



(86) Date de dépôt PCT/PCT Filing Date: 2000/03/17
 (87) Date publication PCT/PCT Publication Date: 2000/09/28
 (45) Date de délivrance/Issue Date: 2010/05/18
 (85) Entrée phase nationale/National Entry: 2001/09/19
 (86) N° demande PCT/PCT Application No.: US 2000/007349
 (87) N° publication PCT/PCT Publication No.: 2000/056771
 (30) Priorité/Priority: 1999/03/19 (US60/125,299)

(51) Cl.Int./Int.Cl. *C07K 16/18* (2006.01),
A61K 39/395 (2006.01), *C07K 16/24* (2006.01),
C12N 15/12 (2006.01), *C12N 5/12* (2006.01)
 (72) Inventeurs/Inventors:
 HO, YEN SEN, US;
 HOLMES, STEPHEN D., GB;
 TAYLOR, ALEXANDER H., US;
 ABDEL-MEGUID, SHERIN S., US
 (73) Propriétaires/Owners:
 SMITHKLINE BEECHAM CORPORATION, US;
 SMITHKLINE BEECHAM PLC, GB
 (74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : ANTAGONISTES D'IL-18 RECOMBINES S'UTILISANT POUR LE TRAITEMENT DE TROUBLES ASSOCIES
 A IL-18
 (54) Title: RECOMBINANT IL-18 ANTAGONISTS USEFUL IN TREATMENT OF IL-18 MEDIATED DISORDERS

(57) **Abrégé/Abstract:**

Chimeric, humanized and other IL-18 mAbs, derived from high affinity neutralizing mAbs, pharmaceutical compositions containing same, methods of treatment and diagnostics are provided.

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁷ : C07K 16/18, A61K 39/395, C12N 5/12, 15/12</p>	<p>A1</p>	<p>(11) International Publication Number: WO 00/56771</p> <p>(43) International Publication Date: 28 September 2000 (28.09.00)</p>
<p>(21) International Application Number: PCT/US00/07349</p> <p>(22) International Filing Date: 17 March 2000 (17.03.00)</p> <p>(30) Priority Data: 60/125,299 19 March 1999 (19.03.99) US</p> <p>(71) Applicants (for all designated States except US): SMITHK-LINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US). SMITHK-LINE BEECHAM PLC [GB/GB]; New Horizons Court, Great West Road, Brentford, Middlesex TW8 9EP (GB).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): HO, Yen, Sen [US/US]; 362 Old State Road, Berwyn, PA 19312 (US). HOLMES, Stephen, D. [GB/GB]; Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD (GB). TAYLOR, Alexander, H. [US/US]; 522 Westfield Drive, Exton, PA 19341 (US). ABDEL-MEGUID, Sherin, S. [US/US]; 236 Autumn Drive, Exton, PA 19341 (US).</p> <p>(74) Agents: KEREKES, Zoltan et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).</p>	<p>(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CZ, EE, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MA, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, TZ, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: RECOMBINANT IL-18 ANTAGONISTS USEFUL IN TREATMENT OF IL-18 MEDIATED DISORDERS</p>		
<p>(57) Abstract</p> <p>Chimeric, humanized and other IL-18 mAbs, derived from high affinity neutralizing mAbs, pharmaceutical compositions containing same, methods of treatment and diagnostics are provided.</p>		

RECOMBINANT IL-18 ANTAGONISTS USEFUL IN TREATMENT OF IL-18
MEDIATED DISORDERS

5 FIELD OF THE INVENTION

The present invention relates generally to the field of antibodies and altered antibodies, useful in the treatment and diagnosis of conditions mediated by IL-18, and more specifically to mAbs, Fabs, chimeric and humanized antibodies.

10 BACKGROUND OF THE INVENTION

Human interleukin-18 is a recently identified cytokine that is synthesized as a biologically inactive 193 amino acid precursor protein (Ushio et al., J. Immunol. 156:4274, 1996). Cleavage of the precursor protein, for example by caspase-1 or caspase-4, liberates the 156 amino acid mature protein (Gu et al., Science 275:206, 15 1997; Ghayur et al., Nature 386:619, 1997), which exhibits biological activities that include the costimulation of T cell proliferation, the enhancement of NK cell cytotoxicity, the induction of IFN- γ production by T cells and NK cells, and the potentiation of T helper type 1 (Th1) differentiation (Okamura et al., Nature 378:88, 1995; Ushio et al., J. Immunol. 156:4274, 1996; Micallef et al., Eur. J. Immunol. 20 26:1647, 1996; Kohno et al., J. Immunol. 158:1541, 1997; Zhang et al., Infect. Immunol. 65:3594, 1997; Robinson et al., Immunity 7:571, 1997). In addition, IL-18 is an efficacious inducer of human monocyte proinflammatory mediators, including IL-8, tumor necrosis factor- α (TNF- α), and prostaglandin E₂ (PGE₂) (Ushio, S. et al., J. Immunol. 156:4274-4279, 1996; and Puren, A.J. et al., J. Clin. Invest. 25 10:711-721, 1997).

The previously cloned IL-1 receptor-related protein (IL-1Rrp) (Parnet et al., J. Biol. Chem. 271:3967, 1996) was recently identified as a subunit of the IL-18 receptor (Kd = 18 nM) (Torigoe et al., J. Biol. Chem. 272:25737, 1997). A second subunit of the IL-18 receptor exhibits homology to the IL-1 receptor accessory protein, and has been termed AcPL (for accessory protein-like). Expression of both 30 IL-1Rrp and AcPL are required for IL-18-induced NF- κ B and JNK activation (Born et al., J. Biol. Chem. 273:29445, 1998). In addition to NF- κ B and JNK, IL-18 signals through IL-1 receptor-associated kinase (IRAK), p56lck (LCK), and mitogen-activated protein kinase (MAPK) (Micallef et al., Eur. J. Immunol. 35 26:1647, 1996; Matsumoto et al., Biophys Biochem. Res. Comm. 234:454, 1997; Tsuji-Takayama et al., Biochem. Biophys. Res. Comm. 237:126, 1997).

Th1 cells, which produce proinflammatory cytokines such as IFN- γ , IL-2 and TNF- β (Mosmann et al., J. Immunol. 136:2348, 1986), have been implicated in mediating many of autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), type 1, or insulin dependent, diabetes (IDDM), inflammatory bowel disease (IBD), and psoriasis (Mosmann and Sad, Immunol. Today 17:138, 1996). Thus, antagonism of a Th1-promoting cytokine such as IL-18 would be expected to inhibit disease development. Il-18 specific mAbs could be used as an antagonist.

The role of IL-18 in the development of autoimmune diseases has been demonstrated. Accordingly, it has been demonstrated that IL-18 expression is significantly increased in the pancreas and spleen of the nonobese diabetic (NOD) mouse immediately prior to the onset of disease (Rothe et al., J. Clin. Invest. 99:469, 1997). Similarly, IL-18 levels have been shown to be markedly elevated in the synovial fluid of rheumatoid arthritis patients (Kawashima et al., Arthritis and Rheumatism 39:598, 1996). Furthermore, it has been demonstrated that IL-18 administration increases the clinical severity of murine experimental allergic encephalomyelitis (EAE), a Th1-mediated autoimmune disease that is a model for multiple sclerosis. In addition, it has been shown that neutralizing anti-rat IL-18 antiserum prevents the development of EAE in female Lewis rats (Wildbaum et al., J. Immunol. 161:6368, 1998). Accordingly, IL-18 is a desirable target for the development of a novel therapeutic for autoimmunity.

Taniguchi et al., J. Immunol. Methods 206:107, describe seven murine and six rat anti-human IL-18 mAbs, which bind to four distinct antigenic sites. One of the murine mAbs (#125-2H), and the six rat mAbs inhibit IL-18-induced IFN- γ production by KG-1 cells, with the rat mAbs exhibiting neutralizing activities 10-fold lower than that of #125-2H. As demonstrated by Western blot analysis, three of the murine mAbs, but none of the rat mAbs, are strongly reactive with membrane-bound human IL-18. In addition, an enzyme-linked immunosorbent assay (ELISA) to detect human IL-18 is described, utilizing #125-2H and a rat mAb. The limit of detection of this ELISA is 10 pg/ml.

European patent application EP 0 712 931 discloses two mouse anti-human IL-18 mAbs, H1 (IgG1) and H2 (IgM). As demonstrated by Western blot analysis, both mAbs react with membrane-bound human IL-18, but not with membrane-bound human IL-12. H1 is utilized in an immunoaffinity chromatography protocol to purify human IL-18, and in an ELISA to measure human IL-18. H2 is utilized in a radioimmunoassay to measure human IL-18.

Neutralizing IL-18 antibodies may potentially be useful in relieving autoimmune diseases and related symptoms in man. Hence there is a need in the art for a high affinity IL-18 antagonist, such as a neutralizing monoclonal antibody to human interleukin 18, which would reduce Th1 differentiation and proliferation and thus autoimmune diseases and related symptoms.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides rodent (e.g., rat and murine) neutralizing monoclonal antibodies specific for human interleukin-18 and having a binding affinity characterized by a dissociation constant equal to or less than about 3.9×10^{-11} M as described in the detailed description. Exemplary of such monoclonal antibodies are the rat monoclonal antibody 2C10 and rat and murine monoclonal antibodies such as 14B7 and 13G9. Another aspect of the invention are hybridomas such as 19522C10(2)F2(1)A1, 195214B7(1)H10 and 187413G9(3)F12

In a related aspect, the present invention provides neutralizing Fab fragments or $F(ab')_2$ fragments thereof specific for human interleukin-18 produced by deleting the Fc region of the rodent neutralizing monoclonal antibodies of the present invention.

In still another related aspect, the present invention provides an altered antibody specific for human interleukin-18 which comprises complementarity determining regions (CDRs) derived from a non-human neutralizing monoclonal antibody (mAb) characterized by a dissociation constant equal to or less than about 3.9×10^{-11} M for human interleukin-18 and nucleic acid molecules encoding the same. When the altered antibody is a humanized antibody, the sequences that encode complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner in which at least one, and preferably all complementarity determining regions (CDRs) of the first immunoglobulin partner are replaced by CDRs from the non-human monoclonal antibody. Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner as well, which comprises all or a part of an immunoglobulin constant chain.

In a related aspect, the present invention provides CDRs derived from non-human neutralizing monoclonal antibodies (mAbs) characterized by a dissociation constant equal to or less than about 3.9×10^{-11} M for human interleukin-18, and nucleic acid molecules encoding such CDRs.

In still another aspect, there is provided a chimeric antibody containing human heavy and light chain constant regions and heavy and light chain variable

regions derived from non-human neutralizing monoclonal antibodies characterized by a dissociation constant equal to or less than about 3.9×10^{-11} M for human interleukin-18.

In yet another aspect, the present invention provides a pharmaceutical composition which contains one (or more) of the above described altered antibodies and a pharmaceutically acceptable carrier.

In a further aspect, the present invention provides a method for treating conditions in humans associated with excess Th1 production, for example autoimmune diseases, by administering to said human an effective amount of the pharmaceutical composition of the invention.

In yet another aspect, the present invention provides methods for, and components useful in, the recombinant production of altered antibodies (e.g., engineered antibodies, CDRs, Fab or F(ab)₂ fragments, or analogs thereof) which are derived from non-human neutralizing monoclonal antibodies (mAbs) characterized by a dissociation constant equal to or less than 3.9×10^{-11} M for human IL-18. These components include isolated nucleic acid sequences encoding same, recombinant plasmids containing the nucleic acid sequences under the control of selected regulatory sequences which are capable of directing the expression thereof in host cells (preferably mammalian) transfected with the recombinant plasmids. The production method involves culturing a transfected host cell line of the present invention under conditions such that an altered antibody, preferably a humanized antibody, is expressed in said cells and isolating the expressed product therefrom.

In yet another aspect of the invention is a method to diagnose conditions associated with excess Th1 production in a human which comprises obtaining a sample of biological fluid from a patient and allowing the antibodies and altered antibodies of the instant invention to come in contact with such sample under conditions such that an IL-18/antibody (monoclonal or altered) complex is formed and detecting the presence or absence of said IL-18/antibody complex.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 [SEQ ID NOS: 1 and 2] illustrates the light chain variable region for the rat antibody 2C10. Fig. 1 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 3-8]. The bolded area indicates the degenerate primer sequence.

Fig. 2. [SEQ ID NOS: 9 and 10] illustrates the heavy chain variable region for the rat antibody 2C10. Fig. 2 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 11-16]. The bolded area indicates the degenerate primer sequence.

5 Fig. 3 [SEQ ID NOS: 17 and 18] illustrates the light chain variable region for the murine antibody 13G9. Fig. 3 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 19-24]. The bolded area indicates the degenerate primer sequence.

10 Fig 4 [SEQ ID NOS: 25 and 26] illustrates the heavy chain variable region for the murine antibody 13G9. Fig. 4 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 27-32]. The bolded area indicates the degenerate primer sequence.

15 Fig 5 [SEQ ID NOS: 33 and 34] illustrates the light chain variable region for the rat antibody 14B7. Fig. 5 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 35-40]. The bolded area indicates the degenerate primer sequence.

20 Fig 6 [SEQ ID NOS: 41 and 42] illustrates the heavy chain variable region for the rat antibody 14B7. Fig. 6 includes sequence data for both strands. The boxed areas indicate the CDR's [SEQ ID NOS: 43-48]. The bolded area indicates the degenerate primer sequence.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a variety of antibodies, altered antibodies and fragments thereof, which are characterized by human IL-18 binding specificity, neutralizing activity, and high affinity for human IL-18 as exemplified in rat monoclonal antibody 2C10, murine monoclonal antibody 13G9 and rat monoclonal antibody 14B7. The antibodies of the present invention were prepared by conventional hybridoma techniques to generate novel neutralizing antibodies. These products are useful in therapeutic and pharmaceutical compositions for treating IL-18-mediated disorders, e.g. autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis (RA), type 1, or insulin dependent, diabetes (IDDM), inflammatory bowel disease (IBD), and psoriasis (Mosmann and Sad, Immunol. Today 17:138, 1996). These products are also useful in the diagnosis of IL-18-mediated conditions by measurement (e.g., enzyme linked immunosorbent assay (ELISA)) of endogenous IL-18 levels in humans or IL-18 released *ex vivo* from activated cells.

I. *Definitions.*

"Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies are engineered antibodies (e.g., chimeric or humanized antibodies) or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, or F(ab)₂ and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding altered antibody of the invention. When the altered antibody is a CDR-grafted or humanized antibody, the sequences that encode the complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Neutralizing" refers to an antibody that inhibits IL-18 activity by preventing the binding of human IL-18 to its specific receptor or by inhibiting the signaling of IL-18 through its receptor, should binding occur. A mAb is neutralizing if it is 90% effective, preferably 95% effective and most preferably 100% effective in inhibiting IL-18 activity as measured in the IL-18 neutralization assay, see Example 1 and Table I.

The term "high affinity" refers to an antibody having a binding affinity characterized by a K_d equal to or less than 3.9×10^{-11} M for human IL-18 as determined by optical biosensor analysis (see Example 2 and Table I).

By "binding specificity for human IL-18" is meant a higher affinity for human IL-18 than murine, or other IL-18.

"Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in

frame or by means of an optional conventional linker sequence (i.e., operatively linked). Preferably it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or F(ab)₂ (i.e., a discrete part of an appropriate human constant region or framework region). Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase, β -galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or F(ab)₂ are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric or humanized antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more)

human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

The term "donor antibody" refers to an antibody (monoclonal, or recombinant) which contributes the nucleic acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody. One donor antibody suitable for use in this invention is a non-human neutralizing monoclonal antibody (i.e., rat) designated as 2C10. The antibody 2C10 is defined as a high affinity, human-IL-18 specific (i.e., does not recognize murine IL-18), neutralizing antibody of isotype IgG_{1,K} having the variable light chain DNA and amino acid sequences of SEQ ID NOs: 1 and 2 and respectively, the variable heavy chain DNA and amino acid sequences of SEQ ID NOs: 9 and 10 on a suitable murine IgG constant region.

The term "acceptor antibody" refers to an antibody (monoclonal, or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but preferably all) of the nucleic acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate).

CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

By sharing the antigen binding specificity or neutralizing ability is meant, for example, that although mAb 2C10 may be characterized by a certain level of antigen

affinity, a CDR encoded by a nucleic acid sequence of 2C10 in an appropriate structural environment may have a lower, or higher affinity. It is expected that CDRs of 2C10 in such environments will nevertheless recognize the same epitope(s) as 2C10. Exemplary light chain CDRs of 2C10 include

5 SEQ ID NO: 3;

SEQ ID NO: 5;

SEQ ID NO: 7;

and exemplary heavy chain CDRs of 2C10 include

SEQ ID NO: 11;

10 SEQ ID NO: 13;

and SEQ ID NO: 15.

A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or
15 neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity and high
20 affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions.

Analogous may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence encoding the amino acid
25 or peptide sequences of the invention. Such variations or modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein carrier molecules to which
30 the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore [Pharmacia] system, or other non-protein substances
35 useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or

radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

II. High Affinity IL-18 Monoclonal Antibodies

5 For use in constructing the antibodies, altered antibodies and fragments of this invention, a non-human species (for example, bovine, ovine, monkey, chicken, rodent (e.g., murine and rat), etc.) may be employed to generate a desirable immunoglobulin upon presentment with native human IL-18 or a peptide epitope therefrom. Conventional hybridoma techniques are employed to provide a
10 hybridoma cell line secreting a non-human mAb to IL-18. Such hybridomas are then screened for binding using IL-18 coated to 96-well plates, as described in the Examples section, or alternatively with biotinylated IL-18 bound to a streptavidin coated plate.

One exemplary, high affinity, neutralizing mAb of this instant invention is
15 mAb 2C10, a rat antibody which can be used for the development of a chimeric or humanized antibody, described in more detail in examples below. The 2C10 mAb is characterized by an antigen binding specificity for human IL-18 of about K_d 3.9×10^{-11} M. This mAb is characterized by being isotype $IgG_{1,K}$.

Another desirable donor antibody is the murine mAb 13G9. This mAb is
20 characterized by being isotype $IgG_{1,K}$. The mAb has a dissociation constant for IL-18 of about 12×10^{-9} M.

Yet, another desirable donor antibody is the rat mAb, 14B7. This mAb is characterized by having a dissociation constant for IL-18 about 1.5×10^{-10} M. 14B7 is also characterized by being isotype $IgG_{1,K}$.

25 This invention is not limited to the use of the 13G9, 2C10, 14B7, or their hypervariable (i.e., CDR) sequences. Any other appropriate high affinity IL-18 antibodies characterized by a dissociation constant equal or less than about 3.9×10^{-11} M for human IL-18 and corresponding anti-IL-18 CDRs may be substituted therefor. Wherever in the following description the donor antibody is identified as 13G9,
30 2C10, 14B7, this designation is made for illustration and simplicity of description only.

III. Antibody Fragments

The present invention also includes the use of Fab fragments or F(ab')₂ fragments derived from mAbs directed against human IL-18. These fragments are useful as agents protective *in vivo* against IL-18 and Th1-mediated conditions or *in vitro* as part of an IL-18 diagnostic. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and an F(ab')₂ fragment is the fragment formed by two Fab fragments bound by disulfide bonds. MAbs 13G9, 2C10, 14B7, and other similar high affinity, IL-18 binding antibodies, provide sources of Fab fragments and F(ab')₂ fragments which can be obtained by conventional means, e.g., cleavage of the mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. These Fab and F(ab')₂ fragments are useful themselves as therapeutic, prophylactic or diagnostic agents, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

The Fab and F(ab')₂ fragments can be constructed via a combinatorial phage library (see, e.g., Winter et al., Ann. Rev. Immunol., 12:433-455 (1994)) or via immunoglobulin chain shuffling (see, e.g., Marks et al., Bio/Technology, 10:779-783 (1992)).

wherein the Fd or V_H immunoglobulin from a selected antibody (e.g., 13G9) is allowed to associate with a repertoire of light chain immunoglobulins, V_L (or V_K), to form novel Fabs. Conversely, the light chain immunoglobulin from a selected antibody may be allowed to associate with a repertoire of heavy chain immunoglobulins, V_H (or Fd), to form novel Fabs.

IV. Anti-IL-18 Amino Acid and Nucleotide Sequences of Interest

The mAb 2C10 or other antibodies described above may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

As one example, the present invention provides variable light chain and variable heavy chain sequences from the IL-18 mAb 2C10 and sequences derived therefrom.

The nucleic acid sequences of this invention, or fragments thereof, encoding the variable light chain and heavy chain peptide sequences are also useful for mutagenic introduction of specific changes within the nucleic acid sequences

encoding the CDRs or framework regions, and for incorporation of the resulting modified or fusion nucleic acid sequence into a plasmid for expression..

Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences of the invention as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. The isolated nucleic acid sequences of this invention, or fragments thereof, encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other engineered antibodies of this invention when operatively combined with a second immunoglobulin partner.

It should be noted that in addition to isolated nucleic acid sequences encoding portions of the altered antibody and antibodies described herein, other such nucleic acid sequences are encompassed by the present invention, such as those complementary to the native CDR-encoding sequences or complementary to the modified human framework regions surrounding the CDR-encoding regions. Useful DNA sequences include those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences. An example of one such stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively an exemplary stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Preferably, these hybridizing DNA sequences are at least about 18 nucleotides in length, i.e., about the size of a CDR.

25

V. Altered Immunoglobulin Molecules And Altered Antibodies

Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions that encode peptides having the antigen specificity of an IL-18 antibody, preferably a high affinity antibody such as provided by the present invention, inserted into a first immunoglobulin partner (a human framework or human immunoglobulin variable region).

Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner. The second immunoglobulin partner is defined above, and may include a sequence encoding a second antibody region of interest, for example an Fc region. Second immunoglobulin partners may also include sequences

35

encoding another immunoglobulin to which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of IL-18 may be designed to elicit enhanced binding with the same antibody.

5 The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the second immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by
10 conventional covalent or ionic bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the
15 effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

In addition, signal sequences for the molecules of the invention may be modified to enhance expression.

An exemplary altered antibody contains a variable heavy and/or light chain
20 peptide or protein sequence having the antigen specificity of mAb 2C10, e.g., the V_H and V_L chains. Still another desirable altered antibody of this invention is characterized by the amino acid sequence containing at least one, and preferably all of the CDRs of the variable region of the heavy and/or light chains of the rat antibody molecule 2C10 with the remaining sequences being derived from a human
25 source, or a functional fragment or analog thereof.

In still a further embodiment, the engineered antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or CH2 CH3 domain of a complete antibody molecule has
30 been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence, for example, having the antigen specificity of rat 2C10. The
35 resulting protein may exhibit both anti-IL-18 antigen specificity and characteristics of the non-immunoglobulin upon expression. That fusion partner characteristic may be, e.g., a functional characteristic such as another binding or receptor domain, or a

therapeutic characteristic if the fusion partner is itself a therapeutic protein, or additional antigenic characteristics.

Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete
5 fragment thereof, such as the Fab or F(ab')₂ fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F_v or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor mAb, e.g., mAb 2C10. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

10 Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable
15 (preferably all) CDRs from the donor antibody, e.g., the anti-IL-18 antibody described herein. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or the donor CDR regions may be made in order to retain donor antibody antigen binding specificity.

20 Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the IL-18 mAb (optionally modified as described) or one or more of the below-identified heavy or light chain CDRs. The engineered antibodies would be expected to be neutralizing, i.e., they desirably block binding to the receptor of the IL-18 protein and they also block or prevent
25 proliferation of IL-18 dependent cells.

Such engineered antibodies may include a humanized antibody containing the framework regions of a selected human immunoglobulin or subtype, or a chimeric antibody containing the human heavy and light chain constant regions fused to the IL-18 antibody functional fragments. A suitable human (or other
30 animal) acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant
35 region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted

that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4),
5 IgM, IgA, and IgE. However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

10 One example of a particularly desirable humanized antibody would contain CDRs of 2C10 inserted onto the framework regions of a selected human antibody sequence. For neutralizing humanized antibodies, one, two or preferably three CDRs from the IL-18 antibody heavy chain and/or light chain variable regions are inserted into the framework regions of the selected human antibody sequence,
15 replacing the native CDRs of the latter antibody.

Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of less than the six CDRs. Preferably all six CDRs are replaced. It is possible to replace the CDRs only in the
20 human heavy chain, using as light chain the unmodified light chain from the human acceptor antibody. Still alternatively, a compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

25 The engineered humanized antibody thus preferably has the structure of a natural human antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use, e.g., treatment of IL-18 mediated inflammatory diseases in man, or for diagnostic uses.

As another example, an engineered antibody may contain CDRs of the
30 variable light chain region of 2C10 and CDRs of the variable heavy chain region of 13G9. The resulting humanized antibody should be characterized by the same antigen binding specificity and high affinity of mAb 2C10.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without
35 necessarily affecting the specificity and high affinity of the donor antibody (i.e., an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both.

In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement (see, e.g., Angal et al., Mol. Immunol., 30:105-108 (1993), Xu et al., J. Biol. Chem., 269:3469-3474 (1994), Winter et al., EP 307,434-B).

An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor antibody heavy chain and light chain variable regions, including framework regions, in association with human immunoglobulin constant regions for both chains. It is anticipated that chimeric antibodies which retain additional non-human sequence relative to humanized antibodies of this invention may elicit a significant immune response in humans.

Such antibodies could be useful in the prevention and treatment of IL-18 mediated disorders, as discussed below.

VI. *Production Of Altered Antibodies And Engineered Antibodies*

Preferably, the variable light and/or heavy chain sequences and the CDRs of mAb 2C10 or other suitable donor mAbs, and their encoding nucleic acid sequences, are utilized in the construction of altered antibodies, preferably humanized antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.

A hybridoma producing a selected donor mAb, e.g., the rat antibody 2C10, is conventionally cloned, and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook *et al.*, (Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory (1989)). The variable heavy and light regions of 2C10 containing at least the CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin can be obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding regions are identified using a known database and by comparison to other antibodies.

A rat/human chimeric antibody may then be prepared and assayed for binding ability. Such a chimeric antibody contains the entire non-human donor antibody V_H and V_L regions, in association with human Ig constant regions for both chains.

A humanized antibody may be derived from the chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody of the invention may be prepared using standard mutagenesis techniques. Thus, the resulting humanized antibody contains human framework regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells. Other humanized antibodies may be prepared using this technique on other suitable IL-18-specific, neutralizing, high affinity, non-human antibodies.

A conventional expression vector or recombinant plasmid can be produced by placing these coding sequences for the altered antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules of this invention.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector used is pUC19, which is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or

Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

5 Similarly, the vectors employed for expression of the engineered antibodies according to this invention may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA
10 sequences which code for the engineered antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for
15 amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

20 The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in
25 the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the engineered antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and
30 other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the engineered antibody or altered antibody of the invention are preferably mammalian cells such as CHO,
35 COS, a fibroblast cell (e.g., 3T3), and myeloid cells, and more preferably a CHO or a myeloid cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be

employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, cited above.

5 Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the
10 bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also
15 available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems. See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors of the invention may be constructed, the transfection methods required to produce the host cells of the
20 invention, and culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the altered antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis
25 and the like. Such techniques are within the skill of the art and do not limit this invention.

Yet another method of expression of the humanized antibodies may utilize expression in a transgenic animal, such as described in U.S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when
30 transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the engineered antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of
35 the engineered antibody to IL-18. Additionally, other *in vitro* assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies

performed to evaluate the persistence of the engineered antibody in the body despite the usual clearance mechanisms.

Following the general procedures described for preparing humanized antibodies, one of skill in the art may also construct humanized antibodies from other donor IL-18 antibodies, variable region sequences and CDR peptides described herein. Engineered antibodies can be produced with variable region frameworks potentially recognized as "self" by recipients of the engineered antibody. Minor modifications to the variable region frameworks can be implemented to effect large increases in antigen binding without appreciable increased immunogenicity for the recipient. Such engineered antibodies may effectively treat a human for IL-18 mediated conditions. Such antibodies may also be useful in the diagnosis of such conditions.

VII. Therapeutic/Prophylactic Uses

This invention also relates to a method of treating humans experiencing autoimmune related symptoms, such as MS, which comprises administering an effective dose of antibodies including one or more of the engineered antibodies or altered antibodies described herein, or fragments thereof.

The therapeutic response induced by the use of the molecules of this invention is produced by the binding to human IL-18 and thus subsequently blocking Th1 stimulation. Thus, the molecules of the present invention, when in preparations and formulations appropriate for therapeutic use, are highly desirable for those persons experiencing autoimmune disease, such as but not limited to MS, RA, IDDM, IBD and psoriasis.

The altered antibodies, antibodies and fragments thereof of this invention may also be used in conjunction with other antibodies, particularly human mAbs reactive with other markers (epitopes) responsible for the condition against which the engineered antibody of the invention is directed.

The therapeutic agents of this invention are believed to be desirable for treatment of autoimmune conditions from about 2 days to 6 months or as needed. For example, longer treatments may be desirable when treating MS or the like. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The altered antibodies, antibodies, engineered antibodies, and fragments thereof, and pharmaceutical

compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, or intranasally.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the engineered (e.g., humanized) antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the engineered antibody of the invention or a cocktail thereof dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an engineered antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat an inflammatory disorder in a human or other animal, one dose of approximately 0.1 mg to approximately 20 mg per 70 kg body weight of a protein or an antibody of this invention should be administered parenterally, preferably *i.v.*

or i.m. (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician during the disease.

The altered antibodies and engineered antibodies of this invention may also be used in diagnostic regimens, such as for the determination of IL-18 mediated disorders or tracking progress of treatment of such disorders. As diagnostic reagents, these altered antibodies may be conventionally labeled for use in ELISA's and other conventional assay formats for the measurement of IL-18 levels in serum, plasma or other appropriate tissue, or the release by human cells in culture. The nature of the assay in which the altered antibodies are used are conventional and do not limit this disclosure.

Thus, one embodiment of the present invention relates to a method for aiding the diagnosis of autoimmune disease and other conditions associated with excess Th1 T cell production in a patient which comprises the steps of determining the amount of human IL-18 in sample (plasma or tissue) obtained from said patient and comparing said determined amount to the mean amount of human IL-18 in the normal population, whereby the presence of a significantly elevated amount of IL-18 in the patient's sample is an indication autoimmune disease and other conditions associated with excess Th1 T cell production.

The antibodies, altered antibodies or fragments thereof described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

The following examples illustrate various aspects of this invention including the construction of exemplary engineered antibodies and expression thereof in suitable vectors and host cells, and are not to be construed as limiting the scope of this invention. All amino acids are identified by conventional three letter or single letter codes. All necessary restriction enzymes, plasmids, and other reagents and materials were obtained from commercial sources unless otherwise indicated. All general cloning ligation and other recombinant DNA methodology were as performed in T. Maniatis *et al.*, cited above, or the second edition thereof (1989), eds. Sambrook *et al.*, by the same publisher ("Sambrook *et al.*").

Example 1 - Production of MAbs to IL-18

A. *Monoclonal antibody generation*

Mice (F1 hybrids of Balb/c and C57BL/6) or rats (Sprague Dawley) were immunised with 30 µg recombinant IL-18 in adjuvant and 4 weeks later with 30 µg

IL-18 in adjuvant. On the basis of a good serum antibody titre to IL-18 animals received a further immunization of 10-30 µg IL-18 (i.p. in saline). Three days following the final immunization a splenectomy was performed. Mouse or rat spleen cells were used to prepare hybridomas by standard procedures. (Zola, H.Ed.,
5 Monoclonal Antibodies. CRC Press Inc. 1987). Positive hybridomas were cloned by the limiting dilution method.

B. Purification of Mabs

Mabs were purified by ProsepA* (Bio Processing, Consett, UK) chromatography respectively using the manufacturer's instructions. Mabs were
10 >95% pure by SDS-PAGE.

C. Isotyping of Mabs

All rat and mouse Mabs were isotyped by commercially available kits (Zymed, Amersham) and found to be IgG1 kappa.

15

Example 2 – Assays

A. Biotinylation Of Il-18

IL-18 was biotinylated using a kit purchased from Molecular Probes Inc. using a 10:1 ratio of biotinylation reagent. Biotinylation had no effect on the
20 biological activity of IL-18

B. Hybridoma Screening Assay

96-well plates were coated with streptavidin (2ug/ml, 100ul/well in PBS) by incubation overnight at 4°C. The solution was then aspirated and non-specific
25 binding sites were blocked with 250µl/well of 1% bovine serum albumin (BSA) in TBS buffer (50mM Tris, 150 mM NaCl, 0.02% Kathon*, pH 7.4) for 5-60 minutes at RT. Following this and each of the following steps, the plate was washed 4 times in wash buffer (10 mM Tris, 150 mM NaCl, 0.05% Tween* 20, 0.02% Kathon, pH 7.4).
To each well, 100 µL biotin IL-18 (100ng/ml) in assay buffer (0.5% BSA, 0.05%
30 bovine gamma globulin, 0.01% Tween 40, 20µM diethylenetriaminepentaacetic in TBS buffer) was added and the plates were incubated for 30 min at RT in a shaker-incubator. To each well 50µl hybridoma medium and 50 µl assay buffer was then

added and incubated for 60 min at RT in a shaker-incubator. To each well was then added 100ul 0.5µg/ml Eu³⁺ labelled anti-mouse or anti-rat antibody in assay buffer. Finally 200 µl /well of enhancer (Wallac) was added and incubated for 5 min at RT and the time-resolved fluorescence measured. Hybridomas having counts >100K
5 were expanded into 24-well plates.

C. *Immunoassay*

To determine the specificity of the anti-IL-18 Mabs) generated 96-well plates were coated, blocked and incubated with biotin IL-18 as above. All the following
10 incubations were performed in a shaker-incubator at RT. After washing the wells 50 µl IL-18 (3 µg/ml) or assay buffer and 50 µl Mab were added and incubated for 60 min. After washing the wells 100ul 0.5µg/ml Eu³⁺ labelled anti-mouse or anti-rat antibody in assay buffer was added for 60 min, the wells washed and then 100 µl /well of enhancer (Wallac) was added and incubated for 5 min at RT and the time-
15 resolved fluorescence measured. All positive hybridomas showed displacement of binding with IL-18.

D. *Neutralization Assay*

PBMC from healthy donors were isolated by Ficol-Paque*(Pharmacia)
20 gradient and cultured in 96 well plates in 10% FBS DMEM/F12 media with 1µg/ml ConA (Sigma) in the presence of IL-18 (5ng/ml) and/or Mabs. After 18h culture at 37°C, 5% CO₂ in air, 90% humidity 25µl media was removed and interferon gamma (IFNg) concentration measured by immunoassay. The results, obtained from an average of three experiments, are summarized in Table I.

25

E. *Affinity Measurements Of Monoclonal Antibody*

The affinity of the purified mAbs was measured in the BIAcore optical biosensor (Pharmacia Biosensor, Uppsala, Sweden) using a flow rate of 30ul/min. Kinetic data was evaluated using relationships described previously (Karlsson et al,
30 J. Immunol. Meth., 145:229-240 (1991).

The mAb (diluted in HBS buffer, 10 mM HEPES, 150 mM NaCl, 0.01%

Tween-20, pH 7.4) was injected over a rabbit anti-mouse IgG Fc or goat anti-rat IgG Fc surface, followed by buffer flow and the RU was recorded. IL-18 (diluted in HBS buffer) was then injected for 180 seconds followed by a buffer flow for 500 seconds and the RU was recorded. The sensor chip surface was regenerated by an injection of 0.1 M phosphoric acid. The on-rates (K_{ass}) and off-rates (K_{diss}) of binding were calculated using BIAcore software and together these yield a calculated equilibrium constant (K_D) of 12×10^{-9} M for mAb 13G9, 3.9×10^{-11} M for mAb 2C10 and 1.5×10^{-10} M for mAb 14B7. See Table I.

10 *F. Epitope Analysis Of Monoclonal Antibody*

The epitope analysis of the purified Mabs was measured in the BIAcore. Using a flow rate of 10 μ l/min, the first Mab (diluted in HBS buffer) was injected over a rabbit anti-mouse IgG Fc or goat anti-rat IgG Fc surface, followed by an injection of IL-18 for 240s, an injection of blocking Mabs for 48s and an injection of the second Mab for 240s. The surface was regenerated by an injection of 0.1M phosphoric acid and the RU was recorded after each injection. It was found that Mabs 13G9, 2C10 and 14B7 have similar or overlapping epitopes.

TABLE I

20 Affinity and neutralizing activity of mAbs reactive with human IL-18

mAb	Kd (pM) ^a	Neutralisation IC50 (nM) ^b
2C10 (rat)	39	0.1
25 14B7 (rat)	150	0.2
13G9 (mouse)	12000	3.0

^a Determined by optical biosensor (BIAcore) analysis (25°C)

^b Inhibition of IFN gamma production (in nM) of PBMC in response to 5ng/ml human IL-18

30

Example 3 – CDR sequences*Gene Cloning and Sequence Analysis:*

The variable heavy and light genes were cloned from hybridoma cells using standard molecular biological methods described briefly as follows. Total RNA was isolated from the hybridoma cells using TRIZOL Reagent (Life Technologies Cat. # 15596-026) according to manufacturer's protocol. The RNA was reverse transcribed with a RT-PCR kit per the manufacturer's instructions (Boehringer Mannheim Cat. No. 1483-188) using a poly-dT oligonucleotide for priming. Following first strand cDNA synthesis, the heavy and light V regions were PCR amplified using 3' constant region specific primers and degenerate 5' primers. The degenerate 5' primer sequences were designed to encode the previously determined N terminal amino acid sequences of the variable heavy or light chain regions. Full length sequences from multiple clones were obtained from each PCR amplification and aligned to provide consensus. Accordingly, the first 17 bases of DNA sequence for both the heavy and light chains are PCR primer generated, however the translated protein sequence is native.

The nucleotide and deduced amino acid sequences for hybridoma antibodies 2C10, 13G9, and 14B7 are shown Figures 1-6. In each case the CDR s and the nucleotide sequences encoding them are *boxed*. The degenerate primer sequences are *bolded*.

p50897SEQ.txt

SEQUENCE LISTING

[1]GENERAL INFORMATION

- [i] APPLICANT:
- [a] NAME: SmithKLine Beecham Corporation
 STREET: One Franklin Plaza
 CITY: Philadelphia
 STATE: Pennsylvania
 ZIP CODE: 19103
- NAME: SmithKLine Beecham plc
 STREET: Great West Road
 CITY: Brentford
 STATE: Middlesex, UK
 ZIP CODE: TW8 9EP
- and NAME Holmes, Stephen D.
 STREET: Cold Harbour Road
 CITY: Harlow
 STATE: Essex, UK
 ZIP CODE: CM19 5AD
- and NAME Ho, Yen Sen
 STREET: 362 Old State Road
 CITY: Berwyn
 STATE: Pennsylvania
 ZIP CODE: 19312
- and NAME Taylor, Alexander
 STREET: 522 Westfield Drive
 CITY: Exton
 STATE: Pennsylvania
 ZIP CODE: 19341
- and NAME Abdel-Meguid, Sherin S.
 STREET: 236 Autumn Drive
 CITY: Exton
 STATE: Pennsylvania
 ZIP CODE: 19341
- [ii] TITLE OF INVENTION: Recombinant IL-18 Antagonists Useful in
 Treatment of IL-18 Mediated Disorders
- [iii] FILE REFERENCE: P50897
- [iv] NUMBER OF SEQUENCES: 48
- [v] COMPUTER READABLE FORM:
 MEDIUM TYPE: Floppy Disk
 COMPUTER: IBM PC Compatible
 OPERATING SYSTEM: PC-DOS/MS-DOC
 SOFTWARE: FastSEQ for Windows Version 4.0
- [vi] CURRENT APPLICATION DATA:
 [A] APPLICATION NUMBER: PCT/US00/07349
 [B] FILING DATE: 17 MARCH 2000
 [C] CLASSIFICATION
- [vi] PRIOR APPLICATION DATA:
 [A] APPLICATION NUMBER: 60/125,299
 [B] FILING DATE: 19 MARCH 1999
- [vii] ATTORNEY/AGENT INFORMATION:
 [A] NAME: Kerekes, Zoltan

p50897SEQ.txt

[B] REGISTRATION NUMBER: 38,938

TELECOMMUNICATION INFORMATION:

[A] TELEPHONE: 610-270-5024

[B] FAX: 610-270-5090

[2] INFORMATION FOR SEQ ID NO. 1

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 324
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION: SEQ. ID NO:1

gac att caa atg acc cag tct cca gct tcc ctg tct gca tct ctg gga	48
gaa act gtc tcc atc gaa tgt ctg gca agt gag gac ata tac act tat	96
tta aca tgg tat cag cag aaa cca ggg aaa tct cct caa ctc ctg atc	144
tat ggt gca aat aag ttg caa gat ggg gtc cca tca cgg ttc agt ggc	192
agt gga tct ggc aca cag tat tct ctc aag atc agc ggc ata caa cct	240
gaa gat gaa ggg gat tat ttc tgt cta cag ggt tcc aag ttt ccg ctc	288
acg ttc ggt tct ggg acc aag ctg gag atc aaa cgg	324

[2] INFORMATION FOR SEQ ID NO. 2

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 108
 [B] TYPE: PRT
 [C] ORGANISM: rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2

Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Leu Gly	
1 5 10 15	
Glu Thr Val Ser Ile Glu Cys Leu Ala Ser Glu Asp Ile Tyr Thr Tyr	
20 25 30	
Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile	
35 40 45	
Tyr Gly Ala Asn Lys Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly	
50 55 60	
Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Gly Ile Gln Pro	
65 70 75 80	
Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Gly Ser Lys Phe Pro Leu	
85 90 95	
Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg	
100 105	

[2] INFORMATION FOR SEQ ID NO. 3

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 33
 [B] TYPE: DNA
 [C] ORGANISM: rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:3

ctg gca agt gag gac ata tac act tat tta aca 33

[2] INFORMATION FOR SEQ ID NO. 4

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH:11
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4

p50897SEQ.txt

Leu Ala Ser Glu Asp Ile Tyr Thr Tyr Leu Thr
 1 5 10

[2] INFORMATION FOR SEQ ID NO. 5

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 21
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:5

ggt gca aat aag ttg caa gat

21

[2] INFORMATION FOR SEQ ID NO. 6

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 7
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:6

Gly Ala Asn Lys Leu Gln Asp
 1 5

[2] INFORMATION FOR SEQ ID NO. 7

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 27
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:7

cta cag ggt tcc aag ttt ccg ctc acg

27

[2] INFORMATION FOR SEQ ID NO. 8

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 9
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:8

Leu Gln Gly Ser Lys Phe Pro Leu Thr
 1 5

[2] INFORMATION FOR SEQ ID NO. 9

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 378
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:9

gag gtc cag cta cag cag tct ggg gct gag ctt gtg aga cct ggg acc 48
 tct gtg aag tta tct tgc aaa gtt tct ggc gaa ata agt aca gga tac 96
 tat ttc cac ttt gtg agg cga agg cct gga cag ggt ctg gaa tgg ata 144
 gga agg att gat cct gag gat gat agt act aaa tat gct gag agg ttc 192
 aaa gac agg gcg acg ctc act gca caa aca tcc tcc aac aca gcc tac 240
 ctg aac ctc agc agc ctg acc tct gag gac act gca act tat ttt tgt 288
 acc aca tgg cgg ata tac cga gat agt tct ggc cgc ccc ttc tat gtt 336

p50897SEQ.txt

atg gat gcc tgg ggt caa gga gct tca gtc act gtc tcc tca

378

[2] INFORMATION FOR SEQ ID NO. 10

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 126
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10

Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Thr
1			5				10							15	
Ser	Val	Lys	Leu	Ser	Cys	Lys	Val	Ser	Gly	Glu	Ile	Ser	Thr	Gly	Tyr
		20					25						30		
Tyr	Phe	His	Phe	Val	Arg	Arg	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35				40						45			
Gly	Arg	Ile	Asp	Pro	Glu	Asp	Asp	Ser	Thr	Lys	Tyr	Ala	Glu	Arg	Phe
	50				55						60				
Lys	Asp	Arg	Ala	Thr	Leu	Thr	Ala	Gln	Thr	Ser	Ser	Asn	Thr	Ala	Tyr
65				70						75					80
Leu	Asn	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Thr	Ala	Thr	Tyr	Phe	Cys
			85						90					95	
Thr	Thr	Trp	Arg	Ile	Tyr	Arg	Asp	Ser	Ser	Gly	Arg	Pro	Phe	Tyr	Val
			100				105						110		
Met	Asp	Ala	Trp	Gly	Gln	Gly	Ala	Ser	Val	Thr	Val	Ser	Ser		
		115					120						125		

[2] INFORMATION FOR SEQ ID NO. 11

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 15
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11

gga tac tat ttc cac

15

[2] INFORMATION FOR SEQ ID NO. 12

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 5
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12

Gly	Tyr	Tyr	Phe	His
1			5	

[2] INFORMATION FOR SEQ ID NO. 13

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 51
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13

agg att gat cct gag gat gat agt act aaa tat gct gag agg ttc aaa
gac48
51

[2] INFORMATION FOR SEQ ID NO. 14

[i] SEQUENCE CHARACTERISTICS

p50897SEQ.txt

[A] LENGTH:16
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:14

Arg Ile Asp Pro Glu Asp Asp Ser Thr Lys Tyr Ala Glu Arg Phe Lys
 1 5 10 15

[2] INFORMATION FOR SEQ ID NO. 15

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 51
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO: 15

tgg cgg ata tac cga gat agt tct ggc cgc ccc ttc tat gtt atg gat 48
 gcc 51

[2] INFORMATION FOR SEQ ID NO. 16

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH:16
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:16

Trp Arg Ile Tyr Arg Asp Ser Ser Gly Arg Pro Phe Tyr Val Met Asp
 1 5 10 15

[2] INFORMATION FOR SEQ ID NO. 17

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 342
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:17

gac gtt gtt atg act caa act cct ctc tcc ctg cct gtc agt ctt gga 48
 gat caa gcc tcc atc tct tgc aga tct agt cag agc ctt gta cac agt 96
 aat gga aac acc tat tta cat tgg tac ctg cag aag cca ggc cag tct 144
 cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca 192
 gac agg ttc agt ggc agt gga tca ggt aca gat ttc aca ctc aag atc 240
 agc aga gtg gag gct gag gat ctg gga gtt tat ttc tgc tct caa agt 288
 aca cat gtt cct ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata 336
 aaa cgg 342

[2] INFORMATION FOR SEQ ID NO. 18

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH:114
 [B] TYPE: PRT
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:18

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1 5 10 15
 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
 20 25 30
 Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

p50897SEQ.txt

```

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50          55          60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65          70          75          80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
          85          90          95
Thr His Val Pro Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
          100          105          110
Lys Arg
    
```

[2] INFORMATION FOR SEQ ID NO. 19

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 48
 [B] TYPE: DNA
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:19

aga tct agt cag agc ctt gta cac agt aat gga aac acc tat tta cat 48

[2] INFORMATION FOR SEQ ID NO. 20

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 16
 [B] TYPE: PRT
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:20

```

Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His
 1          5          10          15
    
```

[2] INFORMATION FOR SEQ ID NO. 21

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 21
 [B] TYPE: DNA
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:21

aaa gtt tcc aac cga ttt tct 21

[2] INFORMATION FOR SEQ ID NO. 22

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 7
 [B] TYPE: PRT
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:22

```

Lys Val Ser Asn Arg Phe Ser
 1          5
    
```

[2] INFORMATION FOR SEQ ID NO. 23

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 30
 [B] TYPE: DNA
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO: 23

p50897SEQ.txt

tct caa agt aca cat gtt cct ccg tac acg

30

[2]INFORMATION FOR SEQ ID NO. 24

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH:10
 [B] TYPE: PRT
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:24

Ser Gln Ser Thr His Val Pro Pro Tyr Thr
 1 5 10

[2]INFORMATION FOR SEQ ID NO. 25

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 369
 [B] TYPE: DNA
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:25

caa gtt act ctt aag gag tct ggc cct ggg ata ttg aag ccc tca cag	48
acc ctc agt ctg act tgt tct ttc tct ggg ttt tct ctg agc act tct	96
ggt atg ggt att gcc tgg gtt cgt cag cct tca ggg aag ggt ctg gag	144
tgg ctg gca gac att tgg tgg gat gat aat aag tat tat aat cca tcc	192
ctg gag agc cag ctc aca atc tcc aag gat acc tcc aga aac cag gta	240
ttc ctc acg atc acc agt gtg gac act gca gat tct gcc act tat tac	288
tgt gct cgt cat cat tac gac ggt agt agc ctc ctg cct atg gac tac	336
tgg ggt caa gga acc tca gtc acc gtc tcc tca	369

[2]INFORMATION FOR SEQ ID NO. 26

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH:123
 [B] TYPE: PRT
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:26

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Lys Pro Ser Gln	
1 5 10 15	
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser	
20 25 30	
Gly Met Gly Ile Ala Trp Val Arg Gln Pro Ser Gly Lys Gly Leu Glu	
35 40 45	
Trp Leu Ala Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser	
50 55 60	
Leu Glu Ser Gln Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val	
65 70 75 80	
Phe Leu Thr Ile Thr Ser Val Asp Thr Ala Asp Ser Ala Thr Tyr Tyr	
85 90 95	
Cys Ala Arg His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr	
100 105 110	
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser	
115 120	

[2]INFORMATION FOR SEQ ID NO. 27

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 21
 [B] TYPE: DNA
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:27

p50897SEQ.txt

act tct ggt atg ggt att gcc

21

[2]INFORMATION FOR SEQ ID NO. 28

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 7
 [B] TYPE: PRT
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:28

Thr Ser Gly Met Gly Ile Ala
 1 5

[2]INFORMATION FOR SEQ ID NO. 29

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 48
 [B] TYPE: DNA
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:29

gac att tgg tgg gat gat aat aag tat tat aat cca tcc ctg gag agc

48

[2]INFORMATION FOR SEQ ID NO. 30

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 16
 [B] TYPE: PRT
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:30

Asp Ile Trp Trp Asp Asp Asn Lys Tyr Tyr Asn Pro Ser Leu Glu Ser
 1 5 10 15

[2]INFORMATION FOR SEQ ID NO. 31

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 39
 [B] TYPE: DNA
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:31

cat cat tac gac ggt agt agc ctc ctg cct atg gac tac

39

[2]INFORMATION FOR SEQ ID NO. 32

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 13
 [B] TYPE: PRT
 [C] ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:32

His His Tyr Asp Gly Ser Ser Leu Leu Pro Met Asp Tyr
 1 5 10

[2]INFORMATION FOR SEQ ID NO. 33

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 324
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

p50897SEQ.txt

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:33

```

gat att caa atg acg cag tct cca gct tcc ctg tct gca tct ctg gga      48
gaa act gtc tcc atc gaa tgt cta gca agt gag gac ata tac agt tat      96
tta gca tgg tat caa cag aag cca ggg aaa tct cct cag ctc ctg atc     144
tat gcc aca aaa agg ttg caa gat ggg gtc cca tca cgg ttc agt ggc     192
agt gga tct ggc aca cag tat tct ctc aaa ata agc gac atg caa cct     240
gaa gat gaa ggg gat tat ttc tgt cta cag aat tcc aag ttt ccg gtc     288
acg ttc ggt tct ggg acc aag ctg gag atc aaa cgg                       324

```

[2] INFORMATION FOR SEQ ID NO. 34

[i] SEQUENCE CHARACTERISTICS

```

[A] LENGTH: 108
[B] TYPE: PRT
[C] ORGANISM: Rattus norvegicus

```

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:34

```

Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Leu Gly
 1           5           10           15
Glu Thr Val Ser Ile Glu Cys Leu Ala Ser Glu Asp Ile Tyr Ser Tyr
 20           25           30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
 35           40           45
Tyr Ala Thr Lys Arg Leu Gln Asp Gly Val Pro Ser Arg Phe Ser Gly
 50           55           60
Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Ser Asp Met Gln Pro
 65           70           75           80
Glu Asp Glu Gly Asp Tyr Phe Cys Leu Gln Asn Ser Lys Phe Pro Val
 85           90           95
Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
 100           105

```

[2] INFORMATION FOR SEQ ID NO. 35

[i] SEQUENCE CHARACTERISTICS

```

[A] LENGTH: 33
[B] TYPE: DNA
[C] ORGANISM: Rattus norvegicus

```

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:35

```

cta gca agt gag gac ata tac agt tat tta gca      33

```

[2] INFORMATION FOR SEQ ID NO. 36

[i] SEQUENCE CHARACTERISTICS

```

[A] LENGTH: 11
[B] TYPE: PRT
[C] ORGANISM: Rattus norvegicus

```

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:36

```

Leu Ala Ser Glu Asp Ile Tyr Ser Tyr Leu Ala
 1           5           10

```

[2] INFORMATION FOR SEQ ID NO. 37

[i] SEQUENCE CHARACTERISTICS

```

[A] LENGTH: 21
[B] TYPE: DNA
[C] ORGANISM: Rattus norvegicus

```

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:37

p50897SEQ.txt

gcc aca aaa agg ttg caa gat

21

[2] INFORMATION FOR SEQ ID NO. 38

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 7
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:38

Ala Thr Lys Arg Leu Gln Asp
 1 5

[2] INFORMATION FOR SEQ ID NO. 39

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 27
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:39

cta cag aat tcc aag ttt ccg gtc acg

27

[2] INFORMATION FOR SEQ ID NO. 40

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 9
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:40

Leu Gln Asn Ser Lys Phe Pro Val Thr
 1 5

[2] INFORMATION FOR SEQ ID NO. 41

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH: 368
 [B] TYPE: DNA
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:41

gag gtt cag ctt cag cag tct ggg gct gag ctt gtg aga cct ggg acc	48
tct gtg aag ttt tct tgc aaa gtt tct ggc gat acc cct aca aca tac	96
tac gtg cac ttt gtg aga caa agg cct gga cag ggt ctg gaa tgg ata	144
gga agg att gat cct gag gat act agt act aaa tat gct gag aag ttc	192
aga aat aag gcg aca ttc act gca gat cca tcc tcc aac aca gcc tac	240
cta aac ctc agc agc ctg acc cct gag gac act gca acc tat ttt tgt	288
acc ata atg cgg tac cat agt acc tat agg gtc tat gtt atg gat ttc	336
tgg ggt caa gga act gca gtc act gtc tcc tc	368

[2] INFORMATION FOR SEQ ID NO. 42

[i] SEQUENCE CHARACTERISTICS
 [A] LENGTH:122
 [B] TYPE: PRT
 [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:42

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Thr

p50897SEQ.txt

```

1           5           10           15
Ser Val Lys Phe Ser Cys Lys Val Ser Gly Asp Thr Pro Thr Thr Tyr
                20                25                30
Tyr Val His Phe Val Arg Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
                35                40                45
Gly Arg Ile Asp Pro Glu Asp Thr Ser Thr Lys Tyr Ala Glu Lys Phe
                50                55                60
Arg Asn Lys Ala Thr Phe Thr Ala Asp Pro Ser Ser Asn Thr Ala Tyr
65                70                75                80
Leu Asn Leu Ser Ser Leu Thr Pro Glu Asp Thr Ala Thr Tyr Phe Cys
                85                90                95
Thr Ile Met Arg Tyr His Ser Thr Tyr Arg Val Tyr Val Met Asp Phe
                100                105                110
Trp Gly Gln Gly Thr Ala Val Thr Val Ser
                115                120

```

[2] INFORMATION FOR SEQ ID NO. 43

- [i] SEQUENCE CHARACTERISTICS
 - [A] LENGTH: 15
 - [B] TYPE: DNA
 - [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:43

aca tac tac gtg cac

15

[2] INFORMATION FOR SEQ ID NO. 44

- [i] SEQUENCE CHARACTERISTICS
 - [A] LENGTH: 5
 - [B] TYPE: PRT
 - [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:44

Thr Tyr Tyr Val His
1 5

[2] INFORMATION FOR SEQ ID NO. 45

- [i] SEQUENCE CHARACTERISTICS
 - [A] LENGTH: 51
 - [B] TYPE: DNA
 - [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:45

agg att gat cct gag gat act agt act aaa tat gct gag aag ttc aga
aat

48
51

[2] INFORMATION FOR SEQ ID NO. 46

- [i] SEQUENCE CHARACTERISTICS
 - [A] LENGTH: 16
 - [B] TYPE: PRT
 - [C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:46

Arg Ile Asp Pro Glu Asp Thr Ser Thr Lys Tyr Ala Glu Lys Phe Arg
1 5 10 15

[2] INFORMATION FOR SEQ ID NO. 47

- [i] SEQUENCE CHARACTERISTICS

p50897SEQ.txt

[A] LENGTH: 42
[B] TYPE: DNA
[C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:47

atg cgg tac cat agt acc tat agg gtc tat gtt atg gat ttc 42

[2] INFORMATION FOR SEQ ID NO. 48

[i] SEQUENCE CHARACTERISTICS
[A] LENGTH: 14
[B] TYPE: PRT
[C] ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:48

Met Arg Tyr His Ser Thr Tyr Arg Val Tyr Val Met Asp Phe
1 5 10

WHAT IS CLAIMED IS:

1. A rat neutralizing monoclonal antibody specific for human interleukin-18 and having a binding affinity characterized by a dissociation constant equal to about 3.9×10^{-11} M.
- 5 2. A rat neutralizing monoclonal antibody according to claim 1, comprising the light chain amino acid sequence of SEQ ID NO:2, and the heavy chain amino acid sequence of SEQ ID NO:10.
3. A hybridoma which produces the monoclonal antibody of claim 1 or claim 2.
- 10 4. A neutralizing Fab fragment or F(ab')₂ fragment thereof, produced by deleting the Fc region of the monoclonal antibody of claim 1 or 2.
5. A monoclonal antibody specific for human interleukin-18, wherein said monoclonal antibody comprises the light chain amino acid sequence of SEQ ID NO:2 and the heavy chain amino acid sequence of SEQ ID NO:10.
- 15 6. A hybridoma which produces the monoclonal antibody of claim 5.
7. A neutralizing Fab fragment or F(ab')₂ fragment thereof, produced by deleting the Fc region of the monoclonal antibody of claim 5.
8. A monoclonal antibody specific for human interleukin-18, wherein said monoclonal antibody comprises immunoglobulin light chain complementarity
20 determining regions (CDR) of SEQ ID NO:4, SEQ ID NO:6, and SEQ ID NO:8; and immunoglobulin heavy chain complementarity determining regions (CDR) of SEQ ID NO:12, SEQ ID NO:14, and SEQ ID NO:16.
9. A nucleic acid molecule encoding the monoclonal antibody of claim 8.

Vk2c10

1 D I Q M T Q S P A S L S A S L 15
GAC ATT CAA ATG ACC CAG TCT CCA GCT TCC CTG TCT GCA TCT CTG
 CTG TAA GTT TAC TGG GTC AGA GGT CGA AGG GAC AGA CGT AGA GAC

CDRI

16 G E T V S I E C L A S E D I Y 30
 GGA GAA ACT GTC TCC ATC GAA TGT CTG GCA AGT GAG GAC ATA TAC
 CCT CTT TGA CAG AGG TAG CTT ACA GAC CGT TCA CTC CTG TAT ATG

31 T Y L T W Y Q Q K P G K S P Q 45
ACT TAT TTA ACA TGG TAT CAG CAG AAA CCA GGG AAA TCT CCT CAA
TGA ATA AAT TGT ACC ATA GTC GTC TTT GGT CCC TTT AGA GGA GTT

CDRII

46 L L I Y G A N K L Q D G V P S 60
 CTC CTG ATC TAT GGT GCA AAT AAG TTG CAA GAT GGG GTC CCA TCA
 GAG GAC TAG ATA CCA CGT TTA TTC AAC GTT CTA CCC CAG GGT AGT

61 R F S G S G S G T Q Y S L K I 75
 CGG TTC AGT GGC AGT GGA TCT GGC ACA CAG TAT TCT CTC AAG ATC
 GCC AAG TCA CCG TCA CCT AGA CCG TGT GTC ATA AGA GAG TTC TAG

76 S G I Q P E D E G D Y F C L Q 90
 AGC GGC ATA CAA CCT GAA GAT GAA GGG GAT TAT TTC TGT CTA CAG
 TCG CCG TAT GTT GGA CTT CTA CTT CCC CTA ATA AAG ACA GAT GTC

CDRIII

91 G S K F P L T F G S G T K L E 105
GGT TCC AAG TTT CCG CTC ACG TTC GGT TCT GGG ACC AAG CTG GAG
CCA AGG TTC AAA GGC GAG TGC AAG CCA AGA CCC TGG TTC GAC CTC

106 I K R 108
 ATC AAA CGG
 TAG TTT GCC

FIGURE 1

VH2C10

1 E V Q L Q Q S G A E L V R P G 15
GAG GTC CAG CTA CAG CAG TCT GGG GCT GAG CTT GTG AGA CCT GGG
 CTC CAG GTC GAT GTC GTC AGA CCC CGA CTC GAA CAC TCT GGA CCC

16 T S V K L S C K V S G E I S T 30
 ACC TCT GTG AAG TTA TCT TGC AAA GTT TCT GGC GAA ATA AGT ACA
 TGG AGA CAC TTC AAT AGA ACG TTT CAA AGA CCG CTT TAT TCA TGT

CDRI

31 **G Y Y F H** F V R R R P G Q G L 45
GGA TAC TAT TTC CAC TTT GTG AGG CGA AGG CCT GGA CAG GGT CTG
CCT ATG ATA AAG GTG AAA CAC TCC GCT TCC GGA CCT GTC CCA GAC

CDRII

46 E W I G **R I D P E D D S T K Y** 60
 GAA TGG ATA GGA **AGG ATT GAT CCT GAG GAT GAT AGT ACT AAA TAT**
 CTT ACC TAT CCT **TCC TAA CTA GGA CTC CTA CTA TCA TGA TTT ATA**

61 **A E R F K D** R A T L T A Q T S 75
GCT GAG AGG TTC AAA GAC AGG GCG ACG CTC ACT GCA CAA ACA TCC
CGA CTC TCC AAG TTT CTG TCC CGC TGC GAG TGA CGT GTT TGT AGG

76 S N T A Y L N L S S L T S E D 90
 TCC AAC ACA GCC TAC CTG AAC CTC AGC AGC CTG ACC TCT GAG GAC
 AGG TTG TGT CGG ATG GAC TTG GAG TCG TCG GAC TGG AGA CTC CTG

91 T A T Y F C T T **W R I Y R D S** 105
 ACT GCA ACT TAT TTT TGT ACC ACA **TGG CGG ATA TAC CGA GAT AGT**
 TGA CGT TGA ATA AAA ACA TGG TGT **ACC GCC TAT ATG GCT CTA TCA**

CDRIII

106 **S G R P F Y V M D A** W G Q G A 120
TCT GGC CGC CCC TTC TAT GTT ATG GAT GCC TGG GGT CAA GGA GCT
AGA CCG GCG GGG AAG ATA CAA TAC CTA CGG ACC CCA GTT CCT CGA

121 S V T V S S 126
 TCA GTC ACT GTC TCC TCA
 AGT CAG TGA CAG AGG AGT

FIGURE 2

Vk13g9

```

1   D   V   V   M   T   Q   T   P   L   S   L   P   V   S   L   15
    GAC GTT GTT ATG ACT CAA ACT CCT CTC TCC CTG CCT GTC AGT CTT
    CTG CAA CAA TAC TGA GTT TGA GGA GAG AGG GAC GGA CAG TCA GAA

                                CDR I
16  G   D   Q   A   S   I   S   C   R   S   S   Q   S   L   V   30
    GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC CTT GTA
    CCT CTA GTT CGG AGG TAG AGA ACG TCT AGA TCA GTC TCG GAA CAT

                                CDR II
31  H   S   N   G   N   T   Y   L   H   W   Y   L   Q   K   P   45
    CAC AGT AAT GGA AAC ACC TAT TTA CAT TGG TAC CTG CAG AAG CCA
    GTG TCA TTA CCT TTG TGG ATA AAT GTA ACC ATG GAC GTC TTC GGT

                                CDR III
46  G   Q   S   P   K   L   L   I   Y   K   V   S   N   R   F   60
    GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT
    CCG GTC AGA GGT TTC GAG GAC TAG ATG TTT CAA AGG TTG GCT AAA

61  S   G   V   P   D   R   F   S   G   S   G   S   G   T   D   75
    TCT GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCA GGT ACA GAT
    AGA CCC CAG GGT CTG TCC AAG TCA CCG TCA CCT AGT CCA TGT CTA

76  F   T   L   K   I   S   R   V   E   A   E   D   L   G   V   90
    TTC ACA CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT
    AAG TGT GAG TTC TAG TCG TCT CAC CTC CGA CTC CTA GAC CCT CAA

                                CDR III
91  Y   F   C   S   Q   S   T   H   V   P   P   Y   T   F   G   105
    TAT TTC TGC TCT CAA AGT ACA CAT GTT CCT CCG TAC ACG TTC GGA
    ATA AAG ACG AGA GTT TCA TGT GTA CAA GGA GGC ATG TGC AAG CCT

106 G   G   T   K   L   E   I   K   R   114
    GGG GGG ACC AAG CTG GAA ATA AAA CGG
    CCC CCC TGG TTC GAC CTT TAT TTT GCC
    
```

FIGURE 3

VH13G9

1	1 0	Q V T L K E S G P G I L K P S	30	15
		CAA GTT ACT CTT AAG GAG TCT GGC CCT GGG ATA TTG AAG CCC TCA		
		GTT CAA TGA GAA TTC CTC AGA CCG GGA CCC TAT AAC TTC GGG AGT		
16	50	Q T L S L T C S F S G F S L S	7 0	90
		CAG ACC CTC AGT CTG ACT TGT TCT TTC TCT GGG TTT TCT CTG AGC		30
		GTC TGG GAG TCA GAC TGA ACA AGA AAG AGA CCC AAA AGA GAC TCG		
31	1 10	T S G M G I A	13 0	45
		ACT TCT GGT ATG GGT ATT GCC W V R Q P S G K		
		TGA AGA CCA TAC CCA TAA CGG ACC CAA GCA GTC GGA AGT CCC TTC		
46	150	G L E W L A	1 70	60
		GGT CTG GAG TGG CTG GCA D I W W D D N K Y		
		CCA GAC CTC ACC GAC CGT GAC ATT TGG TGG GAT GAT AAT AAG TAT		
		CTG TAA ACC ACC CTA CTA TTA TTC ATA		
61	19 0	V N P S L E S	210	75
		TAT AAT CCA TCC CTG GAG AGC Q L T I S K D T		
		ATA TTA GGT AGG GAC CTC TCG CAG CTC ACA ATC TCC AAG GAT ACC		
		GTC GAG TGT TAG AGG TTC CTA TGG		
76	2 30	S R N Q V F L T I T S V D T A	25 0	270
		TCC AGA AAC CAG GTA TTC CTC ACG ATC ACC AGT GTG GAC ACT GCA		90
		AGG TCT TTG GTC CAT AAG GAG TGC TAG TGG TCA CAC CTG TGA CGT		
91	2 90	D S A T Y Y C A R	31 0	105
		GAT TCT GCC ACT TAT TAC TGT GCT CGT H H Y D G S		
		CTA AGA CGG TGA ATA ATG ACA CGA GCA CAT CAT TAC GAC GGT AGT		
		GTA GTA ATG CTG CCA TCA		
106	330	S L L P M D Y	3 50	120
		AGC CTC CTG CCT ATG GAC TAC W G Q G T S V T		
		TCG GAG GAC GGA TAC CTG ATG TGG GGT CAA GGA ACC TCA GTC ACC		
		ACC CCA GTT CCT TGG AGT CAG TGG		
121	123	V S S		
		GTC TCC TCA		
		CAG AGG AGT		

FIGURE 4

Vk14B7

```

1   D   I   Q   M   T   Q   S   P   A   S   L   S   A   S   L   15
   GAT ATT CAA ATG ACG CAG TCT CCA GCT TCC CTG TCT GCA TCT CTG
   CTA TAA GTT TAC TGC GTC AGA GGT CGA AGG GAC AGA CGT AGA GAC

           1 0                               30
16  G   E   T   V   S   I   E   C   L   A   S   E   D   I   Y   30
   GGA GAA ACT GTC TCC ATC GAA TGT CTA GCA AGT GAG GAC ATA TAC
   CCT CTT TGA CAG AGG TAG CTT ACA GAT CGT TCA CTC CTG TAT ATG

           50                               7 0           CDR I           90

31  S   Y   L   A   W   Y   Q   Q   K   P   G   K   S   P   Q   45
   AGT TAT TTA GCA TGG TAT CAA CAG AAG CCA GGG AAA TCT CCT CAG
   TCA ATA AAT CGT ACC ATA GTT GTC TTC GGT CCC TTT AGA GGA GTC

           1 10                               13 0

46  L   L   I   Y   A   T   K   R   L   Q   D   G   V   P   S   60
   CTC CTG ATC TAT GCC ACA AAA AGG TTG CAA GAT GGG GTC CCA TCA
   GAG GAC TAG ATA CGG TGT TTT TCC AAC GTT CTA CCC CAG GGT AGT

           150           CDR II           1 70

61  R   F   S   G   S   G   S   G   T   Q   Y   S   L   K   I   75
   CGG TTC AGT GGC AGT GGA TCT GGC ACA CAG TAT TCT CTC AAA ATA
   GCC AAG TCA CCG TCA CCT AGA CCG TGT GTC ATA AGA GAG TTT TAT

           19 0                               210

76  S   D   M   Q   P   E   D   E   G   D   Y   F   C   L   Q   90
   AGC GAC ATG CAA CCT GAA GAT GAA GGG GAT TAT TTC TGT CTA CAG
   TCG CTG TAC GTT GGA CTT CTA CTT CCC CTA ATA AAG ACA GAT GTC

           2 30                               25 0                               270

           CDR III           2 90                               31 0

91  N   S   K   F   P   V   T   F   G   S   G   T   K   L   E   105
   AAT TCC AAG TTT CCG GTC ACG TTC GGT TCT GGG ACC AAG CTG GAG
   TTA AGG TTC AAA GGC CAG TGC AAG CCA AGA CCC TGG TTC GAC CTC

106 I   K   R   108
    ATC AAA CGG
    TAG TTT GCC

```

FIGURE 5

VH14B7

```

1      E  V  Q  L  Q  Q  S  G  A  E  L  V  R  P  G  15
    GAG GTT CAG CTT CAG CAG TCT GGG GCT GAG CTT GTG AGA CCT GGG
    CTC CAA GTC GAA GTC GTC AGA CCC CGA CTC GAA CAC TCT GGA CCC

      50      70      90
16     T  S  V  K  F  S  C  K  V  S  G  D  T  P  T  30
    ACC TCT GTG AAG TTT TCT TGC AAA GTT TCT GGC GAT ACC CCT ACA
    TGG AGA CAC TTC AAA AGA ACG TTT CAA AGA CCG CTA TGG GGA TGT

      CDR I      1 10      13 0
31     T  Y  Y  V  H  F  V  R  Q  R  P  G  Q  G  L  45
    ACA TAC TAC GTG CAC TTT GTG AGA CAA AGG CCT GGA CAG GGT CTG
    TGT ATG ATG CAC GTG AAA CAC TCT GTT TCC GGA CCT GTC CCA GAC

      150      1 70 CDR II
46     E  W  I  G  R  I  D  P  E  D  T  S  T  K  Y  60
    GAA TGG ATA GGA AGG ATT GAT CCT GAG GAT ACT AGT ACT AAA TAT
    CTT ACC TAT CCT TCC TAA CTA GGA CTC CTA TGA TCA TGA TTT ATA

      19 0      210
61     A  E  K  F  R  N  K  A  T  F  T  A  D  P  S  75
    GCT GAG AAG TTC AGA AAT AAG GCG ACA TTC ACT GCA GAT CCA TTC
    CGA CTC TTC AAG TCT TTA TTC CGC TGT AAG TGA CGT CTA GGT AGG

      2 30      25 0      270
76     S  N  T  A  Y  L  N  L  S  S  L  T  P  E  D  90
    TCC AAC ACA GCC TAC CTA AAC CTC AGC AGC CTG ACC CCT GAG GAC
    AGG TTG TGT CGG ATG GAT TTG GAG TCG TCG GAC TGG GGA CTC CTG

      2 90      31 0
91     T  A  T  Y  F  C  T  I  M  R  Y  H  S  T  Y  105
    ACT GCA ACC TAT TTT TGT ACC ATA ATG CGG TAC CAT AGT ACC TAT
    TGA CGT TGG ATA AAA ACA TGG TAT TAC GCC ATG GTA TCA TGG ATA

      CDR III      330      3 50
106    R  V  Y  V  M  D  F  W  G  Q  G  T  A  V  T  120
    AGG GTC TAT GTT ATG GAT TTC TGG GGT CAA GGA ACT GCA GTC ACT
    TCC CAG ATA CAA TAC CTA AAG ACC CCA GTT CCT TGA CGT CAG TGA

121    V  S  122
    GTC TCC TC
    CAG AGG AG

```

FIGURE 6