INTERLEUKIN-10 ANTIBODIES

The present application claims the benefit of U.S. Provisional Patent Application No. 60/518,999; filed November 10, 2003, which is herein incorporated by reference in its entirety.

Field of the Invention

[0001] The present invention relates generally to interleukin-10 (IL-10) specific antibodies and uses thereof. More specifically, the invention relates to humanized antibodies that recognize human IL-10 and modulate its activity, particularly in autoimmune disorders.

Background of the Invention

[0002] Initially known as cytokine synthesis inhibitor factor or CSIF, interleukin-10 (IL-10) is a potent immunomodulator of hematopoietic cells, particularly immune cells. Cells such as activated Th2 cells, B cells, keratinocytes, monocytes and macrophages produce IL-10. *See, e.g.*, Moore et al., *Annu. Rev. Immunol.* 11:165 (1993). IL-10 inhibits activation and effector functions of a number of cells that include T cells, monocytes and macrophages. In particular, IL-10 inhibits cytokine synthesis, including that of IL-1, IFN-γ, and TNF, by cells such as Th1 cells, natural killer cells, monocytes, and macrophages. *See, e.g.*, Fiorentino et al., *J. Exp. Med.*, 170:2081-2095 (1989); Fiorentino et al., *J. Immunol.* 146:3444 (1991); Hsu et al., *Int. Immunol.* 4:563 (1992); Hsu et al., *Int. Immunol.* 4:563 (1992); D'Andrea et al., *J. Exp. Med.* 178:1041 (1993); de Waal Malefyt et al., *J. Exp. Med.* 174:915 (1991); Fiorentino et al., *J. Immunol.* 147:3815 (1991).

[0003] Multiple pathogens, particularly intracellular pathogens, elicit IL-10 production to slow or completely stall the effective removal of the pathogen by the immune response. Moore et al., *Annu. Rev. Immunol.* 11:165 (1993). For example, in blood lymphocytes from patients with HIV, leprosy, or tuberculosis, peripheral blood lymphocytes are typically anergic or nonresponsive in vitro when challenged with the pathogen. However, the neutralization of IL-10 in these demonstrated that an active effector response,

i.e., Th1 reactivity, was present in these cells. Thus, it is believed that IL-10 is effectively commandeered by the pathogen to facilitate its infective state.

[0004] IL-10 is also associated with autoimmunity *in vivo*. Autoimmunity results from the development from autoantibodies, autoreactive T cells, or some combination thereof that target normal tissue. One example of autoimmune disease is systemic lupus erythematosus (SLE), a chronic rheumatic disease in which connective tissue throughout the body becomes inflamed. Autoantibodies that attack normal body tissue as if it were an outside invade result in the characteristic inflammation. While the precise cause is not fully understood, researchers believe it has both genetic and environmental components. Specifically, B-cell hyperactivity and the presence of various autoantibodies characterize SLE. Typically, IgG autoantibodies reactive to double stranded DNA (IgG anti-dsDNA abs) are elevated in patients with SLE. Between 60 and 70% of SLE patients produce IgG anti-dsDNA abs, some of which are nephrotoxic. SLE is ten times more prevalent in women than men, with symptoms ranging from facial rashes to disabling and potentially life-threatening organ dysfunction. It can develop at any age, but is most common in young adults.

[0005] Numerous studies support a role for IL-10 in the pathology associated with SLE. For example, while IL-10 is typically not produced by cells without appropriate stimulation, both B cells and macrophages from SLE patients spontaneously produce high levels of IL-10 *in vitro*. Llorente, et al., *Arthritis Rheum.* 40:249-60 (1997). In several studies, researchers demonstrated a correlation between serum levels of IL-10 and disease activity. Moreover, both *in vivo* and *in vitro* studies demonstrated that the blockade of IL-10 production can alleviate the clinical manifestations of SLE. *See, e.g.*, Gonzalez-Amaro, et al. *J. Autoimmunity* 11:395-402 (1998).

[0006] To date, one of the manifestations of SLE, lupus nephritis, has been treated with through the use of immunosuppressive therapies, *e.g.*, corticosteriods and cyclophosphamides. Although effective, these therapies are non-specific and substantial toxicities exist which prevent long term therapy. Thus, specific neutralizing antibodies may be effective antagonists of IL-10, permitting the removal of the suppressive effects of IL-10 while leaving the remainder of the immune response network intact.

[0007] The most significant limitation in using antibodies as a therapeutic agent *in vivo* is the immunogenicity of the antibodies. As most monoclonal antibodies are derived

from rodents, repeated use in humans results in the generation of an immune response against the therapeutic antibody. Such an immune response results in a loss of therapeutic efficacy at a minimum and a potential fatal anaphylactic response at a maximum. Initial efforts to reduce the immunogenicity of rodent antibodies involved the production of chimeric antibodies, in which mouse variable regions were fused with human constant regions. Liu et al., *Proc. Natl. Acad. Sci. USA* 84:3439 (1987). However, mice injected with hybrids of human variable regions and mouse constant regions develop a strong anti-antibody response directed against the human variable region, suggesting that the retention of the entire rodent Fv region in such chimeric antibodies may still result in unwanted immunogenicity in patients.

[0008] It is generally believed that complementarity determining region (CDR) loops of variable domains comprise the binding site of antibody molecules. Therefore, the grafting of rodent CDR loops onto human frameworks (*i.e.*, humanization) was attempted to further minimize rodent sequences. Jones et al., *Nature* 321:522 (1986); Verhoeyen et al., *Science* 239:1534 (1988). However, CDR loop exchanges still do not uniformly result in an antibody with the same binding properties as the antibody of origin. Changes in framework residues (FR), residues involved in CDR loop support, in humanized antibodies also are required to preserve antigen binding affinity. Kabat et al., *J. Immunol.* 147:1709 (1991). While the use of CDR grafting and framework residue preservation in a number of humanized antibody constructs has been reported, it is difficult to predict if a particular sequence will result in the antibody with the desired binding, and sometimes biological, properties. *See, e.g.,* Queen et al., *Proc. Natl. Acad. Sci. USA* 86:10029 (1989), Gorman et al., *Proc. Natl. Acad. Sci. USA* 88:4181 (1991), and Hodgson, *Bio/Technology* 9:421 (1991). Moreover, most prior studies used different human sequences for animal light and heavy variable sequences, rendering the predictive nature of such studies questionable. Sequences of known antibodies have been used or, more typically, those of antibodies having known X-ray structures, antibodies NEW and KOL. *See, e.g.*, Jones et al., *supra*; Verhoeyen et al., *supra*; and Gorman et al., *supra*. Exact sequence information has been reported for only a few humanized constructs.

[0009] The present invention provides humanized monoclonal antibodies which recognize human IL-10 and modulate its activity, in particular with regard to autoimmune

disorders. The humanized antibody should provide an alternative therapy choice without the toxicity and non-specificity associated with current treatments.

Brief Summary of the Invention

[0010] Provided herein is a humanized recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising: at least one antibody light chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:1 at CDR1, SEQ ID NO:2 at CDR2, and SEQ ID NO:3 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence; and at least one antibody heavy chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group of SEQ ID NO:6 at CDR1, SEQ ID NO:7 at CDR2, and SEQ ID NO:8 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence. Also provided herein is an antibody, wherein the antibody light chain, or binding fragment thereof, comprises a polypeptide having a variable region of SEQ ID NO:4. In one specific embodiment, the antibody light chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:5. In one specific embodiment, the antibody heavy chain, or binding fragment thereof, comprises a polypeptide having a variable region of SEQ ID NO:9. In another specific embodiment, the antibody heavy chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:10.

[0011] Further provided herein is a chimeric recombinant antibody molecule that binds IL-10 or binding fragment thereof, comprising: at least one antibody light chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:1 at CDR1, SEQ ID NO:2 at CDR2, and SEQ ID NO:3 at CDR3; and at least one antibody heavy chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:6 at CDR1, SEQ ID NO:7 at CDR2, and SEQ ID NO:8 at CDR3.

[0012] Also provided herein is a humanized recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising: at least one antibody light chain, or binding fragment thereof, comprising a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:11 at CDR1, SEQ ID NO:12 at CDR2, and SEQ ID NO:13 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence; and at least one antibody heavy chain, or binding fragment thereof, comprising a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:15 at CDR1, SEQ ID NO:16 at CDR2, and SEQ ID NO:17 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence. In one specific embodiment, the antibody light chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:14. In yet another specific embodiment, the antibody heavy chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:18.

[0013] Further provided herein is a chimeric recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising: at least one antibody light chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:11 at CDR1, SEQ ID NO:12 at CDR2, and SEQ ID NO:13 at CDR3; and at least one antibody heavy chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:15 at CDR1, SEQ ID NO:16 at CDR2, and SEQ ID NO:17 at CDR3.

[0014] In one embodiment, the antibodies described *supra* further comprise a heavy chain constant region, wherein the heavy chain constant region comprises a $\gamma 1$, $\gamma 2$, $\gamma 3$, or $\gamma 4$ human heavy chain constant region or a variant thereof. In one embodiment, the antibodies described above further comprise a light chain constant region, wherein the light chain constant region comprises a lambda or a kappa human light chain constant region. In some embodiments, the binding fragment of these antibodies is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv, $F(ab')_2$, and a diabody.

[0015] Further provided herein is a method of suppressing an immune response in a human subject comprising administering to a subject in need thereof an antibody specific for IL-10, or a binding fragment thereof, in an amount effective to block the biological activity of IL-10, wherein the antibody is an antibody disclosed herein. The immune response suppressed by this method is a humoral or a cellular response. In one embodiment, the subject treated by this method has systemic lupus erythematosus. In another embodiment, the subject has immune thrombocytopenic purpura (ITC). In yet another embodiment, the subject has lupus nephritis. In a further embodiment, the subject has HIV. In another embodiment, the subject has hepatitis C. In one specific embodiment, the method of suppressing an immune response in a human subject comprising administering to a subject in need thereof (1) an antibody specific for IL-10, or a binding fragment thereof, in an amount effective to block the biological activity of IL-10, wherein the antibody is an antibody disclosed herein, and (2) an immunosuppressive agent.

[0016] Provided herein is a composition comprising an antibody, or binding fragment thereof, in combination with a pharmaceutically acceptable carrier or diluent, wherein the antibody is one of the antibodies disclosed *supra*.

[0017] Further provided herein is an isolated nucleic acid encoding the polypeptide of the antibodies disclosed *supra*. Also provided herein is an expression vector comprising the isolated nucleic acid sequence operably linked to control sequences recognized by a host cell transfected with the vector. Provided herein is a host cell comprising the vector comprising the isolated nucleic acid sequence. Further provided herein is a method of producing a polypeptide, comprising culturing the host cell comprising the vector under conditions wherein the nucleic acid sequence is expressed, thereby producing the polypeptide, and recovering the polypeptide from the host cell.

[0018] Provided herein is an isolated nucleic acid sequence encoding an antibody specific for IL-10 comprising a light chain having the nucleic acid sequence of SEQ ID NO:19 and a heavy chain having the nucleic acid sequence of SEQ ID NO:20. In further embodiments, the light chain has an American Type Culture Collection (ATCC) deposit number of PTA-5923 and the heavy chain has an ATCC deposit number of PTA-5922.

[0019] Provided herein is an isolated nucleic acid sequence encoding an antibody specific for IL-10 comprising a light chain having the nucleic acid sequence of SEQ ID NO:21 and a heavy chain having the nucleic acid sequence of SEQ ID NO:22. In a further

embodiment, the light chain has an ATCC deposit number of PTA-5927 and the heavy chain has an ATCC deposit number of PTA-5926.

[0020] Further provided herein is an isolated nucleic acid sequence encoding a binding fragment of the antibody encoded by the above nucleic acid sequences. In one embodiment, the binding fragment is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv, and $F(ab')_2$.

[0021] Provided herein is a method to identify an acceptor germline sequence for a humanized antibody, which method comprises the steps of: a) identifying a non-human antibody that has the desired biological activity; b) determining the amino acid sequence of a non-human antibody V_H and V_L domains; and c) comparing the nonhuman antibody sequence to a group of human germline sequences, wherein the comparison comprises the substeps of: 1) assigning the sequence of non-human V_H and V_L domain sequences residue numbers; 2) delineating the CDR and FR regions in the sequence; 3) assigning a predetermined numerical score at each residue position for which the non-human and human germline sequences are identical; and 4) totaling all of the residue scores to generate a total score for each human germline sequence; and d) identifying the human germline sequence with the highest total residue score as the acceptor germline sequence. In one embodiment, the method further comprises the substeps of: 5) assigning a numerical score of 1 for each residue position for which the non-human and human germline sequences are identical that was not scored in substep (3) to germline sequences with identical total residue scores after substep (4); 6) totaling all of the residue scores to generate a total score for each human germline sequence. In a specific embodiment, the non-human antibody is specific for IL-10 and inhibits the biological activity of IL-10. In a specific embodiment, the numerical scores are assigned to the residues as in Tables 2 and 3 for V_H and V_L regions, respectively.

[0022] Further provided herein is an antibody generated by the above method.

Brief Description of the Drawings

[0023] Figure 1A shows the assignment of residue numbers and numerical scores to the potential acceptor germline sequence relative to the variable light chain of the anti-human IL-10 antibody, 12G8.

[0024] Figure 1B shows the assignment of residue numbers and numerical scores to the potential acceptor germline sequence relative to the variable heavy chain of the anti-human IL-10 antibody, 12G8.

[0025] Figure 1C shows the assignment of residue numbers and numerical scores to the potential acceptor germline sequence relative to the variable light chain of the anti-human IL-10 antibody, 11D8.

[0026] Figure 1D shows the assignment of residue numbers and numerical scores to the potential acceptor germline sequence relative to the variable heavy chain of the anti-human IL-10 antibody, 12D8.

[0027] Figure 2A is a concentration-time profile for 12G8 antibody administered i.v. as described in Example III.

[0028] Figure 2B is a concentration-time profile for 12G8 administered s.c. as described in Example III.

[0029] Figure 3A shows that administration of the humanized anti-IL-10 antibody, SCH708980, confers resistance to Leishmania major infection in IL-10 transgenic mice. Infection was determined by measuring footpad swelling with a caliper at the times indicated. 12G8 antibody was administered as described in Example VI.

[0030] Figure 3B shows that administration of the rat anti-IL-10 antibody, 12G8, confers resistance to Leishmania major infection in IL-10 transgenic mice. Infection was determined by measuring footpad swelling with a caliper at the times indicated. 12G8 antibody was administered as described in Example VI.

Detailed Description of the Invention

[0031] For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections that follow.

A. Definitions

[0032] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications referred to herein are incorporated by reference in their entirety. If a definition

set forth in this section is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth in this section prevails over the definition that is incorporated herein by reference.

[0033] As used herein, "a" or "an" means "at least one" or "one or more."

[0034] As used herein, the term "antibody" refers to any form of antibody or fragment thereof that exhibits the desired biological activity. Thus, it is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

[0035] As used herein, the term "IL-10 binding fragment" or "binding fragment thereof" encompasses a fragment or a derivative of an antibody that still substantially retain its biological activity of inhibiting IL-10 activity. Therefore, the term "antibody fragment" or IL-10 binding fragment refers to a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, e.g., sc-Fv; and multispecific antibodies formed from antibody fragments. Typically, a binding fragment or derivative retains at least 50% of its IL-10 inhibitory activity. Preferably, a binding fragment or derivative retains at least 60%, 70%, 80%, 90%, 95%, 99% or 100% of its IL-10 inhibitory activity. It is also intended that a IL-10 binding fragment can include conservative amino acid substitutions that do not substantially alter its biologic activity.

[0036] The term "monoclonal antibody", as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic epitope. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of antibodies directed against (or specific for) different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention

may be made by the hybridoma method first described by Kohler et al., *Nature* 256: 495 (1975), or may be made by recombinant DNA methods (*see, e.g.*, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., *Nature* 352: 624-628 (1991) and Marks et al., *J. Mol. Biol.* 222: 581-597 (1991), for example.

[0037] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad Sci. USA* 81: 6851-6855 (1984)).

[0038] As used herein, the term "single-chain Fv" or "scFv" antibody refers to antibody fragments comprising the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun, THE PHARMACOLOGY OF MONOCLONAL ANTIBODIES, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

[0039] As used herein, the term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, *e.g.*, EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

[0040] As used herein, the term "humanized antibody" refers to forms of antibodies that contain sequences from non-human (*e.g.*, murine) antibodies as well as human antibodies. Such antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all

or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

[0041] As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and residues 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk, *J. Mol. Biol.* 196: 901-917 (1987)). As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

[0042] As used herein, the term "conservative substitution" refers to substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (*see, e.g.*, Watson, *et al.*, *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Edition 1987)). Such exemplary substitutions are preferably made in accordance with those set forth in TABLE 1 as follows:

TABLE 1

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys
Asn (N)	Gln; His
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; Gln; Glu
Met (M)	Leu; Tyr; Ile
Phe (F)	Met; Leu; Tyr
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

[0043] Other substitutions are also permissible and may be determined empirically or in accord with known conservative substitutions.

[0044] As used herein, the term "isolated nucleic acid molecule" refers to a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the antibody nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

[0045] The expression "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0046] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0047] As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

[0048] As used herein, "polymerase chain reaction" or "PCR" refers to a procedure or technique in which minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in, *e.g.*, U.S. Pat. No. 4,683,195. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers can coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. *See generally* Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.* 51:263 (1987); Erlich, ed., PCR TECHNOLOGY (Stockton Press, N.Y., 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying

a nucleic acid test sample comprising the use of a known nucleic acid as a primer and a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid.

[0049] As used herein, the term "germline sequence" refers to a sequence of unrearranged immunoglobulin DNA sequences. Any suitable source of unrearranged immunoglobulin may be used.

[0050] As used herein, the term "immunosuppressive agent" refers to natural or synthetic agents that suppress or modulate an immune response. The immune response can be a humoral or cellular response.

B. IL-10 specific antibodies

[0051] The compositions and methods disclosed herein relate to the modulation of IL-10 activity, particularly in immune responses. Specifically, the compositions and methods herein employ antibodies specific for the cytokine, IL-10. IL-10 is a potent cytokine that modulates T and B cell responses through the regulation of growth, differentiation, and cytokine synthesis of a variety of cell types involved in immune responses. Notably, IL-10 production is frequently associated with autoimmune diseases and pathogen-induced immunopathology. Therefore, a composition, and methods thereof, that modulates and inhibits IL-10 activity can alter the development and sustainment of autoimmune disease and related symptoms and ameliorate or reduce pathogen-associated immunopathology.

[0052] Targeting IL-10 activity with humanized antibodies offers several unique advantages. First, targeting IL-10 with antibody permits a specific suppression of IL-10 activity while leaving the remainder of the immune response intact. In many cases of pathogen-induced immunopathology, the reduction or elimination of IL-10 activity should permit the desired effector immune response to eliminate with pathogen without further pathology. For the autoimmune patient, the reduction or elimination of IL-10 activity should reduce or eliminate the disease and/or its symptoms while maintaining the patient's immune competence. Second, humanized IL-10 antibodies circumvents the limitation associated with immunogenic rodent antibodies. The use of human sequences eliminates the immunogenicity of the exogenously administered antibodies, allowing therapeutic administration.

[0053] Humanized antibodies contain sequences from non-human as well as human antibodies. Typically, the process of humanization begins with the generation of a non-human antibody that has the desired biological activity, *i.e.*, inhibits IL-10 activity. Once a non-human antibody with the appropriate characteristics is identified, recombinant means are then employed to create a hybrid sequence using non-human and human sequences.

C. Generation of IL-10 Specific Antibodies

[0054] Any suitable method for generating monoclonal antibodies may be used. For example, a recipient may be immunized with IL-10 or a fragment thereof. Any suitable method of immunization can be used. Such methods can include adjuvants, other immunostimulants, repeated booster immunizations, and the use of one or more immunization routes.

[0055] Any suitable source of IL-10 can be used as the immunogen for the generation of the non-human antibody of the compositions and methods disclosed herein. Such forms include, but are not limited whole protein, peptide(s), and epitopes, generated through recombinant, synthetic, chemical or enzymatic degradation means known in the art. IL-10 is an acid-sensitive, noncovalent homodimer of two interpenetrating polypeptide chain. The cytokine is 160 amino acids in length with well conserved sequences that include an α-helical bundle structure similar to interferons and hemopoietic cytokines. Human and murine IL-10 have 73% amino acid homology, with human IL-10 being active on murine and human cells. IL-10 is commercially available or can be produced using well known molecular biology techniques. Genbank cDNA sequences are available for the human, pig-tailed macaque, mangabey, rhesus, and owl monkeys, lemur, mouse, rat, guinea pig, Syrian hamster, rabbit, cat, dog, as well as others. Recombinant human IL-10 is a 17-18 kDa polypeptide that is not N-glycosylated.

[0056] Any form of the antigen can be used to generate the antibody that is sufficient to generate a biologically active antibody. Thus, the eliciting antigen may be a single epitope, multiple epitopes, or the entire protein alone or in combination with one or more immunogenicity enhancing agents known in the art. The eliciting antigen may be an isolated full-length protein, a cell surface protein (*e.g.*, immunizing with cells transfected with at least a portion of the antigen), or a soluble protein (*e.g.*, immunizing with only the

extracellular domain portion of the protein). The antigen may be produced in a genetically modified cell. The DNA encoding the antigen may genomic or non-genomic (*e.g.*, cDNA) and encodes at least a portion of the extracellular domain. As used herein, the term "portion" refers to the minimal number of amino acids or nucleic acids, as appropriate, to constitute an immunogenic epitope of the antigen of interest. Any genetic vectors suitable for transformation of the cells of interest may be employed, including but not limited to adenoviral vectors, plasmids, and non-viral vectors, such as cationic lipids.

[0057] Any suitable method can be used to elicit an antibody with the desired biologic properties to inhibit IL-10. It is desirable to prepare monoclonal antibodies (mAbs) from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, *e.g.*, Stites, et al. (eds.) BASIC AND CLINICAL IMMUNOLOGY (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) ANTIBODIES: A LABORATORY MANUAL CSH Press; Goding (1986) MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY. Thus, monoclonal antibodies may be obtained by a variety of techniques familiar to researchers skilled in the art. Typically, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell. *See* Kohler and Milstein (1976) *Eur. J. Immunol.* 6:511-519. Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. *See, e.g.*, Doyle, et al. (eds. 1994 and periodic supplements) CELL AND TISSUE CULTURE: LABORATORY PROCEDURES, John Wiley and Sons, New York, NY. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according, *e.g.*, to the general protocol outlined by Huse, et al. (1989) *Science* 246:1275-1281.

[0058] Other suitable techniques involve selection of libraries of antibodies in phage or similar vectors. *See, e.g.*, Huse et al., *Science* 246:1275-1281 (1989); and Ward et al., *Nature* 341:544-546 (1989). The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies.

Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, *see* Cabilly U.S. Patent No. 4,816,567; and Queen et al. (1989) *Proc. Nat'l Acad. Sci. USA* 86:10029-10033; or made in transgenic mice, see Mendez et al. (1997) *Nature Genetics* 15:146-156; also see Abgenix and Medarex technologies.

[0059] Antibodies or binding compositions against predetermined fragments of IL-10 can be raised by immunization of animals with conjugates of the polypeptide, fragments, peptides, or epitopes with carrier proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective IL-10. These monoclonal antibodies will usually bind with at least a K_d of about 1 μM, more usually at least about 300 nM, typically at least about 30 nM, preferably at least about 10 nM, more preferably at least about 3 nM or better, usually determined by ELISA. Suitable non-human antibodies may also be identified using the biologic assays described in Section D *infra*.

C. Humanization of IL-10 Specific Antibodies

[0060] Any suitable non-human antibody can be used as a source for the hypervariable region. Sources for non-human antibodies include, but are not limited to, murine, lupine, bovine, and primates. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody

performance of the desired biological activity. For further details, see Jones et al., *Nature* 321: 522-525 (1986); Reichmann et al., *Nature* 332: 323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2: 593-596 (1992).

[0061] Methods for recombinantly engineering antibodies have been described, *e.g.*, by Boss et al. (U.S. Pat. No. 4,816,397), Cabilly et al. (U.S. Pat. No. 4,816,567), Law et al. (European Patent Application Publication No. 438 310) and Winter (European Patent Application Publication No. 239 400).

[0062] Amino acid sequence variants of humanized anti-IL-10 antibody are prepared by introducing appropriate nucleotide changes into the humanized anti-IL-10 antibody DNA, or by peptide synthesis. Such variants include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences shown for the humanized anti-IL-10 F(ab) (*e.g.* as in SEQ ID NO's 5 and 10). Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the humanized anti-IL-10 antibody, such as changing the number or position of glycosylation sites.

[0063] A useful method for identification of certain residues or regions of the humanized anti-IL-10 antibody polypeptide that are preferred locations for mutagenesis is called "alanine scanning mutagenesis," as described by Cunningham and Wells, *Science* 244: 1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with IL-10 antigen. The amino acid residues demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed humanized anti-IL-10 antibody variants are screened for the desired activity.

[0064] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues.

Examples of terminal insertions include humanized anti-IL-10 antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the humanized anti-IL-10 antibody molecule include the fusion to the N- or C-terminus of humanized anti-IL-10 antibody of an enzyme or a polypeptide which increases the serum half-life of the antibody.

[0065] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the humanized anti-IL-10 antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable loops, but FR alterations are also contemplated. Tables 2 and 3 in the method described below provides guidance as to hypervariable region residues which can be altered. Hypervariable region residues or FR residues involved in antigen binding are generally substituted in a relatively conservative manner.

[0066] Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0067] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0068] Nucleic acid molecules encoding amino acid sequence variants of humanized IL-10 specific antibody are prepared by a variety of methods known in the art. These

methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of humanized anti-IL-10 antibody.

[0069] Ordinarily, amino acid sequence variants of the humanized anti-IL-10 antibody will have an amino acid sequence having at least 75% amino acid sequence identity with the original humanized antibody amino acid sequences of either the heavy or the light chain (*e.g.* as in SEQ ID NO:5 and 10), more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the humanized anti-IL-10 residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence shall be construed as affecting sequence identity or homology.

[0070] The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA, and IgE. Preferably, the antibody is a IgG antibody. Any isotype of IgG can be used, including IgG_1, IgG_2, IgG_3, and IgG_4. Variants of the IgG isotypes are also contemplated. The humanized antibody may comprise sequences from more than one class or isotype. Optimization of the necessary constant domain sequences to generate the desired biologic activity is readily achieved by screening the antibodies in the biological assays described below.

[0071] Likewise, either class of light chain can be used in the compositions and methods herein. Specifically, kappa, lambda, or variants thereof are useful in the present compositions and methods.

[0072] Any suitable portion of the CDR sequences from the non-human antibody can be used. The CDR sequences can be mutagenized by substitution, insertion or deletion of at least one residue such that the CDR sequence is distinct from the human and non-human antibody sequence employed. It is contemplated that such mutations would be minimal. Typically, at least 75% of the humanized antibody residues will correspond to

those of the non-human CDR residues, more often 90%, and most preferably greater than 95%.

[0073] Any suitable portion of the FR sequences from the human antibody can be used. The FR sequences can be mutagenized by substitution, insertion or deletion of at least one residue such that the FR sequence is distinct from the human and non-human antibody sequence employed. It is contemplated that such mutations would be minimal. Typically, at least 75% of the humanized antibody residues will correspond to those of the human FR residues, more often 90%, and most preferably greater than 95%.

[0074] CDR and FR residues are determined according to the standard sequence definition of Kabat. Kabat et al., Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda Md. (1987).

[0075] Provided herein is a method to identify an acceptor germline sequence for a humanized antibody, which method comprises the steps of: a) identifying a non-human antibody that has the desired biological activity; b) determining the amino acid sequence of a non-human antibody V_H and V_L domains; and c) comparing the nonhuman antibody sequence to a group of human germline sequences, wherein the comparison comprises the substeps of: 1) assigning the non-human V sequences residue numbers according to Kabat *supra*; 2) delineating the CDR and FR regions in the sequence according to Kabat *supra*; 3) assigning a predetermined numerical score at specific residue position for which the non-human and human antibody germline sequences are identical; and 4) totaling all of the residue scores to generate a total score for each human germline sequence; and d) identifying the human germline sequence with the highest total residue score as the acceptor germline sequence. In one embodiment, the method further comprises the substeps of: 5) assigning a numerical score of 1 for each FR residue position for which the non-human and human antibody germline sequences are identical that was not scored in substep (3) to germline sequences with identical total residue scores after substep (4); 6) totaling all of the residue scores to generate a total score for each human germline sequence. In a specific embodiment, the non-human antibody is specific for IL-10 and inhibits the biological activity of IL-10. Also provided herein is an antibody generated by the above method.

[0076] In one embodiment, the IL-10 antibody is humanized using the following method. First, the non-human V_L and V_H domains of the IL-10 antibody are cloned and sequenced, and the amino acid sequence determined. Then, the non-human V_H sequence are

compared to a group of five human V_H germline amino acid sequences. The five groups contain one representative from the subgroups IGHV1 and IGHV4 and three representatives from subgroup IGHV3. The V_H subgroups are listed in M.-P. Lefranc, *Exp. Clin. Immunogenetics*, 18:100-116 (2001). Specifically, the comparison with the five germline sequences begins with the assignment of residue numbers to the non-human V_H sequence according to the Kabat numbering system. *See* Kabat, et al., U. S. Department of Health and Human Services, NIH Pub. 91-3242 (5th Ed., 1991). The non-human V_H sequence are then aligned with each of the five human germline sequences. Since the V genes only comprise V_H residues 1-94, only these residues are considered in the alignment. Next, the complementarity-determining (CDR) and framework (FR) regions in the sequence are delineated. CDR and FR are delineated according to the combination of the definitions provided in Kabat, et al., U. S. Department of Health and Human Services, NIH Pub. 91-3242 (5th Ed., 1991), and C. Chothia & A.M. Lesk, *J. Mol. Biol.*, 196:901-917 (1987). Therefore, the CDR definition used is residues 26-35 for CDR1, residues 50-65 for CDR2, and CDR3 is residues 95-102 for CDR3 of the V_H domain. The next step involves assigning a numerical score at identified residue position where the non-human and human sequences are identical. One example of this scoring is shown in Table 2 below.

Table 2

Residue #	Score	Reason
2	4	Affects CDR-H1,3*
4	3	Affects CDR-H1,3
24	3	Affects CDR-H1
26	4	Affects CDR-H1*
27	4	Affects CDR-H1,3*
29	4	Affects CDR-H1*
34	4	Affects CDR-H1*
35	2	VH/VL interface
37	2	VH/VL interface
39	2	VH/VL interface
44	2	VH/VL interface
45	2	VH/VL interface
47	4	VH/VL interface, CDR-L3
48	3	Affects CDR-H2
49	3	Affects CDR-H2
50	2	VH/VL interface

51	3	Affects CDR-H2
58	2	VH/VL interface
59	3	Affects CDR-H2
60	2	VH/VL interface
63	3	Affects CDR-H2
67	3	Affects CDR-H2
69	3	Affects CDR-H2
71	4	Affects CDR-H2*
73	3	Affects CDR-H1
76	3	Affects CDR-H1
78	3	Affects CDR-H1
91	2	VH/VL interface
93	3	Affects CDR-H3
94	4	Affects CDR-H3*

max 89

* Noted as affecting CDR conformation in C. Chothia et al., *Nature* 342:877-883, (1989).

[0077] After the residue positions are assigned a numerical score, all of the residue scores are totaled. The acceptor germline sequence is the one with the highest total score. In a case where two or more germline sequences have identical scores, then add 1 to the total for each position where the non-human and human sequences are IDENTICAL for the following residues: 1, 3, 5-23, 25, 36, 38, 40-43, 46, 66, 68, 70, 72, 74, 75, 77, 79-90, and 92 (max 49). The residue scores are totaled again, and the acceptor germline sequence is the one with the highest total score. If two or more germline sequences still have identical scores, either one can be used as the acceptor germline sequence.

[0078] If the V_L sequence is a member of the kappa subclass of V_L, the non-human V_L sequence from the IL-10 specific antibody is compared to a group of four human V_L kappa germline amino acid sequences. The four sequences are comprised of one representative from each of four established human V_L subgroups listed in V. Barbie & M.-P. Lefranc, *Exp. Clin. Immunogenetics* 15:171-183 (1998) and M.-P. Lefranc, *Exp. Clin. Immunogenetics* 18:161-174 (2001). The four sequences also correspond to the four subgroups listed in Kabat et al., U. S. Department of Health and Human Services, NIH Pub. 91-3242, pp. 103-130 (5th Ed., 1991). The comparison of the non-human sequence to the four germline sequences begins with the assignment of residue numbers to the non-human V_L sequence residues according to Kabat et al., U. S. Department of Health and Human Services, NIH Pub. 91-3242 (5th Ed., 1991). The non-human V_L sequences are then aligned with each of the four human germline sequences. Since the V genes only comprise V_L

residues 1-95, only these residues are considered in the alignment. Next, the complementarity-determining (CDR) and framework (FR) regions are delineated in the sequence. CDR and FR are delineated according to the combination of the definitions provided in Kabat et al., U. S. Department of Health and Human Services, NIH Pub. 91-3242 (5th Ed. 1991), and C. Chothia & A.M. Lesk, *J. Mol. Biol.*, 196:901-917 (1987). Therefore, the CDR definition used is residues 24-34 for CDR1, residues 50-56 for CDR2, and residues 89-97 for CDR3 of the V_L domain. The next step involves assigning a numerical score at identified residue position where the non-human and human sequences are identical. One example of this scoring is shown in Table 3 below.

Table 3

	Residue #	Score	Reason
	2	4	Affects CDR-L1,3*
	4	3	Affects CDR-L1,3
	25	4	Affects CDR-L1*
	29	4	Affects CDR-L1,3*
	33	4	Affects CDR-L1,3*
	34	2	VL/VH interface
	36	2	VL/VH interface
	38	2	VL/VH interface
	43	2	VL/VH interface
	44	2	VL/VH interface
	46	4	VL/VH interface, CDR-H3
	47	3	Affects CDR-L2
	48	4	Affects CDR-L2*
	49	2	VL/VH interface
	55	2	VL/VH interface
	58	3	Affects CDR-L2
	62	3	Affects CDR-L2
	64	4	Affects CDR-L2*
	71	4	Affects CDR-L1*
	87	2	VL/VH interface
	89	2	VL/VH interface
	90	4	Affects CDR-L3*
	91	2	VL/VH interface
	94	2	VL/VH interface
	95	4	Affects CDR-L3*

* Noted as affecting CDR conformation in C. Chothia et al., *Nature* 342:877-883, (1989).

[0079] After the residue positions are assigned a numerical score, all of the residue scores are totaled. The acceptor germline sequence is the one with the highest total score.

In a case where two or more germline sequences have identical scores, then add 1 to the total for each position where the non-human and human sequences are IDENTICAL for the following residues: 1, 3, 5-23, 35, 37, 39-42, 57, 59-61, 63, 65-70, 72-86, and 88. The residue scores are totaled again, and the acceptor germline sequence is the one with the highest total score. If two or more germline sequences still have identical scores, either one can be used as the acceptor germline sequence.

[0080] For recombinant production of the antibody, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

[0081] In one embodiment, the antibody is a humanized recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising: at least one antibody light chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:1 (KTSQNIFENLA) at CDR1, SEQ ID NO:2 (NASPLQA) at CDR2, and SEQ ID NO:3 (HQYYSGYT) at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence; and at least one antibody heavy chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:6 (GFTFSDYHMA) at CDR1, SEQ ID NO:7 (SITLDATYTYYRDSVRG) at CDR2, SEQ ID NO:8 (HRGFSVWLDY) at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence. In a specific embodiment, the antibody light chain, or binding fragment thereof, comprises a polypeptide having a variable region of SEQ ID NO:4 (DIQMTQSPSSLSASVGDRVTITCKTSQNIFENLAWYQQKPGKAPKLLIY NASPLQAGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYYSG-YTFGPGTKLELKRT). In one specific embodiment, the antibody light chain, or binding

fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:5. *See* Table 4. In one specific embodiment, the antibody heavy chain, or binding fragment thereof, comprises a polypeptide having a variable region of SEQ ID NO:9 (QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYHMAWV RQAPGKGLEWVASITLDATYTYYRDSVRGRFTISRDNSKNTLYLQMNSLRAEDTAV YYCARHRGFSVWLDYWGQGTLVTVSS). In another specific embodiment, the antibody heavy chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:10. *See* Table 5.

[0082] Plasmids containing the nucleic acids encoding the humanized 12G8 light and heavy chains were deposited with the American Type Culture Collection (ATCC) as deposit numbers PTA-5923 and PTA-5922, respectively.

Table 4. Full length sequences for light chain of the humanized 12G8 antibody

SEQ ID NO:5 Full length amino acid sequence of humanized 12G8 antibody
SEQ ID NO:19 Full length nucleic acid sequence of humanized 12G8 antibody

--> variable light domain

```
 D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R   V   T   I   T
GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT

 C   K   T   S   Q   N   I   F   E   N   L   A   W   Y   Q   Q   K   P   G   K   A   P
TGC AAG ACA AGT CAG AAC ATT TTT GAG AAC TTG GCC TGG TAT CAG CAG AAA CCA GGG AAA GCC CCT

 K   L   L   I   Y   N   A   S   P   L   Q   A   G   V   P   S   R   F   S   G   S   G
AAG CTC CTG ATC TAT AAT GCA AGC CCT TTG CAA GCG GGG GTC CCA TCA AGG TTC AGT GGC AGT GGA

 S   G   T   D   F   T   L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C
TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGT CTG CAA CCT GAA GAT TTT GCA ACT TAC TAC TGT

                                                                        --> human
 H   Q   Y   Y   S   G   Y   T   F   G   P   G   T   K   L   E   L   K   R   T   V   A
CAC CAG TAT TAT AGC GGG TAC ACG TTT GGA CCT GGG ACC AAG CTG GAA CTG AAA CGT ACG GTG GCT
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light constant domain

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 A   P   S   V   F   I   F   P   P   S   D   E   Q   L   K   S   G   T   A   S   V   V
GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG

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

 S   G   N   S   Q   E   S   V   T   E   Q   D   S   K   D   S   T   Y   S   L   S   S
TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC

 T   L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A   C   E   V   T   H   Q
ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG

 G   L   S   S   P   V   T   K   S   F   N   R   G   E   C
GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT
```

Table 5. Full length sequences for heavy chain of the humanized 12G8 antibody

SEQ ID NO: 10 Full length amino acid sequence of humanized 12G8 antibody
SEQ ID NO: 20 Full length nucleic acid sequence of humanized 12G8 antibody

```
 Q  V  Q  L  V  E  S  G  G  G  V  V  Q  P  G  R  S  L  R  L  S  C
CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG AGA CTC TCC TGT

 A  A  S  G  F  T  F  S  D  Y  H  M  A  W  V  R  Q  A  P  G  K  G
GCA GCC TCT GGA TTC ACT TTC AGT GAC TAT CAT ATG GCC TGG GTC CGC CAG GCT CCA GGC AAG GGG

 L  E  W  V  A  S  I  T  L  D  A  T  Y  T  Y  Y  R  D  S  V  R  G
CTG GAG TGG GTG GCA AGC ATT ACT CTT GAT GCT ACC TAC ACT TAC TAT CGC GAC TCC GTG CGC GGC

 R  F  T  I  S  R  D  N  S  K  N  T  L  Y  L  Q  M  N  S  L  R  A
CGC TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCT

 E  D  T  A  V  Y  Y  C  A  R  H  R  G  F  S  V  W  L  D  Y  W  G
GAG GAC ACG GCT GTG TAT TAC TGT GCG AGA CAT CGA GGC TTT AGC GTC TGG CTT GAT TAC TGG GGC

                                    --> human IgG1 constant heavy domains
 Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P
CAA GGC ACC CTG GTC ACC GTC TCG TCG GCT AGC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC

 S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E
TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA

 P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA

 Q  S  S  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q
CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG

 T  Y  I  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG CCC AAA

 S  C  D  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V
TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC

 F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V
TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG

 V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V
GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG

 H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC

 T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L
ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC
```

```
 P   A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E   P   Q   V   Y   T
CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC

 L   P   P   S   R   D   E   L   T   K   N   Q   V   S   L   T   C   L   V   K   G   F
CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC

 Y   P   S   D   I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T
TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG

 P   P   V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V   D   K   S   R
CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG

 W   Q   Q   G   N   V   F   S   C   S   V   M   H   E   A   L   H   N   H   Y   T   Q
TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG

 K   S   L   S   L   S   P   G   K
AAG AGC CTC TCC CTG TCT CCG GGT AAA
```

[0083] In one embodiment, the antibody is a humanized recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising: at least one antibody light chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:11 (RASESVDDYGHSFMH)at CDR1, SEQ ID NO:12 (RASTLES) at CDR2, and SEQ ID NO:13 (QQGNEDPWT) at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence; and at least one antibody heavy chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:15 (GFSLTNYGVH) at CDR1, SEQ ID NO:16 (VIWSGGSTDYNAAFIS) at CDR2, and SEQ ID NO:17 (NRGYDVYFDY) at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence. In one specific embodiment, the antibody light chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:14. *See* Table 6. In yet another specific embodiment, the antibody heavy chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:18. *See* Table 7.

[0084] Plasmids containing the humanized 11D8 heavy and light chains were deposited with the ATCC as deposit numbers PTA-5926 and PTA-5927, respectively.

Table 6. Full length sequences for light chain of the humanized 11D8 antibody

SEQ ID NO: 14 Full length amino acid sequence for humanized 11D8 antibody
SEQ ID NO: 21 Full length nucleotide sequence for humanized 11D8 antibody

```
 E   I   V   L   T   Q   S   P   G   T   L   S   L   S   P   G   E   R   A   T
GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA AGA GCC ACC

 L   S   C   R   A   S   E   S   V   D   D   Y   G   H   S   F   M   H   W   Y
CTC TCC TGC AGA GCC AGT GAA AGT GTT GAT GAT TAT GGC CAT AGT TTT ATG CAC TGG TAC

 Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   R   A   S   T   L   E   S
CAG CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT CGT GCA TCC ACC CTA GAA TCT

 G   I   P   D   R   F   S   G   S   G   S   G   T   D   F   T   L   T   I   S
GGC ATC CCA GAC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT CTC ACC ATC AGC

 R   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q   G   N   E   D   P   W
AGA CTG GAG CCT GAA GAT TTT GCA GTG TAT TAC TGT CAG CAA GGT AAT GAG GAT CCG TGG

 T   F   G   Q   G   T   K   V   E   I   K   R   T   V   A   A   P   S   V   F
ACG TTC GGT CAA GGT ACC AAG GTG GAA ATC AAG CGT ACG GTG GCT GCA CCA TCT GTC TTC

 I   F   P   P   S   D   E   Q   L   K   S   G   T   A   S   V   V   C   L   L
ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG

 N   N   F   Y   P   R   E   A   K   V   Q   W   K   V   D   N   A   L   Q   S
AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG

 G   N   S   Q   E   S   V   T   E   Q   D   S   K   D   S   T   Y   S   L   S
GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC

 S   T   L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A   C   E   V
AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC

 T   H   Q   G   L   S   S   P   V   T   K   S   F   N   R   G   E   C
ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT
```

Table 7. Full length sequences for heavy chain of the humanized 11D8 antibody

SEQ ID NO: 18 Full length amino acid sequence for humanized 11D8 antibody
SEQ ID NO: 22 Full length nucleotide sequence for humanized 11D8 antibody

```
 Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S
CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC

 L   R   L   S   C   A   A   S   G   F   S   L   T   N   Y   G   V   H   W   V
CTG AGA CTC TCC TGT GCA GCC TCT GGT TTC TCA TTA ACA AAC TAT GGT GTA CAC TGG GTC

 R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   S   G   G   S   T
CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTG ATA TGG AGT GGT GGA AGC ACA

 D   Y   N   A   A   F   I   S   R   F   T   I   S   R   D   N   S   K   N   T
GAC TAT AAT GCA GCT TTC ATA TCC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG

 L   Y   L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   R
CTG TAT CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT GCG AGA

 N   R   G   Y   D   V   Y   F   D   Y   W   G   Q   G   T   L   V   T   V   S
AAT AGG GGG TAC GAC GTC TAC TTT GAC TAC TGG GGC CAA GGC ACC CTT GTC ACA GTC TCG

 S   A   S   T   K   G   P   S   V   F   P   L   A   P   S   S   K   S   T   S
TCG GCT AGC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT
```

```
 G   G   T   A   A   L   G   C   L   V   K   D   Y   F   P   E   P   V   T   V
GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG

 S   W   N   S   G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S
TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC

 S   G   L   Y   S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   Q
TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG

 T   Y   I   C   N   V   N   H   K   P   S   N   T   K   V   D   K   K   V   E
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG

 P   K   S   C   D   K   T   H   T   C   P   P   C   P   A   P   E   L   L   G
CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG

 G   P   S   V   F   L   F   P   P   K   P   K   D   T   L   M   I   S   R   T
GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC

 P   E   V   T   C   V   V   V   D   V   S   H   E   D   P   E   V   K   F   N
CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC

 W   Y   V   D   G   V   E   V   H   N   A   K   T   K   P   R   E   E   Q   Y
TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC

 N   S   T   Y   R   V   V   S   V   L   T   V   L   H   Q   D   W   L   N   G
AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC

 K   E   Y   K   C   K   V   S   N   K   A   L   P   A   P   I   E   K   T   I
AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC

 S   K   A   K   G   Q   P   R   E   P   Q   V   Y   T   L   P   P   S   R   D
TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT

 E   L   T   K   N   Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D
GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC

 I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K   T   T   P   P
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC

 V   L   D   S   D   G   S   F   F   L   Y   S   K   L   T   V   D   K   S   R
GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG

 W   Q   Q   G   N   V   F   S   C   S   V   M   H   E   A   L   H   N   H   Y
TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC

 T   Q   K   S   L   S   L   S   P   G   K
ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
```

[0085] In one embodiment, the antibodies described *supra* further comprise a heavy chain constant region, wherein the heavy chain constant region comprises a γ1, γ2, γ3, or γ4 human heavy chain constant region or a variant thereof. In one embodiment, the antibodies described above further comprise a light chain constant region, wherein the light chain constant region comprises a lambda or a kappa human light chain constant region. In some embodiments, the binding fragment of these antibodies is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv, $F(ab')_2$, and a diabody.

[0086] Also provided herein is a chimeric recombinant antibody molecule that binds IL-10 or binding fragment thereof, comprising: at least one antibody light chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:1 at CDR1, SEQ ID NO:2 at CDR2, and SEQ ID NO:3 at CDR3; and at least one antibody heavy chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:6 at CDR1, SEQ ID NO:7 at CDR2, and SEQ ID NO:8 at CDR3.

[0087] In a specific embodiment, the chimeric recombinant antibody molecule has a light chain as set forth in SEQ ID NO: 23 and a heavy chain as set forth in SEQ ID NO:24. *See* Table 8. Nucleic acids encoding the 12G8 chimeric antibody light and heavy chains were deposited at the ATCC as deposit numbers PTA-5925 and PTA-5924, respectively.

Table 8. Sequences of the chimeric 12G8 anti-human IL-10 antibody

SEQ ID NO: 23 Amino acid sequence of light chain
SEQ ID NO: 25 Nucleic acid sequence of light chain

--> signal sequence

```
 M   A   P   V   Q   L   L   G   L   L   V   L   F   L   P   A   M   R   C
ATG GCT CCA GTT CAA CTT TTA GGG CTT TTG GTG CTC TTC CTC CCA GCC ATG AGA TGT
```

mature IgG
--> rat 12G8 light variable domain

```
 D   I   Q   M   T   Q   S   P   S   L   L   S   A   S   V   G   D   R   V   T   L   N
GAC ATC CAG ATG ACC CAG TCT CCT TCA CTC CTG TCT GCA TCT GTG GGA GAC AGA GTC ACT CTC AAC

 C   K   T   S   Q   N   I   F   E   N   L   A   W   Y   Q   Q   K   L   R   E   P   P
TGC AAG ACA AGT CAG AAC ATT TTT GAG AAC TTG GCC TGG TAT CAG CAA AAG CTT AGA GAA CCT CCC

 K   L   L   I   F   N   A   S   P   L   Q   A   G   I   P   S   R   F   S   G   S   G
AAA CTC CTG ATT TTT AAT GCA AGC CCT TTG CAA GCG GGC ATC CCT TCA AGG TTC AGT GGC AGT GGA

 S   G   T   D   F   T   L   T   I   T   S   L   Q   P   E   D   V   A   T   Y   F   C
TCT GGT ACA GAT TTC ACA CTC ACC ATC ACC AGC CTG CAG CCT GAG GAT GTT GCC ACA TAT TTC TGC
```

--> human

H Q Y Y S G Y T F G P G T K L E L K R T V A
CAC CAG TAT TAT AGC GGG TAC ACG TTT GGA CCT GGG ACC AAG CTG GAA CTG AAA CGT ACG GTG GCT

constant light domain

A P S V F I F P P S D E Q L K S G T A S V V
GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG

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

S G N S Q E S V T E Q D S K D S T Y S L S S
TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC

T L T L S K A D Y E K H K V Y A C E V T H Q
ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG

G L S S P V T K S F N R G E C
GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAA

SEQ ID NO: 24 Amino acid sequence of heavy chain
SEQ ID NO: 26 Nucleic acid sequence of heavy chain

M D I R L S L V F L V L F M K D V Q C
ATG GAC ATC AGG CTC AGC TTG GTT TTC CTT GTC CTT TTT ATG AAA GAT GTC CAG TGT

mature IgG
--> rat 12G8 variable heavy domain

E V Q L V E S G G G L V R P G G S L R L S C
GAG GTG CAG TTG GTG GAG TCT GGA GGA GGC TTG GTG CGG CCT GGA GGG TCC CTG AGA CTC TCC TGT

T A S G F T F S D Y H M A W V R Q S P D K G
ACA GCC TCA GGA TTC ACT TTC AGT GAC TAT CAC ATG GCC TGG GTC CGC CAG TCT CCA GAC AAG GGT

L E W V A S I T L D A T Y T Y Y R D S V R G
CTG GAG TGG GTC GCA AGC ATT ACT CTT GAT GCT ACC TAC ACT TAC TAT CGC GAC TCC GTG AGG GGC

R F T I S R N N A K T T L Y L Q M D S L R S
CGA TTC ACC ATC TCC CGA AAT AAT GCA AAA ACC ACC CTT TAC CTG CAA ATG GAC AGT CTG AGG TCT

E D T A T F Y C T R H R G F S V W L D Y W G
GAG GAC ACG GCC ACT TTT TAC TGT ACA AGA CAT CGA GGC TTT AGC GTC TGG CTT GAT TAC TGG GGC

--> human IgG1 heavy chain

Q G V M V T V S S A S T K G P S V F P L A P
CAA GGA GTC ATG GTC ACT GTC TCT TCA GCT AGC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC

S S K S T S G G T A A L G C L V K D Y F P E
TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA

P V T V S W N S G A L T S G V H T F P A V L
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA

Q S S G L Y S L S S V V T V P S S S L G T Q
CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG

T Y I C N V N H K P S N T K V D K K V E P K
ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG CCC AAA

```
 S   C   D   K   T   H   T   C   P   P   C   P   A   P   E   L   L   G   G   P   S   V
TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC

 F   L   F   P   P   K   P   K   D   T   L   M   I   S   R   T   P   E   V   T   C   V
TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG

 V   V   D   V   S   H   E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V
GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG

 H   N   A   K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S   V   L
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC

 T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   K   A   L
ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC

 P   A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E   P   Q   V   Y   T
CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC
```

[0088] Further provided herein is a chimeric recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising: at least one antibody light chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:11 at CDR1, SEQ ID NO:12 at CDR2, and SEQ ID NO:13 at CDR3; and at least one antibody heavy chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:15 at CDR1, SEQ ID NO:16 at CDR2, and SEQ ID NO:17 at CDR3.

[0089] Further provided herein is an isolated nucleic acid encoding the polypeptide of the antibodies disclosed *supra*. Also provided herein is an expression vector comprising the isolated nucleic acid sequence operably linked to control sequences recognized by a host cell transfected with the vector. Provided herein is a host cell comprising the vector comprising the isolated nucleic acid sequence. Further provided herein is a method of producing a polypeptide, comprising culturing the host cell comprising the vector under conditions wherein the nucleic acid sequence is expressed, thereby producing the polypeptide, and recovering the polypeptide from the host cell.

[0090] Provided herein is an isolated nucleic acid sequence encoding an antibody specific for IL-10 comprising a light chain having the nucleic acid sequence of SEQ ID NO:19 and a heavy chain having the nucleic acid sequence of SEQ ID NO:20. *See* Tables 4 and 5.

[0091] Provided herein is an isolated nucleic acid sequence encoding an antibody specific for IL-10 comprising a light chain having the nucleic acid sequence of SEQ ID NO:21 and a heavy chain having the nucleic acid sequence of SEQ ID NO:22. *See* Tables 6 and 7.

[0092] Further provided herein is an isolated nucleic acid sequence encoding a binding fragment of the antibody encoded by the above nucleic acid sequences. In one embodiment, the binding fragment is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv, $F(ab')_2$, and a diabody.

[0093] Bispecific antibodies are also useful in the present methods and compositions. As used herein, the term "bispecific antibody" refers to an antibody, typically a monoclonal antibody, having binding specificities for at least two different antigenic epitopes. In one embodiment, the epitopes are from the same antigen. In another embodiment, the epitopes are from two different antigens. Methods for making bispecific antibodies are known in the art. For example, bispecific antibodies can be produced recombinantly using the co-expression of two immunoglobulin heavy chain/light chain pairs. *See, e.g.,* Milstein *et al., Nature* 305: 537-39 (1983). Alternatively, bispecific antibodies can be prepared using chemical linkage. *See, e.g.,* Brennan, *et al., Science* 229: 81 (1985). Bispecific antibodies include bispecific antibody fragments. *See, e.g.,* Hollinger, *et al., Proc. Natl. Acad. Sci. U.S.A.* 90: 6444-48 (1993), Gruber, *et al., J. Immunol.* 152: 5368 (1994).

D. Biological Activity of Humanized Anti-IL-10 Antibodies

[0094] Antibodies having the characteristics identified herein as being desirable in a humanized anti-IL-10 antibody can be screened for inhibitory biologic activity *in vitro* or suitable binding affinity. To screen for antibodies which bind to the epitope on human IL-10 bound by an antibody of interest (*e.g.*, those which block binding of the cytokine to its receptor), a routine cross-blocking assay such as that described in ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping, *e.g.*, as described in Champe et al., *J. Biol. Chem.* 270: 1388-1394 (1995), can be performed to determine whether the antibody binds an epitope of interest. Antibody affinities (*e.g.* for human IL-10) may be

determined using standard Scatchard analysis. Preferred humanized antibodies are those which bind human IL-10 with a K_d value of no more than about $1x10^{-7}$; preferably no more than about $1x10^{-8}$; more preferably no more than about $1x10^{-9}$; and most preferably no more than about $2x10^{-10}$.

[0095] The antibodies and fragments thereof useful in the present compositions and methods are biologically active antibodies and fragments. As used herein, the term "biologically active" refers to an antibody or antibody fragment that is capable of binding the desired the antigenic epitope and directly or indirectly exerting a biologic effect. Typically, these effects result from the failure of IL-10 to bind its receptor. As used herein, the term "specific" refers to the selective binding of the antibody to the target antigen epitope. Antibodies can be tested for specificity of binding by comparing binding to IL-10 to binding to irrelevant antigen or antigen mixture under a given set of conditions. If the antibody binds to IL-10 at least 10, and preferably 50 times more than to irrelevant antigen or antigen mixture then it is considered to be specific.

[0096] The inhibitory IL-10 specific antibody can inhibit its biological activity in any manner, including but not limited to production of IL-1, IFN-γ, PGE2, IL-12, TNF, CC and CXC chemokines, and the cell surface expression of MHC class II antigens, CD54, CD80, and CD86. The biologic activity of an IL-10 specific antibody can be determined by any useful method. *See, e.g.*, U.S. Patent Nos: 6,239,260 and 6,207,154. In one example, the biologic activity is assessed in cell proliferation assay using the murine mast cell line, MC9/2. *See* Thompson-Snipes et al., *J. Exp. Med.* 173:507-10 (1991). IL-10 stimulates the proliferation of this cell line, and therefore an inhibitory antibody can be identified by its ability to reduce proliferation. The ED_{50} for proliferation of the MC9/2 cell line is typically 0.5-1.0ng/mL. An antibody is inhibitory for proliferation if it inhibits the proliferation of cells relative to the proliferation of cells in the absence of the antibody or in the presence of a non-binding antibody. Proliferation may be quantified using any suitable methods. Typically, the proliferation is determined by assessing the incorporation of radioactive-labeled nucleotides into DNA (*e.g.*, ^{3}H-thymidine). In another embodiment, proliferation is determined by ATP luminescence. Preferably, the antibody useful in the present compositions inhibits 80% of IL-10's biologic activity, more preferably 95%, most preferably 99%.

E. Uses of Humanized Anti-IL-10 Antibodies

[0097] Provided herein is a method of suppressing an immune response in a human subject comprising administering to a subject in need thereof an antibody specific for IL-10, or a binding fragment thereof, in an amount effective to block the biological activity of IL-10, wherein the antibody is a humanized recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising: at least one antibody light chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:1 at CDR1, SEQ ID NO:2 at CDR2, and SEQ ID NO:3 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence; and at least one antibody heavy chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:6 at CDR1, SEQ ID NO:7 at CDR2, and SEQ ID NO:8 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence.

[0098] Further provided herein is a method of suppressing an immune response in a human subject comprising administering to a subject in need thereof an antibody specific for IL-10, or a binding fragment thereof, in an amount effective to block the biological activity of IL-10, wherein the antibody is a humanized recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising: at least one antibody light chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:11 at CDR1, SEQ ID NO:12 at CDR2, and SEQ ID NO:13 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence; and at least one antibody heavy chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:15 at CDR1, SEQ ID NO:16 at CDR2, and SEQ ID NO:17 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence.

[0100] The immune response suppressed by these methods is a humoral or a cellular response. The suppression of the humoral and cellular responses can be determined using

well known methods in the art. For example, in diseases associated with high levels of autoreactive antibodies, *e.g.*, SLE, a reduction in the serum levels of these antibodies relative to the pre-treatment serum levels is an indication of the suppression of the humoral response. Likewise, the suppression of the cellular immune response can be determined using well known *in vitro* analyses, *e.g.*, proliferation and cytotoxicity assays or characterization of activation phenotypes of peripheral blood lymphocytes by, *e.g.*, flow cytometric analysis. *See* CURRENT PROTOCOLS IN IMMUNOLOGY, most recent edition. In one embodiment, the subject treated by this method has systemic lupus erythematosus. In another embodiment, the subject has immune thrombocytopenic purpura (ITC). In yet another embodiment, the subject has lupus nephritis. In a further embodiment, the subject has HIV. In another embodiment, the subject has hepatitis C.

[0101] Provided herein is a composition comprising an antibody, or binding fragment thereof, in combination with a pharmaceutically acceptable carrier or diluent, wherein the antibody is one of the antibodies disclosed herein.

[0102] Any subject that would benefit from treatment with IL-10 specific antibodies can be treated using the compositions and methods provided herein. Any subject can be treated with the methods and compositions provided herein. Such a subject is a mammal, preferably a human, with an autoimmune disease or symptom or pathogen-induced immunopathology. In one specific embodiment, the subject has SLE, lupus nephritis, rheumatoid arthritis, ITC, HIV or hepatitis C. Veterinary uses of the disclosed methods and compositions are also contemplated.

[0103] As used herein, "inhibit" or "treat" or "treatment" includes a postponement of development of the symptoms associated with autoimmune disease or pathogen-induced immunopathology and/or a reduction in the severity of such symptoms that will or are expected to develop. The terms further include ameliorating existing uncontrolled or unwanted autoimmune-related or pathogen-induced immunopathology symptoms, preventing additional symptoms, and ameliorating or preventing the underlying causes of such symptoms. Thus, the terms denote that a beneficial result has been conferred on a vertebrate subject with an autoimmune or pathogen-induced immunopathology disease or symptom, or with the potential to develop such a disease or symptom.

[0104] As used herein, the term "therapeutically effective amount" or "effective amount" refers to an amount of an IL-10 specific antibody that when administered alone or

in combination with an additional therapeutic agent to a cell, tissue, or subject is effective to prevent or ameliorate the autoimmune disease or pathogen-induced immunopathology associated disease or condition or the progression of the disease. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

[0105] An antibody useful in the present methods (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be administered to a subject in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration.

[0106] The pharmaceutical composition of the invention may also contain other immunosuppressive or immunomodulating agents. Any suitable immunosuppressive agent can be employed, including but not limited to anti-inflammatory agents, corticosteroids, cyclosporine, tacrolimus (*i.e.*, FK-506), sirolimus, interferons, soluble cytokine receptors (*e.g.*, sTNRF and sIL-1R), agents that neutralize cytokine activity (*e.g.*, inflixmab, etanercept), mycophenolate mofetil, 15-deoxyspergualin, thalidomide, glatiramer, azathioprine, leflunomide, cyclophosphamide, methotrexate, and the like. The pharmaceutical composition can also be employed with other therapeutic modalities such as phototherapy and radiation.

[0107] In another embodiment, kits are provided that contain the necessary reagents to carry out the assays of the methods provided herein. Specifically provided herein is a compartment kit comprising one or more containers, wherein a first container comprises one

or more antibodies specific to IL-10, and one or more other containers comprising one or more of the following: reconstitution reagents, administration reagents. The containers can be glass, plastic, or strips of plastic or paper. In one embodiment, the kit also contain written instructions.

[0108] In practicing the methods of treatment or use provided herein, a therapeutically effective amount of antibody provided herein is administered to a mammal having a condition suitable for treatment with IL-10. The antibody may be administered in accordance with the methods herein either alone or in combination with other therapies such as treatments employing other immunomodulating factors (*e.g.*, cytokines), immunosuppressive agents, and the like. When co-administered with one or more biologically active agents, the antibody provided herein may be administered either simultaneously with the biologically active agent(s), or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with the biologically active agent(s).

[0109] Toxicity and therapeutic efficacy of the antibody compositions, administered alone or in combination with an immunosuppressive agent, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50}. Antibodies exhibiting high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

[0110] Techniques for formulation and administration of the antibodies of the instant methods may be found in REMINGTON'S PHARMACEUTICAL SCIENCES, Mack Publishing Co., Easton, Pa., latest edition. The mode of administration is not particularly important. Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous,

intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Administration of antibody used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral, intraarterial or intravenous injection. Intravenous administration to the patient is preferred.

[0111] Alternately, one may administer the antibody in a local rather than systemic manner, for example, via injection of the antibody directly into an arthritic joint or pathogen-induced lesion characterized by immunopathology, often in a depot or sustained release formulation. Furthermore, one may administer the antibody in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody, targeting, for example, arthritic joint or pathogen-induced lesion characterized by immunopathology. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

[0112] Pharmaceutical compositions for use in accordance with the present methods thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations that can be used pharmaceutically. These pharmaceutical compositions may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

[0113] When a therapeutically effective amount of antibody of the methods herein is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH,

isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0114] For administration by inhalation, the antibodies for use according to the present methods are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0115] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the

preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.,* sterile pyrogen-free water, before use.

[0116] The amount of antibody useful in the disclosed methods in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments that the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. It is to be expected that the dosage will vary according to the age, weight and response of the individual patient. Initially, the attending physician will administer low doses of antibodies of the present methods and observe the patient's response. Larger doses of antibodies of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the methods herein should contain about 0.01 μg to about 100 mg (preferably about 0.1 μg to about 10 mg, more preferably about 0.1 μg to about 1 mg) of antibody of the present invention per kg body weight. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Therapeutically useful agents other than an antibody of the present methods that may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. *See, e.g.,* Fingl *et al.,* THE PHARMACOLOGICAL BASIS OF THERAPEUTICS (latest edition). Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety sufficient to maintain the desired therapeutic effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data; for example, the concentration necessary to achieve 50-90% inhibition of biologic activity using the assays described herein.

[0117] The antibody provided herein can be administered alone or in combination with other therapeutic modalities. The antibody provided herein also can be administered

alone or in combination with other antibodies identified as inhibitors of IL-10 activity or other immunosuppressive agents.

[0118] Any disease where autoimmunity is implicated can be treated with the present methods. Preferably, autoimmune diseases targeted for treatment with IL-10 specific antibodies are characterized by abnormal IL-10 expression levels and/or a lack of appropriate cellular, i.e., Th1-mediated, responses. Such disease include, but are not limited to systemic lupus erythematosus (SLE), immune thrombocytopenic purpura (ITC), lupus nephritis, diabetes, insulin-dependent diabetes mellitus (IDDM), rheumatoid arthritis (RA).

[0119] Any disease where pathogen-induced immunopathology is implicated can be treated with the present methods. Preferably, pathogen-induced immunopathologies targeted for treatment with IL-10 specific antibodies are characterized by abnormal IL-10 expression levels and/or a lack of appropriate cellular, *i.e.*, Th1-mediated, responses. Such diseases include, but are not limited to HIV, hepatitis C, visceral leishmaniasis, malaria, filariasis, leprosy, tuberculosis, candidiasis, and *M. avium* infections.

[0120] The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

F. Examples

Example I. General Methods

[0121] Some of the standard methods are described or referenced, *e.g.*, in Maniatis, et al. (1982) MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) MOLECULAR CLONING: A LABORATORY MANUAL, (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., BIOLOGY, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene/Wiley, New York. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. *See, e.g.*,

Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in METH. ENZYMOL., vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, *e.g.*, Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments, *e.g.*, to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. *See, e.g.*, Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) GENETIC ENGINEERING, PRINCIPLE AND METHODS 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAEXPRESS: THE HIGH LEVEL EXPRESSION & PROTEIN PURIFICATION SYSTEM, Qiagen, Inc., Chatsworth, CA.

[0122] Computer sequence analysis is performed, *e.g.*, using available software programs, including those from the GCG (U. Wisconsin) and GenBank sources. Public sequence databases were also used, *e.g.*, from GenBank and others.

Example II. Humanization of anti-human IL-10 antibodies

[0123] The humanization of the rat anti-human IL-10 antibody, 12G8, was performed as described in Section C *supra*. Figure 1 shows the assignment of the assignment of residue numbers and corresponding numerical scores for residue positions that are identical to the germline sequences being examined. Calculations are shown for the 12G8 variable regions of the light (Figure 1A) and heavy (Figure 1B) of the 12G8 anti-human IL-10 antibody and for the variable regions of the light (Figure 1C) and heavy (Figure 1D) of the 11D8 anti-human IL-10 antibody.

Example III. Pharmacokinetics of 12G8, an anti-human IL-10 antibody

[0124] *Objective*: To obtain estimates of in-vivo terminal half-lives and subcutaneous bioavailability for the 12F8 antibody in a murine model.

[0125] *Antibody:* The antibody is administered in a vehicle of 10 mM Na acetate, 8% sucrose, pH 5.25.

[0126] *Mice:* Crl:CD-1®(ICR)BR female mice were purchased from Charles River Laboratories.

[0127] *Experimental Design:* Mice received a single bolus injection of antibody either intravenously (i.v. in lateral tail vein) or subcutaneously (s.c. at nape of neck or mid-

scapular or lateral flank). Antibody doses included 0.03, 0.3, 3.0, and 30 mg/kg per mouse. The mice were observed for up to 28 days post-injection. During this time period, mice were weighed and serum samples taken. Serum samples for the 12G8 (SCH 708980) groups (Groups 1-8) were taken at 0.5, 1, 3, 6, 10, 16 hrs, Day 1, 2, 3, 5, 7, 10, 14, 21, and 28 post-injection using 5 mice/time point. In the vehicle group (Group 9), serum samples were taken at pre-injection, 1hr post-injection, 14 day or 21 day only using 5 mice/time point. Serum IL-10 levels and serum 12G8 antibody levels were determined using specific ELISAs.

[0128] *Pharmacokinetic Parameter determinations.* All parameters were estimated or calculated using WinNonlin Pro v 4.0. For noncompartmental analyses, Model 200 (SC) or Model 201 (IV) was used. Input data were dose-normalized group arithmetic mean concentration-time data. Input doses were nominal doses for Groups 2-4 and 6-8. Input dose for Groups 1 and 5 was 0.014 mg/kg. For compartmental analyses, Model 3 (SC) or Model 7 (IV) was used. Input data were dose-normalized individual animal concentration-time data. All fits used uniform weighting (wt=1) for individual data points. Input doses were nominal doses for Groups 2-4 and 6-8. Input dose for Groups 1 and 5 was 0.014 mg/kg. Good of fit was evaluated using visual inspection, comparisons of SE's for estimated/calculated parameters, residuals, and AIC & SBC criteria.

A summary of the dosing solution recovery is shown in the Table below.

Table 9. Summary of Body Weights, Dose Levels, and Dosing Solution Concentrations (mean ±SD

Group	Route	Body Weights (g)	Nominal Dose Level[B] (mg/kg)	Actual Dose Level (mg/kg)	Nominal Dosing Soln Conc[D] (mg/mL)	Actual Dosing Soln Conc (mg/mL)	Actual Dose % of Nominal
1	IV	28.2 ± 2.61	0.03	11.4 ±1.02	~0.0075	0.0032	38.0
2	IV	29.7 ± 2.83	0.3	251 ±23.4	~0.075	0.074	83.7
3	IV	24.5 ± 2.00	3.0	3410 ±268	~0.75	0.83	114
4	IV	30.0 ± 3.38	30.0	28300 ±3040	Min 7.5	8.40	94.3
5	SC	28.4 ±2.38	0.03	11.4 ±0.954	~0.0075	0.0032	38.0
6	SC	29.9 ±2.87	0.3	250 ±23.1	~0.075	0.074	83.3
7	SC	24.7 ±1.95	3.0	3380 ±263	~0.75	0.83	113
8	SC	29.7 ±2.61	30.0	28500 ±248	Min 7.5	8.4	95.0

[0129] The tables shown below summarize the data from the groups receiving the 12G8 antibody via i.v. injection.

Table 10. Noncompartmental Method Parameters for IV Bolus Dosing Groups

Group (fold incr)	Dose Level (mg/kg)	Parameters				
		$AUC_{0\text{-last}}$	V_z	CL	Initial $t_{1/2}$	Term $t_{1/2}$
		(day*ug/mL)	(mL/kg)	(mL/day/kg)	(day)	(day)
1	0.014	1.97	87.3	4.68	NC	12.9
2 (21.4x)	0.3	44.1 (22.3x)	99.6	5.08	NC	13.6
3 (10x)	3.0	404 (9.2x)	127	5.08	NC	17.3
4 (10x)	30	4000 (9.9x)	101	6.36	NC	11.0

Table 11. Compartmental Method Parameters for IV Bolus Dosing Groups

Group (fold incr)	Dose Level (mg/kg)	Parameters				
		$AUC_{0\text{-inf}}$	V_i	CL	Initial $t_{½}$	Term $t_{1/2}$
		(day*ug/mL)	(mL/kg)	(mL/day/kg)	(day)	(day)
1	0.014	2.22	31.4	5.13	0.041	10.5
2 (21.4x)	0.3	53.5 (24.1x)	48.7	5.60	0.105	11.6
3 (10x)	3.0	550 (10.3x)	55.7	5.46	0.103	15.0
4 (10x)	30	4500 (8.18)	45.8	6.67	0.08	9.79

[0130] The tables shown below summarize the data from the groups receiving the 12F8 antibody via s.c. injection.

Table 12. Noncompartmental Method Parameters for SC Bolus Dosing Groups

Group (fold incr)	Dose Level (mg/kg)	Parameters					
		$AUC_{0\text{-last}}$	C_{max}	T_{max}	F	t ½ abs	t ½ elim
		(day*ug/mL)	(ug/mL)	(day)	(%)	(day)	(day)
1	0.03	1.56	0.113	2.0	77.8	NC	18.9
2 (21.4x)	0.3	44.9 (28.8x)	3.56 (31.5x)	0.417	100	NC	13.0
3 (10x)	3.0	343 (7.6x)	24.4 (6.85x)	1.00	84.8	NC	13.6
4 (10x)	30	3170 (9.2x)	247 (10.1x)	0.667	79.3	NC	8.76

* Bioavailability may be high due to underestimating IV AUC.

Table 13. Compartmental Method Parameters for SC Bolus Dosing Groups

		Parameters					
Group	**Dose Level (mg/kg)**	**$AUC_{0\text{-last}}$**	**C_{max}**	**T_{max}**	**F**	**t ½ abs**	**t ½ elim**
(fold incr)		**(day*ug/mL)**	**(ug/mL)**	**(day)**	**(%)**	**(day)**	**(day)**
1	0.03	2.00	0.107	1.44	90.0	0.254	11.9
2 (21.4x)	0.3	53.5 (26.8x)	3.11 (29.1x)	1.31	100	0.229	11.0
3 (10x)	3.0	450 (8.41x)	22.4 (7.20x)	1.59	81.7	0.284	12.7
4 (10x)	30	3210 (7.1x)	256 (11.4x)	1.25	71.3	0.241	7.78

[0131] Concentration-time profiles are shown for 12F8 antibody using various dosages and routes in Figure 2.

[0132] *Conclusions:* The doses were within 20% of nominal for all groups except lowest dose level. Lower than expected concentrations, probably due to presence of anti-SCH708980 (humanized 12G8) antibodies were observed from Day 10 post-injection in groups 7 or 8. (A) IV Bolus Pharmacokinetics. Half-lives, clearance and distribution volumes are typical of those seen for other IgG1 monoclonal antibodies. Distribution volume is approximately equal to or slightly larger than serum volume suggesting minimal extravascular distribution. The terminal half-lives ranged from 10 to 17 days. The increase in AUC was generally dose-proportional suggesting linear PK over the dose range tested. (B) SC Bolus Pharmacokinetics. The maximum concentrations were generally dose-proportional and were reached by 1-2 days postdose suggesting consistent rates and extents of absorption over the dose range tested. The increase in AUC was generally dose-proportional suggesting linear PK. The terminal elimination half-lives ranged from 8-14 days, similar to other IgG1 monoclonal antibodies. The absolute bioavailability was high, range= 70-100%, although the estimates > 90% may be high due to underestimation of IV AUC.

Example IV. Determining the Equilibrium Dissociation Constant (Kd) for humanized anti- human IL-10 antibody SCH 708980 (12G8) using KinExA technology.

[0133] The equilibrium dissociation constant (Kd) for humanized antibody SCH 708980 was determined using KinExA 3000 (Sapidyne Instruments Inc.). KinExA uses the principle of the Kinetic Exclusion Assay method based on measuring the concentration of uncomplexed antibody in a mixture of antibody, antigen and antibody- antigen complex. The concentration of free antibody was measured by exposing the mixture to a solid- phase immobilized antigen for a very brief period of time. In practice, this was accomplished by flowing the solution phase antigen- antibody mixture past antigen coated particles trapped in a flow cell. Data generated by the instrument were analyzed using custom software. Equilibrium constants were calculated using a mathematical theory based on the following assumptions:

1. The binding follows the reversible binding equation for equilibrium:

$$K_{on}\,[Ab]\,[Ag] = K_{off}[AbAg]$$

2. Antibody and antigen bind 1 : 1 , and total antibody equals antigen- antibody complex plus free antibody

3. Instrument signal is linearly related to free antibody concentration

[0134] *Materials used:* Monoclonal humanized antibody SCH 708980 to recombinant human IL-10 (h12G8); recombinant human IL-10 (hIL-10); recombinant mouse IL-10 (mIL-10), recombinant cyno IL-10 (cyno IL-10); PMMA particles , 98 micron (Sapidyne, Cat No. 440198); Neutravidin (Pierce, Cat No. 31000); EZ- link TFP PEO-Biotin (Pierce, Cat No. 21219); Biotinylated rhIL-10; and Cy5 conjugated Goat anti- HuIgG (H + L) (Jackson Immunoresearch Laboratories Cat. No 109-175-088, lot 49069).

[0135] PMMA particles were coated with biotinylated rhIL5 according to manufacturer's protocols. For biotinylation of rhIL5 EZ-link TFP PEO-biotin was used according to manufacturer's recommendations (Pierce bulletin 0874). All experimental procedures were done according to the KinExA 3000 manual.

[0136] *Experimental conditions:* All runs were done in duplicate. For hIL-10 runs the following conditions were used:

Sample volume:	1.5 ml
Sample flow rate:	0.25 ml/min
Label volume:	0.5 ml
Label flow rate:	0.25 ml/min

mAb conc.: 0.1 nM
Highest Ag (hIL-10) conc.: 4.0 nM
Lowest Ag (hIL-10) conc. : 3.91 pM

[0137] Two- fold serial dilutions of the antigen were prepared and mixed with the antibody at constant concentration. The mixture was incubated for 2 hours at room temperature (RT) to equilibrate.

[0138] For mIL-10 runs the following conditions were used:

Sample volume: 0.5 ml
Sample flow rate: 0.25 ml/min
Label volume: 0.5 ml
Label flow rate: 0.25 ml/min
mAb conc. : 1 nM
Highest Ag (mIL-10) conc. : 50 nM
Lowest Ag (mIL-10) conc. : 48.8 pM

[0139] Two- fold serial dilutions of the antigen were prepared and mixed with the antibody at constant concentration. The mixture was incubated for 2 hours at RT. to equilibrate.

[0140] For cyno IL-10 runs the following conditions were used:

Sample volume: 2 ml
Sample flow rate: 0.25 ml/min
Label volume: 1 ml
Label flow rate: 0.25 ml/min
mAb conc. : 0.1 nM
Highest Ag (mIL-10) conc. : 5.0 nM
Lowest Ag (mIL-10) conc. : 4.88 pM

[0141] Two- fold serial dilutions of the antigen were prepared and mixed with the antibody at constant concentration. The mixture was incubated for 2 hours at RT to equilibrate.

[0142] The results are shown in the Table below.

Table 14. Equilibrium Dissociation constant (Kd) for 12F8 Antibody using KinExA technology

Antigen	Antigen monomer M.W.(kDa)	Antibody	Kd (Standard)	Error (%)
hIL-10 #1	19.5	hz x hIL-10 mAb 12G8-1	2.738e-11	2.18%
hIL-10 #2	19.5	hz x hIL-10 mAb 12G8, SCH708980	3.232e-11	4.4%
hIL-10 #3	19.5	hz x hIL-10 mAb 12G8, SCH708980	1.553e-11	3.7%

mIL-10 #1	19.5	hz x hIL-10 mAb 12G8-1	2.82e-10	1.42%
mIL-10 #2	19.5	hz x hIL-10 mAb 12G8, SCH708980	2.673e-10	1.65%
mIL-10 #3	19.5	hz x hIL-10 mAb 12G8, SCH708980	3.078e-10	1.97%
Cyno IL-10 #1	19.5	hz x hIL-10 mAb 12G8-1	3.97e-11	1.93%
Cyno IL-10 #2	19.5	hz x hIL-10 mAb 12G8, SCH708980	9.657e-11	1.37%
Cyno IL-10 #3	19.5	hz x hIL-10 mAb 12G8, SCH708980	9.245e-11	3.5%

Example V. Application of competitive electrochemiluminescence assay (ECLA) to measure binding of anti-hIL-10 monoclonal antibodies and hIL-10-Ra to recombinant IL-10 of different origin.

[0143] *Summary of technology.* Electrochemiluminescence technology was developed by IGEN, Inc (Gaithersburg, MD) and is employed in the M-series M8/384 analyzer used in this work. Electrochemiluminescence technology utilizes a stable ruthenium metal chelate (Ori-TAG) which, in the presence of tripropylamine (TPA), generates electrochemiluminescence upon voltage application. Paramagnetic beads, microns in diameter, act as the solid phase and facilitate rapid assay kinetics. The bead/complex is channeled through a flow cell and captured at an electrode by magnetic application. Voltage is applied and resulting electrochemiluminescence is measured.

[0144] *Materials used.* 96 well Polypropylene plates (Costar, Cat. No. 3365, Fisher Sci. Cat. No. 07200697); assay buffer of 0.1 % BSA, 0.05% tween 20, PBS pH 7.5; paramagnetic beads (Streptavidin-Dynabeads, Igen, Inc., Cat .No. 110029); recombinant human IL-10 dimer (hIL-10-dimer); recombinant human IL-10-monomer (hIL-10-mono); recombinant mouse IL-10 (mIL-10); recombinant cyno IL-10 (cyno IL-10); and recombinant hIL-10Ra (hIL-10Ra): FLAG-tagged protein. Ori-Tag labeled anti-FLAG M2 monoclonal antibodies were prepared using Ori-Tag-NHS ester (Igen, Inc. Cat. No. 110034) according to manufacturer's protocol (OriTag label: IgG challenge ratio 8:1). Anti-Flag M2 monoclonal antibodies were purchased from Sigma(Cat. No. F3165). Ori-Tag labeled anti hIgG 1A2 monoclonal antibodies were prepared as above using rat anti hIgG monoclonal antibodies. Ori-Tag labeled anti rat IgG antibodies were prepared as above from polyclonal Goat anti rat IgG (H+L) antibodies (Jackson Immunoresearch Laboratories, Inc. PA, Cat. No. 112-005-143). Biotinylated recombinant human IL-10 (

hIL-10-biotin) was prepared using TFP-PEO-biotin (Pierce, Cat. No. 21219) according to manufacturer's recommendations (Pierce bulletin 0874). The rat anti hIL-10 mAb 12G8 (r12G8): JES3.12G8 and humanized anti hIL-10 mAb 12G8 (h12G8-1) were prepared as described herein.

[0145] *Protocol.* 1/3 serial dilutions in 50 microliters of the assay buffer were made in 96-well microtiter plate for all unlabeled IL-10 preparations (mIL-10, cyno IL-10, hIL-10 dimer, hIL-10 mono) starting with 3 μg/ml in the first well. All samples were run in duplicates. 50 μl of hIL-10-biotin at 25 ng/ml was added to each well, followed by the addition of either hIL-10Ra (50 μl at 100 ng/ml) or anti hIL-10 mAb (50 μl at 10 ng/ml). 50 microliters of Ori-Tag conjugated secondary antibodies was added to each well at 500 ng/ml conc. For hIL-10Ra , r12G8 and h12G8 the following Ori-Tag conjugated were used accordingly: anti-FLAG M2-OriTag, anti-rat IgG–OriTag and anti hIgG 1A2–OriTag. Finally to each well 50 μl of Streptavidin-Dynabeads at 0.1 mg/ml was added. After a one hour incubation at room temperature the plate was processed by the M-series M8/384 analyzer. Percent inhibition of the signal by unlabeled IL-10 preparations was calculated relative to the control samples. To plot the data and calculate IC_{50} the GraphPad Prism Software was used.

[0146] Results are shown in the Table below.

Table 15. Binding affinity determination using ECLA

Antigen	Antigen monomer M.W.(kDa)	Antibody/Receptor	IC_{50}(nM)	SD
mIL-10	19.5	Rat x hIL-10 mAb JES3.12G8	37.9	17.8
Cyno IL-10	19.5	Rat x hIL-10 mAb JES3.12G8	6.6	1.5
hIL-10 (dimer)	19.5	Rat x hIL-10 mAb JES3.12G8	4.7	0.8
hIL-10 (monomer)	19.5	Rat x hIL-10 mAb JES3.12G8	7.1	0.6
mIL-10	19.5	hz x hIL-10 mAb 12G8-1	53.0	8.2
Cyno IL-10	19.5	hz x hIL-10 mAb 12G8-1	4.9	0.9
hIL-10 (dimer)	19.5	hz x hIL-10 mAb 12G8-1	4.0	0.5
hIL-10 (monomer)	19.5	hz x hIL-10 mAb 12G8-1	5.8	1.0
mIL-10	19.5	huIL-10Ra	no binding	-

Cyno IL-10	19.5	huIL-10Ra	17.6	7.0
hIL-10 (dimer)	19.5	huIL-10Ra	2.9	0.4
hIL-10 (monomer)	19.5	huIL-10Ra	7.2	1.1

[0147] The results of the characterization of the rat 12G8 antibody and the humanized 12G8 antibody (SCH708980) are summarized in the Table below.

TABLE 16

Rat 12G8

	IGEN IC_{50}(nM) mean±S.D. (n)	Kinexa Kd(pM) mean±S.D.(n)	Biacore Kd(pM) mean±S.D.(n)	Bioassay IC_{50}(pM) mean±S.D.(n)
mouse IL-10	38±18 (2)		7400±2500 (9)	
cyno IL-10	6.6±1.5 (2)		330±60 (9)	
hu IL-10	4.7±0.8 (2)	23 (1)	277±39 (7)	66±9 (5)

SCH708980 (hu12G8)

	IGEN IC_{50}(nM) mean±S.D. (n)	Kinexa Kd(pM) mean±S.D.(n)	Biacore Kd(pM) mean±S.D.(n)	Bioassay IC_{50}(pM) mean±S.D.(n)
mouse IL-10	53±8 (2)	286±21 (3)	8600±600 (8)	
cyno IL-10	4.9±0.9 (2)	76±32 (3)	659±71 (8)	
hu IL-10	4.0±0.5 (2)	25±9 (3)	511±68 (11)	93±9 (5)

Example VI. Neutralizing effects of humanized anti-human IL-10 antibody *in vivo*

[0148] *In vivo* neutralizing efficacy of SCH 708980 and JES.12G8 was evaluated in the *Leishmania major* model in mice. In this model, CB6F1 mice normally resistant to parasite infection were rendered susceptible by heterozygosity for a human IL-10 transgene under the control of the MHC class II promoter. CB6F1 or CB6F1-huIL-10Tg mice were injected s.c. with SCH 708980 or JES.12G8 weekly beginning three days before s.c. footpad challenge with $15x10^6$ stationary phase *L. major* parasites. Disease progression was monitored by weekly measurements of footpad swelling. Fig. 3 shows that both SCH708980 (the humanized 12G8) and the parental rat 12G8 neutralized the protective effect of IL-10 in a dose-dependent manner.

[0149] Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

[0150] Citation of the above publications or documents is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents. U.S. patents and other publications referenced herein are hereby incorporated by reference.

CLAIMS

1. A humanized recombinant antibody molecule that binds IL-10 or binding fragment thereof, comprising:

at least one antibody light chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:1 at CDR1, SEQ ID NO:2 at CDR2, and SEQ ID NO:3 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence; and

at least one antibody heavy chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:6 at CDR1, SEQ ID NO:7 at CDR2, and SEQ ID NO:8 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence.

2. The antibody of claim 1, further comprising a heavy chain constant region, wherein the heavy chain constant region comprises a γ1, γ2, γ3, or γ4 human heavy chain constant region or a variant thereof.

3. The antibody of claim 1, further comprising a light chain constant region, wherein the light chain constant region comprises a lambda or a kappa human light chain constant region.

4. The antibody of claim 1, wherein the binding fragment is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv, $F(ab')_2$, and a diabody.

5. The antibody of claim 1, wherein the antibody light chain, or binding fragment thereof, comprises a polypeptide having a variable region of SEQ ID NO:4.

6. The antibody of claim 1, wherein the antibody light chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:5.

7. The antibody of claim 1, wherein the antibody heavy chain, or binding fragment thereof, comprises a polypeptide having a variable region of SEQ ID NO:9.

8. The antibody of claim 1, wherein the antibody heavy chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:10.

9. A chimeric recombinant antibody molecule that binds IL-10 or binding fragment thereof, comprising:

at least one antibody light chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group of SEQ ID NO:1 at CDR1, SEQ ID NO:2 at CDR2, and SEQ ID NO:3 at CDR3; and

at least one antibody heavy chain variable region, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group of SEQ ID NO:6 at CDR1, SEQ ID NO:7 at CDR2, and SEQ ID NO:8 at CDR3.

10. A humanized recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising:

at least one antibody light chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of SEQ ID NO:11 at CDR1, SEQ ID NO:12 at CDR2, and SEQ ID NO:13 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence; and

at least one antibody heavy chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence selected of SEQ ID NO:15 at CDR1, SEQ ID NO:16 at CDR2, and SEQ ID NO:17 at CDR3; and a framework region, wherein the amino acid sequence of framework region is all or substantially all of a human immunoglobin amino acid sequence.

11. The antibody of claim 10, further comprising a heavy chain constant region, wherein the heavy chain constant region comprises a γ1, γ2, γ3, or γ4 human heavy chain constant region or a variant thereof.

12. The antibody of claim 10, further comprising a light chain constant region, wherein the light chain constant region comprises a lambda or a kappa human light chain constant region.

13. The antibody of claim 10, wherein the binding fragment is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv, $F(ab')_2$, and diabody.

14. The antibody of claim 10, wherein the antibody light chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:14.

15. The antibody of claim 10, wherein the antibody heavy chain, or binding fragment thereof, comprises a polypeptide having a variable region and a constant region of SEQ ID NO:18.

16. A chimeric recombinant antibody molecule that binds IL-10, or binding fragment thereof, comprising:

at least one antibody light chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence of SEQ ID NO:11 at CDR1, SEQ ID NO:12 at CDR2, and SEQ ID NO:13 at CDR3; and

at least one antibody heavy chain, or binding fragment thereof, comprising a polypeptide having at least one amino acid sequence of SEQ ID NO:15 at CDR1, SEQ ID NO:16 at CDR2, and SEQ ID NO:17 at CDR3.

17. A method of suppressing an immune response in a human subject comprising administering to a subject in need thereof an antibody specific for IL-10, or a binding fragment thereof, in an amount effective to block the biological activity of IL-10, wherein the antibody is the antibody of claim 1 or 9.

18. The method of claim 17, wherein the immune response is a humoral response.

19. The method of claim 17, wherein the subject has systemic lupus erythematosus.

20. The method of claim 17, wherein the subject has immune thrombocytopenic purpura (ITC).

21. The method of claim 17, wherein the subject has lupus nephritis.

22. The method of claim 17, wherein the subject has HIV.

23. The method of claim 17, wherein the subject has hepatitis C.

24. The method of claim 17, further comprising administering an immunosuppressive agent.

25. A method of suppressing an immune response in a human subject comprising administering to a subject in need thereof, an antibody specific for IL-10, or a binding fragment thereof, in an amount effective to block the biological activity of IL-10, wherein the antibody is the antibody of claim 10 or 16.

26. The method of claim 25, wherein the immune response is a humoral response.

27. The method of claim 25, wherein the subject has systemic lupus erythematosus.

28. The method of claim 25, wherein the subject has immune thrombocytopenic purpura (ITC).

29. The method of claim 25, wherein the subject has lupus nephritis.

30. The method of claim 25, wherein the subject has HIV.

31. The method of claim 25, wherein the subject has hepatitis C.

32. The method of claim 25, further comprising administering an immunosuppressive agent.

33. A composition comprising an antibody, or binding fragment thereof, in combination with a pharmaceutically acceptable carrier or diluent, wherein the antibody is the antibody of claim 1 or 9.

34. The composition of claim 33, further comprising an immunosuppressive agent.

35. A composition comprising an antibody, or binding fragment thereof, in combination with a pharmaceutically acceptable carrier or diluent, wherein the antibody is the antibody of claim 10 or 16.

36. The composition of claim 35, further comprising an immunosuppressive agent.

37. An isolated nucleic acid encoding the polypeptide of claim 1 or 9.

38. An isolated nucleic acid encoding the polypeptide of claim 10 or 16.

39. An expression vector comprising the nucleic acid sequence of claim 37 operably linked to control sequences recognized by a host cell transfected with the vector.

40. A host cell comprising the vector of claim 38.

41. A method of producing a polypeptide, comprising culturing the host cell of claim 40 under conditions wherein the nucleic acid sequence is expressed, thereby producing the polypeptide, and recovering the polypeptide from the host cell.

42. An expression vector comprising the nucleic acid sequence of claim 38 operably linked to control sequences recognized by a host cell transfected with the vector.

43. A host cell comprising the vector of claim 42.

44. A method of producing a polypeptide, comprising culturing the host cell of claim 43 under conditions wherein the nucleic acid sequence is expressed, thereby producing the polypeptide, and recovering the polypeptide from the host cell.

45. An isolated nucleic acid sequence encoding an antibody specific for IL-10 comprising a light chain having the nucleic acid sequence of SEQ ID NO:21 and a heavy chain having the nucleic acid sequence of SEQ ID NO:22.

46. The nucleic acid of claim 45, wherein the light chain has an American Type Culture Collection (ATCC) deposit number of PTA-5923 and the heavy chain has an ATCC deposit number of PTA-5922.

47. An isolated nucleic acid sequence encoding an antibody specific for IL-10 comprising a light chain having the nucleic acid sequence of SEQ ID NO:23 and a heavy chain having the nucleic acid sequence of SEQ ID NO:24.

48. The nucleic acid of claim 47, wherein light chain has an ATCC deposit number of PTA-5927 and the heavy chain has an ATCC deposit number of PTA-5926.

49. An isolated nucleic acid sequence encoding a binding fragment of the antibody encoded by the nucleic acid sequence of claim 45.

50. An isolated nucleic acid sequence encoding a binding fragment of the antibody encoded by the nucleic acid sequence of claim 47.

51. The nucleic acid of claim 49 or 50, wherein the binding fragment is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv, $F(ab')_2$, and a diabody.

52. A method to identify an acceptor germline sequence for a humanized antibody, which method comprises the steps of:

a) identifying a non-human antibody that has the desired biological activity;

b) determining the amino acid sequence of a non-human antibody V_H and V_L domains; and

c) comparing the nonhuman antibody sequence to a group of human germline sequences, wherein the comparison comprises the substeps of:

1) assigning the sequence of non-human V_H and V_L domain sequences residue numbers;

2) delineating the CDR and FR regions in the sequence;

3) assigning a predetermined numerical score at each residue position for which the non-human and human germline sequences are identical; and

4) totaling all of the residue scores to generate a total score for each human germline sequence; and

d) identifying the human germline sequence with the highest total residue score as the acceptor germline sequence.

53. The method of claim 52, further comprising the substeps of :

a) assigning a numerical score of 1 for each residue position for which the non-human and human germline sequences are identical that was not scored in substep (3) to germline sequences with identical total residue scores after substep (4);

b) totaling all of the residue scores to generate a total score for each human germline sequence.

54. The method of claim 52, wherein the V_H region is scored as in Table 2.

55. The method of claim 52, wherein the V_L region is scored as in Table 3.

56. The method of claim 52, wherein the non-human antibody is specific for IL-10 and inhibits the biological activity of IL-10.

57. An antibody generated by the method of claim 52.