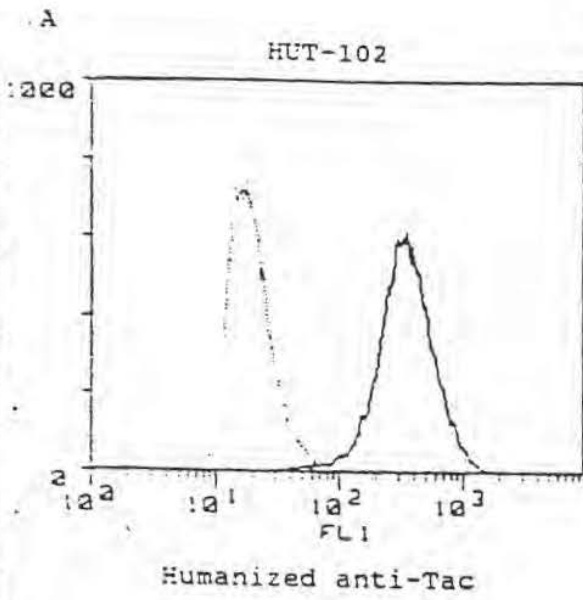
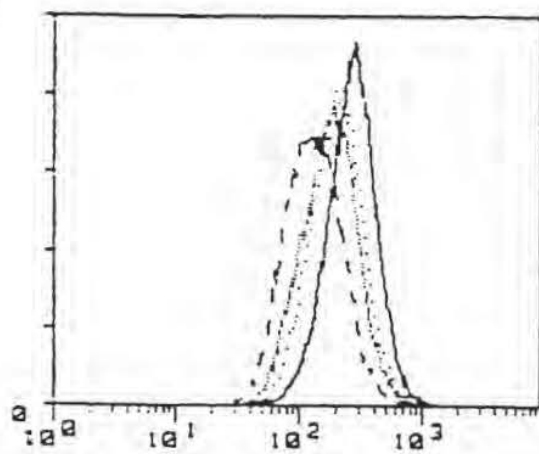


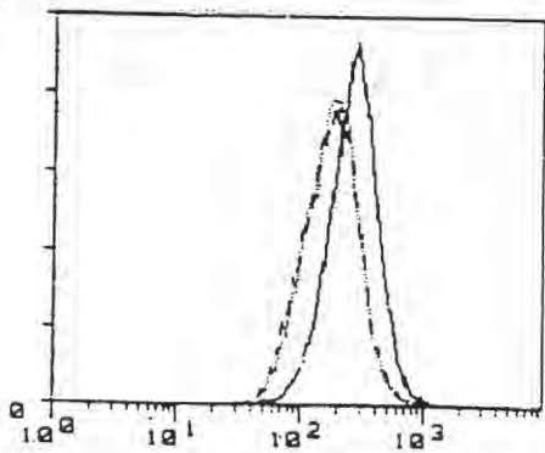
FIGURE 10

A



— 0 ng anti-Tac  
..... 10 ng  
- - - - 20 ng  
- · - · 40 ng

B



— 0 ng anti-Tac  
..... 20 ng anti-Tac  
- - - 20 ng humanized anti-Tac

02/11/99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206	Group Art Unit: 1642 Examiner: J. Reeves
Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	<p style="text-align: center;"><b>CERTIFICATE OF HAND DELIVERY</b></p> <p style="text-align: center;">I hereby certify that this correspondence is being hand delivered in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on</p> <p style="text-align: center;">February 1, 1999</p> <p style="text-align: center;"><i>R. H. Mitchell</i></p>

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

3/26/1999 TGRAY1 00000002 070630 08146206  
 Assistant Commissioner of Patents  
 1 FC:126 240.00 CH  
 Washington, D.C. 20231

Sir:

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

This Information Disclosure Statement:

- (a)  accompanies the new patent application submitted herewith. 37 CFR §1.97(a).
- (b)  is filed within three months after the filing date of the application or within three months after the date of entry of the national stage of a PCT application as set forth in 37 CFR §1.491.
- (c)  as far as is known to the undersigned, is filed before the mailing date of a first Office action on the merits.
- (d)  is filed after the first Office Action and more than three months after the application's filing date or PCT national stage date of entry filing but, as far as is known to the undersigned, prior to the mailing date of either a final rejection or a notice of allowance, whichever occurs first, and is accompanied by either the fee (\$240) set forth in 37 CFR §1.17(p) or a statement as specified in 37 CFR §1.97(e), as checked below. Should any fee be due, the U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of \$240.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment



should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

- (e)  is filed after the mailing date of either a final rejection or a notice of allowance, whichever occurred first, and is accompanied by the fee (\$130) set forth in 37 CFR §1.17(i) and a statement as specified in 37 CFR §1.97(e), as checked below. This document is to be considered as a petition requesting consideration of the information disclosure statement. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 in the amount of \$130.00 to cover the cost of this Information Disclosure Statement. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.
- (f) (x)  is filed after the mailing date of a final rejection, but a request to withdraw the finality thereof under 37 CFR § 1.129(a) was submitted on August 24, 1998. The U.S. Patent and Trademark Office is hereby authorized to charge Deposit Account No. 07-0630 to cover the cost of this Information Disclosure Statement in the event that any fees are due. A duplicate of this sheet is enclosed.

(If either of boxes (d) or (e) is checked above, the following statement under 37 CFR §1.97(e) may need to be completed.) The undersigned states that:

- Each item of information contained in the information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this information disclosure statement.
- No item of information contained in this information disclosure statement was cited in a communication mailed from a foreign patent office in a counterpart foreign application and, to the knowledge of the undersigned after making reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this information disclosure statement.

A list of the patent(s) or publication(s) is set forth on the attached Form PTO-1449 (Modified). A copy of the items on PTO-1449 is supplied herewith:

(x) each  none  only those listed below:

A concise explanation of relevance of the items listed on PTO-1449 is:

- (x)  not given
- given for each listed item
- given for only non-English language listed item(s) (Required)
- in the form of an English language copy of a Search Report from a foreign patent

office, issued in a counterpart application, which refers to the relevant portions of the references.

The Examiner is reminded that a "concise explanation of the relevance" of the submitted prior art "may be nothing more than identification of the particular figure or paragraph of the patent or publication which has some relation to the claimed invention," MPEP §609.

While the information and references disclosed in this Information Disclosure Statement may be "material" pursuant to 37 CFR §1.56, it is not intended to constitute an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

In accordance with 37 CFR §1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR §1.56(a) exists. It is submitted that the Information Disclosure Statement is in compliance with 37 CFR §1.98 and MPEP §609 and the Examiner is respectfully requested to consider the listed references.

Respectfully submitted,

GENENTECH, INC.

By: 

Wendy M. Lee  
Reg. No. 40,378

Date: January 29, 1999

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Fax: (650) 952-9881



Handwritten initials 'AF' and 'CP1642' above the printed text 'Patent Docket P0709P1'.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

<p>In re Application of Paul J. Carter et al. Serial No.: 08/146,206</p>	<p>Group Art Unit: 1642 Examiner: J. Reeves</p>
<p>Filed: November 17, 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES</p>	<p><b>CERTIFICATE OF MAILING</b> I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail with sufficient postage in an envelope addressed to: Assistant Commissioner of Patents, Washington, D.C. 20231 on March 9, 1999 <i>Ann Savelli</i></p>

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner of Patents  
Washington, D.C. 20231

13/26/1999 TGRAY1 00000003 070630 08146206

11 FC:126 Sir: 240.00 CH

TECH CENTER 150-2200  
99 MAR 16 PM 2:40  
GROUP 180

Applicants submit herewith patents, publications or other information (attached hereto and listed on the attached Form PTO-1449) of which they are aware, which they believe may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56.

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Respectfully submitted,

GENENTECH, INC.

By: 

Wendy M. Lee  
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Date: March 9 1999

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Fax: (650) 952-9881



**UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/146,206	11/17/93	CARTER	P 709P1

GENENTECH, INC.  
1 DNA WAY  
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HM22/0329

EXAMINER

REEVES, J

ART UNIT	PAPER NUMBER
1642	H8

DATE MAILED:


03/29/99

**Please find below and/or attached an Office communication concerning this application or proceeding.**

**Commissioner of Patents and Trademarks**

# Office Action Summary

Application No. <b>08/146,206</b>	Applicant(s) <b>Carter et al</b>
Examiner <b>Julie E. Reeves, Ph.D.</b>	Group Art Unit <b>1642</b>



Responsive to communication(s) filed on Aug 26, 1998

This action is **FINAL**.

Since this application is 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.

A shortened statutory period for response to this action is set to expire zero month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

### Disposition of Claims

Claim(s) 43-128 is/are pending in the application.

Of the above, claim(s) \_\_\_\_\_ is/are withdrawn from consideration.

Claim(s) \_\_\_\_\_ is/are allowed.

Claim(s) \_\_\_\_\_ is/are rejected.

Claim(s) \_\_\_\_\_ is/are objected to.

Claims 43-128 are subject to restriction or election requirement.

### Application Papers

See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.

The proposed drawing correction, filed on \_\_\_\_\_ is  approved  disapproved.

The specification is objected to by the Examiner.

The oath or declaration is objected to by the Examiner.

### Priority under 35 U.S.C. § 119

Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

All  Some\*  None of the CERTIFIED copies of the priority documents have been received.

received in Application No. (Series Code/Serial Number) \_\_\_\_\_

received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\*Certified copies not received: \_\_\_\_\_

Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

### Attachment(s)

Notice of References Cited, PTO-892

Information Disclosure Statement(s), PTO-1449, Paper No(s). \_\_\_\_\_

Interview Summary, PTO-413 Paper # 43

Notice of Draftsperson's Patent Drawing Review, PTO-948

Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

Art Unit: 1642

1. Restriction is required under 35 U.S.C. 121 and 372.
2. This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

The species are as follows:

Species A: 4L

Species B: 38L

Species C: 43L

Species D: 44L

Species E: 46L

Species F: 58L

Species G: 62L

Species H: 65L

Species I: 66L

Species J: 67L

Species K: 68L

Species L: 69L

Species M: 73L

Species N: 85L

Species O: 98L



Art Unit: 1642

Species P: 2H

Species Q: 4H

Species R: 36H

Species S: 39H

Species T: 43H

Species U: 45H

Species V: 69H

Species W: 70H

Species X 74H

Species Y 75H

Species Z: 76H

Species AA: 78H

Species BB: 92H

Species CC: noncovalently binds antigen directly

Species DD: interacts with a CDR

Species EE: comprises a glycosylation site which affects the antigen binding or affinity of the antibody

Species FF: participates in the VL-VH interface by affecting the proximity or orientation of the VL and VH regions with respect to one another.

Species GG 24H

Art Unit: 1642

Species HH 73H

Species II 76H

Species JJ 78H

Species KK 93H

Applicant is required, in reply to this action, to elect a single species to which the claims shall be restricted if no generic claim is finally held to be allowable. The reply must also identify the claims readable on the elected species, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered non-responsive unless accompanied by an election.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise include all the limitations of an allowed generic claim as provided by 37 CFR 1.141. If claims are added after the election, applicant must indicate which are readable upon the elected species. MPEP § 809.02(a).

3. The claims are deemed to correspond to the species A-BB listed above in the following manner: Claims 47-70 and claims 76-103 are limited to one of Species A-BB, respectively. Claims 107-110 are limited to one of the species CC-FF, respectively.

The following claim(s) are generic:

Claims 43-46, 71-75, 104-105 are generic for Species A-BB.

Claims 106, 111-114, 128 are generic for Species CC-FF.

Art Unit: 1642

Claims 115-118, 124-126 are generic for Species GG-KK

4. The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: each of the particular amino acid substitution positions recited in Species A-BB or GG-KK or each of the functional definitions of amino acid substitution changes recited in Species CC-FF result in different primary amino acid structure which would result in different secondary, tertiary, and quaternary structure yielding a protein with different biological, physiological and immunological properties, including different immunogenicity and antigen binding functions. Further, species EE, for example, recites the addition of a glycosylation site, which would involve the presence of a carbohydrate moiety and its affect on amino acid structure. The examination of all species would require the consideration of different patentability issues.

Applicant is advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

5. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).