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(54) Abstract Title
TRX1 antibody

(57) A humanised antibody, TRX1 includes modified constant regions and light and heavy chain framework of a human antibody and CDR regions derived from a mouse monoclonal antibody. TRX1 binds to an epitope of a CD4 antigen on CD4 positive human T-cells or monocytes. The antibody is designed such that (i) it includes CDRs that are free from glycosylation sites and (ii) does not bind to the Fc region of the receptor. Also claimed is a method of treating a graft transplant patient by administering TRX1 thereby inhibiting an immune response and preventing graft rejection.

GB 2 376 466 A

At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

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ATG GAG ACA GAC ACA ATC CTG CTA TGG GTG CTG CTG CTC TGG GTT CCA GGC TCC ACT GGT GAC ATT GTG ATG ACC CAA TCT CCA GAT TCT TTG
M   E   T   D   T   I   L   L   W   V   L   L   L   W   V   P   G   S   T   G   D   I   V   M   T   Q   S   P   D   S   L
<-------------------------------Leader----------------------------------------> <-----------------------FR1---------------

GCT GTG TCT CTA GGT GAG AGG GCC ACC ATC AAC TGC AAG GCC AGC CAA AGT GTT GAT TAT GAT GGT GAT AGT TAT ATG AAC TGG TAT CAA CAG
A   V   S   L   G   E   R   A   T   I   N   C   K   A   S   Q   S   V   D   Y   D   G   D   S   Y   M   N   W   Y   Q   Q
-----------------------------------------------><------------------------CDR1---------------------------> <--------------

AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT GTT GCA TCC AAT CTA GAG TCT GGG GTC CCA GAC AGG TTT AGT GGC AGT GGG TCT GGG ACA
K   P   G   Q   P   P   K   L   L   I   Y   V   A   S   N   L   E   S   G   V   P   D   R   F   S   G   S   G   S   G   T
------------------FR2--------------------> <----------CDR2----------> <--------------------------FR3-------------------

GAC TTC ACC CTC ACC ATC AGT TCT CTG CAG GCG GAG GAT GTT GCA GTC TAT TAC TGT CAG CAA AGT CTT CAG GAC CCT CCG ACG TTC GGT GGA
D   F   T   L   T   I   S   S   L   Q   A   E   D   V   A   V   Y   Y   C   Q   Q   S   L   Q   D   P   P   T   F   G   G
-------------------------------------------------------------------------> <---------------CDR3-------------> <----------

GGT ACC AAG GTG GAA ATC AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT
G   T   K   V   E   I   K   R   T   V   A   A   P   S   V   F   I   F   P   P   S   D   E   Q   L   K   S   G   T   A   S
------------FR4-----------> <-------------------------------------Constant---------------------------------------------

GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT
V   V   C   L   L   N   N   F   Y   P   R   E   A   K   V   Q   W   K   V   D   N   A   L   Q   S   G   N   S   Q   E   S
-----------------------------------------------------------------------------------------------------------------------

GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC
V   T   E   Q   D   S   K   D   S   T   Y   S   L   S   S   T   L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A
-----------------------------------------------------------------------------------------------------------------------

TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG
C   E   V   T   H   Q   G   L   S   S   P   V   T   K   S   F   N   R   G   E   C   *
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FIGURE 1A
TRX1 Light Chain

ATGGAGACAGACACAATCCTGCTATGGGTGCTGCTGCTCTGGGTTCCAGGC
TCCACTGGTGACATTGTGATGACCCAATCTCCAGATTCTTTGGCTGTGTCT
CTAGGTGAGAGGGCCACCATCAACTGCAAGGCCAGCCAAAGTGTTGATTAT
GATGGTGATAGTTATATGAACTGGTATCAACAGAAACCAGGACAGCCACCC
AAACTCCTCATCTATGTTGCATCCAATCTAGAGTCTGGGGTCCCAGACAGG
TTTAGTGGCAGTGGGTCTGGGACAGACTTCACCCTCACCATCAGTTCTCTG
CAGGCGGAGGATGTTGCAGTCTATTACTGTCAGCAAAGTCTTCAGGACCCT
CCGACGTTCGGTGGAGGTACCAAGGTGGAAATCAAACGAACTGTGGCTGCA
CCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACT
GCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA
CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTC
ACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACG
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC
CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
TAG

FIGURE 1B
TRX1 Light Chain Nucleic Acid Sequence

With leader sequence:

METDTILLWVLLLWVPGSTGDIVMTQSPDSLAVSLGERATINC**KASQSVDYDG**
DSYMNWYQQKPGQPPKLLIY**VASNLES**GVPDRFSGSGSGTDFTLTISSLQAED
VAVYYC**QQSLQDPPT**FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL
LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK
HKVYACEVTHQGLSSPVTKSFNRGEC

Without leader sequence:

DIVMTQSPDSLAVSLGERATINC**KASQSVDYDGDSYMN**WYQQKPGQPPKLLIY
VASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QQSLQDPPT**FGGGT
KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC

FIGURE 1C
TRX1 Light Chain Amino Acid Sequence
with CDRs Highlighted

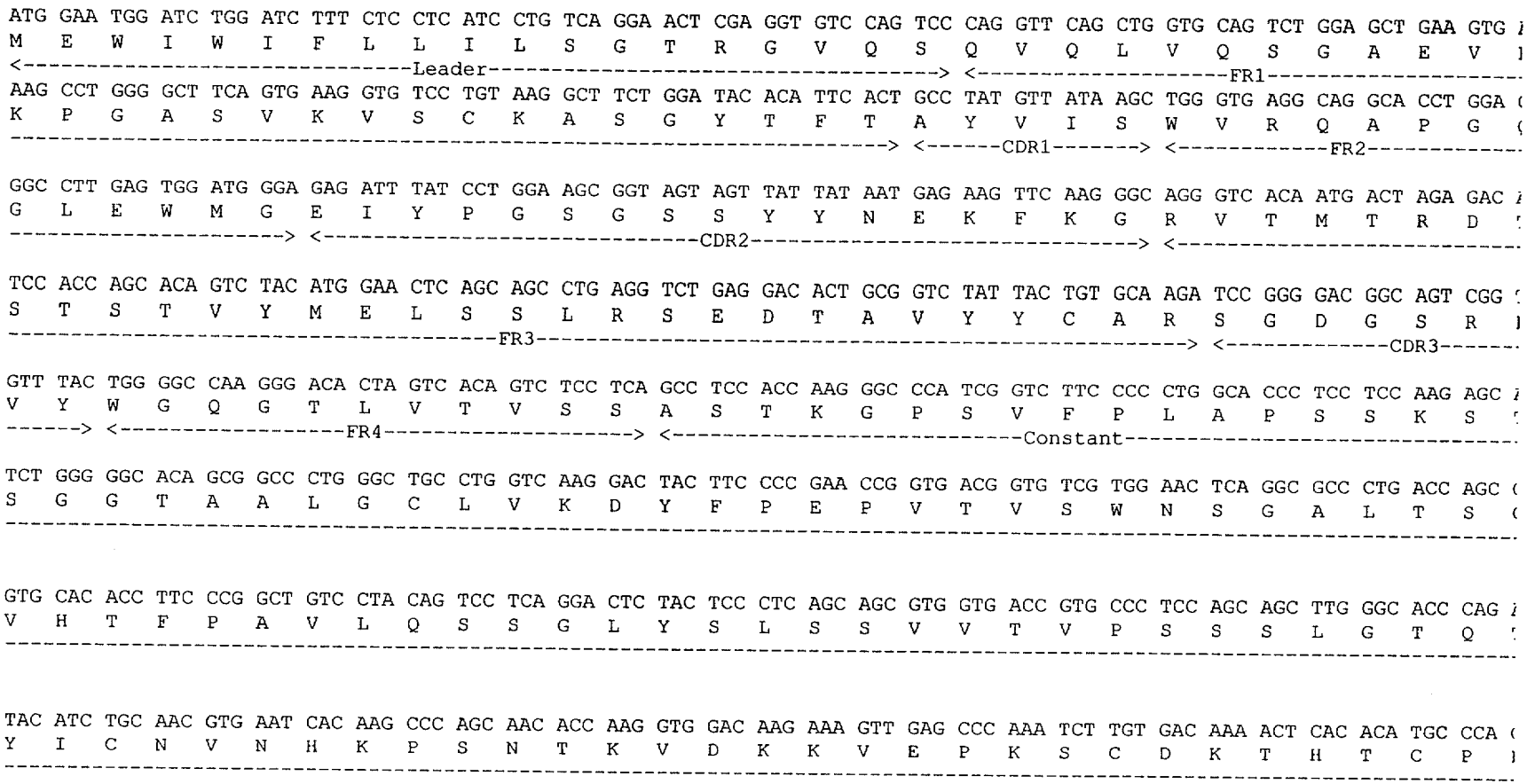

FIGURE 1D
TRX1 Light Chain

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TGC CCA GCA CCT GAA CTC GCG GGG GCA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC
C   P   A   P   E   L   A   G   A   P   S   V   F   L   F   P   P   K   P   K   D   T   L   M   I   S   R   T   P   E   V
-----------------------------------------------------------------------------------------------------------------------

ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG
T   C   V   V   V   D   V   S   H   E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N   A   K   T   K
-----------------------------------------------------------------------------------------------------------------------

CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC
P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S   V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C
-----------------------------------------------------------------------------------------------------------------------

AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC
K   V   S   N   K   A   L   P   A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E   P   Q   V   Y   T   L   P
-----------------------------------------------------------------------------------------------------------------------

CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC
P   S   R   D   E   L   T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D   I   A   V   E   W   E   S
-----------------------------------------------------------------------------------------------------------------------

AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG
N   G   Q   P   E   N   N   Y   K   T   T   P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V   D   K
-----------------------------------------------------------------------------------------------------------------------

AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG
S   R   W   Q   Q   G   N   V   F   S   C   S   V   M   H   E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   P
-----------------------------------------------------------------------------------------------------------------------

GGT AAA TGA
G   K   *
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FIGURE 1D (cont)
TRX1 Light Chain

ATGGAATGGATCTGGATCTTTCTCCTCATCCTGTCAGGAACTCGAGGTGTC
CAGTCCCAGGTTCAGCTGGTGCAGTCTGGAGCTGAAGTGAAGAAGCCTGGG
GCTTCAGTGAAGGTGTCCTGTAAGGCTTCTGGATACACATTCACTGCCTAT
GTTATAAGCTGGGTGAGGCAGGCACCTGGACAGGGCCTTGAGTGGATGGGA
GAGATTTATCCTGGAAGCGGTAGTAGTTATTATAATGAGAAGTTCAAGGGC
AGGGTCACAATGACTAGAGACACATCCACCAGCACAGTCTACATGGAACTC
AGCAGCCTGAGGTCTGAGGACACTGCGGTCTATTACTGTGCAAGATCCGGG
GACGGCAGTCGGTTTGTTTACTGGGGCCAAGGGACACTAGTCACAGTCTCC
TCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAG
AGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
CCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG
CACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC
GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAA
TCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCGCG
GGGGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATG
ATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAAT
GCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG
TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC
AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCC
CGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC
TTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC
TTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
AGCCTCTCCCTGTCTCCGGGTAAATGA

FIGURE 1E
TRX1 Heavy Chain Nucleic Acid Sequence

With leader sequence:

MEWIWIFLLILSGTRGVQSQVQLVQSGAEVKKPGASVKVSCKASGYTFT**AY**
VISWVRQAPGQGLEWMG**EIYPGSGSSYYNEKFKG**RVTMTRDTSTSTVYMEL
SSLRSEDTAVYYCAR**SGDGSRFVY**WGQGTLVTVSSASTKGPSVFPLAPSSK
STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELA
GAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPGK

Without leader sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFT**AYVIS**WVRQAPGQGLEWMG**EI**
YPGSGSSYYNEKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR**SGDG**
SRFVYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAGAPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FIGURE 1F
TRX1 Heavy Chain Amino Acid Sequence with CDRs Highlighted

TRX1 ANTIBODY AND USES THEREFOR

This invention relates to an antibody (or fragments or derivatives thereof) and preferably, to an antibody (or fragments or derivatives thereof) which binds to human lymphocytes. This invention also relates to treatment with a therapeutic agent that is to treat or prevent a disease wherein administration of the therapeutic agent produces in the host an immune response against the agent. The present invention is applicable to inhibiting, preventing, or ameliorating an immune response against such an agent by administering the above-mentioned antibody (or fragments or derivatives thereof) to a host. Such inhibiting, preventing, or ameliorating an immune response against the agent includes inducing tolerance to the agent. This invention also relates to tolerance induction and/or preventing or inhibiting T cell activation and proliferation through the administration of such antibody or fragments or derivatives thereof to a patient.

BACKGROUND OF THE INVENTION

Tolerance to foreign antigen or tissue, or self antigen or tissue is a state whereby an otherwise normal, mature immune system is specifically unable to respond aggressively to that antigen/tissue which it therefore treats like a normal (non-diseased) body tissue/component, yet at the same time it can respond aggressively to foreign or diseased antigens/tissues to which it has not been specifically made tolerant by the natural process of self tolerance or by

therapeutic tolerance induction procedures. A test for tolerance usually requires a demonstration that the tolerant individual fails to become immune to the specific antigen/tissue when one or preferably more attempts to immunize are made at a later time when the same individual can be shown to respond to an irrelevant antigen/tissue.

Monoclonal antibodies (mAb) against the murine CD4 (L3T4) antigen have proven to be potent immunosuppressive agents for the control of humoral immunity, transplant rejection and autoimmunity. In addition, CD4 mAbs have been shown to create a tolerance-permissive environment *in vivo* with which can be achieved tolerance to certain soluble protein antigens as well as transplantation antigens.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the amino acid sequences of the heavy and light chains of the TRX1 antibody, as well as the CDR and framework regions of the heavy and light chains.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with an aspect of the present invention, there is provided a molecule (preferably a humanized antibody or fragment thereof) which binds to the same epitope (or a portion thereof) on human lymphocytes as the humanized antibody shown in Figure 1. The humanized antibody shown in Figure 1 means the humanized antibody whose light chain and heavy chain are as shown in Figures 1A to 1F.

The antibody is hereinafter sometimes referred to as TRX1. The term "molecule" or "antibody that binds the same epitope as TRX1" includes TRX1. The term "TRX1" includes the antibody shown in Figure 1 and those identical thereto which may be produced, for example, by recombinant technology.

Although the preferred antibody is TRX1, from the teachings herein, one skilled in the art can produce antibodies that are equivalent to TRX1. As representative but non-limiting examples of such equivalent TRX1 antibodies there may be mentioned:

1) humanized antibodies that bind to the same epitope as TRX1;

2) humanized antibodies that have the same CDRs as TRX1 but which have a different humanized framework and/or a different human constant region;

3) humanized antibodies that bind to the same epitope as TRX1 in which one or more amino acids of one or more of the CDRs of TRX1 have been changed (preferably but not necessarily a conservative amino acid substitution) and in which the framework may be the same framework as TRX1 or have a different humanized framework or in which one or more of the amino acids of the framework region of TRX1 have been changed and/or in which the constant region may be the same as or different from TRX1;

4) humanized antibodies that bind to the same epitope as TRX1 wherein the antibody does not bind to the Fc ~~receptor~~ region of the receptor.

5) humanized antibodies that bind to the same epitope as TRX1 wherein the CDRs thereof do not include a glycosylation site;

6) humanized antibodies that bind to the same epitope as TRX1 and that do not bind to the Fc receptor and the CDRs do not include a glycosylation site; region of the

7) a chimeric antibody that binds to the same epitope as TRX1; and

8) a murine antibody that binds to the same epitope as TRX1.

The antibodies that are equivalent to TRX1 may be used in the same manner and for the same purposes as TRX1.

The molecules or antibodies of the present invention may be used in a method for treating an animal, in particular a human, especially for use in inhibiting, ameliorating, or reducing an immune response to an antigen, which may be a foreign antigen or a self antigen, including inducing tolerance to an antigen. The molecules or antibodies may be used to inhibit, ameliorate, or reduce an immune response to a Class I presented antigen and/or to a Class II presented antigen. The molecules or antibodies may be used to inhibit,

ameliorate, or reduce an immune response to such antigens. In the case of a transplant, for example, Class I and Class II major histocompatibility (MHC) antigens and non-MHC or minor histocompatibility antigens may be presented. Apart from transplantation antigens, the molecules or antibodies may be used to inhibit, ameliorate, or reduce an immune response to globular proteins, glycoproteins such as immunoglobulins, materials carried on particles such as pollen proteins, polypeptides intended for therapeutic use such as interferon, Interleukin-2 or tumor necrosis factor, or hormone replacements such as lutenizing hormone, its analogues and antagonists. Further specific antigens to which an immune response can be inhibited, ameliorated, or reduced include synthetic peptide analogues of protein therapeutic agents which are used to aid in receptor blocking, and alloantigens. Alloantigens may be responsible for rejection of foreign tissue in tissue transplants or skin grafts. The term "antigen" as used herein is a compound or material that induces an immune response in an animal, in particular a human animal. The immune response may be a T-cell response which may or may not be accompanied by a humoral response.

The molecules or antibodies of the present invention inhibit and/or alter T-cell activation and proliferation and Applicant has found that such inhibition can be effected when adding the molecule or antibody either before or after an agent which stimulates T-cell activation.

The molecules or antibodies of the present invention have the characteristics of binding to an epitope of a CD4 antigen (CD4 positive human T-cells), but it is to be understood, however, that although the antibody is believed to function by binding to a CD4 antigen on T-cells, the antibody may function by binding to a CD4 antigen on other cells; e.g., monocytes. As a result, the ability of such molecules or antibodies to inhibit and/or alter T-cell activation or proliferation may or may not be effected through binding to CD4 positive cells, although Applicant presently believes that the mechanism of action involves binding of the molecule or antibody to CD4 positive cells.

In accordance with another aspect of the present invention, there is provided a method of preventing and/or inhibiting an on-going immune response in human patients through the administration to the patient of an antibody, hereafter referred to as TRX1 (or fragment or derivative thereof) or any

molecule that mimics such antibody or derivative or fragment thereof, i.e., binds to the same epitope as TRX1.

Although Applicants do not want to limit the invention to any theoretical reasoning, it is believed that the mechanism which enables the monoclonal antibody of this invention to inhibit or prevent or reduce or ameliorate the severity of an immune response, and to inhibit and/or alter the activation and proliferation of T-cells, is the fact that the TRX1 antibody either decreases the density of CD4 expressed on T-cell surfaces and thus decreases the number of functional CD4 +T lymphocytes; and/or affects signal transduction and thus decreases the number of functional CD4 + T lymphocytes. It is believed that these mechanisms of action are responsible for not only the prevention of immune responses, but also the reduction in severity of on-going immune responses. In addition, the TRX1 antibody inhibits natural killer (NK) cell activity in vitro. This is pertinent to the present invention because it is believed that a non-MHC restricted cytotoxic mechanism such as NK cell activity has been implicated in graft versus host disease.

The term "inhibit" as used herein throughout this application is intended to mean prevention, or inhibition, or reduction in severity, or amelioration of an immune response to one or more antigens. The antigen may be a foreign antigen or a self antigen. The term "graft" as used herein for purposes of this application shall mean any and all transplantation, including but not limited to, allograft and xenograft transplantation. Such transplantation may by way of example include, but not be limited to, transplantation of cells, bone marrow, tissue, solid-organ, bone, etc.

The term "immune response(s)" as used herein is intended to mean immune responses dependent upon T cell activation and proliferation which includes both cellular effects and T cell dependent antibodies which may be elicited in response to, by way of example and not limitation: (i) grafts, (ii) graft versus host disease, and (iii) autoantigens resulting in autoimmune diseases, which by way of example include but are not limited to rheumatoid arthritis, systemic lupus, multiple sclerosis, diabetes mellitus, etc.

The molecule employed in the present invention is one which binds to the same epitope (or a part of that epitope) as the TRX1 humanized antibody. The term "binds to the same epitope as TRX1 humanized antibody" is intended to

describe not only the TRX1 humanized antibody but also describes other antibodies, fragments or derivatives thereof or molecules which bind to the same such epitope as the TRX1 humanized antibody.

Such molecules are preferably antibodies. In a preferred embodiment, the antibody does not bind to the Fc receptor and the CDRs do not include a glycosylation site.

Such other antibodies include, by way of example and not by limitation, rat, murine, porcine, bovine, human, chimeric, humanized antibodies, or fragments or derivatives thereof.

The term "fragment" as used herein means a portion of an antibody, by way of example such portions of antibodies shall include but not be limited to CDR, Fab, or such other portions, which bind to the same epitope or any portion thereof as recognized by TRX1.

The term "antibody" as used herein includes polyclonal and monoclonal antibodies as well as antibody fragments and derivatives, as well as antibodies prepared by recombinant techniques, such as chimeric or humanized antibodies, single chain or bispecific antibodies which bind to the same epitope or a portion thereof as recognized by the humanized antibody TRX1. The term "molecules" includes by way of example and not limitation, peptides, oligonucleotides or other such compounds derived from any source which mimic the antibody or binds to the same epitope or a portion thereof as the antibody fragment or derivative thereof.

Another embodiment of the present invention provides for a method of treating a patient who is to receive or has received a graft transplant with an effective amount of at least one member selected from the group consisting of TRX1 antibody, or an antibody, or derivative or fragment thereof or molecules which bind to the same epitope (or a portion thereof) as the TRX1 antibody. The treatment is preferably effected with the whole or intact TRX1 antibody.

In one embodiment, the antibody is TRX1 which is a humanized antibody that includes modified constant regions of a human antibody, and light and heavy chain framework and CDR regions, in which the framework regions of the light and heavy chain variable regions correspond to the framework regions of the

light and heavy chain variable region of a human antibody, and the CDRs derived from a mouse monoclonal antibody designated NSM4.7.2.4. The TRX1 antibody is shown in Figure 1. Figure 1A shows the amino acid and DNA sequences for the TRX1 light chain. Figure 1B shows the TRX1 light chain nucleic acid sequence. Figure1C shows the TRX1 light chain amino acid sequence with the CDRs highlighted. Figure 1D shows the amino acid and DNA sequences for the TRX1 heavy chain. Figure 1E shows the TRX1 heavy chain nucleotide sequence. Figure 1F shows the TRX1 heavy chain amino acid sequences with the CDRs highlighted. In the figures, amino acid residue 1 is the first amino acid, in each of the heavy and light chains, after the leader sequence. It also is the first residue in FR1 in both sequences.

The preparation of TRX1 humanized antibody suitable for the purposes of the present invention should be apparent to those skilled in the art from the teachings herein. Such antibody may be prepared by recombinant techniques known to those skilled in the art.

The antibodies of the present invention may be used to inhibit an immune response in an animal by administering the antibody (or fragment thereof) in an amount effective to inhibit such immune response.

For example, in some cases, treatment with a therapeutic agent includes an immune response against the therapeutic agent. As representative examples of such therapeutic agents there may be mentioned monoclonal antibodies such as ReoPro and OKT3, enzymes for replacement therapy such as, but not limited to, glucocerebrosidase for Gaucher's disease and clotting factors such as Factor VIII, and products of gene therapy and gene therapy delivery vehicles such as adenovirus derived vectors.

In accordance with an aspect of the present invention, an antibody as hereinabove described (or fragment of such antibody) is administered to a patient that is to be treated with such therapeutic agent, with the antibody (or fragment) being administered in an amount effective to inhibit the immune response against the therapeutic agent. The antibody may be administered prior to, in combination with, or subsequent to administration of the therapeutic agent. The method of administration is dependent on a variety of factors, including, but not limited to, the specific indication, specific therapeutic agent and optimal dosing schedule If administered prior to the administration of the

therapeutic agent, the antibody is administered from about 1 hour to about 10 days prior to the administration of the therapeutic agent, preferably from about 1 hour to about 24 hours prior to the administration of the therapeutic agent. If administered after the administration of the therapeutic agent, the antibody is administered from about 1 hour to about 10 days after the administration of the therapeutic agent, preferably from about 1 hour to about 24 hours after the administration of the therapeutic agent.

The amount of antibody administered, the dosing schedule and the number of times that the antibody is administered is dependent upon the therapeutic agent and the regimen used for treating a patient with the therapeutic agent.

In general, the antibody may be used in an amount from 0.1 milligram to 3 grams per dose.

The antibody of the present invention may also be used to inhibit an immune response against a self-antigen and/or against a transplant (for example, transplant rejection) and/or to inhibit or ameliorate an immune response of a graft against a host.

The antibody of the present invention may also be used to inhibit an immune response against gene therapy products as well as an immune response against gene therapy delivery vehicles such as adenovirus derived vectors which limit the effectiveness of the gene therapy.

Thus, an immune response to an antigen in a host can be inhibited, ameliorated, or reduced by administering TRX1 antibody along with the antigen. A patient may be given a tissue transplant such as an organ transplant or a bone marrow transplant and may be given TRX1 antibody along with the transplant to inhibit rejection thereof. Also, tolerance may be induced to an antigen already possessed by a patient. Long-term specific tolerance can be induced to a self-antigen or antigens in order to treat autoimmune diseases.

Persistent or periodic antigen presence is required to maintain tolerance. A tissue graft, for example, supplies the antigen to maintain

tolerance to itself. In the case of extraneous foreign antigens such as allergens, antigen "reminders" can be given at regular intervals.

An antibody or fragment thereof or molecule of the type hereinabove described may be administered in vivo in accordance with the present invention to inhibit the activation and proliferation of T-cells, and decrease the density of CD4 expression on the cell surface and/or affect signal transduction thereby reducing the functionality of CD4+ T lymphocytes and/or the number of $CD4^+$ T lymphocytes.

Thus, for example, in an in vivo procedure, such antibodies are administered to prevent and/or inhibit an immune response and thereby inhibit T cell activation and proliferation.

An antibody or fragment thereof or molecule of the type hereinabove described may be administered ex vivo in accordance with the present invention to decrease the density of $CD4^+$ expression on the cell surface and/or affect signal transduction, thus reducing the functionality of CD4+ T lymphocytes and/or the number of $CD4^+$ cells of the donor cells. By way of example and not limitation, in an ex vivo procedure, such antibodies or fragments or derivatives thereof or molecules would be infused into donor bone marrow prior to transplantation to prevent the onset of graft versus host disease upon transplantation.

The antibody or fragment thereof is generally administered in a pharmaceutically acceptable carrier. As representative examples of such carriers, there may be mentioned normal saline solution, buffers, etc. Such pharmaceutical carriers are well known in the art and the selection of a suitable carrier is deemed to be within the scope of those skilled in the art from the teachings contained herein.

The TRX1 antibody or other antibody of the present invention may be administered in vivo intravenously, subcutaneously, or by intramuscular administration, etc.

As hereinabove indicated, TRX1 antibody or other antibody of the present invention is administered in vivo in an amount effective to inhibit an immune response against an antigen(s). The term "an effective amount" for purposes

of this Application shall mean that amount of antibody capable of producing the desired effect. In general, such antibody is administered in an amount of at least 0.1 milligram per dose. It is to be understood that lower amounts could be used. In addition after the initial treatment, the hereinabove described amounts may be reduced for subsequent treatments, if any. Thus the scope of the invention is not limited by such amounts.

The TRX1 antibody or other antibody of the present invention may be employed to induce tolerance to an antigen. The term "tolerance", as used herein, means that a T-cell non-response persists against an antigen after stopping the antibody treatment, even in the case of challenge. If needed, however, booster or reinforcing doses of the antibody may be given in order to maintain such tolerance.

The techniques of the present invention for inhibiting the activation of T-cells may be employed alone or in combination with other techniques, drugs or compounds for inhibiting the activation of T-cells or inhibiting graft rejection or graft versus host disease or in treating various autoimmune diseases Examples may include drugs such as rapamycin and cyclosporin, or other immunomodulatory compounds including monoclonal antibodies directed against co-stimulatory molecules such as CD2, CD8 and CD28, as well as monoclonal antibodies directed against adhesion molecules.

The antibodies of the present invention also may be employed in a method of selecting for or determining the presence of CD4 positive cells in a sample, such a blood sample, for example. In such method, a sample is contacted with the molecule or antibody, and the presence of CD4 positive cells is determined, and/or CD4 positive cells then can be selected or isolated form the sample.

EXAMPLES

The invention now will be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

EXAMPLE 1

A cDNA library was constructed from the mouse hybridoma NSM 4.7.2.4 using the Superscript plasmid system (Gibco/BRL, cat. no. 82485A) according

to the manufacturer's suggested protocol. Heavy and light chain cDNAs were cloned from the library by DNA hybridization using as probes rat heavy and light chain gene cDNAs from the rat hybridoma YTS 177.

The rat heavy and light chain gene cDNAs of YTS 177 were isolated from the expression vector pHA Pr-1 as BamH1/Sal 1 fragments and labeled with ^{32}P and used independently to screen the NSM 4.7.2.4. cDNA library using standard molecular biology techniques (Sambrook, et al., Molecular Cloning, A. Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (2001); Ausubel, et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York (2001).) Sequence analysis of the cDNAs derived from the NSM 4.7.2.4 cDNA library confirmed the NSM 4.7.2.4 heavy chain to be mouse gamma-1 subclass and the NSM 4.7.2.4 light chain to be kappa. The NSM 4.7.2.4 heavy and light V regions (VH and VL, respectively) were reshaped to the human VH and VL regions with the "best fit" or highest sequence similarity in the framework regions to that of the mouse. For the light chain, human antibody HSIGKAW (from EMBL) with a sequence similarity of 79%, was used (LA Spatz et al., 1990 *J. Immunol.* 144:2821-8). The sequence of HSIGKAW VL is:

MVLQTQVFISLLLWISGAYGDIVMTQSPDSLAVSLGERATINCKSSQSLLYS
SNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISS
LQAEDVAVYYCQQYYSTPPMFGQGTKVEIKRT

D start of framework 1
Q changed to G

For the heavy chain, human antibody A32483 (From GenBank) with a sequence similarity of 74%, was used (Larrick, et al., Biochem. Biophys. Res. Comm., Vol. 160, pgs. 1250-1256 (1989)). The sequence of A32483 VH is:

LLAVAPGAHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQ
APGQGLEWMGIINPSGNSTNYAQKFQGRVTMTRDTSTSTVYMELSSLRSE
DTAVYYCAREKLATTIFGVLI ITGMDYWGQGTLVTVSSGSASA

Q start of framework 1

For the humanization process, anti-CD4 light chain clone 77.53.1.2 (insert size 1kb) and anti-CD4 heavy chain clone 58.59.1 (insert size 1.7kb) were chosen from the cDNA library and inserts isolated from the pSport vector as Sal I/Not I fragments and cloned into M13mp18 vector to produce single stranded DNA for sequencing and template for mutagenesis. The humanization of NSM 4.7.2.4 was performed by site-directed mutagenesis of the mouse cDNA using a kit from Amersham International (RPN 1523) according to the manufacturer's suggested protocol.

Mutagenesis of the VL gene framework regions was performed using five oligonucleotides ranging in length from 29 to 76 bases. The oligos used were:

Primer #1998 76 bases

5'-TGA CAT TGT GAT GAC CCA ATC TCC AGA TTC TTT GGC TGT
GTC TCT AGG TGA GAG GGC CAC CAT CAA CTG CAA GGC
C

Primer #1999 29 bases

5'-TGA ACT GGT ATC AAC AGA AAC CAG GAC AG

Primer #2000 28 bases

5'-AGA GTC TGG GGT CCC AGA CAG GTT TAG T

Primer #2001 42 bases

5'-GTC TTC AGG ACC CTC CGA CGT TCG GTG GAG GTA CCA AGC
TGG

Primer #2008 52 bases

5'-CAC CCT CAC CAT CAG TTC TCT GCA GGC GGA GGA TGT TGC
AGT CTA TTA GTG T

The oligos were phosphorylated and mutagenesis performed in three steps using no more than two oligos per step to introduce changes according to the following procedure:

(1) Annealing phosphorylated mutant oligos to ssDNA template
(2) Polymerization
(3) Filtration to remove single-stranded DNA

(4) Nicking non mutant strand with Nci I
(5) Digestion of non-mutant strand with Exo III
(6) Repolymerization of gapped DNA
(7) Transformation of competent JM101
(8) Sequencing of clones

Mutations were confirmed by single strand DNA sequencing using M13 primers -20 and -40 and also the mutagenic primers # 1999 and # 2000.

A Sal I site at the 5' end of the variable region was changed to Hind III by linker oligos #2334 and #2335 to allow cloning of the variable region as a Hind III/Kpn I fragment into the light chain constant region of CAMPATH-1H.

Primer #2334 24 bases

5'-AGC TTT ACA GTT ACT GAG CAC ACA

Primer #2335 24 bases

5'-TCG ATG TGT GCT CAG TAA CTG TAA

Mutagenesis of the VH gene framework regions was performed using five oligonucleotides ranging in length from 24 to 75 bases. The oligos used were:

Primer #2003 75 bases

5'-GGT TCA GCT GGT GCA GTC TGG AGC TGA AGT GAA GAA
GCC TGG GGC TTC AGT GAA GGT GTC CTG TAA GGC TTC
TGG

Primer # 2004 52 bases

5'-AGC TGG GTG AGG CAG GCA CCT GGA CAG GGC CTT GAG
TGG ATG GGA GAG ATT T

Primer #2005 60 bases

5'-CAA GGG CAG GGT CAC AAT GAC TAG AGA CAC ATC CAC CAG
CAC AGT CTA CAT GGA ACT CAG

Primer #2006 44 bases

5'CAG CCT GAG GTC TGA GGA CAC TGC GGT CTA TTA CTG TGC AAG A

Primer #2007 24 bases

5'-GCC AAG GGA CAC TAG TCA CTG TGT

Mutagenesis was carried out as described above for the light chain again using no more than two oligos at a time to introduce the changes. Mutations were confirmed by single strand DNA sequencing using M13 primers -20 and -40 as well as the mutagenic primers #2002 and #2004.

Primer #2002 was used to correct a reading frame error in starting clone 58.59.1.

Primer #2002 39 bases

5'-ACT CTA ACC ATG GAA TGG ATC TGG ATC TTT CTC CTC ATC

Primer #2380 was used to correct extra mutation added by #2004 which was missed in the first sequencing.

Primer #2380 39 bases

5'-TCA CTG CCT ATG TTA TAA GCT GGG TGA GGC AGG CAC CTG

As with the light chain, the heavy chain 5' Sal I site was changed to Hind III using linker oligo's #2334 and #2335 to allow cloning of the heavy chain variable region as Hind III/ Spe I (site introduced by primer #2007) fragment into the heavy chain constant region of CAMPATH-1H.

Construction of heavy chain

The following samples of DNA were used

1. Plasmid 1990

Human gamma-1 heavy chain constant region gene cloned into pUC18 (obtained from Martin Sims, Wellcome Foundation Ltd).

2. Plasmid 2387

Reshaped heavy chain of NSM 4.7.2.4 containing human framework regions and mouse gamma 1 constant region.

A Sal I site in the reshaped CD4 heavy chain was altered to a Hind III site. The variable region gene was excised by digestion with Hind III/Spe I and ligated with the constant region gene in plasmid 1990 to give a complete humanized heavy chain (plasmid 2486). The heavy chain gene was cut out of this plasmid with Hind III/EcoR I and ligated with the expression vector pEE6.

Construction of light chain

The following samples of DNA were used.

1. Plasmid 2028

CAMPATH-1H light chain gene cloned into M13mp18 at Sal I/BamH I restriction site.

2. Plasmid 2197

Reshaped light chain of NSM 4.7.2.4 containing human framework regions and mouse kappa constant region. A Kpn I site already had been introduced between variable and constant portions of this gene.

A Kpn I restriction site was introduced into the CAMPATH 1H light chain gene corresponding to the site in plasmid 2197 and an EcoR I site was introduced at the 3' end of the constant region. The constant region gene was excised from this plasmid (2502) by digestion with Hind III/Kpn I.

Meanwhile a Sal I site in plasmid 2197 was changed to a Hind III site (this step had to be repeated because a frame-shift mutation inadvertently was introduced the first time). The new plasmid (2736) was digested with Hind

III/Kpn I. The CD4 variable region fragment was cloned into a plasmid containing the kappa constant region gene from plasmid 2502 to give a complete humanized light chain (plasmid 2548). The light chain gene was cut out from this plasmid with Hind III/EcoR I and ligated with the expression vector pEE12 to give plasmid 2798.

Ligation of heavy and light chains and expression in NSO cells

The heavy chain gene was excised from the pEE6 vector by digestion with Sal I/Bgl II and cloned into the light chain pEE12 vector which had been digested with BamH I/Sal I.

The final vector construct was checked by restriction digests with Hind III, EcoR I, Sal I, BamH I, Bgl II and Spe I for the presence of the expected fragments, including 700 bp light chain, 1400 bp heavy chain, 2300 bp fragment of pEE6 and 7000 bp fragment of pEE12.

The pEE12 vector was linearised by digestion with Sal I and transferred into NSO cells by electroporation, following a standard protocol (Celltech 1991) except that the selection medium was slightly modified, being based on IMDM rather than DMEM. Transfectants were selected in medium lacking glutamine, supplemented with dialysed FCS, ribonucleosides, glutamic acid, and asparagine as recommended.
The transfection mixes were cultured in three 96-well plates, and of 36 growing wells which were tested, 5 were strongly positive for production of human heavy and light chains (18 others were positive for one or other, or weakly positive for both).

A clone, designated SDG/B7B.A.7 was selected and stored frozen but no further characterization has been done on this wild type antibody.

Construction of mutant IgG1 antibody designated to abolish effector functions

Due to concerns about side effects of other CD4 antibodies reported in various clinical trials, it was considered desirable to avoid the possibility of engaging Fc regions of receptors. Human IgG4 is thought to have minimal Fc binding or complement-activating ability. However, experiments have show that it does engage Fc regions of receptors in some individuals (Greenwood et al., Eur. J. Immunol., Vol. 23, pgs. 1098-1104, 1993), and clinical studies with a human IgG4 variant to

CAMPATH-1H have demonstrated an ability to kill cells *in vivo* (Isaacs et al., Clin. Exp. Immunol., Vol. 106, pgs. 427-433 (1996)). To eliminate the possibility of binding Fc receptors, constructs were made with mutations in the IgG1 heavy chain constant region.

TRX 1 has the mutations Leu^{236} to Ala and Gly^{238} to Ala, as shown in Figures 1D and 1E. These particular residues were chosen because they were predicted to disrupt maximally binding to all three types of human Fc receptors for IgG. Either mutation is sufficient to reduce binding to Fc(RI (Woof, et al., Mol. Immunol, Vol. 332, pgs. 563-564, 1986; Duncan, et al., Nature, Vol. 332, pgs. 563-564 1988; Lund, et al., J. Immunol, Vol. 147, pgs. 2657-2662 1991) or Fc(RII (Lund et al., 1991; Sarmay et al., Mol. Immunol., Vol. 29, pgs. 633-639 1992) whereas Gly^{238} to Ala has the biggest effect on binding to Fc(RIII (Sarmay et al., 1992).

The following samples of DNA were used.

1. Plasmid 2555 and Plasmid 2555 Mut.

Humanized V_H region of NSM 4.7.2.4 cloned into pEE6 expression vector at a Hind III/Spe I restriction site. Plasmid 2555 then was mutated by site directed mutagenesis such that amino acid residue Asn^{101} was changed to Asp^{101}, as shown in Figures 1D and 1E. The resulting plasmid is plasmid 2555 Mut.

2. Plasmid 2798

Humanized V_H region of NSM 4.7.2.4 joined to human kappa constant regions to give approx 700 bp fragment cloned into pEE12 expression vector at a Hind III/EcoR I.

3. Plasmid MF4260

Human IgG1 heavy chain associated with the humanized CD18 V_H region, having the mutations Leu^{236} to Ala and Gly^{238} to Ala as well as a Spe I restriction site introduced into framework region 4, cloned into pUC18.

The purpose of the Spe I restriction site is to allow separation and recombination of different variable regions.

The CD18 V_H region gene was excised from plasmid MF 4260 by digestion with Spe I and Hind III and the remaining vector, now having only the relevant heavy chain constant region, was purified using Geneclean. It was ligated with the humanized V_H region DNA of NSM 4.7.2.4 which had been isolated from plasmid 2555 Mut in the same way. The product was used to transform "Sure" cells and colonies were checked for the presence of the expected 1400 bp complete heavy chain insert.

The complete V_H and constant region insert was excised from the pUC vector by digestion with Hind III and EcoR I. The 1400 bp fragment was purified using QiaexII (Qiagen) and then ligated in turn into the vector pEE6, which had previously been cut with the same enzymes.

The next step was to excise the CD4 heavy chain genes from the pEE6 vector and clone them into pEE12, already containing the humanized CD4 light chain gene (plasmid 2798). The pEE6 vector was digested with Sal I and Bgl II and the pEE12 vector was digested with Sal I and BamH I to create the appropriate sites for re-ligation.

The final vector construct was checked by restriction digests with Hind III, EcoR I, Sal I and Spe I for the presence of the expected fragment, *i.e.*, 700 bp light chain, 1400 bp heavy chain, 2300 bp fragment of pEE6, and 7000 bp fragment of pEE12.

The pEE12 vector was linearized by digestion with Sal I and transfected into NSO cells by electroporation as above. The transfection mixes were cultured in six 96-well plates, and of 90 growing wells which were tested, all were positive for production of human heavy and light chains. At this stage a sample of the pEE12 vector DNA was digested with Sal I, precipitated with ethanol and transferred to the Therapeutic Antibody Centre (TAC).

Target Cells For Final Transfection

NSO cells were obtained directly from the ECACC (clone CB1782, accession number 85110503). A master cell bank (MCB) was prepared at the Therapeutic Antibody Centre, Churchill Hospital, Oxford, England.

Transfection and Selection Of Final Transfectant

The pEE12 vector was transfected into NSO cells from the MCB by electroporation as hereinabove described. A total of $2x10^7$ cells were transfected with 80 μg of linearized plasmid DNA in a final volume of 2.0ml. The transfection mix was plated out in twelve 96-well plates and fed with selective medium according to the standard protocol (The Cell Tech Glutamine Synthetase Gene Expression System, Version 2 - Expression from Myeloma Cells, Revision 6.) Six plates received selective medium containing 10(M methionine sulfoximine (MSX).

Purification of the antibody

Culture supernatant is purified by using a Biopilot chromatography system (Pharmacia) in three steps as follows:

(1) Affinity chromatography on a column of Protein A-Sepharose Fast Flow

(2) Ion exchange chromatography on S-Sepharose Fast Flow

(3) Size exclusion chromatography on Superdex 20.

The purified product was filtered and pooled into a single biocontainer.

Throughout the purification process, precautions are taken to ensure that the system remains aseptic. All buffers and reagents are passed through a 0.2 micron membrane filter and the purified product is also passed through a 0.2 micron filter before being pooled. After a batch of antibody has been processed, the entire chromatography system and columns are sanitized with 0.5M NaOH, washed with sterile PBS and stored in 20% ethanol. Before it is used again, the ethanol is washed out with sterile PBS and a complete trial run is carried out. Samples of buffers and column eluates are checked for endotoxin level.

Example 2

Construction of TRX1 Antibody Starting from Nucleotide Sequence

Cloning of Human Constant Regions

Heavy Chain Constant Region

The human gamma 1 heavy chain constant region (IgG1) is amplified from human leukocyte cDNA (QUICK-Clone™ cDNA Cat. No. 7182-1, Clontech) using the following primer set and cloned into pCR-Script (Stratagene). The plasmid containing the human gamma 1 heavy chain constant region in pCR-Script is designated pHCγ-1.

primer hcγ-1

Spe I

5' primer: 5'- *ACT AGT* CAC AGT CTC CTC AGC

primer hcγ-2

EcoR I

3' primer: 5'- *GAA TTC* ATT TAC CCG GAG ACA G

Non-Fc binding mutations ($Leu^{236}Ala$, $Gly^{238}Ala$) are made in the heavy chain constant region by site-directed mutagenesis using the following primer and the Transformer™ Site-Directed Mutagenesis Kit from Clontech (Cat. No. K1600-1). The plasmid containing the human gamma 1 heavy chain non-Fc binding mutant constant region in pCR-Script is designated pHCγ-1Fcmut.

primer hcγ-3

Fc mut oligo: 5'- CCG TGC CCA GCA CCT GAA CTC
GCG GGG GCA CCG TCA GTC TTC
CTC CCC C

Light Chain Constant Region

The human kappa light chain constant region is amplified from human leukocyte cDNA (QUICK-Clone™ cDNA Cat. No. 7182-1, Clontech) using the following primer set and cloned into pCR-Script (Stratagene). The plasmid containing the human kappa light chain constant region in pCR-Script is designated pLCκ-1.

primer lcκ-1

Kpn I

5' primer: 5'- *GGT ACC* AAG GTG GAA ATC AAA CGA AC

primer lcκ-2

Hind III

3' primer: 5'- *AAG CTT* CTA ACA CTC TCC CCT GTT G

Synthesis, Construction and Cloning of TRX1 Variable Regions

The heavy and light chain variable regions are constructed from a set of partially overlapping and complementary synthetic oligonucleotides encompassing the entire variable regions. The oligonucleotide set used for each variable region is shown below.

Heavy Chain Variable Region Synthetic Oligonucleotides

Coding Strand Heavy Chain Variable Region Primers

***primer hv-1* (1 – 72) + 6 nucleotide linker**
5'- *aagctt* ATG GAA TGG ATC TGG ATC TTT CTC CTC ATC CTG TCA GGA ACT CGA GGT GTC CAG TCC CAG GTT CAG CTG GTG

***primer hv-2* (120 – 193)**
5'- C TGT AAG GCT TCT GGA TAC ACA TTC ACT GCC TAT GTT ATA AGC TGG GTG AGG CAG GCA CCT GGA CAG GGC CTT G

***primer hv-3* (223 – 292)**
5'- GGT AGT AGT TAT TAT AAT GAG AAG TTC AAG GGC AGG GTC ACA ATG ACT AGA GAC ACA TCC ACC AGC ACA G

***primer hv-4* (322 – 399)**
5'- GAG GAC ACT GCG GTC TAT TAC TGT GCA AGA TCC GGG GAC GGC AGT CGG TTT GTT TAC TGG GGC CAA GGG ACA CTA GT

Non-Coding Strand Heavy Chain Variable Region Primers

***primer hv-5* (140 – 51)**
5'- GTG TAT CCA GAA GCC TTA CAG GAC ACC TTC ACT GAA GCC CCA GGC TTC TTC ACT TCA GCT CCA GAC TGC ACC AGC TGA ACC TGG GAC TGG

***primer hv-6* (246 – 170)**
5'- CTT CTC ATT ATA ATA ACT ACT ACC GCT TCC AGG ATA AAT CTC TCC CAT CCA CTC AAG GCC CTG TCC AGG TGC CTG CC

***primer hv-7* (342 – 272)**
5'- GTA ATA GAC CGC AGT GTC CTC AGA CCT CAG GCT GCT GAG TTC CAT GTA GAC TGT GCT GGT GGA TGT GTC TC

Light Chain Variable Region Synthetic Oligonucleotides

Coding Strand Light Chain Variable Region Primers

***primer lv-1* (1 - 63) + 6 nucleotide linker**
5'- gaattc ATG GAG ACA GAC ACA ATC CTG CTA TGG GTG CTG CTG CTC TGG GTT CCA GGC TCC ACT GGT GAC

***primer lv-2* (93 - 158)**
5'- GGC TGT GTC TCT AGG TGA GAG GGC CAC CAT CAA CTG CAA GGC CAG CCA AAG TGT TGA TTA TGA TGG

***primer lv-3* (184 - 248)**
5'- CAG AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT GTT GCA TCC AAT CTA GAG TCT GGG GTC CC

***primer lv-4* (275 - 340)**
5'- GGA CAG ACT TCA CCC TCA CCA TCA GTT CTC TGC AGG CGG AGG ATG TTG CAG TCT ATT ACT GTC AGC

Non-Coding Strand Light Chain Variable Region Primers

***primer lv-5* (109-43)**
5'- CAC CTA GAG ACA CAG CCA AAG AAT CTG GAG ATT GGG TCA TCA CAA TGT CAC CAG TGG AGC CTG GAA C

***primer lv-6* (203-138)**
5'- GGT GGC TGT CCT GGT TTC TGT TGA TAC CAG TTC ATA TAA CTA TCA CCA TCA TAA TCA ACA CTT TGG

***primer lv-7* (294-228)**
5'- GGT GAG GGT GAA GTC TGT CCC AGA CCC ACT GCC ACT AAA CCT GTC TGG GAC CCC AGA CTC TAG ATT G

***primer lv-8* (378-319)**
5'- GGT ACC TCC ACC GAA CGT CGG AGG GTC CTG AAG ACT TTG CTG ACA GTA ATA GAC TGC AAC

After HPLC purification and removal of organic solvents the oligonucleotides are resuspended in TE pH8.0 and phosphorylated. An aliquot of each oligonucleotide in the respective variable region set then are combined in equal molar amounts. The oligonucleotide mixtures are heated to 68(C for 10 minutes and allowed to cool slowly to room temperature. The annealed oligonuceotides then are extended to produce double stranded variable region DNA fragments. For the extension, dNTPs are added to a final concentration of 0.25 mM followed by an appropriate volume of 5X T4 DNA polymerase buffer [165 mM Tris acetate, pH 7.9, 330 mM sodium acetate, 50mM magnesium acetate, 500 (g/ml BSA, 2.5mM DTT] and 4 units of T4 DNA polymerase. The mixture is incubated at 37(C for 1 hour followed by heat inactivation of the T4 DNA polymerase at 65(C for 5 minutes.

The double stranded DNA is ethanol precipitated and resuspended in the same volume of TE pH 8.0. An appropriate volume of 5X T4 DNA ligase buffer [250mM Tris-HCl, pH7.6, 50mM $MgCl_2$, 5mM ATP, 5mM DTT, 25% w/v polyethylene glycol-8000] then is added to the double stranded DNA followed by 2 units of T4 DNA ligase and the mixture incubated for 1 hour at 37_C to ligate the extended fragments. The T4 DNA ligase then is heat inactivated at 65_C for 10 minutes. The variable region DNA fragments then are phenol extracted, ethanol precipitated, and resuspended in TE, pH 8.0 and cloned into pCR-Script (Stratagene). The resulting plasmid containing the heavy chain variable region is designated pHV-1 and the plasmid containing the light chain variable region was designated pLV-1.

The final heavy and light chain expression vectors are constructed in pcDNA 3.1 (Invitrogen). For the heavy chain expression vector, the Fc mutated constant region is released from plasmid pHC-1Fcmut by digestion with Spe I and EcoR I and isolated by agarose gel electrophoresis. The heavy chain variable region is released from plasmid pHV-1 by digestion with Hind III and Spe I and isolated by agarose gel electrophoresis. The two fragments in equal molar amounts are ligated into the Hind III/EcoR I sites of pcDNA3.1(+) (Invitrogen) using standard molecular biology techniques. The resulting TRX1 heavy chain expression vector is designated pTRX1/HC.

Similarly, for the light chain expression vector, the light chain constant region is released from plasmid pLC-1 by digestion with Kpn I and Hind III followed by agarose gel purification. The light chain variable region is released from pLV-1

by digestion with EcoR I and Kpn I followed by agarose gel purification. The two light chain fragments in equal molar amounts are ligated into the EcoR I/Hind III sites of pcDNA3.1(-) (Invitrogen) using standard molecular biology techniques yielding the TRX1 light chain expression vector pTRX1/LC.

For production of TRX1 antibody, the TRX1 heavy chain and TRX1 light chain expression plasmids are cotransfected into CHO cells using standard molecular biology techniques.

The disclosures of all patents, publications (including published patent applications), depository accession numbers, and database accession numbers are hereby incorporated by reference to the same extent as if each patent, publication, depository accession number, and database accession number were specifically and individually incorporated herein by reference.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

WHAT IS CLAIMED IS:

1. An antibody that binds to the same epitope as the humanized antibody shown in Figure 1.

2. The antibody of claim 1 wherein said antibody is a humanized antibody or fragment thereof.

3. The antibody of Claim 1 wherein said antibody does not bind to the Fc region of the receptor.

4. The antibody of Claim 1 wherein said antibody includes CDRs that are free of a glycosylation site.

5. The antibody of Claim 1 wherein said antibody is a humanized antibody identical to the humanized antibody shown in Figure 1.

6. The antibody of Claim 1 wherein said antibody is a humanized antibody having the same CDRs as the antibody shown in Figure 1.

7. The antibody of Claim 1 wherein said antibody is a chimeric antibody that includes the CDRs shown in Figure 1.

8. A composition, comprising:

(a) the antibody of Claim 1; and

(b) an acceptable pharmaceutical carrier.

9. A process for inducing tolerance to an antigen in a patient, comprising:

administering to said patient an effective amount of the antibody of Claim 1.

10. A process for inhibiting an immune response in a patient comprising:

administering to said patient an effective amount of the antibody of Claim 1.

11. A process for inhibiting the rejection of a graft in a human patient, comprising:

administering to said patient the antibody of Claim 1, wherein said antibody is administered in an amount effective to inhibit rejection of said graft.

12. The process of Claim 11 wherein said graft is an organ.

Application No: GB 0114517.6 **Examiner:** Dr Jeremy Kaye
Claims searched: 1-8 **Date of search:** 28 January 2002

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.T):

Int Cl (Ed.7):

Other: Online: EPODOC, WPI, PAJ, BIOSIS, CAPLUS, EMBASE, GENBANK, MEDLINE, SCISEARCH

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	WO 92/05274 A1 (GORMAN ET AL.) p.1, l.1 - p.5, l.10; p.11, ll.14-29; p.12, l.18 - p.15, l.16.	1-8
X	US 6136310 (HANNA ET AL.) col.5, l.42 - col.6, l.20; col.7, l.61 - col.16, l.44.	1-8
X	US 5859205 (ADAIR ET AL.) col.1, ll.8-20; col.3, l.64 - col.8, l.36; col.11, l.65 - col.27, l.30.	1-8
X	Transplantation, Vol.65, No.5, 1998, Mourad, G.J et al., "Humanized IgG1 and IgG4 anti-CD4...", pp.632-641.	1-8
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X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.