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61/047,358 23 April 2008 (23.04.2008) US(71) Applicant (for all designated States except US): EPITO-
MICS, INC. [US/US]; 863 Mitten Road, Suite 103,
Burlingame, CA 94010-1303 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): KE, Yaohuang
[US/US]; 2323 18th Avenue, San Francisco, CA 94116
(US). YU, Guo-Liang [US/US]; 242 Gravatt Drive,
Berkeley, CA 94705 (US).(74) Agent: KEDDIE, James, S.; Bozicevic, Field & Francis
LLP, 1900 University Avenue, Suite 200, East Palo Alto,
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VH: <-----FR1----->CDR1 -----FR2-----
      QQLQESGPGSLVKPSETISITCAVSGFSLSRVGINWVRQAPGSGLEW
      SVK EG F CD T T VS N Q
      A T

--> CDR2 <-----FR3-----
      IGAIETGRAYVASWAKSRSTISGRDTSKNQVSLKMGSVTAADTAVY
      T A S F N R V C N N L T T R C L Y
      T V T
      E

---> CDR3 <---FR4--->
      FCARGELFNNGMGAFNIWGPGTMVTVSS
      V L
      E

VK: <-----FR1-----> CDR1 <---FR2--->
      AYQMCQSPSSLSASVGRVTICKQASESTIYSSLAWYQQKPGKPPKL
      D T A V EP GT N TG Q

--> CDR2 <-----FR3----->
      LIYSASTLASGVPSRFSGSGSDTFTLTISIQPEDFATYYCQQGEF
      Q S K EE A DVECA A S
      G A

      CDR3 <---FR4--->
      GTSNVENPFGGGTKVEIK
      A N SE VV

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Fig. 1

(57) Abstract: An antibody that binds TNF α and neutralizes its activity is provided. In certain cases, the antibody comprises: a) a heavy chain variable domain comprising an amino acid sequence that is at least 95 % identical to the amino acid sequence of SEQ ID NO. 1, and b) a light chain variable domain comprising an amino acid sequence that is at least 95 % identical to the amino acid sequence of SEQ ID No. 2.

ANTI-TNF α ANTIBODY

BACKGROUND

5 Human tumor necrosis factor-alpha (TNF α) is a homotrimer consisting of three 17 kDa protein subunits (Eck M. J. et al., JBC, 267: 2119-2122, 1992; Smith R. A. et al., JBC, 262: 6951-6954, 1987). TNF α is an inflammatory cytokine secreted from macrophages and monocytes, and functions as a signal transmitter in several cellular reactions such as necrosis and apoptosis (Beyaert R. et al., FEBS Lett., 340: 9-16, 1994).

10 TNF α causes a pro-inflammatory action leading to tissue destruction, such as breakdown of the cartilage and bone (Saklatvala, Nature, 322: 547-549, 1986), induction of procoagulation activity in vascular endothelial cells (Pober J S et al., J. Immunol., 136: 1680-1687, 1986), and increase in the adherence of neutrophils and lymphocytes (Pober et al., J. Immunol. 138: 3319-3324, 1987).

15 The pathology of a variety of disorders is attributed to excessive amounts of TNF α , either locally or systemically. For example, there is strong evidence that abnormally high production and release from cells of TNF α contributes to disease initiation and progression in rheumatoid arthritis, systemic inflammatory syndromes, diabetes, and multiple sclerosis. In every one of these conditions, the initiating and
20 sustaining pathophysiologic action is directly a result of an immediate local release and synthesis of massive amounts of TNF α from several types of cells at or adjacent to the site of tissue damage. The locally released TNF α is followed by additional synthesis and release of TNF α by invading macrophages drawn to the site of tissue damage by a cascade of chemotactic cytokines released locally from cells in response to the greatly
25 elevated TNF α concentrations.

There is a constant demand in the art for new antibodies that bind and neutralize TNF α .

SUMMARY

30 A TNF α neutralizing antibody is provided. In certain embodiments, an antibody may comprise a heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 1, and a light chain

variable domain comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 2. The antibody may be, for example, monoclonal, monovalent, bivalent, or single chain antibody. Methods of using a subject antibody to inhibit TNF α activity, methods of treatment using a subject antibody and kits containing the same are also provided. The subject antibody finds use in a variety of research and medical applications.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the amino acid sequence of the heavy (VH, SEQ ID NO.1) and the light (VK, SEQ ID NO. 2) chain of a subject antibody. FR regions are labeled and CDRs are boxed and labeled. Underneath these sequences are potential amino acid substitutions that would be expected to result in an active antibody.

Fig. 2A is a graph showing the RA scores for mice subjected to different treatments. Fig. 2B shows representative sections through the ankles of those animals. Human TNF α transgenic mice with rheumatoid arthritis (RA) were treated with Humira or HZD RabMAb, and the RA score was compared to the non treatment group (A). Normal mice were used as a control. At the end of the treatment, histopathological studies on ankle joints were performed (B). Treatment with HZD RabMAb decreased RA scores toward to that for the normal mice and the ankle joints were also found as healthy as the normal mice.

Fig. 3 is a graph showing the body weight for mice subjected to different treatments. No significant difference in body weight was observed between the non treatment and treated groups (Humira and HZD RabMAb) at the same time points in spite of some gain with time (C).

Fig. 4 shows the amino acid sequence of HZD-M RabMAb (SEQ ID NOS: 9 and 10). The leader sequences of the heavy and light chains are underlined, the variable domains are shown in bold, and the constant regions are underlined and bolded. The

variable domains of the HZD RabMAb contain the amino acid sequences of SEQ ID NO:1 and 2.

Fig. 5 shows the amino acid sequence of HZD-N-Nterm RabMAb (SEQ ID NOS: 11 and 12). The leader sequences of the heavy and light chains are underlined, the variable domains are shown in bold, and the constant regions are underlined and bolded. The variable domains of the HZD RabMAb are identical the amino acid sequences of SEQ ID NO:1 and 2, except for amino acid changes at the N-terminus.

DEFINITIONS

Before the present subject invention is described further, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" includes a plurality of such antibodies and reference to "a framework region" includes reference to one or more framework regions and equivalents thereof known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue

of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

The terms "antibody" and "immunoglobulin" are used interchangeably herein.

5 These terms are well understood by those in the field, and refer to a protein consisting of one or more polypeptides that specifically binds an antigen. One form of antibody constitutes the basic structural unit of an antibody. This form is a tetramer and consists of two identical pairs of antibody 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
10 to an antigen, and the constant regions are responsible for the antibody effector functions.

The recognized immunoglobulin polypeptides include the kappa and lambda light chains and the alpha, gamma (IgG₁, IgG₂, IgG₃, IgG₄), delta, epsilon and mu heavy chains or equivalents in other species. Full-length immunoglobulin "light chains" (of about 25 kDa or about 214 amino acids) comprise a variable region of about 110 amino
15 acids at the NH₂-terminus and a kappa or lambda constant region at the COOH-terminus. Full-length immunoglobulin "heavy chains" (of about 50 kDa or about 446 amino acids), similarly comprise a variable region (of about 116 amino acids) and one of the aforementioned heavy chain constant regions, e.g., gamma (of about 330 amino acids).

The terms "antibodies" and "immunoglobulin" include antibodies or
20 immunoglobulins of any isotype, fragments of antibodies which retain specific binding to antigen, including, but not limited to Fab, Fv, scFv, and Fd fragments, chimeric antibodies, humanized antibodies, single-chain antibodies, fusion proteins comprising an antigen-binding portion of an antibody and a non-antibody protein, and antibodies that have post-translational modifications. The antibodies may be detectably labeled, e.g.,
25 with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, and the like. The antibodies may be further conjugated to other moieties, such as members of specific binding pairs, e.g., biotin (member of biotin-avidin specific binding pair), and the like. The antibodies may also be bound to a solid support, including, but not limited to, polystyrene plates or beads, and the like. Also encompassed by the term
30 are Fab', Fv, F(ab')₂, and or other antibody fragments that retain specific binding to antigen, and monoclonal antibodies.

Antibodies may exist in a variety of other forms including, for example, Fv, Fab, and (Fab')₂, as well as bi-functional (i.e. bi-specific) hybrid antibodies (e.g., Lanzavecchia et al., Eur. J. Immunol. 17, 105 (1987)) and in single chains (e.g., Huston et al., Proc. Natl. 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),).

An immunoglobulin light or heavy chain variable region consists of a "framework" region (FR) interrupted by three hypervariable regions, also called "complementarity determining regions" or "CDRs". The extent of the framework region and CDRs have been precisely defined (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, (1991)). The numbering of all antibody amino acid sequences discussed herein conforms to the Kabat system. 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 CDRs. The CDRs 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 antibody variable and constant region genes belonging to different species. For example, the variable segments of the genes from a rabbit monoclonal antibody may be joined to human constant segments, such as gamma 1 and gamma 3. An example of a therapeutic chimeric antibody is a hybrid protein composed of the variable or antigen-binding domain from a rabbit antibody and the constant or effector domain from a human antibody (e.g., the anti-Tac chimeric antibody made by the cells of A.T.C.C. deposit Accession No. CRL 9688), although other mammalian species may be used.

As used herein, the term "humanized antibody" or "humanized immunoglobulin" refers to a non-human (e.g., mouse or rabbit) antibody containing one or more amino acids (in a framework region, a constant region or a CDR, for example) that have been substituted with a correspondingly positioned amino acid from a human antibody. In

general, humanized antibodies may produce a reduced immune response in a human host, as compared to a non-humanized version of the same antibody.

It is understood that the humanized antibodies designed and produced by the present method may have additional conservative amino acid substitutions which have substantially no effect on antigen binding or other antibody functions. By conservative substitutions is intended combinations such as those from the following groups: gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr. Amino acids that are not present in the same group are “substantially different” amino acids.

The term “specific binding” refers to the ability of an antibody to preferentially bind to a particular analyte that is present in a homogeneous mixture of different analytes. In certain embodiments, a specific binding interaction will discriminate between desirable and undesirable analytes in a sample, in some embodiments more than about 10 to 100-fold or more (e.g., more than about 1000- or 10,000-fold).

In certain embodiments, the affinity between a capture agent and analyte when they are specifically bound in a capture agent/analyte complex is characterized by a K_D (dissociation constant) of less than 10^{-6} M, less than 10^{-7} M, less than 10^{-8} M, less than 10^{-9} M, less than 10^{-9} M, less than 10^{-11} M, or less than about 10^{-12} M or less.

A “variable region” of a heavy or light antibody chain is an N-terminal mature domain of the chains. All domains, CDRs and residue numbers are assigned on the basis of sequence alignments and structural knowledge. Identification and numbering of framework and CDR residues is as described in by Chothia and others (Chothia, Structural determinants in the sequences of immunoglobulin variable domain. J Mol Biol 1998; 278: 457-79).

VH is the variable domain of an antibody heavy chain. VL is the variable domain of an antibody light chain, which could be of the kappa (K) or of the lambda isotype. K-1 antibodies have the kappa-1 isotype whereas K-2 antibodies have the kappa-2 isotype and VL is the variable lambda light chain. The variable domain of an antibody contains a heavy chain variable domain and a light chain variable domain.

As used herein, the terms “determining,” “measuring,” and “assessing,” and “assaying” are used interchangeably and include both quantitative and qualitative determinations.

The terms "polypeptide" and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; fusion proteins with detectable fusion partners, e.g., fusion proteins including as a fusion partner a fluorescent protein, β -galactosidase, luciferase, etc.; and the like. Polypeptides may be of any size, and the term "peptide" refers to polypeptides that are 8-50 residues (e.g., 8-20 residues) in length.

As used herein the term "isolated," when used in the context of an isolated antibody, refers to an antibody of interest that is at least 60% free, at least 75% free, at least 90% free, at least 95% free, at least 98% free, and even at least 99% free from other components with which the antibody is associated with prior to purification.

The terms "treatment", "treating" and the like are used herein to refer to any treatment of any disease or condition in a mammal, e.g., particularly a human or a mouse, and includes: a) preventing a disease, condition, or symptom of a disease or condition from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; b) inhibiting a disease, condition, or symptom of a disease or condition, e.g., arresting its development and/or delaying its onset or manifestation in the patient; and/or c) relieving a disease, condition, or symptom of a disease or condition, e.g., causing regression of the condition or disease and/or its symptoms.

The terms "subject", "host", "patient" and "individual" are used interchangeably herein to refer to any mammalian subject for whom diagnosis or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on.

"Corresponding amino acids" are amino acid residues that are at an identical position (i.e., they lie across from each other) when two or more amino acid sequences are aligned. Methods for aligning and numbering antibody sequences are set forth in great detail in Chothia, *supra*, Kabat *supra*, and others. As is known in the art (see, e.g., Kabat 1991 Sequences of Proteins of Immunological Interest, DHHS, Washington, DC),

sometimes one, two or three gaps and/or insertions of up to one, two, three or four residues, or up to about 15 residues (particularly in the L3 and H3 CDRs) may be made to one or both of the amino acids of an antibody in order to accomplish an alignment.

A “natural” antibody is an antibody in which the heavy and light immunoglobulins of the antibody have been naturally selected by the immune system of a multi-cellular organism, as opposed to unnaturally paired antibodies made by e.g., phage display, or humanized antibodies. As such, the subject parental antibodies do not usually contain any viral (e.g., bacteriophage M13)-derived sequences. Spleen, lymph nodes and bone marrow are examples of tissues that produce natural antibodies.

A “substitutable position” is a particular position of an antibody that may be substituted by different amino acids without significantly decreasing the binding activity of the antibody. Methods for identifying substitutable positions, and how they may be substituted, are described in much greater detail below. A substitutable positions may also be referred to as “variation tolerant position”.

A “parent” antibody is an antibody that is the target of amino acid substitutions. In certain embodiments, amino acids may be “donated” by a “donor” antibody to the parent antibody to produce an altered antibody.

“Related antibodies” are antibodies that have a similar sequence and produced by cells that have a common B cell ancestor. Such a B cell ancestor contains a genome having a rearranged light chain VJC region and a rearranged heavy chain VDJC region, and produces an antibody that has not yet undergone affinity maturation. “Naïve” or “virgin” B cells present in spleen tissue are exemplary B cell common ancestors. Related antibodies bind to the same epitope of an antigen and are typically very similar in sequence, particularly in their L3 and H3 CDRs. Both the H3 and L3 CDRs of related antibodies have an identical length and a near identical sequence (i.e., differ by 0, 1 or 2 residues). Related antibodies are related via a common antibody ancestor, the antibody produced in the naïve B cell ancestor. The term “related antibodies” is not intended to describe a group of antibodies that do not have a common antibody ancestor produced by a B-cell.

The term “TNF α ” or its non-abbreviated form “tumor necrosis factor- α ”, as used herein, is intended to refer to a human cytokine that exists as a 17 kD secreted form and a

26 kD membrane associated form, the biologically active form of which is composed of a trimer of noncovalently bound 17 kD molecules. The structure of TNF α is described further in, for example, Pennica et al. (Nature 1984 312:724-729), Davis et al.

(Biochemistry 1987 26:1322-1326) and Jones et al. (Nature 1989 338:225-228). The term

5 TNF α is intended to include recombinant TNF α molecules, which can be prepared by standard recombinant expression methods or purchased commercially (R & D Systems, Catalog No. 210-TA, Minneapolis, Minn.), as well as fusion proteins containing a TNF α molecule. Amino acid sequences of exemplary TNF α s that may be employed herein are found in the NCBI's Genbank database and a full description of human TNF α and its role
10 in various diseases and conditions is found in NCBI's Online Mendelian Inheritance in Man database.

A "TNF α neutralizing antibody", "antibody that neutralizes TNF α activity" or any grammatical equivalent thereof, is intended to refer to an antibody whose binding to TNF α results in inhibition of a biological activity of TNF α . This inhibition of the
15 biological activity of TNF α can be assessed by measuring one or more indicators of TNF α biological activity, such as TNF α -induced cytotoxicity (either in vitro or in vivo), TNF α -induced cellular activation or TNF α binding to a TNF α receptor. TNF α biological activity can be assessed by one or more of several standard in vitro or in vivo assays known in the art.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A TNF α neutralizing antibody is provided. In certain embodiments, an antibody may comprise a heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO. 1, and a light chain
25 variable domain comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID No. 2. The antibody may be, for example, monoclonal, monovalent, bivalent, or single chain antibody. Methods of using a subject antibody to inhibit TNF α activity, methods of treatment using a subject antibody and kits containing the same are also provided. The subject antibody finds use in a variety of research and
30 medical applications.

In certain embodiments, a monoclonal antibody may comprise a variable domain comprising: a heavy chain variable domain comprising the CDR1 (RYGIN; SEQ ID NO: 3), CDR2 (AIGETGRAYYASWAKS; SEQ ID NO: 4), and CDR3 (GELFNNGWGAFNI; SEQ ID NO: 5)) regions of SEQ ID NO: 1; and a light chain variable domain comprising the CDR1 (QASESIYSSLA; SEQ ID NO: 6), CDR2 (SASTLAS; SEQ ID NO: 7), and CDR3 (QQGFGTSNVENP; SEQ ID NO: 8) regions of SEQ ID NO: 2; or a variant of the variable domain that is otherwise identical to the variable domain except for up to 6 amino acid substitutions (i.e., 1, 2, 3, 4, 5 or 5 substitutions) in the CDR regions (i.e., the 6 CDR regions, collectively, may contain up to a total of 6 amino acid substitutions), where the monoclonal antibody neutralizes TNF α activity.

In other embodiments, the antibody may comprise a) a heavy chain variable domain that differs in amino acid sequence from SEQ ID NO: 1 by up to about 6 amino acid substitutions, e.g., 1, 2, 3, 4, 5 or 6 substitutions, and b) a light chain variable domain that differs in amino acid sequence from SEQ ID NO: 2 by up to about 6 amino acid substitutions, e.g., 1, 2, 3, 4, 5 or 6 substitutions. Exemplary amino acid substitutions are shown below. A subject antibody may have any one or combination of these substitutions.

Table 1. Exemplary amino acid substitutions in the heavy chain variable domain.

Amino acid position	SEQ ID No. 1	Substitution
2 (FR1)	Q	S
3 (FR1)	L	V
4 (FR1)	Q	K
7 (FR1)	G	E
8 (FR1)	P	G
11 (FR1)	V	F
14 (FR1)	S	T/A
15 (FR1)	E	D
18 (FR1)	S	T
22 (FR1)	A	T
33 (CDR1)	I	V
34 (CDR1)	N	S/T

42 (FR2)	S	N
45 (FR2)	E	Q
49 (CDR2)	A	T
53 (CDR2)	T	A
56 (CDR2)	A	S
58 (CDR2)	Y	F
60 (CDR2)	S	N
63 (CDR2)	K	R
68 (FR3)	I	V
69 (FR3)	S	T
71 (FR3)	D	N/T
73 (FR3)	S	N
74 (FR3)	K	L/V/E
76 (FR3)	Q	T
78 (FR3)	S	T
80 (FR3)	K	R/T
82 (FR3)	S	T
84 (FR3)	V	L
91 (FR3)	V	T
99 (CDR3)	L	V/E
115 (FR4)	M	L

Table 2. Exemplary amino acid substitutions in the light chain variable domain.

Amino acid position	SEQ ID No. 2	Substitution
3 (FR1)	Q	D
7 (FR1)	S	T
9 (FR1)	S	A
11 (FR1)	L	V
13 (FR1)	A	E
14 (FR1)	S	P
17 (FR1)	D	G
18 (FR1)	R	T
28 (CDR1)	S	N
31 (CDR1)	S	T
32 (CDR1)	S	G

42 (FR2)	K	Q
50(CDR2)	S	Q
59 (FR3)	P	S
63 (FR3)	S	K
70 (FR3)	D	E
71 (FR3)	F	E
74 (FR3)	T	A
77 (FR3)	S	D/G
78 (FR3)	L	V
79 (FR3)	Q	E/A
80 (FR3)	P	C
81 (FR3)	E	A
83 (FR3)	F	A
85 (FR3)	T	S
93 (CDR3)	G	A
95 (CDR3)	S	N
105 (FR4)	T	S
106(FR4)	K	E
108 (FR4)	E	V
109 (FR4)	I	V

These exemplary amino acid substitutions are also shown in Fig. 1. An antibody having any of these substitutions should neutralize TNF α activity as antibodies with all of these substitutions have been shown to neutralize TNF α activity. TNF α -neutralizing antibodies containing amino acids at these positions are disclosed in US patent application publication no. 20060216293, which is herein incorporated by reference. In certain embodiments, subject antibody may be a humanized version of the antibodies disclosed in US patent application no. 20060216293.

The amino acid substitutions may be in both the frame work regions and the CDRs, or solely in the frame work regions or the CDRs. Thus, in certain embodiments the frame work regions of the heavy chain variable domain may collectively differ in amino acid sequence from SEQ ID NO. 1 by up to about 6 amino acid substitutions, e.g., 1, 2, 3, 4, 5 or 6 substitutions, and the frame work regions of the light chain variable

domain may collectively differ in amino acid sequence from SEQ ID NO. 2 by up to about 6 amino acid substitutions, e.g., 1, 2, 3, 4, 5 or 6 substitutions.

In some antibodies, the amino acid substitutions may all be in the CDRs. Thus the CDRs of the heavy chain variable domain may collectively differ in amino acid sequence from SEQ ID NO. 1 by up to about 6 amino acid substitutions, e.g., 1, 2, 3, 4, 5 or 6 substitutions, and the CDRs of the light chain variable domain may collectively differ in amino acid sequence from SEQ ID NO. 2 by up to about 6 amino acid substitutions, e.g., 1, 2, 3, 4, 5 or 6 substitutions.

In particular embodiments, the antibody may comprise a) a heavy chain variable domain comprising an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO. 1 and b) a light chain variable domain comprising an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO. 2.

In particular embodiments, the antibody may comprise a) a heavy chain variable domain that is at least about 95% identical in amino acid sequence to SEQ ID NO. 1 and b) a light chain variable domain that is at least about 95% identical in amino acid sequence to SEQ ID NO. 2. Thus, the subject antibody might have a) a heavy chain variable domain that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical in amino acid sequence to SEQ ID NO. 1 and b) a light chain variable domain that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical in amino acid sequence to SEQ ID NO. 2.

In addition to the amino acid substitutions described above, a subject antibody may have additional amino acids at either end of the heavy and light chains. For example, a subject antibody may contain at least 1, 2, 3, 4, 5, or 6 or more at the C or N-terminal end of the heavy and/or light chains, independently. In certain cases, a subject antibody may be shorter than the exemplary antibody described herein, by 1, 2, 3, 4, 5, or 6 amino acids at either end of the heavy and light chains, independently.

The subject antibody may be humanized. In general, humanized antibodies are made by substituting amino acids in the framework regions of a parent antibody to produce a modified antibody that may be less immunogenic in a human than the parent antibody. Antibodies can be humanized using a variety of techniques known in the art

including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska. et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332). In certain embodiments, framework substitutions are identified by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions (see, e.g., U.S. Pat. No. 5,585,089; Riechmann et al., *Nature* 332:323,1988). Antibodies can be humanized in accordance to methods described set forth in great detail in U.S. patent application 10/984,473, filed on November 8, 2004 and entitled "Methods for antibody engineering", which application is incorporated by reference in its entirety. In general, this humanization method involves identifying a substitutable position of an antibody by comparing sequences of antibodies that bind to the same antigen, and replacing the amino acid at that position with a different amino acid that is present at the same position of a similar human antibody. In these methods, the amino acid sequence of a parental antibody is compared to (i.e., aligned with) the amino acid sequences of other related antibodies to identify variation tolerant positions. The amino acid sequence of the variable domain of the parental antibody is usually compared to a database of human antibody sequences, and a human antibody that has an amino acid sequence that is similar to that of the parental antibody is selected. The amino acid sequences of the parental antibody and the human antibody are compared (e.g., aligned), and amino acids at one or more of the variation tolerant positions of the parental antibody are substituted by correspondingly positioned amino acids in the human antibody.

The above-discussed variation tolerant position substitution methods are readily incorporated into any known humanization method and are also readily employed to produce humanized antibodies containing CDR regions that are altered with respect to the CDR regions of the parent antibody. Accordingly humanized TNF α neutralizing antibodies containing altered versions of the CDRs of the parent antibodies are provided.

ANTIBODIES THAT NEUTRALIZE TNF α ACTIVITY

In certain embodiments, a subject antibody may be employed to bind TNF α and neutralize its activity. A TNF α neutralizing antibody inhibits at least one activity of TNF α in the range of about 20% to 100%, e.g., by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, usually up to about 70%, up to about 80%, up to about 90% or more. In any of these assays, a subject antibody inhibits TNF α activity with an IC₅₀ of 1 x 10⁻⁷ M or less (e.g., 1 x 10⁻⁷ M or less, 1 x 10⁻⁸ M or less, 1 x 10⁻⁹ M or less, usually to 1 x 10⁻¹² M or 1 x 10⁻¹³ M). In assays in which a mouse is employed, a subject antibody typically has an ED₅₀ of less than 1 μ g/mouse (e.g., 10 ng/mouse to 1 μ g/mouse).

TNF α activity can be assayed in a variety of ways, including, but not limited to: assays for TNF α -induced cytotoxicity (either in vitro or in vivo) using suitable cells, e.g., L929 cells; assays for binding of TNF α to its receptor using suitable cells, e.g., U-937 cells; assays for inhibition of endothelial cell leukocyte adhesion molecule 1 (ELAM-1) expression on human umbilical vein endothelial (HEVEC) cells; or in vivo assays using D-galactosamine sensitized mice. Such assays are described in great detail in U.S. 6,090,382, which is incorporated by reference herein for that purpose.

Certain embodiments of the subject antibody have the following general characteristics:

- a) high affinity for TNF α (e.g., a K_d of 10⁻⁸ or less);
- b) slow off rate for dissociation with TNF α (e.g., a K_{off} of 10⁻³ sec⁻¹ or less); and
- c) TNF α neutralizing activity.

Methods for measuring binding affinity, off rate and other antibody binding kinetics are well known in the art, and may be employed to determine whether an antibody has a high affinity and a slow off rate for TNF α . In many methods and as is well known in the art, antibody binding kinetics may be measured by ELISA methods or by measuring surface plasmon resonance using, for example, a BIACORETM biosensor sold by Pharmacia (now Pfizer). Methods for measuring binding of antigens to antibodies using surface plasmon resonance are well known in the art (see, e.g., Methods of Dev. Biol. 2003 112:141-51 and J. Mol. Recognit. 1999 12:310-5) and are readily adapted for use herein.

The antibody may be a full-length natural antibody or any chimera thereof, for example. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison et al (Science 1985 229:1202); Oi et al (BioTechniques 1986 4:214); Gillies et al. (J. Immunol. Methods 1989 125:191-202) and U.S. Pat. Nos. 5,807,715, 4,816,567 and 4,816,397, which are incorporated herein by reference in their entirety. In certain embodiments, the subject antibody may be monoclonal, monovalent, bivalent, or single chain antibody.

METHODS OF USING ANTIBODIES TO INHIBIT TNF α ACTIVITY

Subject antibody may be employed in a method of inhibiting TNF α activity. The subject antibody may be employed in a variety of protocols described below.

The protocols that may be employed in these methods are numerous, and include but are not limited to cell-free assays, e.g., binding assays to a TNF α receptor; cellular assays in which a cellular phenotype is measured, e.g., gene expression or cytotoxicity; and in vivo assays that involve a particular animal (which, in certain embodiments may be an animal model for a TNF α -related condition).

Such assays, including those described above, are well known in the art and are described in a variety of publications, including 20040151722, 20050037008, 20040185047, 20040138427, 20030187231, 20040002589, 20030199679, 6,090,382 and Balazovich (Blood 1996 88: 690-696).

METHODS FOR PRODUCING ANTIBODIES

In many embodiments, the nucleic acids encoding a subject antibody are introduced directly into a host cell, and the cell incubated under conditions sufficient to induce expression of the encoded antibody.

Any cell suitable for expression of expression cassettes may be used as a host cell. For example, yeast, insect, plant, etc., cells. In many embodiments, a mammalian host cell line that does not ordinarily produce antibodies is used, examples of which are as follows: monkey kidney cells (COS cells), monkey kidney CVI cells transformed by SV40 (COS-7, ATCC CRL 165 1); human embryonic kidney cells (HEK-293, Graham et al. J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10);

chinese hamster ovary-cells (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. (USA) 77:4216, (1980); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CVI ATCC CCL 70); african green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL 51); TRI cells (Mather et al., Annals N. Y. Acad. Sci 383:44-68 (1982)); NIH/3T3 cells (ATCC CRL-1658); and mouse L cells (ATCC CCL-1). Additional cell lines will become apparent to those of ordinary skill in the art. A wide variety of cell lines are available from the American Type Culture Collection, 10801 University Boulevard, Manassas, Va. 20110-2209.

Methods of introducing nucleic acids into cells are well known in the art. Suitable methods include electroporation, particle gun technology, calcium phosphate precipitation, direct microinjection, and the like. The choice of method is generally dependent on the type of cell being transformed and the circumstances under which the transformation is taking place (i.e. in vitro, ex vivo, or in vivo). A general discussion of these methods can be found in Ausubel, et al, Short Protocols in Molecular Biology, 3rd ed., Wiley & Sons, 1995. In some embodiments lipofectamine and calcium mediated gene transfer technologies are used.

After the subject nucleic acids have been introduced into a cell, the cell is typically incubated, normally at 37°C, sometimes under selection, for a period of about 1-24 hours in order to allow for the expression of the antibody. In most embodiments, the antibody is typically secreted into the supernatant of the media in which the cell is growing in.

In mammalian host cells, a number of viral-based expression systems may be utilized to express a subject antibody. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of

expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

5 For long-term, high-yield production of recombinant antibodies, stable expression may be used. For example, cell lines, which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with immunoglobulin expression cassettes and a selectable marker. Following the introduction of the foreign DNA, engineered cells may
10 be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into a chromosome and grow to form foci which in turn can be cloned and expanded into cell lines. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that
15 interact directly or indirectly with the antibody molecule.

 Once an antibody molecule of the invention has been produced, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation,
20 differential solubility, or by any other standard technique for the purification of proteins. In many embodiments, antibodies are secreted from the cell into culture medium and harvested from the culture medium.

ANTIBODY CONJUGATES

25 In certain embodiments, subject antibody may be conjugated to an agent. Any agent would be suitable so long as its conjugation to the antibody does not substantially reduce a desired function and/or characteristic of the antibody. For example, in some embodiments, an immunoconjugate comprises an agent which is a cytotoxic agent. In some embodiments, said cytotoxic agent is selected from the group consisting of a
30 radioactive isotope, a chemotherapeutic agent and a toxin. In some embodiments, said toxin is selected from the group consisting of doxorubicin, methotrexate, maytansine,

ricin, diphtheria toxin and trichothene. The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) *Anticancer Research* 19:605-614; Niculescu-Duvaz and Springer (1997) *Adv. Drg Del. Rev.* 26:151-172; U.S. Pat. No. 4,975,278) theoretically allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin et al., (1986) *Lancet* pp. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in *Monoclonal Antibodies 84: Biological And Clinical Applications*, A. Pinchera et al. (ed.s), pp. 475-506). For selective destruction of the tumor, the antibody may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At.sup.211, I.sup.131, I.sup.125, Y.sup.90, Re.sup.186, Re.sup.188, Sm.sup.153, Bi.sup.212, P.sup.32, Pb.sup.212 and radioactive isotopes of Lu. When the conjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc.sup.99m or I.sup.123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

The radio- or other labels may be incorporated in the conjugate in using a number of methods known in the art. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as tc.sup.99m or I.sup.123, Re.sup.186, Re.sup.188 and In.sup.111 can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker et al (1978) *Biochem. Biophys. Res. Commun.* 80: 49-57 can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-

pyridyldithio)propionate (SPDP), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Pat. No. 5,208,020) may be used.

In some embodiments, an immunoconjugate comprises an agent which is a detectable marker. In some embodiments, said detectable marker is selected from the group consisting of a radioactive isotope, a member of a ligand-receptor pair, a member of an enzyme-substrate pair and a member of a fluorescence resonance energy transfer pair.

FORMULATIONS AND ADMINISTRATION

The antibodies of the invention may be administered in any manner which is medically acceptable. This may include injections, by parenteral routes such as intravenous, intravascular, intraarterial, subcutaneous, intramuscular, intratumor, intraperitoneal, intraventricular, intraepidural, or others as well as oral, nasal, ophthalmic, rectal, or topical. Sustained release administration is also specifically included in the invention, by such means as depot injections or erodible implants. Localized delivery is particularly contemplated, by such means as delivery via a catheter to one or more arteries, such as the renal artery or a vessel supplying a localized tumor.

The subject antibodies may be a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" means one or more organic or inorganic ingredients, natural or synthetic, with which the antibody is combined to facilitate its

application. A suitable carrier includes sterile saline although other aqueous and non-aqueous isotonic sterile solutions and sterile suspensions known to be pharmaceutically acceptable are known to those of ordinary skill in the art. An "effective amount" refers to that amount which is capable of ameliorating or delaying progression of the diseased,
5 degenerative or damaged condition. An effective amount can be determined on an individual basis and will be based, in part, on consideration of the symptoms to be treated and results sought. An effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

In one embodiment a subject antibody is administered to a patient by intravenous,
10 intramuscular or subcutaneous injection. An antibody may be administered within a dose range between about 0.1 mg/kg to about 100 mg/kg; between about 1 mg/kg to 75 mg/kg; or about 10 mg/kg to 50 mg/kg. The antibody may be administered, for example, by bolus injection or by slow infusion. Slow infusion over a period of 30 minutes to 2 hours may be used.

UTILITY

The subject antibodies are useful for treating a TNF α -mediated disorder. In one embodiment, the invention provides a method of treating a subject for a TNF α -related condition. The method generally involves administering a subject antibody a subject
20 having a TNF α -related disorder in an amount effective to treat at least one symptom of the TNF α -related disorder.

The term "TNF α -mediated disorder" refers to any disorder or disease state in which TNF- α plays a direct role, e.g., by excessive production or release of TNF α itself or by TNF α -induced production or release of another agent that produces a pathological
25 effect. As such, the subject methods are useful for treating any fibrotic disorder, including obliterative bronchiolitis, interstitial lung disease, fibrotic lung disease (e.g., idiopathic pulmonary fibrosis (IPF), pulmonary fibrosis of a known etiology, cystic fibrosis, adult respiratory distress, syndrome (ARDS), tumor stroma in lung disease, systemic sclerosis, Hermansky-Pudlak syndrome (HPS), coal worker's pneumoconiosis
30 (CWP), asbestosis, sarcoidosis, silicosis, black lung disease, chronic pulmonary hypertension, AIDS associated pulmonary hypertension, and the like), human kidney

disease (e.g., nephrotic syndrome, Alport's syndrome, HIV-associated nephropathy, polycystic kidney disease, Fabry's disease, diabetic nephropathy, and the like), glomerular nephritis, nephritis associated with systemic lupus erythematosus, fibrotic vascular disease, arterial sclerosis, atherosclerosis, varicose veins, coronary infarcts, cerebral infarcts, musculoskeletal fibrosis, post-surgical adhesions, cutis keloid formation, progressive systemic sclerosis, primary sclerosing cholangitis (PSC), renal fibrosis, scleroderma (local and systemic), diabetic retinopathy, glaucoma, Peyronie's disease, penis fibrosis, arterostenosis after test using cystoscope, inner accretion after surgery, myelofibrosis, idiopathic retroperitoneal fibrosis, fibrosis incident to microbial infection (e.g. viral, bacterial, fungal, parasitic, etc.), fibrosis incident to inflammatory bowel disease (including stricture formation in Crohn's disease and microscopic colitis), fibrosis induced by chemical or environmental insult (e.g., cancer chemotherapy, pesticides, radiation (e.g. cancer radiotherapy), and the like), peritoneal fibrosis, liver fibrosis, myocardial fibrosis, pulmonary fibrosis, Grave's ophthalmopathy, drug induced ergotism, cardiovascular disease, fibrosis incident to benign or malignant cancer (including desmoid tumor), Alzheimer's disease, scarring, scleroderma, glioblastoma in Li-Fraumeni syndrome, sporadic glioblastoma, myeloid leukemia, acute myelogenous leukemia, myelodysplastic syndrome, myeloproliferative syndrome, fibrosis incident to benign or malignant gynecological cancer (e.g., ovarian cancer, Lynch syndrome, and the like), Kaposi's sarcoma, Hansen's disease, inflammatory bowel disease (including stricture formation in Crohn's disease and microscopic colitis), Crohn's disease, ulcerative colitis, multiple sclerosis, Type II diabetes, rheumatoid arthritis, asthma, chronic bronchitis, atopic dermatitis, urticaria, allergic rhinitis, allergic conjunctivitis, chronic obstructive pulmonary disease, graft rejection, graft-versus-host disease, sepsis, and the like.

Some of these disorders are described in greater detail below.

CNS disorders Evidence exists in the literature that TNF α has effects on cells of the central nervous systems (CNS). Evidence for CNS production of TNF α , involvement of TNF α in brain injury, the role of polymorphonuclear leukocytes (PMNs) in brain injury, the role of adhesion molecules in brain injury, and potential TNF α directed

therapeutic strategies for prevention of brain injury have been reviewed in the literature. See, e.g., Babak Arvin et al. (1995) Ann. N.Y. Acad. Sciences 765: 62-71.

The prevention of brain edema by anti-TNF α antibodies in experimental meningitis provides firm evidence for the involvement of TNF α in the breakdown of the
5 Blood Brain Barrier. TNF α can also trigger the infiltration of neutrophils into the tissue with consequent induction of secondary mediators in local areas. See, e.g., "Cytokines and CNS," Edit: R. M. Ransohoff and E. N. Benevise, CRC Press, Page 193, 1996).

Closed head injury (CHI) in rats triggers the production of TNF α in the contused brain hemisphere, and it was shown that a decrease in TNF α levels or inhibition of its
10 activity is accompanied by significantly reduced brain damage. Shohami et al. (1996) J. Cerebral Blood Flow Metab., 16:378-384.

Multiple Sclerosis Multiple sclerosis (MS) plaques within the CNS are infiltrated by peripheral blood mononuclear cells. In patients, TNF α , but not lymphotoxin, is overproduced by peripheral blood mononuclear cells during MS relapse. Glabinski et al.
15 (1995) Neurol Scand. 91:276-279. TNF α has an ability to cause cell death of oligodendrocytes *in vitro*. Robbins et al. (1987) J. Immunol., 139:2593. This aspect of TNF α activity may contribute directly to myelin damage and/or the demyelination process observed in diseases such as multiple sclerosis (MS). TNF α has been shown to play a central role in the demyelination of the CNS in MS. Serum levels of TNF α are
20 elevated in patients with active MS, and TNF α producing macrophages, microglia and astrocytes are present at active lesion sites. In *in vitro* experiments, TNF α directly mediates oligodendrocyte damage and suppresses myelin formation, and it stimulates astrocytes, which are then responsible for the CNS scarring plaques in MS (Owens and Sriram, Neurological Clinics, 13:51, 1995).

Serum levels of TNF α are elevated in patients with active MS (M. Chofflon et al., Eur. Cytokine Net., 3:523, 1991; Sharief, M. K. and Hentgen, N. E. Jour. Med., 325:467, 1991). TNF α producing macrophages/microglia and astrocytes are present at active lesion sites (K. Selmaj et al., Jour. Clin. Invest., 87:949, 1991). In *in vitro* experiments, TNF α directly mediates oligodendrocyte damage and suppresses myelin formation (K. Selmaj et al., J. Immunol., 147:1522, 1990); T. Tsumamoto et al., Acta Neurol. Scand., 91:71,
30

1995), and it stimulates astrocytes, which are responsible for the scarring plaques (K. Selmaj et al., J. Immunol., 144:129, 1990).

An increase in TNF α expression preceding MS exacerbation attacks has been shown. ("Cytokines and the CNS," Edit: R. M. Ransohoff and E. N. Benveniste, CRC Press, 1996, p.232). *In vivo* studies of murine, rat and human demyelinating diseases indicate that TNF α participates in the inflammatory reactions that take place within the CNS. TNF α positive astrocytes and macrophages have been identified in the brains of MS patients, particularly in the plaque region (F. M. Hofman et al., J. Exp. Med., 170:607, 1991, and Selmaj et al., J. Clin. Invest., 87:949, 1991) have determined that both TNF α and TNF- β are present in MS plaque regions, and that TNF α is localized within astrocytes, whereas TNF α is associated with microglia and T-cells. Increased serum and cerebrospinal fluid levels of TNF α have been documented in patients with MS (Sharief, M. K., M. Phil, and R. Hentges, N. Engl. J. Med., 325:467, 1991), and a strong correlation exists between cerebrospinal fluid levels of TNF α , disruption of the blood brain barrier, and high levels of circulating ICAM-1 in patients with active MS.

Alzheimer's Disease Alzheimer's disease (AD), the most common dementing disorder of late life, is a major cause of disability and death in the elderly. The disease is manifested by the appearance of abnormalities in the brain, particularly involving the hippocampus, amygdala, thalamus and neocortex. Lesions in these regions are associated with dysfunction/death of neurons and deafferentation of targets. The principal pathological hallmarks of AD are deposits of the amyloid- β protein (A β) in extracellular parenchyma and cerebral vessels, and neurofibrillary tangles.

TNF α has been generally elevated in the serum of AD patients based upon both antibody assays and bioassays. In one study almost half of the AD cases had elevated TNF α , but none of the controls had a similar elevation. The blood-brain barrier does not normally permit passage of cytokines. However, there is evidence to suggest that the blood-brain barrier may not be intact in AD.

Respiratory disorders TNF α has been shown to play a role in pulmonary fibrosis induced by bleomycin and silica (Piguet et al., Jour. Exper. Med., 170:655-663, 1989, and Nature, 344:245-247, 1990; Everson and Chandler, Amer. Jour. Path., 140:503-512, 1992; Phan and Kunkel, Exp. Lung Res. 18:29-43, 1992; also, Warren et al., Jour. Clin.

Invest., 84:1873-1882, 1989; Denis et al., Amer. Jour. Cell Mol. Biol., 5:477-483, 1991). TNF α has been reported to orchestrate its proinflammatory effects by regulating the compartmentalized release of secondary messenger cytokines. Investigations have shown that nude mice exposed to chronic *in vivo* TNF α develop pulmonary inflammation and
5 fibrosis (ARRD 145:A307, 1992).

Asthma It has been reported that levels of TNF α are increased in bronchoalveolar lavage (BAL) fluid from patients with allergic asthma. Cirelli, et al. (1995) Amer. Jour. Resp. Critical Care Med., 151:345A; Redington et al., (1995) Amer. Jour. Respir. Crit. Care Med., 151: 702A. These findings indicate an increased tissue level of TNF α in
10 asthma and that this may contribute to the pathophysiology of the condition.

Chronic Obstructive Pulmonary Disease (COPD) Another disease state in which TNF α plays a role in the pathophysiology is chronic obstructive pulmonary disease. In silicosis, a disease of progressive respiratory failure caused by a fibrotic reaction, antibody to TNF α completely blocked the silica-induced lung fibrosis in mice (Piguet et
15 al., Nature, 344:245-247, 1990). High levels of TNF α production (in the serum and in isolated macrophages) have been demonstrated in animal models of silica and asbestos induced fibrosis (Bissonnette et al., Inflammation, 13:329-339, 1989).

Adult Respiratory Distress Syndrome (ARDS) Excessive TNF α concentrations, in excess of 12,000 pg/ml, have been detected in pulmonary aspirates from ARDS patients
20 (Millar et al., Lancet, 2(8665):712-714, 1989). Systemic infusion of recombinant TNF α was shown to result in changes typically seen in ARDS (Ferrai-Baliviera et al., Arch. Surg., 124:1400-1405, 1989).

Lung Sarcoidosis Alveolar macrophages from pulmonary sarcoidosis patients have been found to spontaneously release massive quantities of TNF α as compared with
25 macrophages from normal donors (Baughman et al., Jour. Lab. Clin. Med., 115:36-42, 1990). TNF- α also implicated in other acute disease states such as the pathophysiologic responses which follows subsequent reperfusion. It is involved in reperfusion injury, and is a major cause of tissue damage after loss of blood flow. (Vedder et al., Proc. Nat. Acad. Sci., 87:2643-2646, 1990).

Sepsis Overproduction of TNF- α has been implicated in the pathogenesis of
30 endotoxin induced septic shock, (see Carswell et al., Proc. Nat. Acad. Sci., 2:3666-3670,

1975). Endotoxin is the lipopolysaccharide component of the cell wall of gram-negative bacteria, and is a macrophage activator which induces the synthesis and enhanced secretion of TNF- α and other biologically active cytokine molecules. TNF- α is recognized as a central mediator of sepsis, septic shock and multiple organ failure. These host reactions are associated with increased blood levels of TNF- α , due to increased TNF- α production. (F. Stuber et al., Jour. Inflam., 46:42-50, 1996).

Liver disorders Because of its central role in metabolism and host defense mechanisms, the liver is thought to be major organ responsible for initiation of the multiple organ failure during sepsis. The depression in hepatocellular function in early, hyperdynamic stages of sepsis does not appear to be due to any reduction in hepatic perfusion, but is associated with elevated levels of circulating cytokines such as TNF- α . Furthermore, administration of recombinant TNF- α at doses that do not reduce cardiac output or hepatic perfusion, produces hepatocellular dysfunction. (P. Wang et al., Amer. Jour. Physiol., 270:5, 1996).

The role of TNF- α in induction of hepatic apoptosis under transcriptional arrest, activation of the 55 kDa receptor in the induction of hepatic apoptosis, the glycosylation step in TNF-induced hepatic apoptosis, hepatic injury induction by T cell-initiated cytokine release, and T cell-dependent TNF-mediated liver injury without transcriptional arrest has been reported. (A. Wendel et al., Cell. Biol. Mol. Basis Liver Transp., Int., Ringberg Conf. Hepatic Transp., 2nd, 1995, Pages 105-111.).

Diabetes TNF- α plays a central role in the state of insulin resistance associated with obesity. It has been previously shown that one important mechanism by which TNF- α interferes with insulin signaling is through serine phosphorylation of insulin receptor substrate-1 (IRS-1), which can function as an inhibitor of the tyrosine kinase activity of the insulin receptor (IR). The data strongly suggest that TNF- α inhibits signaling via a stimulation of p55 TNFR, and sphingomyelinase activity, which results in the production of an inhibitory form of IRS-1 (Peraldi et al., J. Biol. Chem. 271:13018-13022, 1996).

Crohn's disease TNF- α levels are elevated in Crohn's disease. In one study, TNF- α concentration was measured in stool samples from normal children, infants with diarrhea, and children with inflammatory bowel disease in active and inactive phases. Compared with diarrhea controls, stool TNF- α concentrations were significantly

increased in children with active Crohn's disease. In patients with inactive Crohn's disease, either as a result of surgery, or treatment with steroids, the concentration of stool TNF- α fell to the level of the controls (C. P. Braegger et al., Lancet, 339:89-91, 1992).

Pre-Eclampsia Pre-eclampsia is an endothelial disorder, and TNF- α has

5 fundamental effects on endothelial cells by several means, including alteration of the balance between oxidant and anti-oxidant, changing the pattern of prostaglandin production, and affecting the expression of several cell surface components. In patients, results show that TNF- α mRNA expression is significantly elevated in preeclamptic patients compared to the control groups. These observations are consistent with a major
10 role for TNF- α in the development of eclampsia (G. Chen et al., Clin. Exp. Immunol. 104:154-159, 1996).

Dermal Burns The protein catabolic rate and TNF- α content of the soleus muscle of the scalded region and remote region were dynamically determined in the first week after the rats were inflicted with 37% TBSA full thickness scalding. The TNF- α content
15 of skeletal muscles was far greater in the scalded region than in the remote region. TNF- α increase was also significantly correlated to the protein catabolic rate of the skeletal muscles (Li et al., Jour. Med. Coll., PLA 10:262-267, 1995; C.A. 125:938, 1245:8156a, 1996).

Bone Resorption TNF- α is increased in bone resorption diseases, including
20 arthritis, wherein it has been determined that when activated, leukocytes will produce a bone reabsorbing activity. Data indicate that TNF- α enhances this activity (Bertolini et al., Nature, 319:516-518, 1986, and Johnson et al., Endocrinology, 124:1424-1427, 1989). TNF- α stimulates bone resorption and inhibits bone formation in vitro and in vivo through stimulation of osteoclast formation and activation combined with inhibition of
25 osteoblast function. TNF- α may be involved in many bone resorption diseases, including arthritis.

Rheumatoid Arthritis Analysis of cytokine mRNA and protein in human
rheumatoid arthritis tissue revealed that many proinflammatory cytokines such as TNF- α
are abundant in all patients regardless of therapy. In rheumatoid joint cell cultures that
30 spontaneously produce IL1, TNF- α was the major dominant regulator of IL1. Subsequently, other proinflammatory cytokines were also inhibited if TNF- α was

neutralized, leading to the concept that the proinflammatory cytokines were linked in a network with TNF- α at its apex. This led to the concept that TNF- α was of major importance in rheumatoid arthritis. This has been successfully tested in animal models of collagen-induced arthritis, and these studies have provided the rationale for clinical trials of anti-TNF- α therapy in patients with long-standing rheumatoid arthritis. Several clinical trials using a chimeric anti-TNF- α antibody have shown marked clinical benefit, verifying the concept that TNF- α is of major importance in rheumatoid arthritis. Re-treatment clinical studies have also shown benefit in repeated relapses, indicating that the disease remains TNF- α dependent (M. Feldmann, Annual Rev. Immunol., 14:397-440, 1996.).

Vascular disorders TNF- α alters the properties of endothelial cells and has various pro-coagulant activities, such as production of an increase in tissue factor procoagulant activity and suppression of the anticoagulant protein C pathway as well as down-regulating the expression of thrombomodulin (Sherry et al., Jour. Cell. Biol., 107:1269-1277, 1988). TNF- α has activities which, together with its early production (during the initial stages of a trauma or injury event), make it a mediator of response to tissue injury in several important disorders including, but not limited to myocardial infarction, stroke and circulatory shock. Of specific importance may be TNF- α induced expression of adhesion molecules, such as intercellular adhesion molecule (ICAM) or endothelial leukocyte adhesion molecule on endothelial cells (Munro et al., Am. Jour. Path., 135:121-132, 1989).

Cardiac disorders Evidence indicates that the current top suspects in heart failure are noradrenaline, angiotensin, vasopressin, endothelin, and tumor-necrosis factor (TNF- α) (N.E. J. Med., 323:236-241, 1990). It has been reported that concentrations of TNF- α , which cause cachexia in chronic inflammatory disorders, infections, cancer and other diseases, are elevated in patients with severe heart failure, especially those with the more severe manifestations of the disease, such as cardiac cachexia.

Graft vs. host disease In graft versus host reactions, increased serum TNF- α levels have been associated with major complications following acute allogeneic bone marrow transplants (Holler et al., Blood, 75:1011-1016, 1990).

An subject antibody modulates, i.e., reduces or increases a symptom of the animal model disease or condition by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%,
5 at least about 80%, at least about 90%, or more, when compared to a control in the absence of the antibody. In general, a subject antibody will cause a subject animal to be more similar to an equivalent animal that is not suffering from the disease or condition. Monoclonal antibodies that have therapeutic value that have been identified using the methods and compositions of the invention are termed "therapeutic" antibodies.

10

KITS

Also provided by the subject invention are kits for practicing the subject methods, as described above. The subject kits at least include one or more of: a subject antibody, a nucleic acid encoding the same, or a cell containing the same. The subject antibody may
15 be humanized. Other optional components of the kit include: buffers, etc., for administering the antibody or for performing a TNF α activity assay. The nucleic acids of the kit may also have restrictions sites, multiple cloning sites, primer sites, etc. to facilitate their ligation to antibody nucleic acids. The various components of the kit may be present in separate containers or certain compatible components may be precombined
20 into a single container, as desired.

In addition to above-mentioned components, the subject kits typically further include instructions for using the components of the kit to practice the subject methods. The instructions for practicing the subject methods are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as
25 paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging) etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, etc. In yet other embodiments, the actual instructions
30 are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a

web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

Also provided by the subject invention are kits including at least a computer readable medium including programming as discussed above and instructions. The instructions may include installation or setup directions. The instructions may include directions for use of the invention with options or combinations of options as described above. In certain embodiments, the instructions include both types of information.

Providing the software and instructions as a kit may serve a number of purposes. The combination may be packaged and purchased as a means for producing antibodies that are less immunogenic in a host than a parent antibody, or nucleotide sequences them.

The instructions are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging), etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g., CD-ROM, diskette, etc, including the same medium on which the program is presented.

EXAMPLES

Male human TNF α transgenic mice (with rheumatoid arthritis, RA), 7-8 weeks old, were purchased from Taconic and male normal mice, 6-7 weeks olds were obtained from the Animal Center, China Medical University. The transgenic mice were divided into three groups, and the mice of each of the three groups were either untreated (5 mice), treated with Humira from Abbot Laboratories (7 mice), or HZD RabMAb (7 mice). The HZD-M RAbMAb was produced by transient expression of cDNAs encoding SEQ ID NOS: 9 and 10 (Fig. 4) in HEK 293-6E cell and purified through a protein A column.

The mice were intraperitoneally injected with Humira (1mg/kg), HZD-M RabMAb (1mg/kg) or PBS (Non treatment and Normal group) 3 times a week (Monday, Wednesday and Friday) for 6 weeks. Body weight and arthritis scores were recorded weekly from one week before treatment to one week after the treatment completed.

Arthritis was evaluated in ankle joints in a blind manner using a semiquantitative arthritis score as described previously.

Results are shown in Figs. 2A, 2B and 3. The score ranges from 0-3: 0 = no arthritis (normal appearance and grip strength); 1 = mild arthritis (joint swelling); 2 = moderated arthritis (severe joint swelling and digit deformation, no grip strength); and 3 = severe arthritis (ankylosis detected on flexion and severely impaired movement).

All mice were sacrificed one week after the treatment completed and ankle joints were removed for histology. The ankle joints were fixed in 10% buffered formalin overnight, decalcified in 30% formic acid for 4 days, and embedded in paraffin. Sections were stained with hematoxylin and eosin, and the histopathologic score was evaluated microscopically in a blinded manner using a scoring system as follows (Douni et al Attenuation of inflammatory polyarthritis in TNF transgenic mice by Diacerein comparative analysis with dexamethasone methotrexate and anti-TNF protocol 2004 *Arthritis Res Ther* 6 (1) R65-R72; Wooley P. H. (1988) Collagen-induced arthritis in the mouse. *Methods Enzymol.* 162 361-373): 0 = no detectable pathology; 1 = hyperplasia of the synovial membrane and presence of polymorphonuclear infiltrates; 2 = pannus and fibrous tissue formation and focal subchondral bone erosion; 3 = articular cartilage destruction and bone erosion; and 4 = extensive articular cartilage destruction and bone erosion; and 4 =extensive articular cartilage destruction and bone erosion.

An additional TNF- α -neutralizing antibody, HZD-M-Nterm, was constructed. This antibody was produced using human leader sequences and contained the variable domains of SEQ ID NOS:1 and 2 except for changes to amino acids at the N-terminus. The heavy chain variable domain of the HZD-M-Nterm antibody contains the amino acid sequence "QVQ" at the N-terminus rather than "QQ" as found in HZD-M. The light chain variable domain of the HZD-M-Nterm antibody contains the amino acid sequence "DIQ" at the N-terminus than "AYQ" as found in HZD-M. In certain cases, an antibody may have variable domains that are identical to those of HZD-M-Nterm.

It is evident from the above discussion that the subject invention provides an important new TNF- α -neutralizing antibody. Accordingly, the present invention represents a significant contribution to the art.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt
5 a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

What is claimed is:

1. An antibody, comprising:
 - a) a heavy chain variable domain comprising an amino acid sequence that is at
5 least 95% identical to the amino acid sequence of SEQ ID NO. 1; and
 - b) a light chain variable domain comprising an amino acid sequence that is at least
95% identical to the amino acid sequence of SEQ ID No. 2,
wherein the antibody binds TNF α .
- 10 2. The antibody of claim 1, wherein said antibody is a monovalent antibody.
3. The antibody of claim 1, wherein said antibody is a bivalent antibody.
4. The antibody of claim 1, wherein said antibody is a single chain antibody.
15
5. The antibody of claim 1, wherein said antibody is a monoclonal antibody.
6. The antibody of claim 1, wherein said antibody comprises a single antigen
binding arm and an Fc region.
20
7. The antibody of claim 1, wherein said antibody is humanized.
8. The antibody of claim 1, wherein the antibody is conjugated to an agent.
- 25 9. The antibody of claim 1, wherein said heavy chain variable domain comprises
CDR regions that are identical to those shown in Fig. 1 and said light chain variable
domain comprises CDR regions that are identical to those shown in Fig. 1.
10. The antibody of claim 1, where said antibody comprises CDRs that are identical
30 to the CDRs of the antibody shown in Fig. 1, except for up to four amino acid amino acid
substitutions.

11. A method of blocking binding of TNF to its receptor comprising:
administering an antibody of claim 1 to a subject, wherein said antibody binds to
TNF in said subject and blocks binding of said receptor to TNF.

5

12. The method of claim 11, wherein said subject is a human.

13. A pharmaceutical composition comprising the antibody of claim 1 and a
pharmaceutically acceptable carrier.

10

VH: <-----FR1----->CDR1 -----FR2-----
QQLQESGPGLVKPFSETLSLTCAVSGFSLSS**RYGIN**WVRQAPGSGLEW
SVK EG F TD T T VS N Q
A T

-> CDR2 <-----FR3-----
IG**AIGETGRAYYASWAKS**RSTISRDTSKNQVSLKMSSVTAADTAVY
T A S F N R VT N NL T T R T L Y
T V T
E

----> CDR3 <----FR4---->
FCAR**GELFNNGWGAFN**LWGPGTMVTVSS
V L
E

VK: <-----FR1-----> CDR1 <-----FR2-----
AYQMTQSPSSLSASVGDRTIK**QASEIYSSLA**WYQQKPGKPKL
D T A V EP GT N TG Q

--> CDR2 <-----FR3----->
LIY**SASTLAS**GVPSRFSGSGGTFTLTISSLQPEDFATY**CCQGF**
Q S K EE A DVECA A S
G A

CDR3 <--FR4-->
GTSNVENPFGGGTKVEIK
A N SE VV

Fig. 1

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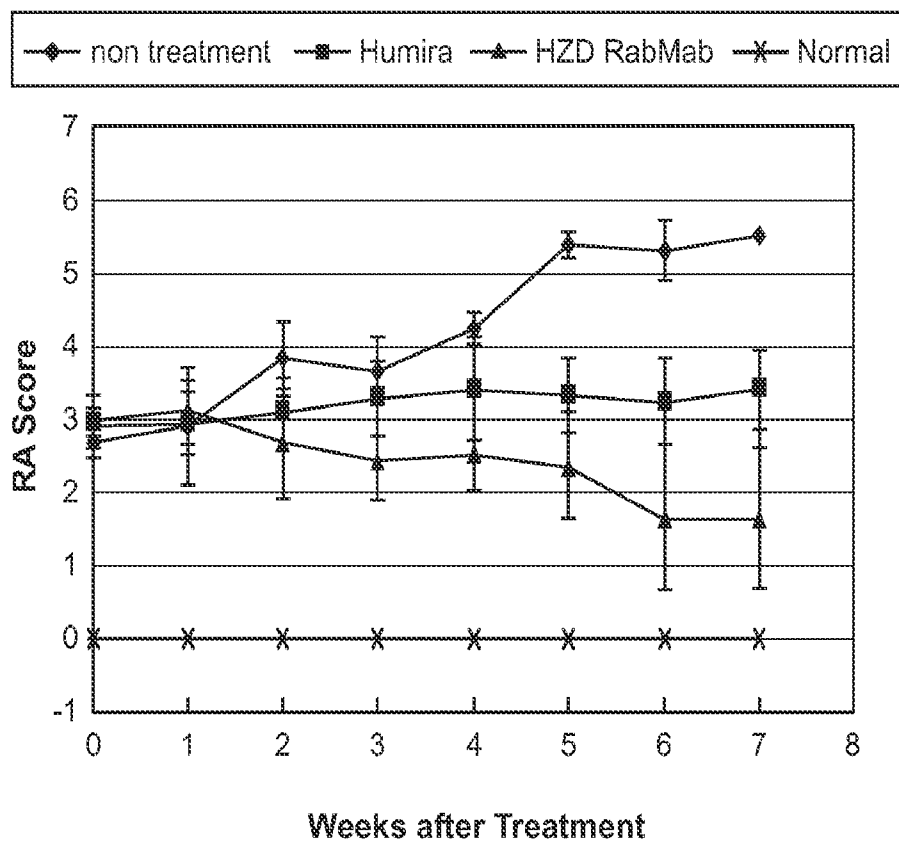


FIG. 2A

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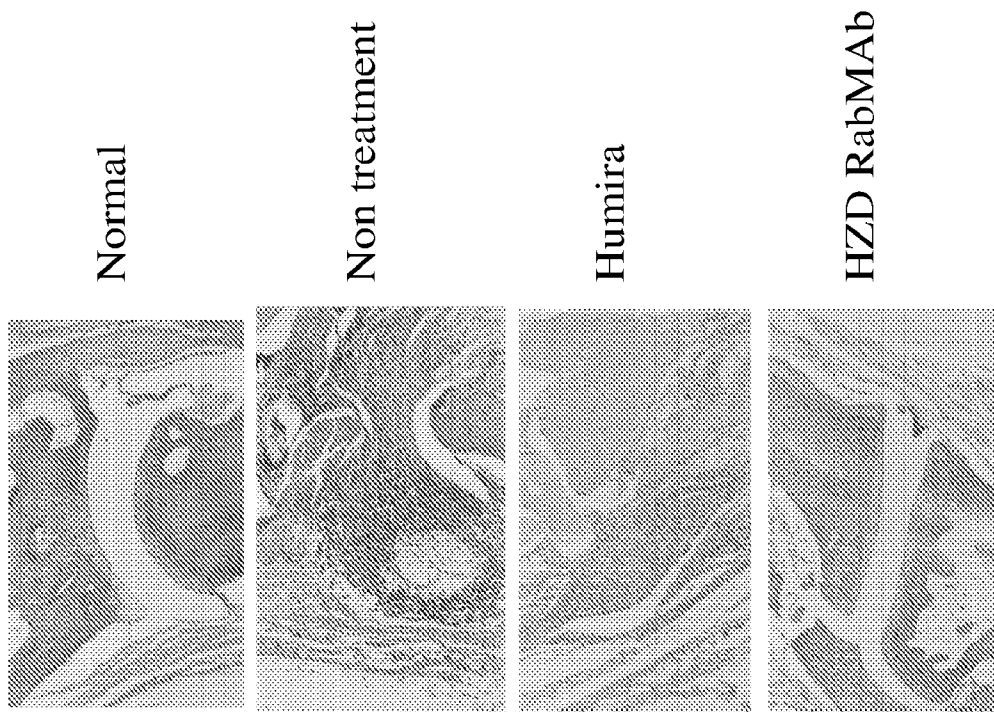


Fig. 2B

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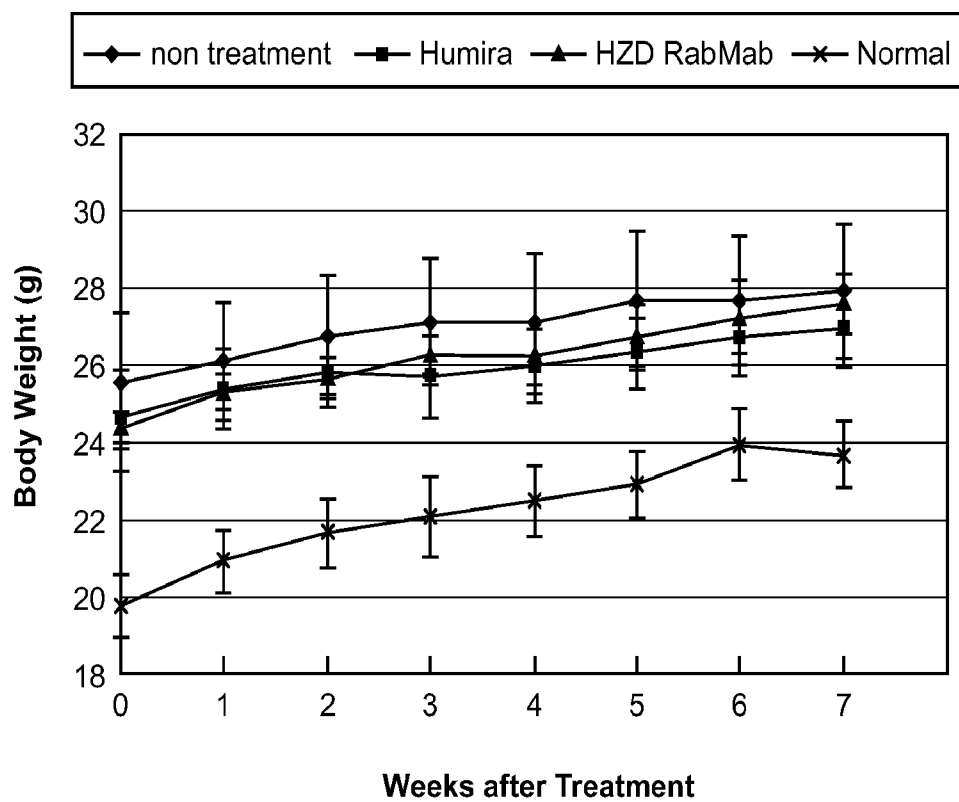


FIG. 3

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HZ3-M, H chain
 METGLRWLLLVAVLKGVCQQQLQESGPGLVKPKSETLSLTCAVSGFSLSRYGINWVRQAPG
 SGLEWIGAIGETGRAYYASWAKSRSTISRDT'SKNQVSLKMSVTAADTAVYFCARGELFN
 NGWAFNIWGP GTMVTVSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
 NSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVKDKVERK
 SCDKTHTCPPCPAPELLGGSVFLEPPPKD TLMISRPEVTCVVVDVSHEDDEVKFNWY
 VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
 AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLI
 DSDGSEFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK

HZ3-M, Kappa L chain
 MDTRAPTQLLGLLILLIWLPGARCA YQMTQSPSSLSASVGDRTVTKCQASESIYSSLAWYQQ
 KPGKPPKLLIYSASTLASGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQGFGTSNVE
 NPFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
 SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Fig. 4

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HZ3-M-Nterm, H chain
Leader sequence
MKHLWFFLLLVAAPRWVLSQVQLQESGPGLVKPSSETISLTCAVSGFSLSRYGINWVRQAPG
SGLEWIGAIGETGRAYYASWAKSRSTISRDTSKNQVSLKMSSVTAADTAVYFCARGELEFN
NGWAFNIWGPGTMTVTVSSASTKGPSVEFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTOTYICNVNHKPSNTKYVDKKEPK
SCDKTHTCPPCPAPELLGGPSVFLFPPPKKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPV
LSLSPGK

HZ3-M-Nterm, Kappa L chain
MDMRVPAQLLGLLLWLRGARCDIQMTQSPSSLSASVGDRTTIKCQASESIYSSLAWYQQ
KPGKPPKLLIYASTLASGVPSRFSGSGSGTDFTLTITSSLPQEDFATYYCQQGFGTSNVE
NPFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/41302

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/00; C07K 16/00; C07K 17/00 (2009.01)

USPC - 530/387.1; 530/389.1; 530/391.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC- 530/387.1; 530/389.1; 530/391.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 435/69.1; 435/325 435/326 435/328 435/331 435/335 435/336 435/358 435/363 435/365 435/366 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Electronic Data Bases: (PBPB, EPAB, JPAB, USPT); Google Scholar: tumor necrosis factor alpha (TNF alpha), anti-TNF, antibody, monoclonal, single chain antibody, humanized antibody, CDR, pharmaceutical composition. GenCore 6.3:SEQ ID NO:1-2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US2006/0216293 A1 (COUTO et al.) 28 Sep 2006 (28.09.2006); SEQ ID NO: 31, SEQ ID NO: 76	1-13

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

3 June 2009 (03.06.2009)

Date of mailing of the international search report

30 JUN 2009

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/41302

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:

a. type of material



a sequence listing



table(s) related to the sequence listing

b. format of material



on paper



in electronic form

c. time of filing/furnishing



contained in the international application as filed



filed together with the international application in electronic form



furnished subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: