Anti-IL-12 antibodies, compositions, methods and uses

The present invention relates to at least one novel anti-IL-12 antibodies, including isolated nucleic acids that encode at least one anti-IL-12 antibody, IL-12, vectors, host cells, transgenic animals or plants, and methods of making and using thereof, including therapeutic compositions, methods and devices.
The present invention relates to antibodies, including specified portions or variants, specific for at least one Interleukin-12 (IL-12) protein or fragment thereof, as well as nucleic acids encoding such anti-IL-12 antibodies, complementary nucleic acids, vectors, host cells, and methods of making and using thereof, including therapeutic formulations, administration and devices.

INTERIEUKIN-12 (IL-12) is a heterodimeric cytokine consisting of glycosylated polypeptide chains of 35 and 40 kD which are disulfide bonded. The cytokine is synthesized and secreted by antigen presenting cells including dendritic cells, monocytes, macrophages, B cells, Langerhans cells and keratinocytes as well as natural killer (NK) cells. IL-12 mediates a variety of biological processes and has been referred to as NK cell stimulatory factor (NKSF), T-cell stimulating factor, cyto toxic T-lymphocyte maturation factor and EBV-transformed B-cell line factor (Burks, J.H.A.J., et al., Clinical Microbiology Reviews, 10:7.42-780 (1997)). In particular, IL-12 is vital for the generation of cytokytic cells (e.g., NK, CTL) and for mounting a cellular immune response (e.g., a Th1 mediated immune response). Thus, IL-12 is critically important in the generation and regulation of both protective immunity (e.g., eradication of infections) and pathological immune responses (e.g., autoimmunity) (Hendrzak, J.A. and Brunda, M.J., Laboratory Investigation, 72:619-637 (1995)). Accordingly, an immune response (e.g., protective or pathogenic) can be enhanced, suppressed or prevented by manipulation of the biological activity of IL-12 in vivo, for example, by means of an antibody.

Non-human mammalian, chimeric, polyclonal (e.g., anti-sera) and/or monoclonal antibodies (Mabs) and fragments (e.g., proteolytic digestion or fusion protein products thereof) are potential therapeutic agents that are being investigated in some cases to attempt to treat certain diseases. However, such antibodies or fragments can elicit an immune response when administered to humans. Such an immune response can result in an immune complex-mediated clearance of the antibodies or fragments from the circulation, and make repeated administration unsuitable for therapy, thereby reducing the therapeutic benefit to the patient and limiting the readministration of the antibody or fragment. For example, repeated administration of antibodies or fragments comprising non-human portions can lead to serum sickness and/or anaphalaxis. In order to avoid these and other problems, a number of approaches have been taken to reduce the immunogenicity of such antibodies and portions thereof, including chimerization and humanization, as well known in the art. These and other approaches, however, still can result in antibodies or fragments having some immunogenicity, low affinity, low avidity, or with problems in cell culture, scale up, production, and/or low yields. Thus, such antibodies or fragments can be less than ideally suited for manufacture or use as therapeutic proteins.

Accordingly, there is a need to provide anti-IL-12 antibodies or fragments that overcome one or more of these problems, as well as improvements over known antibodies or fragments thereof.

SUMMARY OF THE INVENTION

The present invention provides isolated human, primate, rodent, mammalian, chimeric, humanized and/or CDR-grafted anti-IL-12 antibodies, immunoglobulins, cleavage products and other specified portions and variants thereof, as well as anti-IL-12 antibody compositions, encoding or complementary nucleic acids, vectors, host cells, compositions, formulations, devices, transgenic animals, transgenic plants, and methods of making and using thereof, as described and enabled herein, in combination with what is known in the art.

The present invention also provides at least one isolated anti-IL-12 antibody as described herein. An antibody according to the present invention includes at least one protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into an antibody of the present invention. An antibody of the invention can include or be derived from any mammal, such as but not limited to...
The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding at least one IL-12 anti-idiotype antibody, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising said IL-12 anti-idiotype antibody encoding nucleic acid molecules. host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or using such anti-idiotype antibody nucleic acids, vectors and/or host cells.

The present invention also provides at least one method for expressing at least one anti-IL-12 antibody, or IL-12 anti-idiotype antibody, in a host cell, comprising culturing a host cell as described herein under conditions wherein at least one anti-IL-12 antibody is expressed in detectable and/or recoverable amounts.

The present invention further provides at least one composition comprising (a) an isolated anti-IL-12 antibody encoding nucleic acid and/or antibody as described herein; and (b) a suitable carrier or diluent. The carrier or diluent can optionally be pharmaceutically acceptable, according to known carriers or diluents. The composition can optionally further comprise at least one further compound, protein or composition.

The present invention also provides at least one composition, device and/or method of delivery of a therapeutically or prophylactically effective amount of at least one anti-IL-12 antibody, according to the present invention. The present invention further provides at least one composition, device and/or method of delivery for diagnosing of at least one anti-IL-12 antibody, according to the present invention.

DESCRIPTION OF THE FIGURES

Figures 1A and 1B are graphs showing concentration-dependent binding of human anti-IL-12 mAbs to immobilized human IL-12. Anti-IL-12 antibodies were serially diluted in 1% BSA/PBS and incubated on rhIL-12 coated plates for 1 hour at 37°C. Plates were washed twice with 0.02% Tween 20 (polyoxyethylene(20) sorbitan monolaurate), 0.15M saline and then probed with horse radish peroxidase (HRP) labeled goat anti-human IgG kappa specific antibody for 1 hour at room temperature. Plates were again washed, developed with o-phenylenediamine (OPD) substrate and the optical density (OD) of each well was measured at 490 nm.

Figure 2: Lanes from left to right in Figures A and B contain human IL-12, human IL-12 p40, murine IL-12, and prestained molecular weight markers. Figure 2A shows bands stained from total protein. The primary bands in each lane are human IL-12 (75 kd), p40 human IL-12 (40 kd), and murine IL-12 (75 kd). Figure 2B shows a western blot prepared from a gel identical to that shown in Figure 2A. Blot was reacted with C340 followed by HRP labeled goat anti-human IgG and specifically detected human IL-12 (monomer and multimers) and human IL-12 p40 only. A control blot (not shown) reacted with HRP labeled goat anti-human IgG did not display any bands.

Figure 3: Reverse transcription-PCR analysis of IFNγ gene expression in human PBL’s treated with IL-2, IL-12, IL-2+IL-12 with and without anti-IL-12 antibody C340, 8.6.2, isotype control antibody. Total RNA was reverse transcribed, amplified by PCR using gene-specific primers. The level of β-actin mRNA in each sample was also determined which served as a control for mRNA integrity and content.

Figure 4 is a histogram showing that human anti-IL-12 mAb (C340) inhibits production of interferon-γ (IFNγ) by monocyte depleted CD3+ peripheral blood mononuclear cells (PBMC) stimulated with IL-2 plus IL-12. PBMC were cultured for five hours in control media (no added cytokines), media supplemented with IL-12 (0.1 ng/ml) plus IL-2 (50 IU/ml) (IL-12/IL-2), control media containing mAb C340 (10 µg/ml) and IL-12/IL-2 media containing mAb C340 (10 µg/ml). Intracellular IFNγ was measured by two color immunostaining with CD3-PE and IFNγ-FITC. Data are shown for one donor.

Figure 5 is a graph showing dose-dependent inhibition of IFNγ secretion by IL-2 plus IL-12 stimulated peripheral blood lymphocytes with two different lots of a human anti-IL-12 mAb (C340). Human PBL (8 x 106/ml) were cultured for 24 hours with 10 U/ml IL-2, IL-2 plus 400 pg/ml IL-12, or IL-2 plus IL-12 and mAb C340 as indicated. The culture supernatants were removed and assayed for IFNγ by EIA.

Figure 6 is a histogram showing dose-dependent inhibition of IL-12 plus IL-2 induced LAK cell cytotoxicity by a human anti-IL-12 mAb (C340). LAK effector cells (human PBL, 8 x 106/ml) were cultured for 24 hours with IL-12 (400 pg/ml) plus IL-2 (10 U/ml) and mAb C340 (5000 ng/ml or 50 ng/ml as indicated). The LAK effector cells were
As used herein, the term “human antibody” refers to an antibody in which substantially every part of the protein is of human, primate, rodent, mammalian, chimeric, humanized or CDR-grafted origin. Such antibodies include human, primate, rodent, or mammalian full-length antibodies, as well as compositions and encoding nucleic acid molecules comprising at least one polynucleotide encoding at least one antibody or anti-idiotypic antibody. The present invention further includes, but is not limited to, methods of making and using such nucleic acids and antibodies and anti-idiotype antibodies, including diagnostic and therapeutic compositions, methods and devices.

The present invention provides isolated, recombinant and/or synthetic anti-IL-12 human, primate, rodent, mammalian, chimeric, humanized or CDR-grafted, antibodies and IL-12 anti-idiotypic antibodies thereto, as well as compositions and encoding nucleic acid molecules comprising at least one polynucleotide encoding at least one anti-IL-12 antibody or anti-idiotypic antibody. The present invention further includes, but is not limited to, methods of making and using such nucleic acids and antibodies and anti-idiotypic antibodies, including diagnostic and therapeutic compositions, methods and devices.

As used herein, an “anti-Interleukin-12 antibody,” “anti-IL-12 antibody,” “anti-IL-12 antibody portion,” or “anti-IL-12 antibody variant” includes any protein or peptide containing at least one IL-12 activity or binding, or with IL-12 receptor activity or binding, in vitro, in situ and/or in vivo. Such fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a combination gene encoding a F(ab’)2 heavy chain portion can be designed to include DNA sequences encoding the CH1 domain in which one or more stop codons have been introduced upstream of the natural stop site. For example, a combination gene encoding a F(ab’)2 heavy chain portion can be designed to include DNA sequences encoding the CH1 domain in which one or more stop codons have been introduced upstream of the natural stop site.
(e.g., C_{H1}, C_{H2}, C_{H3}) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

![Image](https://via.placeholder.com/150)

**Citations**

All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention and/or to provide description and enablement of the present invention.
Antibodies of the Present Invention


[0027] Human antibodies that are specific for human IL-12 proteins or fragments thereof can be raised against an appropriate immunogenic antigen, such as isolated and/or IL-12 protein or a portion thereof (including synthetic molecules, such as synthetic peptides). Other specific or general mammalian antibodies can be similarly raised. Preparation of immunogenic proteins, and monoclonal antibody production, can be performed using any suitable technique.

[0028] In one approach a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-Ag14, NSO, NS1, NS2, AE-1, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U937, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art. See, e.g., www.atcc.org, www.lifetech.com., and the like, with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, as either endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hRNA, mRNA, RNA, single, double or triple stranded, hybridized, and the like or any combination thereof. See, e.g., Ausubel, supra, and Colligan, Immunology, supra, chapter 2, entirely incorporated herein by reference.

[0029] Antibody producing cells can also be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).

[0030] Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like. display library; e.g., as available from Cambridge antibody Technologies, Cambridgeshire, UK; MorphoSys, Martinsreid/Planegg, DE; Bioviation, Aberdeen, Scotland, UK; Biolvent. Lund, Sweden; Dyax Corp., Enzon, Affymax/Biosite; Xoma, Berkeley, CA; Ixsys. See. e.g., EP 368,684, PCT/GB91/01134; PCT/GB92/01755; PCT/GB92/002240; PCT.GB92.00883; PCT/GB93/00605; US 08/350260 (5/12/94); PCT/GB94/01422; PCT/GB94/02662; PCT/GB97/01835; (CAT/MRC); WO90/14443; WO90/14424; WO90/14430; PCT/US94/1234; WO92/18619; WO96/07754; (Scirpps); EP 614 989 (MorphoSys); WO95/6027 (Biolvent); WO88/06630; PCT/WO90/3809 (Dyax); US 4,704,692 (Enzon); PCT/US91/02989 (Affymax); WO89/06283; EP 371 998; EP 500 400; (Xoma); EP 229 046; PCT/US91/07149 (Ixsys); or stochastically generated peptides or proteins - US 5723323, 5763192, 5814476, 5817483, 525414, 5976862, WO 86/05803, EP 590 689 (Ixsys, now Applied Molecular Evolution (AME), each entirely incorporated herein by reference) or that rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al., Microbiol. Immunol. 41:901-907 (1997); Sandhu et al., Crit. Rev. Biotechnol. 16:95-118 (1996); Eren et al., Immunol. 93:154-161 (1998), each entirely incorporated by reference as well as related patents and application) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al., Proc. Natl. Acad. Sci. USA, 94: 4937-4942 (May 1997); Hanes et al., Proc. Natl. Acad. Sci. USA, 95:14130-14135 (Nov. 1998)); single cell antibody producing technologies (e.g., selected lymphocyte antibody method (“SLAM” (US pat. No. 5,627,052, Wen et al., J.
Methods for engineering or humanizing non-human or human antibodies can also be used and are well known in the art. Generally, a humanized or engineered antibody has one or more amino acid residues from a source which is non-human, e.g., but not limited to mouse, rat, rabbit, non-human primate or other mammal. These human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable, constant or other domain of a known human sequence. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez/query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/online-comp.html; www.public.iastate.edu/~pedro/research-tools.html; www.mgen.uni-heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH05/kuby03.htm; www.library.thinkquest.org/12429/Immune/Antibody.html; www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/~mrc7/mikeimages.html; www.antibodyresource.com/mcb.harvard.edu/BioLinks/Immunology.html; www.immunochemistrylink.com/; pathbox.wustl.edu/~hcenter/index.html; www.biotech.ufl.edu/~hcl/; www.pebio.com/pa/340913/340913.html; www.nal.usda.gov/awic/pubs/antibody/; www.m.ehime-u.ac.jp/~yasuhito/Elsa.html; www.biosdesign.com/table.asp; www.icnet.uk/apx/facs/davies/links.html; www.biotech.ufl.edu/~fccl/protocol.html; www.is-ac-net.org/sites_geo.html; aximitml.uni-marburg.de/~reki/AEPstart.html; baserv.uci.knl/jraats/links.html; www.recab.uni-hd.delimmuno.bmu.nrw.de/; www.mrc-cpe.cam.ac.uk/imb-doc/public/INTRO.html; www.ibt.unam.mx/irx/IY_mice.html; imgnt.cnusc.fr:8104/; www.biochem.ucl.ac.uk/~martin/abs/index.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/~honegger/AHOSeminar/Slide01.html; www.cryst.bbk.ac.uk/~ubcg07s/; www.nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm; www.path.cam.ac.uk/~mrc7/humanisation/TAHBP.html; www.ibt.unam.mx/irx/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.cryst.bioc.cam.ac.uk/~f-molina/Web-pages/Pep/spottech.html; www.jerini.de/fr_products.htm; www.patents.ibm.com/ibm.html.Kabat et al., Sequences of Proteins of Immunological Interest. U.S. Dept. Health (1983), each entirely incorporated herein by reference. Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids. antibodies can also optionally be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, humanized antibodies can be optionally prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, Fv residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Humanization or engineering of antibodies of the present invention can be performed using any known method, such as but not limited to those described in, Winter (Jones et al., Nature 321:522 (1986); Riechmann et al., Nature 322:265 (1986); Verhoeven et al., Science 239:1534 (1988)), Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk. J. Mol. Biol. 196:901 (1987), Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4265 (1992); Presta et al., J. Immunol. 151:2623 (1993), US patent Nos: 5732332, 5976862, 5824514, 5817483, 5814476, 5763192, 5732332, 5,768868, 5714352, 6204023, 6180370, 5585089, 5225539; 4816567, PCT: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB98/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, each entirely incorporated herein by reference, included references cited therein. 

Anti-IL-12 antibody can also be optionally generated by immunization of a transgenic animal (e.g., mouse, rat, hamster, non-human primate, and the like) capable of producing a repertoire of human antibodies, as described herein and/or as known in the art. Cells that produce a human anti-IL-12 antibody can be isolated from such animals and immortalized using suitable methods, such as the methods described herein. Transgenic mice that can produce a repertoire of human antibodies that bind to human antigens can be produced by known methods (e.g., but not limited to, U.S. Pat. Nos: 5,770,428, 5,569,825, 5,545,806, 5,625,126, 5,625,825, 5,633,425, 5,661,016 and 5,789,650 issued to Lonberg et al.; Jakobovits et al. WO 98/50433, Jakobovits et al. WO
Screening antibodies for specific binding to similar proteins or fragments can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. Antibody screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 25 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT Patent Publication Nos. 91/17271, 91/18880, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent Publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Patent Nos. 5,658,754; and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, CA), and Cambridge Antibody Technologies (Cambridgeshire, UK). See, e.g., U.S. Pat. Nos. 4704692, 4939666, 4946778, 5260203, 5455030, 5518889, 5534621, 5565708, 5763733, 5767260, 5856456, assigned to Enzon; 5223409, 5403484, 5571698, 5837500, assigned to Dyax, 5427908, 5580717, assigned to Affymax; 5885793, assigned to Cambridge Antibody Technologies; 5750373, assigned to Genentech, 5618920, 5595898, 5576195, 5698435, 5693493, 5698417, assigned to Xoma, Colligan, supra; Ausubel, supra; or Sambrook, supra, each of the above patents and publications entirely incorporated herein by reference.

Antibodies of the present invention can also be prepared using at least one anti-IL-12 antibody encoding nucleic acid to provide transgenic animals or mammals; such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. Such animals can be provided using known methods. See, e.g., but not limited to, US patent nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

Antibodies of the present invention can additionally be prepared using at least one anti-IL-12 antibody encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco and maize) that produce such antibodies. Specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant antibodies have been successfully used to provide large amounts of recombinant proteins. E. g., using an inducible promoter. See, e. g., Cramer et al., Curr. Top. Microbiol. Immunol. 240: 95-118 (1999) and references cited therein. Also, transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e. g., Hood et al., Adv. Exp. Med. Biol. 464:127-147 (1999) and references cited therein. Antibodies have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv’s), including tobacco seeds and potato tubers. See, e.g., Conrad et al., Plant Mol. Biol. 38:101-109 (1998) and reference cited therein. Thus, antibodies of the present invention can also be produced using transgenic plants, according to known methods. See also, e.g., Fischer et al., Biotechnol. Appl. Biochem. 30:99-108 (Oct., 1999), Ma et al., Trends Biotechnol. 13:522-7 (1995); Ma et al., Plant Physiol. 109:341-6 (1995); Whitelam et al., Biochem. Soc. Trans. 22:940-944 (1994); and references cited therein. See, also generally for plant expression of antibodies, but not limited to, each of the above references is entirely incorporated herein by reference.

The antibodies of the invention can bind human IL-12 with a wide range of affinities (Kd). In a preferred embodiment, at least one human mAb of the present invention can optionally bind human IL-12 with high affinity. For example, a human mAb can bind human IL-12 with a Kd equal to or less than about 10⁻⁷ M, such as but not limited to, 0.1-9.9 (or any range or value therein) x 10⁻⁷, 10⁻⁸, 10⁻⁹, 10⁻¹⁰, 10⁻¹¹, 10⁻¹², 10⁻¹³ or any range or value therein. The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, et al., “Antibody-Antigen Interactions,” In Fundamental Immunology, Paul, W. E., Ed., Raven Press: New York, NY (1984); Kuby, Janis Immunology, W. H. Freeman and Company: New York, NY (1992); and methods described herein). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g., Kd, Ka, Kd) are preferably made with standardized solutions of antibody and antigen, and a standardized
buffer, such as the buffer described herein.

**Nucleic Acid Molecules**

[0039] Using the information provided herein, such as the nucleotide sequences encoding at least 70-100% of the contiguous amino acids of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, specified fragments, variants or consensus sequences thereof, or a deposited vector comprising at least one of these sequences, a nucleic acid molecule of the present invention encoding at least one anti-1L-12 antibody can be obtained using methods described herein or as known in the art.

[0040] Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combinations thereof. The DNA can be single-stranded, double-stranded or single-stranded or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand. It can be the non-coding strand, also referred to as the anti-sense strand.

[0041] Isolated nucleic acid molecules of the present invention can include nucleic acid molecules comprising an open reading frame (ORF), optionally with one or more introns, e.g., but not limited to, at least one specified portion of at least one CDR, as CDR1, CDR2 and/or CDR3 of at least one heavy chain (e.g., SEQ ID NOS: 1-3) or light chain (e.g., SEQ ID NOS: 4-8). nucleic acid molecules comprising the coding sequence for an anti-IL-12 antibody or variable region (e.g., SEQ ID NOS: 7-8); and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode at least one anti-IL-12 antibody as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code for specific anti-IL-12 antibodies of the present invention. See, e.g., Ausubel, et al., supra, and such nucleic acid variants are included in the present invention. Non-limiting examples of isolated nucleic acid molecules of the present invention include SEQ ID NOS: 10-15, corresponding to non-limiting examples of a nucleic acid encoding, respectively, HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, LC CDR3, HC variable region and LC variable region.

[0042] In another aspect, the invention provides isolated nucleic acid molecules encoding an antibody having an amino acid sequence as encoded by the nucleic acid contained in the plasmid deposited as designated clone names ________________________ and ATCC Deposit Nos. __________________________, respectively, deposited on ________________________.

[0043] As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding an anti-IL-12 antibody can include, but are not limited to, those encoding the amino acid sequence of an antibody fragment, by itself, the coding sequence for the entire antibody or a portion thereof: the coding sequence for an antibody, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional non-coding sequences, including but not limited to, non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example - ribosome binding and stability of mRNA): an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding an antibody can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused antibody comprising an antibody fragment or portion.

**Polynucleotides Which Selectively Hybridize to a Polynucleotide as Described Herein**

[0044] The present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library.

[0045] Preferably, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

[0046] Optionally, polynucleotides of this invention will encode at least a portion of an antibody encoded by the poly-
nucleotides described herein. The polynucleotides of this invention embrace nucleic acid sequences that can be employed for selective hybridization to a polynucleotide encoding an antibody of the present invention. See, e.g., Ausubel, supra; Colligan, supra, each entirely incorporated herein by reference.

Construction of Nucleic Acids

[0047] The isolated nucleic acids of the present invention can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art.

[0048] The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention - excluding the coding sequence - is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention.

[0049] Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression. To aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (See, e.g., Ausubel, supra; or Sambrook, supra)

Recombinant Methods for Constructing Nucleic Acids

[0050] The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA; or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries, is well known to those of ordinary skill in the art. (See, e.g., Ausubel, supra; or Sambrook, supra)

Nucleic Acid Screening and Isolation Methods

[0051] A cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the present invention, such as those disclosed herein. Probes can be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by one or more of temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100%, or 70-100%, or any range or value therein. However, it should be understood that minor sequence variations in the probes and primers can be compensated for by reducing the stringency of the hybridization and/or wash medium.

[0052] Methods of amplification of RNA or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein.

[0053] Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Patent Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; 4,795,699 and 4,921,794 to Tabor, et al.; 5,142,033 to Innis; 5,122,464 to Wilson, et al.; 5,091,310 to Innis; 5,066,584 to Gyllensten, et al.; 4,889,818 to Gelfand, et al; 4,994,370 to Silver, et al; 4,766,067 to Biswas; 4,656,134 to Ringold) and RNA mediated amplification that uses anti-sense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Patent No. 5,130,238 to Malek, et al, with the tradename NASBA), the entire contents of which references are incorporated herein by reference. (See, e.g., Ausubel, supra; or Sambrook, supra.)

[0054] For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in

Synthetic Methods for Constructing Nucleic Acids

[0055] The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by known methods, e.g., Ausubel, et al., supra. Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

Recombinant Expression Cassettes

[0056] The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence of the present invention, for example a cDNA or a genomic sequence encoding an antibody of the present invention, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention.

[0057] In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in intron) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered in vivo or in vitro by mutation, deletion and/or substitution.

Vectors And Host Cells

[0058] The present invention also relates to vectors that include isolated nucleic acid molecules of the present invention, host cells that are genetically engineered with the recombinant vectors, and the production of at least one anti-IL-12 antibody by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., supra; Ausubel, et al., supra; each entirely incorporated herein by reference.

[0059] The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid: If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[0060] The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating site, a sequence encoding an antibody of the present invention, and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

[0061] Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but not limited to, methotrexate (MTX), dihydrofolate reductase (DHFR, US Pat. Nos. 4,399,216; 4,634,665; 4,656,134; 4,956,288; 5,149,636; 5,179,017), ampicillin, neomycin (G418), mycophenolic acid, or glutamine synthetase resistance genes for culturing in E. coli and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other known methods. Such methods are described in the art, such as Sambrook, supra, Chapters 1-4 and 16-18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

[0062] At least one antibody of the present invention can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of an antibody to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide
moieties can be added to an antibody of the present invention to facilitate purification. Such regions can be removed prior to final preparation of an antibody or at least one fragment thereof. Such method are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.29-17.42 and 18.1-18.74; Ausubel, supra, Chapters 16, 17 and 18.

[0063] Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention.

[0064] Alternatively, nucleic acids of the present invention can be expressed in a host cell by turning on (by manipulation) in a host cell that contains endogenous DNA encoding an antibody of the present invention. Such methods are well known in the art, e.g., as described in US patent Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

[0065] Illustrative of cell cultures useful for the production of the antibodies, specified portions or variants thereof, are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions or bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610) and BSC-1 (e.g., ATCC CRL-26) cell lines, Cos-7 cells, CHO cells, hep G2 cells, P3X63Ag8.653, SP2/0-Ag04, 293 cells, HeLa cells and the like, which are readily available from, for example, American Type Culture Collection, Manassas, Va (www.atcc.org). Preferred host cells include cells of lymphoid origin such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851). In a particularly preferred embodiment, the recombinant cell is a P3X63Ag8.653 or a SP2/0-Ag14 cell.

[0066] Expression vectors for these cells can include one or more of the following expression control sequences. such as, but not limited to an origin of replication; a promoter (e.g., late or early SV40 promoters, the CMV promoter (US Pat.Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (US Pat.No. 5,266,491). at least one human immunoglobulin promoter; an enhancer. and/or processing information sites. such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., supra; Sambrook, et al., supra. Other cells useful for production of nucleic acids or proteins of the present invention are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (www.atcc.org) or other known or commercial sources.

[0067] When eukaryotic host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

**Purification of an antibody**

[0068] An anti-IL-12 antibody can be recovered and purified from recombinant cell cultures by well-known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin chromatography. High performance liquid chromatography (“HPLC”) can also be employed for purification. See, e.g., Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001), e.g., Chapters 1, 4, 6, 8, 9, 10, each entirely incorporated herein by reference.

[0069] Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the antibody of the present invention can be glycosylated or can be non-glycosylated, with glycosylated preferred. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Sections 17.37-17.42; Ausubel, supra, Chapters 10, 12, 13, 16, 18 and 20, Colligan, Protein Science, supra, Chapters 12-14, all entirely incorporated herein by reference.

**Anti-IL-12 Antibodies**

[0070] The isolated antibodies of the present invention comprise an antibody encoded by any one of the polynucleotides or the present invention as discussed more fully herein, or any isolated or prepared antibody. Preferably, the human antibody or antigen-binding fragment binds human IL-12 and, thereby partially or substantially neutralizes at least one biological activity of the protein. An antibody, or specified portion or variant thereof, that partially or preferably substantially
neutralizes at least one biological activity of at least one IL-12 protein or fragment can bind the protein or fragment and
thereby inhibit activities mediated through the binding of IL-12 to the IL-12 receptor or through other IL-12-dependent or
mediated mechanisms. As used herein, the term "neutralizing antibody" refers to an antibody that can inhibit an IL-12-
dependent activity by about 20-120%, preferably by at least about 10, 20, 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91,
92, 93, 94, 95, 96, 97, 98, 99, 100% or more depending on the assay. The capacity of an anti-IL-12 antibody to inhibit
an IL-12-dependent activity is preferably assessed by at least one suitable IL-12 protein or receptor assay, as described
herein and/or as known in the art. A human antibody of the invention can be of any class (IgG, IgA, IgM, IgE, IgD, etc.)
or isotype and can comprise a kappa or lambda light chain. In one embodiment, the human antibody comprises an IgG
heavy chain or defined fragment, for example, at least one of isotypes, IgGI, IgG2, IgG3 or IgG4. Antibodies of this type
can be prepared by employing a transgenic mouse or other transgenic non-human mammal comprising at least one
human light chain (e.g., IgG, IgA and IgM (e.g., γ1, γ2, γ3, γ4) transgenes as described herein and/or as known in the
art. In another embodiment, the anti-human IL-12 human antibody comprises an IgGI heavy chain and a IgGl light chain.

[0071] At least one antibody of the invention binds at least one specified epitope specific to at least one IL-12 protein,
subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one antibody
binding region that comprises at least one portion of said protein, which epitope is preferably comprised of at least one
extracellular, soluble, hydrophillic, external or cytoplasmic portion of said protein. The at least one specified epitope can
comprise any combination of at least one amino acid sequence of at least 1-3 amino acids into the entire specified portion
of contiguous amino acids of the SEQ ID NO:9.

[0072] Generally, the human antibody or antigen-binding fragment of the present invention will comprise an antigen-
binding region that comprises at least one human complementarity determining region (CDR1, CDR2 and CDR3) or
variant of at least one heavy chain variable region and at least one human complementarity determining region (CDR1,
CDR2 and CDR3) or variant of at least one light chain variable region. As a non-limiting example, the antibody or antigen-
binding portion or variant can comprise at least one of the heavy chain CDR3 having the amino acid sequence of SEQ
ID NO:3, and/or a light chain CDR3 having the amino acid sequence of SEQ ID NO:6. In a particular embodiment, the
antibody or antigen-binding fragment can have an antigen-binding region that comprises at least a portion of at least
one heavy chain CDR (i.e.. CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs
1, 2 and/or 3 (e.g. SEQ ID NOS:1, 2, and/or 3). In another particular embodiment, the antibody or antigen-binding portion
or variant can have an antigen-binding region that comprises at least a portion of at least one light chain CDR (i.e.,
CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs 1, 2 and/or 3 (e.g., SEQ ID
NOS: 4, 5, and/or 6). In a preferred embodiment the three heavy chain CDHRs and the three light chain CDHRs of the
antibody or antigen-binding fragment have the amino acid sequence of the corresponding CDR of at least one of mAb
12B75, C340, or any others as described herein. Such antibodies can be prepared by chemically joining together the
various portions (e.g., CDRs, framework) of the antibody using conventional techniques, by preparing and expressing
a (i.e., one or more) nucleic acid molecule that encodes the antibody using conventional techniques of recombinant
data technology or by using any other suitable method.

[0073] The anti-IL-12 antibody can comprise at least one of a heavy or light chain variable region having a defined
amino acid sequence. For example, in a preferred embodiment, the anti-IL-12 antibody comprises at least one of at
least one heavy chain variable region, optionally having the amino acid sequence of SEQ ID NO:7 and/or at least one
light chain variable region, optionally having the amino acid sequence of SEQ ID NO:8: antibodies that bind to human
IL-12 and that comprise a defined heavy or light chain variable region can be prepared using suitable methods, such
as phage display (Katsube, Y., et al., Int J Mol. Med, 1(5):863-868 (1998)) or methods that employ transgenic animals,
as know in the art and/or as described herein. For example, a transgenic mouse, comprising a functionally rearranged
human immunoglobulin heavy chain transgene and a transgene comprising DNA from a human immunoglobulin light
chain locus that can undergo functional realignment, can be immunized with human IL-12 or a fragment thereof to
elicit the production of antibodies. If desired, the antibody producing cells can be isolated and hybridomas or other
immortalized antibody-producing cells can be prepared as described herein and/or as known in the art. Alternatively,
the antibody, specified portion or variant can be expressed using the encoding nucleic acid or portion thereof in a suitable
host cell.

[0074] The invention also relates to antibodies, antigen-binding fragments, immunoglobulin chains and CDRs com-
prising amino acids in a sequence that is substantially the same as an amino acid sequence described herein. Preferably,
such antibodies or antigen-binding fragments and antibodies comprising such chains or CDRs can bind human IL-12
with high affinity (e.g., K<sub>D</sub> less than or equal to about 10<sup>-9</sup> M). Amino acid sequences that are substantially the same as
the sequences described herein include sequences comprising conservative amino acid substitutions, as well as amino
acid deletions and/or insertions. A conservative amino acid substitution refers to the replacement of a first amino acid
by a second amino acid that has chemical and/or physical properties (e.g., charge, structure, polarity, hydrophobicity/
hydrophilicity) that are similar to those of the first amino acid. Conservative substitutions include replacement of one
amino acid by another within the following groups: lysine (K), arginine (R) and histidine (H); aspartate (D) and glutamate
(E); asparagine (N), glutamine (Q), serine (S), threonine (T), tyrosine (Y), K, R, H, D and E; alanine (A), valine (V),...
leucine (L), isoleucine (I), proline (P), phenylalanine (F), tryptophan (W), methionine (M), cysteine (C) and glycine (G); F, W and Y; C, S and T.

Amino Acid Codes

The amino acids that make up anti-IL-12 antibodies of the present invention are often abbreviated. The amino acid designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B., et al., Molecular Biology of The Cell, Third Ed., Garland Publishing, Inc., New York, 1994):

<table>
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<tr>
<th>SINGLE LETTER CODE</th>
<th>THREE LETTER CODE</th>
<th>NAME</th>
<th>THREE NUCLEOTIDE CODON(S)</th>
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<tr>
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<td>Ala</td>
<td>Alanine</td>
<td>GCA, GCC, GCG, GCU</td>
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<tr>
<td>C</td>
<td>Cys</td>
<td>Cysteine</td>
<td>UGC, UGU</td>
</tr>
<tr>
<td>D</td>
<td>Asp</td>
<td>Aspartic acid</td>
<td>GAC, GAU</td>
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<td>Glu</td>
<td>Glutamic acid</td>
<td>GAA, GAG</td>
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<tr>
<td>F</td>
<td>Phe</td>
<td>Phenylalanine</td>
<td>UUC, UUU</td>
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<tr>
<td>G</td>
<td>Gly</td>
<td>Glycine</td>
<td>GGA, GCC, GGG, GGU</td>
</tr>
<tr>
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<td>His</td>
<td>Histidine</td>
<td>CAC, CAU</td>
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<td>Isoleucine</td>
<td>AUA, AUC, AUU</td>
</tr>
<tr>
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<td>Lys</td>
<td>Lysine</td>
<td>AA, AAG</td>
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<td>UUA, UUG, CU, CUC, CUG, CU</td>
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<td>P</td>
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<td>Tyr</td>
<td>Tyrosine</td>
<td>UAC, UAU</td>
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</table>

An anti-IL-12 antibody of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein.

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions, insertions or deletions for any given anti-IL-12 Ig-derived protein, fragment or variant will not be more than 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, such as 1-30 or any range or value therein, as specified herein.

Amino acids in an anti-IL-12 antibody of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, supra, Chapters 8, 15; Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to at least one IL-12 neutralizing activity. Sites that are critical for antibody binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899-904 (1992) and de Vos, et al., Science 255:306-312 (1992)).

Anti-IL-12 antibodies of the present invention can include, but are not limited to, at least one portion, sequence or combination selected from 5 to all of the contiguous amino acids of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, 6.

IL-12 antibodies or specified portions or variants of the present invention can include, but are not limited to, at
least one portion, sequence or combination selected from at least 3-5 contiguous amino acids of SEQ ID NO: 1, 5-17 contiguous amino acids of SEQ ID NO:2, 5-10 contiguous amino acids of SEQ ID NO:3, 5-11 contiguous amino acids of SEQ ID NO:4, 5-7 contiguous amino acids of SEQ ID NO:S: 5-9 contiguous amino acids of SEQ ID NO:6; Leu21, Lys76, Met83, Ser85 of SEQ ID NO:7.

[0081] A(n) anti-IL-12 antibody can further optionally comprise a polypeptide of at least one of 70-100% of 5, 17, 10, 11, 7, 9, 119, or 108 contiguous amino acids of at least one of SEQ ID NO:S:1, 3, 4, 5, 6, 7 or 8.

[0082] In one embodiment, the amino acid sequence of an immunoglobulin chain, or portion thereof (e.g., variable region, CDR) has about 70-100% identity (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) to the amino acid sequence of the corresponding chain of at least one of SEQ ID NO:S:7, 8. For example, the amino acid sequence of a light chain variable region can be compared with the sequence of SEQ ID NO:8, or the amino acid sequence of a heavy chain CDR3 can be compared with SEQ ID NO:3. Preferably, 70-100% amino acid identity (i.e., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) is determined using a suitable computer algorithm, as known in the art.

[0083] Exemplary heavy chain and light chain variable regions sequences are provided in SEQ ID NO:S: 7 and 8. The antibodies of the present invention, or specified variants thereof, can comprise any number of contiguous amino acid residues from an antibody of the present invention, wherein that number is selected from the group of integers consisting of from 10- 100% of the number of contiguous residues in an anti-IL-12 antibody. Optionally, this subsequence of contiguous amino acids is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250 or more amino acids in length, or any range or value therein. Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as at least 2, 3, 4, or 5.

[0084] As those of skill will appreciate, the present invention includes at least one biologically active antibody of the present invention. Biologically active antibodies can have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95%-100% of that of the native (non-synthetic), endogenous or related and known antibody. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity, are well known to those of skill in the art.

[0085] In another aspect, the invention relates to human antibodies and antigen-binding fragments, as described herein, which are modified by the covalent attachment of an organic moiety. Such modification can produce an antibody or antigen-binding fragment with improved pharmacokinetic properties (e.g., increased in vivo serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

[0086] The modified antibodies and antigen-binding fragments of the invention can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the antibody. Each organic moiety that is bonded to an antibody or antigen-binding fragment of the invention can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term "fatty acid" encompasses mono-carboxylic acids and dicarboxylic acids. A "hydrophilic polymeric group," as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, an antibody modified by the covalent attachment of polylysine is encompassed by the invention. Hydrophilic polymers suitable for modifying antibodies of the invention can be linear or branched and include, for example, polyalkane glycols (e.g., PEG, monomethoxy-polyethylene glycol imPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polysapartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrrolidone. Preferably, the hydrophilic polymer that modifies the antibody of the invention has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example PEG_{4000} and PEG_{20,000}, wherein the subscript is the average molecular weight of the polymer in Daltons, can be used. The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (e.g., activated with N,N-carbonvi diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

[0087] Fatty acids and fatty acid esters suitable for modifying antibodies of the invention can be saturated or can contain one or more unsaturation. Fatty acids that are suitable for modifying antibodies of the invention include, for example, n-dodecanoate (C_{12}, laurate), n-tetradecanoate (C_{14}, myristate), n-octadecanoate (C_{18}, stearate), n-eicosanoate (C_{20}, arachidate), n-docosanoate (C_{22}, behenate), n-triacontanoate (C_{30}), n-tetracontanoate (C_{40}), cis-Δ9-octadecanoate (C_{18}, oleate), all cis-Δ5,8,11,14-eicosatetraenoate (C_{20}, arachidonate), octadecanoic acid, tetradecanoic acid, octadecanedic acid, docosanedic acid, and the like. Suitable fatty acid esters include mono-esters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve,
The modified human antibodies and antigen-binding fragments can be prepared using suitable methods, such as by reaction with one or more modifying agents. A "modifying agent" as the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty acid ester) that comprises an activating group. An "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acryloyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimidate linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., Bioconjugate Techniques, Academic Press: San Diego, CA (1996)). An activating group can be bonded directly to the organic group (e.g., hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, for example a divalent C1-C12 wherein one or more carbon atoms can be replaced by a heteroatom such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example, tetraethylene glycol, -(CH2)2-NH-(CH2)6-NH-, -(CH2)2-NH- and -CH2-O-CH2-CH2-O-CH2-CH2-O-CH-NH2. Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkylamine (e.g., mono-Boc-ethylamine, mono-Boc-diaminohexane) with a fatty acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate as described, or can be reacted with maleic anhydride and the resulting product cyclized to produce an activated maleimido derivative of the fatty acid. (See, for example, Thompson, et al., WO 92/16221 the entire teachings of which are incorporated herein by reference.)

The modified antibodies of the invention can be produced by reacting a human antibody or antigen-binding fragment with a modifying agent. For example, the organic moieties can be bonded to the antibody in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified human antibodies or antigen-binding fragments can also be prepared by reducing disulfide bonds (e.g., intra-chain disulfide bonds) of an antibody or antigen-binding fragment. The reduced antibody or antigen-binding fragment can then be reacted with a thiol-reactive modifying agent to produce the modified antibody of the invention. Modified human antibodies and antigen-binding fragments comprising an organic moiety that is bonded to specific sites of an antibody of the present invention can be prepared using suitable methods, such as reverse proteolysis (Fisch et al., Bioconjugate Chem., 3:147-153 (1992); Werlen et al, Bioconjugate Chem., 5:411-417 (1994); Kumaran et al., Protein Sci. 6(10):2233-2241 (1997); Itoh et al., Bioorg. Chem., 24(1):59-68 (1996); Capellas et al., Biotechnol. Bioeng., 56(4):456-463 (1997)), and the methods described in Hermanson, G. T., Bioconjugate Techniques, Academic Press: San Diego, CA (1996).

ANTICDIOTYPE ANTIBODIES TO ANTI-IL-12 IG DERIVED PROTEIN COMPOSITIONS

In addition to monoclonal or chimeric anti-IL-12 antibodies, the present invention is also directed to an anti-idiotypic (anti-Id) antibody specific for such antibodies of the invention. An anti-Id antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding region of another antibody. The anti-Id can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the Id antibody with an immunogen to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody.

ANTI-IL-12 IG DERIVED PROTEIN COMPOSITIONS

The present invention also provides at least one anti-IL-12 antibody composition comprising at least one, at least two, at least three, at least four, at least five, at least six or more anti-IL-12 antibodies thereof, as described herein and/or as known in the art that are provided in a non-naturally occurring composition, mixture or form. Such compositions comprise non-naturally occurring compositions comprising at least one or two full length, C- and/or N-terminally deleted variants, domains, fragments, or specified variants, of the anti-IL-12 antibody amino acid sequence selected from the group consisting of 70-100% of the contiguous amino acids of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 or 8, or specified fragments, domains or variants thereof. Preferred anti-IL-12 derived protein, fragment or variant compositions include at least one or two full length, fragments, domains or variants as at least one CDR containing portions of the anti-IL-12 antibody sequence of 70-100% of SEQ ID NOS: 1, 2, 3, 4, 5, 6, or specified fragments, domains or variants thereof. Further preferred compositions comprise 40-99% of at least one of 70-100% of SEQ ID NOS: 1,2,3,4,5,6, or specified fragments, domains or variants thereof. Such composition percentages are by weight, volume, concentration, molarity, or molality.
as liquid or dry solutions, mixtures, suspension, emulsions or colloids, as known in the art or as described herein.

**[0092]** Anti-IL-12 antibody compositions of the present invention can further comprise at least one of any suitable and effective amount of a composition or pharmaceutical composition comprising at least one anti-IL-12 antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy, optionally further comprising at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antihematogetic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, lefunomide, sulfa-salazine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic a neuromuscular blocker, an antimicrobial (e.g., aminglycoside, an antifungal, an antiparasitic an antiviral a carbapenem, cephalosporin a florourquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antiportisporic, a corticosteroid, an anabolic steroid a diabetes related agent, a mineral a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antialucer, a laxative, an anticoagulant an erythropoietin (e.g., epoetin alpha), a filgrastin (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antiaminic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromoly, an epinephrine or analog, tramazone alpha (Pulmozyme), a cytokine or a cytokine antagonist. Such anti-cancer or anti-infectives can also include toxin molecules that are associated, bound. co-formulated or co-administered with at least one antibody of the present invention. The toxin can optionally act to selectively kill the pathologic cell or tissue. The pathologic cell can be a cancer or other cell. Such toxins can be, but are not limited to, purified or recombinant toxin or toxin fragment comprising at least one functional cytotoxic domain of toxin. e.g., selected from at least one of ricin, diphtheria toxin, a venom toxin, or a bacterial toxin. The term toxin also includes both endotoxins and exotoxins produced by any naturally occurring, mutant or recombinant bacteria or viruses which may cause any pathologic condition in humans and other mammals, including toxin shock, which can result in death. Such toxins may include, but are not limited to, enterotoxigenic E. coli heat-labile enterotoxin (LT), heat-stable enterotoxin (ST), Shigella cytotoxin, Aeromonas enterotoxins, toxic shock syndrome toxin-1 (TSST-1), Staphylococcal enterotoxin A (SEA), B (SEB), or C (SEC), Streptococcal enterotoxins and the like. Such bacteria include, but are not limited to, strains of a species of enterotoxigenic E. coli (ETEC), enterohemorrhagic E. coli (e.g., strains of serotype O157:H7), Staphylococcus species (e.g., Staphylococcus aureus, Staphylococcus pyogenes), Shigella species (e.g., Shigella dysenteriae, Shigella flexneri, Shigella boydii, and Shigella sonnei), Salmonella species (e.g., Salmonella typhi, Salmonella cholera-suis, Salmonella enteritidis), Clostridium species (e.g., Clostridium perfringens, Clostridium difficile, Clostridium botulinum), Camphobacter species (e.g., Camphobacter jejuni, Camphobacter fetus), Helicobacter species, (e.g., Helicobacter pylori), Aeromonas species (e.g., Aeromonas sobria, Aeromonas hydrophila, Aeromonas caviae), Pleisomonas shigelloides, Yersinia enterocolitica, Vibris species (e.g., Vibrios cholerae, Vibrios parahaemolyticus), Klebsiella species, Pseudomonas aeruginosa, and Streptococci. See, e.g., Stein, ed., KLEBSIELLA species, Pseudomonas aeruginosa and Streptococci. See, e.g., Stein, ed., INTERNAL MEDICINE, 3rd ed., pp 1-13, Little, Brown and Co., Boston, (1990); Evans et al., eds., Bacterial Infections of Humans: Epidemiology and Control, 2d. Ed.; pp 239-254, Plenum Medical Book Co., New York (1991); Mandell et al, Principles and Practice of Infectious Diseases, 3d. Ed., Churchill Livingstone. New York (1990); Berkow et al, eds., The Merck Manual, 16th edition, Merck and Co., Rahway, N.J., 1992; Wood et al, FEMS Microbiology Immunology, 76:121-134 (1991); Marrack et al, Science, 248:705-711 (1990), the contents of which references are incorporated entirely herein by reference. Anti-IL-12 antibody compounds, compositions or combinations of the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, buffer, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmacologically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions arcw well known in the art, such as, but limited to, Gennaro, Ed., Remington’s Pharmaceutical Sciences, 18th Edition, Mack Publishing Co. (Easton, PA) 1990. Pharmacologically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the anti-IL-12 antibody, fragment or variant composition as well known in the art or as described herein.

**[0095]** Pharmaceutical excipients and additives useful in the present composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditos, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising or in combination comprising 1-99.99% by weight or
Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextran, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myo-inositol, and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

Anti-IL-12 antibody compositions can also include a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

Additionally, anti-IL-12 antibody compositions of the invention can include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polyethoxylates such as "TWEEN 20" and "TWEEN 80"), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

These and additional known pharmaceutical excipients and/or additives suitable for use in the anti-IL-12 antibody, portion or variant compositions according to the invention are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), and in the "Physician’s Desk Reference", 52nd ed., Medical Economics, Montvale, NJ (1998), the disclosures of which are entirely incorporated herein by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

Formulations

As noted above, the invention provides for stable formulations, which is preferably a phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multiple-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one anti-IL-12 antibody in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrate, phenoxethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzetonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1-2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

As noted above, the invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one anti-IL-12 antibody with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising lyophilized at least one anti-IL-12 antibody, and a second vial comprising an aqueous diluent of prescribed buffer or preservative wherein said packaging material comprises a label that instructs a patient to reconstitute the at least one anti-IL-12 antibody in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

The at least one anti-IL-12 antibody used in accordance with the present invention can be produced by recombinant means, including from mammalian cell or transgenic preparations, or can be purified from other biological sources, as described herein or as known in the art.
[0104] Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient to yield an anti-microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

[0105] Other excipients, e.g. isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably the formulations of the present invention have pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably sodium phosphate, particularly phosphate buffered saline (PBS).

[0106] Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan mono-palmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants such as polysorbate 20 or 80 or poloxamer 184 or 188, Pluronic® polyols, other block co-polymers, and chelators such as EDTA and EGTA can optionally be added to the formulations or compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the formulation. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.

[0107] The formulations of the present invention can be prepared by a process which comprises mixing at least one anti-IL-12 antibody and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one anti-IL-12 antibody and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one anti-IL-12 antibody in buffered solution is combined with the desired preservative in a buffered solution in quantities sufficient to provide the protein and preservative at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

[0108] The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-IL-12 antibody that is reconstituted with a second vial containing water, a preservative and/or excipients, preferably a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus can provide a more convenient treatment regimen than currently available.

[0109] The present claimed articles of manufacture are useful for administration over a period of immediately to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient. Formulations of the invention can optionally be safely stored at temperatures of from about 2 to about 40°C and retain the biologically activity of the protein for extended periods of time, thus, allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. If preserved diluent is used, such label can include use up to 1-12 months, one-half, one and a half, and/or two years.

[0110] The solutions of at least one anti-IL-12 antibody in the invention can be prepared by a process that comprises mixing at least one antibody in an aqueous diluent. Mixing is carried out using conventional dissolution and mixing procedures. To prepare a suitable diluent, for example, a measured amount of at least one antibody in water or buffer is combined in quantities sufficient to provide the protein and optionally a preservative or buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

[0111] The claimed products can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-IL-12 antibody that is reconstituted with a second vial containing the aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

[0112] The claimed products can be provided indirectly to patients by providing to pharmacies, clinics, or other such institutions and facilities, clear solutions or dual vials comprising a vial of lyophilized at least one anti-IL-12 antibody that is reconstituted with a second vial containing the aqueous diluent. The clear solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one antibody solution can be retrieved one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their customers.
The present invention also provides a method for modulating or treating at least one immune related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of rheumatoid arthritis, juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, psoriatic arthritis, ankylosing spondilitis, gastric ulcer, seronegative arthropathies, osteoarthritis, inflammatory bowel disease, ulcerative colitis, systemic lupus erythematosus, antiphospholipid syndrome, iridocyclitis/uveitis/optic neuritis, idiopathic pulmonary fibrosis, systemic vasculitis/wegener’s granulomatosis, sarcoidosis, orchiitis/vasectomy reversal procedures, allergic/atopic diseases, asthma/allergic rhinitis, eczema, allergic contact dermatitis, allergic conjunctivitis, hypersensitivity pneumonitis, transplants, organ transplant rejection, graft-versus-host disease, systemic inflammatory response syndrome, sepsis syndrome, gram positive sepsis, gram negative sepsis, culture negative sepsis, fungal sepsis, neutropenic fever, urosepsis, meningococcemia, trauma/hemorrhage, burns, ionizing radiation exposure, acute pancreatitis, adult respiratory distress syndrome, rheumatoid arthritis. alcohol-induced hepatitis, chronic inflammatory pathologies, sarcoidosis. Crohn’s pathology, sickle cell anemia, diabetes, nephrosis, atopic diseases, hypersensitivity reactions. allergic rhinitis, hay fever, perennial rhinitis, conjunctivitis, endometriosis, asthma, urticaria, systemic anaphalaxis, dermatitis, pernicious anemia, hemolytic disesease, thrombocytopenia. graft rejection of any organ or tissue, kidney transplant rejection, heart transplant rejection, liver transplant rejection, pancreas transplant rejection, lung transplant rejection, bone marrow transplant (BMT) rejection, skin allograft rejection, cartilage transplant rejection, bone graft rejection, small bowel transplant rejection, fetal thymus implant rejection, parathyroid transplant rejection, xenograft rejection of any organ or tissue, allotransplant rejection. anti-receptor hypersensitivity reactions, Graves disease, Raynoud’s disease, type B insulin-resistant diabetes, asthma, myasthenia gravis, antibody-mediated cytotoxicity, type III hypersensitivity reactions, systemic lupus erythematosus, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes syndrome, antiphospholipid syndrome, pemphigus, scieroderma, mixed connective tissue disease, idiopathic Addison’s disease, diabetes mellitus, chronic active hepatitis, primary biliary cirrhosis, vitiligo, vasculitis, post-MI cardiomyopathy, type IV

[0119] The present invention also provides a method for modulating or treating at least one cardiovascular disease in a cell, tissue, organ, animal, or patient, including, but not limited to, concurrently, and/or after, at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an anti-inflammatory (e.g., methotrexate, auranofin, aurothiol-glucois, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalazine), a muscle relaxant a narcotic, a non-steroid anti-inflammatory drug (NSAID), an anaglytic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a tetracycline, macrolide, a penicillin, a sulfonamide, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiabetic, an antihypertensive, an anti-platelet, a laxative, an anticoagulant, an ertapenem, an erythromycin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplic, an alkylation agent, an antimitobolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, an antianimal agent, an antipsychotic, an antiepileptic, an hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an antihistamine, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2nd Edition, Appleton and Lange, Stamford, CT (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, CA (2000), each of which references are entirely incorporated herein by reference.

[0120] TNF antagonists suitable for compositions, combination therapy; co-administration, devices and/or methods of the present invention (further comprising at least one anti body, specified portion and variant thereof, of the present invention), include, but are not limited to, anti-TNF antibodies, antigen-binding fragments thereof, and receptor molecules which bind specifically to TNF; compounds which prevent and/or inhibit TNF synthesis, TNF release or its action on target cells, such as thalidomide, tenida, phosphodiesterase inhibitors (e.g. pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers; compounds which prevent and/or inhibit TNF receptor signaling, such as mitogen activated protein (MAP) kinase inhibitors; compounds which block and/or inhibit membrane TNF cleavage, such as metalloproteinase inhibitors; compounds which block and/or inhibit membrane TNF production and/or synthesis, such as MAP kinase inhibitors.

[0121] As used herein, a “tumor necrosis factor antibody,” “TNF antibody,” “TNF antibody,” or fragment and the like decreases, blocks, inhibits, abrogates or interferes with TNF activity in vitro, in situ and/or preferably in vivo. For example, a suitable TNF human antibody of the present invention can bind TNFα and includes anti-TNF antibodies, antigen-binding fragments thereof, and specified mutants or domains thereof that bind specifically to TNFα. A suitable TNF antibody or fragment can also decrease block, abrogate, interfere, prevent and/or inhibit TNF RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane TNF cleavage, TNF activity, TNF production and/or synthesis.

[0122] Chimeric antibody cA2 consists of the antigen binding variable region of the high-affinity neutralizing mouse anti-human TNFα IgG1 antibody, designated A2, and the constant regions of human IgGl, kappa immunoglobulin. The human IgGl Fc region improves allogeneic antibody effector function, increases the circulating serum half-life and decreases the immunogenicity of the antibody. The avidity and epitope specificity of the chimeric antibody cA2 is derived from the variable region of the murine antibody A2. In a particular embodiment, a preferred source for nucleic acids encoding the variable region of the murine antibody A2 is the A2 hybridoma cell line.

[0123] Chimeric A2 (cA2) neutralizes the cytotoxic effect of both natural and recombinant human TNFα in a dose dependent manner. From binding assays of chimeric antibody cA2 and recombinant human TNFα, the affinity constant of chimeric antibody cA2 was calculated to be 1.04x10^10M^-1. Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow, et al., antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988; Coligan et al., eds., Current Protocols in Immu-

[0124] In a particular embodiment, murine monoclonal antibody A2 is produced by a cell line designated c134A. Chimeric antibody cA2 is produced by a cell line designated c168A.


TNF Receptor Molecules

[0126] Preferred TNF receptor molecules useful in the present invention are those that bind TNF with high affinity (see, e.g., Feldmann et al., International Publication No. WO 92/07076 (published April 30, 1992); Schall et al., Cell 61: 361-370 (1990); and Loetscher et al., Cell 61:351-359 (1990), which references are entirely incorporated herein by reference) and optionally possess low immunogenicity. In particular, the 55 kDa (p55 TNF-R) and the 75 kDa (p75 TNF-R) TNF cell surface receptors are useful in the present invention. Truncated forms of these receptors, comprising the extracellular domains (ECD) of the receptors or functional portions thereof (see, e.g., Corcoran et al., Eur. J. Biochem. 232:831-840 (1994)), are also useful in the present invention. Truncated forms of the TNF receptors, comprising the ECD, have been detected in urine and serum as 30 kDa and 40 kDa TNF inhibitory binding proteins (Engelmann, H. et al., J. Biol. Chem. 265:1531-1536 (1990)). TNF receptor multimeric molecules and TNF immunoreceptor fusion molecules, and derivatives and fragments or portions thereof, are additional examples of TNF receptor molecules which are useful in the methods and compositions of the present invention. The TNF receptor molecules which can be used in the invention are characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, can contribute to the therapeutic results achieved.

[0127] TNF receptor multimeric molecules useful in the present invention comprise all or a functional portion of the ECD of two or more TNF receptors linked via one or more polypeptide linkers or other nonpeptide linkers, such as polyethylene glycol (PEG). The multimeric molecules can further comprise a signal peptide of a secreted protein to direct expression of the multimeric molecule. These multimeric molecules and methods for their production have been described in U.S. Application No. 08/437,533 (filed May 9, 1995), the content of which is entirely incorporated herein by reference.

[0128] TNF immunoreceptor fusion molecules useful in the methods and compositions of the present invention comprise at least one portion of one or more immunoglobulin molecules and all or a functional portion of one or more TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. An example of such a TNF immunoreceptor fusion molecule is TNF receptor/IgG fusion protein. TNF immunoreceptor fusion molecules and methods for their production have been described in the art (Lesslauer et al., Eur. J. Immunol 11:2883-2886 (1991); Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Peppel et al., J. Exp. Med 174:1483-1489 (1991); Kolls et al., Proc. Natl. Acad. Sci. LSA 91:215-219 (1994); Butler et al., Cytokine 6(6):616-623 (1994); Baker et al., Eur. J. Immunol. 24: 2040-2048 (1994); Beutler et al., U.S. Patent No. 5,447,851; and U.S. Application No. 08/442,133 (filed May 16, 1995), each of which references are entirely incorporated herein by reference). Methods for producing immunoreceptor fusion molecules can also be found in Capon et al., U.S. Patent No. 5,116,964; Capon et al., U.S. Patent No. 5,225,538; and Capon et al., Nature 337:525-531 1 (1989), which references are entirely incorporated herein by reference.

[0129] A functional equivalent, derivative, fragment or region of TNF receptor molecule refers to the portion of the TNF receptor molecule, or the portion of the TNF receptor molecule sequence which encodes TNF receptor molecule, that is of sufficient size and sequences to functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF with high affinity and possess low immunogenicity). A functional equivalent of TNF receptor molecule also includes modified TNF receptor molecules that functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF with high affinity and possess low immunogenicity). For example, a functional equivalent of TNF receptor molecule can contain a "SILENT" codon or one or more amino acid substitutions, deletions or additions (e.g., substitution of one acidic amino acid for another acidic amino acid; or substitution of one codon encoding the same or different hydrophobic amino acid for another codon encoding a hydrophobic amino acid). See Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and-Wiley-Interscience, New York (1987-2000).
Cytokines include any mown cytokine. See, e.g., www.CopewithCytokines.com. Cytokine antagonists include, but are not limited to, any antibody, fragment or mimetic, any soluble receptor, fragment or mimetic, any small molecule antagonist, or any combination thereof.

**Therapeutic Treatments.** Any method of the present invention can comprise a method for treating a IL-12 mediated disorder, comprising administering an effective amount of a composition or pharmaceutical composition comprising at least one anti-IL-12 antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one anti-IL-12 antibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one of at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalazine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminglycoside, an antifungal, an antiparasitic, an antiviral, a carbenapenem, cephalosporin, a fluorourquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antiarheal, an antiulcer, a laxative, an antiinflammatory, an antiarthritic, a anticoagulant, an erythropoietin (e.g., epoetin alpha), a fibrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an antirheumatic agent, an anticonvulsion, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, an antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma agent, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a fibrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an antirheumatic agent, an anticonvulsion, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, an antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist.

Typically, treatment of pathologic conditions is effected by administering an effective amount or dosage of at least one anti-IL-12 antibody composition that total, on average, a range from at least about 0.01 to 500 milligrams of at least one anti-IL-12 antibody per kilogram of patient per single or multiple administration, or any range, value or fraction thereof, or to achieve a serum concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9 and/or 100-500 mg/kg/dose, or any range, variety or fraction thereof, to achieve a serum concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.5, 1.9, 2.0, 2.5, 2.9, 3.0, 3.5, 3.9, 4.0, 4.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 10.9, 11.5, 11.9, 12.5, 12.9, 13.0, 13.5, 13.9, 14.0, 14.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 10.0, 10.5, 10.9, 11.5, 11.9, 12.5, 12.9, 13.2, 13.9, 14.3, 14.9, 15.4, 15.9, 16.5, 16.9, 17.5, 17.9, 18.5, 18.9, 19.5, 19.9, 20.0, 20.5, 20.9, 21.2, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and/or 5000 μg/ml serum concentration per single or multiple administration, or any range, value or fraction thereof.

Alternatively, the dosage administered can vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.1 to 50, and preferably 0.1 to 10 milligrams per kilogram per administration or in sustained release form is effective to obtain desired results.

As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one antibody of the present invention 0.1 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2.0, 3.5, 4.5, 5.6, 7.8, 9.10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 56, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively or additionally, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52, or alternatively or additionally, at least one of 1, 2, 3, 4, 5, 6, 7,
Alternative Administration

Many known and developed modes of can be used according to the present invention for administering pharmaceutically effective amounts of at least one anti-IL-12 antibody according to the present invention. While pulmonary administration is used in the following description, other modes of administration can be used according to the present invention with suitable results.

Alternative Delivery

The invention further relates to the administration of at least one anti-IL-12 antibody by parenteral, subcutaneous, intramuscular, intravenous, intratrigeminal, intrategmental, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intradermal, intramuscular, intravenous, intrarticular, intrabranchial, intraabdominal, intracapsular. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 1-10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

Parenteral Formulations and Administration

Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usuable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent, or suspending solvent, sterile involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or semisynthetic mono- or di- or triglycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressurized needleless injection device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446 entirely incorporated herein by reference.

For parenteral administration, the antibody can be formulated as a solution, suspension, emulsion or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer’s solution, dextrose solution, and 1-10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

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The invention further relates to the administration of at least one anti-IL-12 antibody by parenteral, subcutaneous, intramuscular, intravenous, intratrigeminal, intrategmental, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intradermal, intramuscular, intravenous, intrarticular, intrabranchial, intraabdominal, intracapsular. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 1-10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

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Pulmonary/Nasal Administration

[0143] For pulmonary administration, preferably at least one anti-IL-12 antibody composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses. According to the invention, at least one anti-IL-12 antibody can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Other devices suitable for directing the pulmonary or nasal administration of antibodies are also known in the art. All such devices can use of formulations suitable for the administration for the dispensing of antibody in an aerosol. Such aerosols can be comprised of either solutions (both aqueous and non aqueous) or solid particles. Metered dose inhalers like the Ventolin® metered dose inhaler, typically use a propellent gas and require actuation during inspiration (See, e.g., WO 94/16970, WO 98/35888). Dry powder inhalers like Turbuhaler™ (Astra), Rotahaler® (Glaxo), Diskus® (Glaxo), Spiros™ inhaler (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler® powder inhaler (Fisons), use breath-actuation of a mixed powder (US 4668218 Astra, EP 237507 Astra. WO 97/25086 Glaxo. WO 94/08552 Dura. US 5458135 Inhalo, WO 94/06498 Fisons, entirely incorporated herein by reference). Nebulizers like AERx™ Aradigm, the Ultravent® nebulizer (Mallinckrodt), and the Acorn II® nebulizer (Marquest Medical Products) (US 5404871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. These specific examples of commercially available inhalation devices are intended to be a representative of specific devices suitable for the practice of this invention, and are not intended as limiting the scope of the invention. Preferably, a composition comprising at least one anti-IL-12 antibody is delivered by a dry powder inhaler or a sprayer. There are a several desirable features of an inhalation device for administering at least one antibody of the present invention. For example, delivery by the inhalation device is advantageous reliable, reproducible, and accurate. The inhalation device can optionally deliver small dry particles, e.g. less than about 10 um, preferably about t \( \leq 5 \) \( \mu m \), for good respirability.

Administration of IL-12 antibody Composition as a Spray

[0144] A spray including IL-12 antibody composition protein can be produced by forcing a suspension or solution of at least one anti-IL-12 antibody through a nozzle under pressure.

[0145] The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of at least one anti-IL-12 antibody composition protein delivered by a sprayer have a particle size less than about 10 um, preferably in the range of about 1 \( \mu m \) to about 5 \( \mu m \), and most preferably about 2 \( \mu m \) to about 3 \( \mu m \).

[0146] Formulations of at least one anti-IL-12 antibody composition protein suitable for use with a sprayer typically include antibody composition protein in an aqueous solution at a concentration of about 0.1 mg to about 100 mg of at least one anti-IL-12 antibody composition protein per ml of solution or mg/gm, or any range or value therein, e.g., but not limited to, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/ml or mg/gm. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the antibody composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating antibody composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating antibody composition proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like. The antibody composition protein formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the antibody composition protein caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 14% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as IL-12 antibodies, or specified portions or variants, can also be included in the formulation.

Administration of IL-12 antibody compositions by a Nebulizer

[0147] antibody composition protein can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of antibody composition protein through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types...
can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, highfrequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of antibody composition protein either directly or through a coupling fluid, creating an aerosol including the antibody composition protein. Advantageously, particles of antibody composition protein delivered by a nebulizer have a particle size less than about 10 μm, preferably in the range of about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of at least one anti-IL-12 antibody suitable for use with a nebulizer, either jet or ultrasonic, typically include a concentration of about 0.1 mg to about 100 mg of at least one anti-IL-12 antibody protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, a solubilizer such as sorbitol. The formulation can also include an excipient or agent for stabilization of the at least one anti-IL-12 antibody composition protein, such as a buffer, a reducing agent, a surfactant, or a carbohydrate. Bulk proteins useful in formulating at least one anti-IL-12 antibody composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one anti-IL-12 antibody include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one anti-IL-12 antibody formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one anti-IL-12 antibody caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan mono-oleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as antibody protein can also be included in the formulation.

Administration of IL-12 antibody compositions By A Metered Dose Inhaler

In a metered dose inhaler (MDI), a propellant, at least one anti-IL-12 antibody, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10 μm, preferably about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm. The desired aerosol particle size can be obtained by employing a formulation of antibody composition protein produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

Formulations of at least one anti-IL-12 antibody for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one anti-IL-12 antibody as a suspension in a non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluoroalkane-134a), HFA-227 (hydrofluoroalkane-227), or the like. Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one anti-IL-12 antibody as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for formulation of a protein such as protein can also be included in the formulation.

One of ordinary skill in the art will recognize that the methods of the current invention can be achieved by pulmonary administration of at least one anti-IL-12 antibody compositions via devices not described herein.

Oral Formulations and Administration

Formulations for oral rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl(polyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFP) and trasyloil) to inhibit enzymatic degradation. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, -tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc.

Tablets and pills can be further processed into enteric-coated preparations. The liquid preparations for oral administration include emulsion, syrup, elixir, suspension and solution preparations allowable for medical use. These preparations can contain inactive diluting agents ordinarily used in said field, e.g., water. Liposomes have also been
described as drug delivery systems for insulin and heparin (U.S. Pat. No. 4,239,754). More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No. 4,925,673). Furthermore, carrier compounds described in U.S. Pat. No. 5,879,681 and U.S. Pat. No. 5,5,871,753 are used to deliver biologically active agents orally are known in the art.

Mucosal Formulations and Administration

[0154] For absorption through mucosal surfaces, compositions and methods of administering at least one anti-IL-12 antibody include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving mucocoadhesion of the emulsion particles (U.S. Pat. Nos. 5,514,670). Mucous surfaces suitable for application of the emulsions of the present invention can include corneal, conjunctival, buccal, sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration. Formulations for vaginal or rectal administration, e.g. suppositories, can contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for intranasal administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily solutions of nasal drops. For buccal administration excipients include sugars, calcium stearate, magnesium stearate, pregelatined starch, and the like (U.S. Pat. Nos. 5,849,695). Transdermal Formulations and Administration

[0155] For transdermal administration the at least one anti-IL-12 antibody is encapsulated in a delivery device such as a liposome or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated). A number of suitable devices are known, including microparticles made of synthetic polymers such as polyhydroxy acids such as polyactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes and natural polymers such as collagen, polyanino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U.S. Pat. Nos. 5,814,599).

Prolonged Administration and Formulations

[0156] It can be sometimes desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from a single administration. Various slow release, depot or implant dosage forms can be utilized. For example, a dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzyl-ethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) e.g. a zinc tannate salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. Pat. No. 3,773,919. The compounds or, preferably, relatively insoluble salts such as those described above can also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow release, depot or implant formulations, e.g. gas or lipid liposomes are known in the literature (U.S. Pat. Nos. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

[0157] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

[0158] The vector pC4 is used for the expression of IL-12 antibody. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (e.g., alpha minus MEM, Life Technologies, Gaithersburg, MD) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al., J. Biol. Chem. 253:1357-1370 (1978); J. L. Hamlin and C. Ma, Biochem. et Biophys. Acta 1097:107-143 (1990); and M. J. Page and M. A. Sydenham, Biotechnology 9:64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained that contain the amplified gene integrated into one or more chromosome(s) of the host cell.
Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, et al., Cell 41:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3’ intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human b-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV. Clontech’s Tet-Off and Tet-On gene expression systems and similar systems can be used to express the IL-12 in a regulated way in mammalian cells (M. Gossen, and H. Bujard, Proc. Natl. Acad. Sci. USA 89: 5547-5551 (1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete IL-12 antibody is used, e.g., as presented in SEQ ID NOS: INSERT MAB AA SEQ ID 1, and INSERT MAS AA SEQ ID NO2, corresponding to HC and LC variable regions of a IL-12 antibody of the present invention, according to known method steps. Isolated nucleic acid encoding a suitable human constant region (i.e., HC and LC regions) is also used in this construct (e.g., as provided in vector p1351: INSERT ATCC ACCESSION NUMBER AND ADDITIONAL HILC plasmids).

The isolated variable and constant region encoding DNA and the dephosphorylated vector are then ligated with T4 DNA ligase. E. coli HB 101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5 g of the expression plasmid pC4 is cotransfected with 0.5 g of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 g/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 g/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained that grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

Example 2: Generation of High Affinity Human IgG Monoclonal Antibodies Reactive With Human IL-12 Using Transgenic Mice

Summary

Transgenic mice have been used that contain human heavy and light chain immunoglobulin genes to generate high affinity, completely human, monoclonal antibodies that can be used therapeutically to inhibit the action of IL-12 for the treatment of one or more IL-12-mediated disease. (CBA/J x C57/BL6/J) F, hybrid mice containing human variable and constant region antibody transgenes for both heavy and light chains are immunized with human recombinant IL-12 (Taylor et al., Intl. Immunol. 6:579-591 (1993); Lonberg, et al., Nature 368:856-859 (1994); Neuberger, M., Nature Biotech. 14:826 (1996); Fishwild, et al., Nature Biotechnology 14:845-851 (1996)); Several fusions yielded one or more panels of completely human IL-12 reactive IgG monoclonal antibodies. The completely human anti-IL-12 antibodies are further characterized. All are IgGI . Such antibodies are found to have affinity constants somewhere between 1x109 and 9x1012. The unexpectedly high affinities of these fully human monoclonal antibodies make them suitable candidates for therapeutic applications in IL-12 related diseases. pathologies or disorders.

Abbreviations

BSA - bovine serum albumin
CO2 - carbon dioxide
DMSO - dimethyl sulfoxide
Materials and Methods

Animals

Transgenic mice that can express human antibodies are known in the art (and are commercially available (e.g., from GenPharm International, San Jose, CA; Abgenix, Freemont, CA, and others) that express human immunoglobulins but not mouse IgM or IgG. For example, such transgenic mice contain human sequence transgenes that undergo \( v(D)J \) joining, heavy-chain class switching, and somatic mutation to generate a repertoire of human sequence immunoglobulins (Lonberg, et al., Nature 368:856-859 (1994)). The light chain transgene can be derived, e.g., in part from a yeast artificial chromosome clone that includes nearly half of the germline human V region. In addition, the heavy-chain transgene can encode both human \( \mu \) and human 1 (Fishwild, et al., Nature Biotechnology 14:845-851 (1996)) and/or 3 constant regions. Mice derived from appropriate genotypic lineages can be used in the immunization and fusion processes to generate fully human monoclonal antibodies to IL-12.

Immunization

One or more immunization schedules can be used to generate the anti-IL-12 human hybridomas. The first several fusions can be performed after the following exemplary immunization protocol, but other similar known protocols can be used. Several 14-20 week old female and/or surgically castrated transgenic male mice are immunized IP and/or ID with 1-1000 \( \mu \)g of recombinant human IL-12 emulsified with an equal volume of TITERMAX or complete Freund’s adjuvant in a final volume of 100-400 \( \mu \)L (e.g., 200). Each mouse can also optionally receive 1-10 \( \mu \)g in 100 \( \mu \)L physiological saline at each of 2 SQ sites. The mice can then be immunized 1-7, 5-12, 17-25 and/or 21-34 days later IP (1-400 \( \mu \)g) and SQ (1-400 \( \mu \)g x 2) with IL-12 emulsified with an equal volume of TITERMAX or incomplete Freund’s adjuvant. Mice can be bled 12-25 and 25-40 days later by retro-orbital puncture without anti-coagulant. The blood is then allowed to clot at RT for one hour and the serum is collected and titered using an IL-12 EIA assay according to known methods. Fusions are performed when repeated injections do not cause titers to increase. At that time, the mice can be given a final IV booster injection of 1-400 \( \mu \)g IL-12 diluted in 100 \( \mu \)L physiological saline. Three days later, the mice can be euthanized by cervical dislocation and the spleens removed aseptically and immersed in 10 mL of cold phosphate buffered saline (PBS) containing 100 U/mL penicillin, 100 \( \mu \)g/mL streptomycin, and 0.25 \( \mu \)g/ml amphotericin B (PSA). The splenocytes are harvested by sterilely perfusing the spleen with PSA-PBS. The cells are washed once in cold PSA-PBS, counted using Trypan blue dye exclusion and resuspended in RPMI 1640 media containing 25 mM Hepes.

Cell Fusion

Fusion can be carried out at a 1:1 to 1:10 ratio of murine myeloma cells to viable spleen cells according to known methods, e.g., as known in the art. As a non-limiting example, spleen cells and myeloma cells can be pelleted together. The pellet can then be slowly resuspended, over 30 seconds, in 1 mL of 50% (w/v) PEG/PBS solution (PEG molecular weight 1.450, Sigma) at 37 C. The fusion can then be stopped by slowly adding 10.5 mL of RPMI 1640 medium containing 25 mM Hepes (37 C) over 1 minute. The fused cells are centrifuged for 5 minutes at 500-1500 rpm. The cells
are then resuspended in HAT medium (RPMI 1640 medium containing 25 mM Hepes, 10% Fetal Clone I serum (HyClone), 1 mM sodium pyruvate, 4 mM L-glutamine, 10 μg/mL gentamicin, 2.5% Origen culturing supplement (Fisher), 10% 653-conditioned RPMI 1640/Hepes media, 50 μM 2-mercaptoethanol, 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine) and then plated at 200 μL/well in fifteen 96-well flat bottom tissue culture plates. The plates are then placed in a humidified 37 C incubator containing 5% CO₂ and 95% air for 7-10 days.

Detection of Human IgG Anti-IL-12 Antibodies in Mouse Serum

[0169] Solid phase EIA’s can be used to screen mouse sera for human IgG antibodies specific for human IL-12. Briefly, plates can be coated with IL-12 at 2 μg/mL in PBS overnight. After washing in 0.15M saline containing 0.02% (v/v) Tween 20, the wells can be blocked with 1% (w/v) BSA in PBS. 200 μL/well for 1 hour at RT. Plates are used immediately or frozen at -20 C for future use. Mouse serum dilutions are incubated on the IL-12 coated plates at 50 μL/well at RT for 1 hour. The plates are washed and then probed with 50 μL/well HRP-labeled goat anti-human IgG, Fc specific diluted 1:30,000 in 1% BSA-PBS for 1 hour at RT. The plates can again be washed and 100 μL/well of the citrate-phosphate substrate solution (0.1M citric acid and 0.2M sodium phosphate, 0.01% H₂O₂ and 1 mg/mL OPD) is added for 15 minutes at RT. Stop solution (4N sulfuric acid) is then added at 25 μL/well and the OD’s are read at 490 nm via an automated plate spectrophotometer.

Detection of Completely Human Immunoglobulins in Hybridoma Supernates

[0170] Growth positive hybridomas secreting fully human immunoglobulins can be detected using a suitable EIA. Briefly, 96 well pop-out plates (VWR, 610744) can be coated with 10 μg/mL goat anti-human IgG Fc in sodium carbonate buffer overnight at 4 C. The plates are washed and blocked with 1% BSA-PBS for one hour at 37°C and used immediately or frozen at -20 C. Undiluted hybridoma supernatants are incubated on the plates for one hour at 37°C. The plates are washed and probed with HRP labeled goat anti-human kappa diluted 1:10,000 in 1% BSA-PBS for one hour at 37°C. The plates are then incubated with substrate solution as described above.

Determination of Fully Human Anti-IL-12 Reactivity

[0171] Hybridomas, as above, can be simultaneously assayed for reactivity to IL-12 using a suitable RIA or other assay. For example, supernatants are incubated on goat anti-human IgG Fc plates as above, washed and then probed with radiolabeled IL-12 with appropriate counts per well for 1 hour at RT. The wells are washed twice with PBS and bound-radiolabeled IL-12 is quantitated using a suitable counter.

[0172] Human IgG1 anti-IL-12 secreting hybridomas can be expanded in cell culture and serially subcloned by limiting dilution. The resulting clonal populations can be expanded and cryopreserved in freezing medium (95% FBS, 5% DMSO) and stored in liquid nitrogen.

Isotyping

[0173] Isotype determination of the antibodies can be accomplished using an EIA in a format similar to that used to screen the mouse immune sera for specific titers. IL-12 can be coated on 96-well plates as described above and purified antibody at 2 μg/mL can be incubated on the plate for one hour at RT. The plate is washed and probed with HRP labeled goat anti-human IgG₁ or HRP labeled goat anti-human IgG₃ diluted at 1:4000 in 1% BSA-PBS for one hour at RT. The plate is again washed and incubated with substrate solution as described above.

Binding Kinetics of Human Anti-Human IL-12 Antibodies With Human IL-12

[0174] Binding characteristics for antibodies can be suitably assessed using an IL-12 capture EIA and BIACore technology. For example. Graded concentrations of purified human IL-12 antibodies can be assessed for binding to EIA plates coated with 2 μg/mL of IL-12 in assays as described above. The OD’s can be then presented as semi-log plots showing relative binding efficiencies.

[0175] Quantitative binding constants can be obtained, e.g., as follows, or by any other known suitable method. A BIACore CM-5 (carboxymethyl) chip is placed in a BIACore 2000 unit. HBS buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% w/v P20 surfactant, pH 7.4) is flowed over a flow cell of the chip at 5 μL/minute until a stable baseline is obtained. A solution (100 μL) of 15 mg of EDC (N-ethyl-N’-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride) in 200 μL water is added to 100 μL of a solution of 2.3 mg ofNHS (N-hydroxysuccinimide) in 200 μL water. Forty (40) μL of the resulting solution is injected onto the chip. Six μL of a solution of human IL-12 (15 μg/mL in 10 mM sodium acetate, pH 4.8) is injected onto the chip, resulting in an increase of ca. 500 RU. The buffer is changed to TBS/Ca/Mg/BSA.
running buffer (20 mM Tris, 0.15 M sodium chloride, 2 mM calcium chloride, 2 mM magnesium acetate, 0.5% Triton X-100, 25 μg/mL BSA, pH 7.4) and flowed over the chip overnight to equilibrate it and to hydrolyze or cap any unreacted succinimide esters.

Antibodies are dissolved in the running buffer at 33.33, 16.67, 8.33, and 4.17 nM. The flow rate is adjusted to 30 μL/min and the instrument temperature to 25 C. Two flow cells are used for the kinetic runs, one on which IL-12 had been immobilized (sample) and a second, underivatized flow cell (blank). 120 μL of each antibody concentration is injected over the flow cells at 30 μL/min (association phase) followed by an uninterrupted 360 seconds of buffer flow (dissociation phase). The surface of the chip is regenerated (interleukin-12 /antibody complex dissociated) by two sequential injections of 30 μL each of 2 M guanidine thiocyanate.

Analysis of the data is done using BIA evaluation 3.0 or CLAMP 2.0, as known in the art. For each antibody concentration the blank sensogram is subtracted from the sample sensogram. A global fit is done for both dissociation (k_d sec^-1) and association (k_a, mol^{-1} sec^{-1}) and the dissociation constant (K_D, mol) calculated (k_d/k_a). Where the antibody affinity is high enough that the RUs of antibody captured are >100, additional dilutions of the antibody are run.

Results and Discussion

Generation of Anti-Human IL-12 Monoclonal Antibodies

Several fusions are performed and each fusion is seeded in 15 plates (1440 wells/fusion) that yield several dozen antibodies specific for human IL-12. Of these, some are found to consist of a combination of human and mouse Ig chains. The remaining hybridomas secret anti-IL-12 antibodies consisting solely of human heavy and light chains. Of the human hybridomas all are expected to be IgG1.

Binding Kinetics of Human Anti-Human IL-I2 Antibodies

ELISA analysis confirms that purified antibody from most or all of these hybridomas bind IL-12 in a concentration-dependent manner. Figures 1-2 show the results of the relative binding efficiency of these antibodies. In this case, the avidity of the antibody for its cognate antigen (epitope) is measured. It should be noted that binding IL-12 directly to the ELA plate can cause denaturation of the protein and the apparent binding affinities cannot be reflective of binding to undenatured protein. Fifty percent binding is found over a range of concentrations.

Quantitative binding constants are obtained using BIAcore analysis of the human antibodies and reveals that several of the human monoclonal antibodies are very high affinity with K_D in the range of 1x10^{-9} to 7x10^{-12}.

Conclusions

Several fusions are performed utilizing splenocytes from hybrid mice containing human variable and constant region antibody transgenes that are immunized with human IL-12. A set of several completely human IL-12 reactive IgG monoclonal antibodies of the IgG1 isotype are generated. The completely human anti-IL-12 antibodies are further characterized. Several of generated antibodies have affinity constants between 1x10^{9} and 9x10^{12}. The unexpectedly high affinities of these fully human monoclonal antibodies make them suitable for therapeutic applications in IL-12-dependent diseases, pathologies or related conditions.

Example 3: C340 is a Neutralizing Human Monoclonal Antibody

The bioactivity of IL-12 was shown to be neutralized by C340 in a variety of IL-12 dependent activity assays. Since IL-12 enhances IFN GAMMA production by NK cells and T lymphocytes, the effect of C340 antibody on the upregulation of IFN GAMMA mRNA and the effect of C340 on the production of IFN GAMMA protein was examined (Trinchieri, G., Current Opinion in Immunology, 9:17-23 (1997), Morris, S.C., et al., Journal of Immunology, 152:1047-1056 (1994)). The ability of C340 to neutralize IL-12 driven induction of lymphokine activated killer (LAK) cell activity was also investigated in these studies (Kutza, J. and Murasko, D.M., Mechanism of Ageing and Development, 90:209-222 (1996), Stern, A.S., et al., Proceedings of the National Academy of Sciences of the U.S.A., 87:6808-6812 (1990)). Lastly, the effect of C340 on IL-12-mediated upregulation of CD95 cell surface expression on T and NK cells was tested (Medvedev, A.E., et al., Cytokine, 9:394-404 (1997)).

Inhibition of IFN gamma mRNA Transcription

To determine whether C340 inhibits IL-12. IL-2 induced IFN GAMMA gene transcription in human PBL, a reverse transcription-PCR assay was performed. Specific primers for β-actin (a control for mRNA integrity and content)
Inhibition of Intracellular IFN GAMMA as Measured by Flow Cytometry

[0184] In response to various signals and as a measure of activation, T cells and NK cells can be induced to secrete cytokines. More specifically, PBL treated with IL-2 and IL-12 initiate substantial synthesis of IFN gamma within 4-8 hours after stimulation. This production can be detected in the cytoplasm of Brefeldin-A treated PBL by flow cytometry. Figure 4 demonstrates a 60% reduction in IFN GAMMA production in such cultures when C340 IL-12 was added in conjunction with IL-12 for five hours.

Inhibition of IL-12 induced IFN GAMMA Secretion

[0185] Figure 5 clearly shows that two different lots of C340 inhibited the secretion of IFN GAMMA by peripheral blood lymphocytes in a dose-dependent fashion. Four hundred picograms of IL-12 were premixed with varying amounts of C340 and then added to IL-2 stimulated cultures of PBL's. When IFN GAMMA was measured by EIA after an 18-24 hour incubation, markedly diminished amounts of IFN GAMMA were detected with as little as 1 ?g/mL of C340 antibody.

Inhibition of IL-12 Induced LAK Cell Cytotoxicity

[0186] Raji cells, an IL-12 sensitive Burkitt lymphoma derived cell line; is an NK cell resistant. LAK cell sensitive cell line. Raji cells, in triplicate, were cultured for four hours with LAK cells which had been activated with 400 pg/mL IL-12 and 10 U/mL IL-2 in the presence or absence of the human monoclonal antibody C340 (5000 ng/mL or 50 ng/mL). Figure 6 shows the results from three normal, healthy donors. IL-12 + IL-2 activation of effector cells resulted in an increasing cytotoxic activity over that of cells activated with IL-2 alone. The C340 antibody inhibited this IL-12 dependent effect. The magnitude of inhibition was related to antibody concentration, with the highest concentration tested reducing cytotoxicity to background levels.

Inhibition of CD95 Upregulation

[0187] Reports have described IL-12-induced upregulation of CD95 on the surface of highly purified CD56+ PBL. As can be seen in Figure 7A and 7B, distributional flow cytometric analysis revealed that CD95 expression was significantly upregulated on CD3+ T cells and CD56+ NK cells after treatment with IL-12 plus IL-2 for 72 hours. Concomitant anti-IL-12 treatment inhibited CD95 expression in both CD3+ and CD56+ populations. CD3+ cells were inhibited by ∼50% (Figure 7A), whereas CD56+ cells were inhibited by ∼85% (Figure 7B), as evidenced by a diminished MFI index (percent greater then unstimulated control).

Example 4: Gene cloning and characterization

[0188] Genomic DNA fragments containing either the C340 heavy chain gene or the C340 light chain were cloned and purified. Genomic DNA purified from C340 hybridoma cells was partially digested with Sau3A restriction enzyme and size-selected by centrifugal fractionation through a 10-40% sucrose gradient. DNA fragments in the size range of 15-23 kb were cloned into the bacteriophage vector, EMBL3, [commercially available ?] and packaged into phage particles. Several packaging reactions resulted in a library of 1 million bacteriophage clones. Approximately 600,000 clones from the library were screened by plaque hybridization using 32P-labeled genomic DNA fragments that contained either human IgG1 heavy chain constant region sequences or human kappa light chain constant region sequences as probe. Thirteen heavy chain and nine light chain clones were detected. Of these, three heavy chain clones and four light chain clones were purified by two more rounds of screening. One of the heavy chain clones and two of the light chain clones were shown to contain the 5’ and 3’ ends of the coding sequences by PCR analysis of bacteriophage DNA. The DNA insert in heavy chain (HC) clone H4 was 16 kb in size and includes 3.6 kb of 5’ flanking and at least 2 kb of 3’ flanking sequence. The DNA insert in light chain (LC) clone LCI was 15 kb in size and included 4.4 kb of 5’ flanking and 6.0 kb of 3’ flanking sequence. The complete inserts were removed from the bacteriophage vector as Sall fragments and cloned between the Xhol and Sall sites of plasmid expression vector p1351, which provided a gpt selectable marker gene. Because there was an internal Sall site in the heavy chain variable region coding sequence, two Sall fragments had to be transferred from bacteriophage H4 to the p1351 expression vector. The resulting heavy and light chain expression plasmids were termed p1560 and p1558, respectively. The orientations of the heavy and light chain genes in these two plasmids relative to the p1351 vector sequences were determined using restriction enzyme analysis and PCR, respectively. In both cases, the orientations were such that the 5’ end of the Ab gene fragment was proximal to the 3’ end of
the gpt gene. Both strands of the coding regions of the cloned genes were sequenced. The sequences of plasmids p1560 and p1558 are presented in Figures 11A-11K and Figures 13A-13J, respectively.

**Example 5: Preparation of recombinant cell lines**

**[0189]** Heavy chain plasmid p1560 was linearized by digestion with PvuI restriction enzyme and light chain plasmid p1558 was linearized using SalI restriction enzyme. p3X63Ag8.653 (653) and SP2/0-Ag14 (SP2/0) cells were separately transfected with the premixed linearized plasmids by electroporation and cells cultured and transfectants selected using mycophenolic acid as described (Knight, et al., Molecular Immunology 30:1443 (1993)). Cell supernatants from mycophenolic acid-resistant colonies were assayed approximately two weeks later for human IgG (i.e., recombinant C340 (rC340)). For this, cell supernatants were incubated on 96-well ELISA plates that were coated with goat antibodies specific for the Fc portion of human IgG. Human IgG which bound to the coated plate was detected using alkaline phosphatase-conjugated goat anti-human IgG (heavy chain + light chain) antibody and alkaline phosphatase substrates as described (Knight, et al., Molecular Immunology 30:1443 (1993)). Cells of the higher producing clones were transferred to 24-well culture dishes in standard media and expanded (IMDM, 5% FBS, 2 mM glutamine, mycophenolic acid selection mix). The amount of antibody produced (i.e., secreted into the media of spent cultures) was carefully quantified by ELISA using purified C340 mAb as the standard. Selected clones were then expanded in T75 flasks and the production of human IgG by these clones was quantified by ELISA. Based on these values, six independent 653 transfectants and three independent SP2/0 transfectants were subcloned (by seeding an average of one cell per well in 96 well plates), the quantity of antibody produced by the subclones was determined by assaying (ELISA) supernatants from individual subclone colonies. Three subclones, 653 transfectant 19-20 (C379B) and the SP2/0 transfectants 84-81 (C381A) and 22-56 (C389A), were selected for further analysis.

**Assay for rC340 antigen binding.**

**[0190]** Prior to subcloning selected cell lines as described above, cell supernatants from three parental lines (653 transfectants clone 2 and clone 18 and SP2/0 transfectant clone 1) were used to test the antigen binding characteristics of rC340. The concentrations of rC340 in the three cell supernatant samples were first determined by ELISA. Titrating amounts of the supernatant samples, or purified C340 positive control, were then incubated in 96-well plates coated with 2 µg/ml of human IL-12. Bound mAb was then detected with alkaline phosphatase-conjugated goat anti-human IgG (heavy chain + light chain) antibody and the appropriate alkaline phosphatase substrates. As shown in Figure 8, rC340 bound specifically to human IL-12 in a manner indistinguishable from the original C340 mAb.

**Characterization of selected cell lines.**

**[0191]** Growth curve analyses were performed on C379B, C381A, and C389A by seeding T75 flasks with a starting cell density of 2 X 10^5 cells/ml in standard media or SFM-5 serum-free media and then monitoring cell number and rC340 concentration on a daily basis until the cultures were spent. The results of cultures in standard media are shown in Figures 9A - 9C. Maximal C340 mAb production levels for C379B, C381A, and C389A were 135 µg/ml, 150 µg/ml, and 110 µg/ml, respectively. Attempts to adapt C379B cells to SFM-5 media were not successful. C381A cells produced the same amount of rC340 in SFM-5 media as in standard media, whereas C389A cells produced only half as much rC340 in SFM-5 media as in standard media.

**[0192]** The stability of rC340 mAb production over time for the three subclones was assessed by culturing cells in 24-well dishes with standard media or standard media without mycophenolic acid selection for varying periods of time. Lines C379B and C381A were observed to stably produce rC340 in the presence or absence of selection for a period of 30 days (the maximum time tested) and 75 days, respectively. Line C389A was unstable and after 43 days of culture produced just 20% as much antibody as at the beginning of the study.

**[0193]** It will be clear that the invention can be practiced otherwise than as particularly described in the foregoing description and examples.

**[0194]** Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

**[0195]** The invention further provides the following numbered embodiments:

1. At least one isolated mammalian anti-IL-12 antibody, comprising at least one variable region comprising SEQ ID NO: 7 or 8.

2. An IL-12 antibody according to embodiment 1, wherein said antibody binds IL-12 with an affinity of at least one selected from at least 10^-9 M, at least 10^-10 M, at least 10^-11 M, or at least 10^-12 M.
3. An IL-12 antibody according to embodiment 1, wherein said antibody substantially neutralizes at least one activity of at least one IL-12 protein.

4. An isolated nucleic acid encoding at least one isolated mammalian anti-IL-12 antibody having at least one variable region comprising SEQ ID NO: 7 or 8.

5. An isolated nucleic acid vector comprising an isolated nucleic acid according to embodiment 4.

6. A prokaryotic or eukaryotic host cell comprising an isolated nucleic acid according to embodiment 5.

7. A host cell according to embodiment 6, wherein said host cell is at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, G2, 653, SP2/0, 293, HeLa, myeloma, or lymphom cells, or any derivative, immortalized or transformed cell thereof.

8. A method for producing at least one anti-IL-12 antibody, comprising translating a nucleic acid according to embodiment 4 under conditions in vitro, in vivo or in situ, such that the IL-12 antibody is expressed in detectable or recoverable amounts.

9. A composition comprising at least one isolated mammalian anti-IL-12 antibody having at least one variable region comprising SEQ ID NO: 7 or 8, and at least one pharmaceutically acceptable carrier or diluent.

10. A composition according to embodiment 9, further comprising at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an anti-inflammatory drug, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, an estrogen, a growth hormone, or a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

11. An anti-idiotype antibody or fragment that specifically binds at least one isolated mammalian anti-IL-12 antibody having at least one variable region comprising SEQ ID NO: 7 or 8.

12. A method for diagnosing or treating a IL-12 related condition in a cell, tissue, organ or animal, comprising

   (a) contacting or administering a composition comprising an effective amount of at least one isolated mammalian anti-IL-12 antibody having at least one variable region comprising SEQ ID NO: 7 or 8, with, or to, said cell, tissue, organ or animal.

13. A method according to embodiment 12, wherein said effective amount is 0.001-50 mg/kilogram of said cells, tissue, organ or animal.

14. A method according to embodiment 12, wherein said contacting or said administering is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavity, intracelialis, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intranodal, intrapelvic, intraperitoneal, intraplateal, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intruterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

15. A method according to embodiment 12, further comprising administering, prior, concurrently or after said (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an anti-inflammatory drug, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsychotic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.
16. A method according to embodiment 12, wherein said IL-12 related condition is psoriasis.

17. A method according to embodiment 12, wherein said IL-12 related condition is multiple sclerosis.

18. A medical device, comprising at least one isolated mammalian anti-IL-12 antibody having at least one variable region comprising SEQ ID NO: 7 or 8, wherein said device is suitable to contacting or administering said at least one anti-IL-12 antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intrapelvic, intrapericardiac, intraperitoneal, intrapulmonary, intrarectal, intrarenal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

19. A method for producing at least one isolated mammalian anti-IL-12 antibody having at least one variable region comprising SEQ ID NO: 7 or 8, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts said antibody.

20. At least one anti-IL-12 antibody produced by a method according to embodiment 19.

21. At least one isolated mammalian anti-IL-12 antibody, comprising either (i) all of the heavy chain complementarity determining regions (CDR) amino acid sequences of SEQ ID NOS: 7, 8, and 9; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NOS: 10, 11, and 12.

22. An IL-12 antibody according to embodiment 21, wherein said antibody binds IL-12 with an affinity of at least one selected from at least 10^-9 M, at least 10^-10 M, at least 10^-11 M, or at least 10^-12 M.

23. An IL-12 antibody according to embodiment 21, wherein said antibody substantially neutralizes at least one activity of at least one IL-12 protein.

24. An isolated nucleic acid encoding at least one isolated mammalian anti-IL-12 antibody either (i) all of the heavy chain CDR amino acid sequences of SEQ ID NOS: 7, 8, and 9; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NOS: 10, 11, and 12.

25. An isolated nucleic acid vector comprising an isolated nucleic acid according to embodiment 4.

26. A prokaryotic or eukaryotic host cell comprising an isolated nucleic acid according to embodiment 25.

27. A host cell according to embodiment 26, wherein said host cell is at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphom cells, or any derivative, immortalized or transformed cell thereof.

28. A method for producing at least one anti-IL-12 antibody, comprising translating a nucleic acid according to embodiment 24 under conditions in vitro, in vivo or in situ, such that the IL-12 antibody is expressed in detectable or recoverable amounts.

29. A composition comprising at least one isolated mammalian anti-IL-12 antibody having either (i) all of the heavy chain CDR amino acid sequences of SEQ ID NOS: 7, 8, and 9; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NOS: 10, 11, and 12, and at least one pharmaceutically acceptable carrier or diluent.

30. A composition according to embodiment 29, further comprising at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.
31. An anti-idiotypic antibody or fragment that specifically binds at least one isolated mammalian anti-IL-12 antibody having either (i) all of the heavy chain CDR amino acid sequences of SEQ ID NOS: 7, 8, and 9; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NOS: 10, 11, and 12.

32. A method for diagnosing or treating a IL-12 related condition in a cell, tissue, organ or animal, comprising

(a) contacting or administering a composition comprising an effective amount of at least one isolated mammalian anti-IL-12 antibody having either (i) all of the heavy chain CDR amino acid sequences of SEQ ID NOS: 7, 8, and 9; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NOS: 10, 11, and 12, with, or to, said cell, tissue, organ or animal.

33. A method according to embodiment 32, wherein said effective amount is 0.001-50 mg/kilogram of said cells, tissue, organ or animal.

34. A method according to embodiment 32, wherein said contacting or said administrating is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracebellar, intracerebroventricular, intracolic, intracr.inal, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrathoracic, intrasynovial, intranasal, or transdermal.

35. A method according to embodiment 32, further comprising administering, prior, concurrently or after said (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antiinflammatory, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antispasmodic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

36. A method according to embodiment 32, wherein said IL-12 related condition is psoriasis.

37. A method according to embodiment 32, wherein said IL-12 related condition is multiple sclerosis.

38. A medical device, comprising at least one isolated mammalian anti-IL-12 antibody having either (i) all of the heavy chain CDR amino acid sequences of SEQ ID NOS: 7, 8, and 9; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NOS: 10, 11, and 12, wherein said device is suitable to contacting or administering said at least one anti-IL-12 antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracebellar, intracerebroventricular, intracolic, intracr.inal, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrasynovial, intrathoracic, intracel. iner, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

39. A method for producing at least one isolated mammalian anti-IL-12 antibody having either (i) all of the heavy chain CDR amino acid sequences of SEQ ID NOS: 7, 8, and 9; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NOS: 10, 11, and 12, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts said antibody.

40. At least one anti-IL-12 antibody produced by a method according to embodiment 39.

41. At least one isolated mammalian anti-IL-12 antibody, comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6.

42. An IL-12 antibody according to embodiment 41, wherein said antibody binds IL-12 with an affinity of at least one selected from at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M, or at least 10^{-12} M.

43. An IL-12 antibody according to embodiment 41, wherein said antibody substantially neutralizes at least one
activity of at least one IL-12 protein.

44. An isolated nucleic acid encoding at least one isolated mammalian anti-IL-12 antibody having at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6.

45. An isolated nucleic acid vector comprising an isolated nucleic acid according to embodiment 44.

46. A prokaryotic or eukaryotic host cell comprising an isolated nucleic acid according to embodiment 45.

47. A host cell according to embodiment 46, wherein said host cell is at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphom cells, or any derivative, immortalized or transformed cell thereof.

48. A method for producing at least one anti-IL-12 antibody, comprising translating a nucleic acid according to embodiment 44 under conditions in vitro, in vivo or in situ, such that the IL-12 antibody is expressed in detectable or recoverable amounts.

49. A composition comprising at least one isolated mammalian anti-IL-12 antibody having at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6, and at least one pharmaceutically acceptable carrier or diluent.

50. A composition according to embodiment 49, further comprising at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

51. An anti-idiotype antibody or fragment that specifically binds at least one isolated mammalian anti-IL-12 antibody having at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6.

52. A method for diagnosing or treating a IL-12 related condition in a cell, tissue, organ or animal, comprising

(a) contacting or administering a composition comprising an effective amount of at least one isolated mammalian anti-IL-12 antibody having at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6, with, or to, said cell, tissue, organ or animal.

53. A method according to embodiment 52, wherein said effective amount is 0.001-50 mg/kilogram of said cells, tissue, organ or animal.

54. A method according to embodiment 52, wherein said contacting or said administrating is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intrabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intrapleural, intraprostastic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

55. A method according to embodiment 52, further comprising administering, prior, concurrently or after said (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.
56. A method according to embodiment 52, wherein said IL-12 related condition is psoriasis.

57. A method according to embodiment 52, wherein said IL-12 related condition is multiple sclerosis.

58. A medical device, comprising at least one isolated mammalian anti-IL-12 antibody having at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6, wherein said device is suitable to contacting or administering said at least one anti-IL-12 antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracebellar, intracerebroventricular, intracolic, intracervical, intragastric, intramyocardial, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

59. A method for producing at least one isolated mammalian anti-IL-12 antibody having at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts said antibody.

60. At least one anti-IL-12 antibody produced by a method according to embodiment 59.

61. At least one isolated mammalian anti-IL-12 antibody that binds to the same region of a IL-12 protein as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6.

62. An IL-12 antibody according to embodiment 61, wherein said antibody binds IL-12 with an affinity of at least one selected from at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M, or at least 10^{-12} M.

63. An IL-12 antibody according to embodiment 61, wherein said antibody substantially neutralizes at least one activity of at least one IL-12 protein.

64. An isolated nucleic acid encoding at least one isolated mammalian anti-IL-12 antibody that binds to the same region of a IL-12 protein as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6.

65. An isolated nucleic acid vector comprising an isolated nucleic acid according to embodiment 64.

66. A prokaryotic or eukaryotic host cell comprising an isolated nucleic acid according to embodiment 65.

67. A host cell according to embodiment 66, wherein said host cell is at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, myeloma, or lymphom cells, or any derivative, immortalized or transformed cell thereof.

68. A method for producing at least one anti-IL-12 antibody, comprising translating a nucleic acid according to embodiment 64 under conditions in vitro, in vivo or in situ, such that the IL-12 antibody is expressed in detectable or recoverable amounts.

69. A composition comprising at least one isolated mammalian anti-IL-12 antibody that binds to the same region of a IL-12 protein as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6, and at least one pharmaceutically acceptable carrier or diluent.

70. A composition according to embodiment 69, further comprising at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antihemophilic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.
71. An anti-idiotypic antibody fragment that specifically binds at least one isolated mammalian anti-IL-12 antibody that binds to the same region of a IL-12 protein as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6.

72. A method for diagnosing or treating a IL-12 related condition in a cell, tissue, organ or animal, comprising:

(a) contacting or administering a composition comprising an effective amount of at least one isolated mammalian anti-IL-12 antibody that binds to the same region of a IL-12 protein as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6, with, or to, said cell, tissue, organ or animal.

73. A method according to embodiment 72, wherein said effective amount is 0.001-50 mg/kilogram of said cells, tissue, organ or animal.

74. A method according to embodiment 72, wherein said contacting or said administering is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intraterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

75. A method according to embodiment 72, further comprising administering, prior, concurrently or after said (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antiinflammatory, muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytochrome, or a cytokine antagonist.

76. A method according to embodiment 72, wherein said IL-12 related condition is psoriasis.

77. A method according to embodiment 72, wherein said IL-12 related condition is multiple sclerosis.

78. A medical device, comprising at least one isolated mammalian anti-IL-12 antibody that binds to the same region of a IL-12 protein as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6, wherein said device is suitable to contacting or administering said at least one anti-IL-12 antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intramyocardial, intraoesstral, intrapelvic, intrapericardiac, intraperitoneal, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intraterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

79. A method for producing at least one isolated mammalian anti-IL-12 antibody that binds to the same region of a IL-12 protein as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, or 6, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts said antibody.

80. At least one anti-IL-12 antibody produced by a method according to embodiment 79.

81. At least one isolated mammalian anti-IL-12 antibody, comprising at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO: 9.

82. An IL-12 antibody according to embodiment 81, wherein said antibody binds IL-12 with an affinity of at least one selected from at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M, or at least 10^{-12} M.

83. An IL-12 antibody according to embodiment 81, wherein said antibody substantially neutralizes at least one
activity of at least one IL-12 protein.

84. An isolated nucleic acid encoding at least one isolated mammalian anti-IL-12 antibody having at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO: 9.

85. An isolated nucleic acid vector comprising an isolated nucleic acid according to embodiment 84.

86. A prokaryotic or eukaryotic host cell comprising an isolated nucleic acid according to embodiment 85.

87. A host cell according to embodiment 86, wherein said host cell is at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphoma cells, or any derivative, immortalized or transformed cell thereof.

88. A method for producing at least one anti-IL-12 antibody, comprising translating a nucleic acid according to embodiment 84 under conditions in vitro, in vivo or in situ, such that the IL-12 antibody is expressed in detectable or recoverable amounts.

89. A composition comprising at least one isolated mammalian anti-IL-12 antibody having at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO: 9, and at least one pharmaceutically acceptable carrier or diluent.

90. A composition according to embodiment 89, further comprising at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

91. An anti-idiotype antibody or fragment that specifically binds at least one isolated mammalian anti-IL-12 antibody having at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO: 9.

92. A method for diagnosing or treating a IL-12 related condition in a cell, tissue, organ or animal, comprising

(a) contacting or administering a composition comprising an effective amount of at least one isolated mammalian anti-IL-12 antibody having at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO: 9, with, or to, said cell, tissue, organ or animal.

93. A method according to embodiment 92, wherein said effective amount is 0.001-50 mg/kilogram of said cells, tissue, organ or animal.

94. A method according to embodiment 92, wherein said contacting or said administrating is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, inraabdominal, intracapsular, intracartilaginous, intracavitary, intracell, intracebellar, intracerebroventricular, intracolic, intracerebral, intragastric, intrahepatic, intramyocardial, intraoestinal, intrapelvic, intrapericardial, intraperitoneal, intraperitoneal, intrapleural, intraprostotic, intrapulmonary, intraretinal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intraterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

95. A method according to embodiment 92, further comprising administering, prior, concurrently or after said (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone re-
placement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

96. A method according to embodiment 92, wherein said IL-12 related condition is psoriasis.

97. A method according to embodiment 92, wherein said IL-12 related condition is multiple sclerosis.

98. A medical device, comprising at least one isolated mammalian antiIL-12 antibody having at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO: 9, wherein said device is suitable to contacting or administering said at least one anti-IL-12 antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraoletal, intrapericardiac, intraperitoneal, intrapleural, intraprostastic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intraterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

99. A method for producing at least one isolated mammalian anti-IL-12 antibody having at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO: 9, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts said antibody.

100. At least one anti-IL-12 antibody produced by a method according to embodiment 99.

101. Any invention described herein.
SEQUENCE LISTING

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Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu
260  265  270
Ser His Ser Leu Leu Leu Leu His Lys Glu Asp Gly Ile Trp Ser
275  280  285
Thr Asp Ile Leu Lys Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe Leu
290  295  300
Arg Cys Glu Ala Lys Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp Leu
305  310  315  320
Thr Thr Ile Ser Thr Asp Leu Thr Phe Ser Val Lys Ser Ser Arg Gly
325  330  335
Ser Ser Asp Pro Gln Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala
340  345  350
Glu Arg Val Arg Gly Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu Cys
355  360  365
Gln Glu Asp Ser Ala Cys Pro Ala Ala Glu Ser Leu Pro Ile Glu
370  375  380
Val Met Val Asp Ala Val His Lys Leu Tyr Glu Asn Tyr Thr Ser
385  390  395  400
Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu
405  410
Gln Leu Lys Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu
420  425  430
Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe
435  440  445
Cys Val Gin Val Gin Gly Lys Ser Lys Glu Arg Lys Gly Lys Asp Arg Val
450  455  460
Phe Thr Asp Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser
465  470  475  480
Ile Ser Val Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu
485  490  495
Trp Ala Ser Val Pro Cys Ser
500
Claims

1. An isolated nucleic acid encoding at least one isolated mammalian anti-IL-12 antibody having at least one variable region comprising SEQ ID NO:7 or SEQ ID NO:8.

2. An isolated nucleic acid encoding at least one isolated mammalian anti-IL-12 antibody having either (i) all of the heavy chain CDR amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

3. An isolated nucleic acid encoding at least one isolated mammalian anti-IL-12 antibody having at least one heavy
chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.

4. An isolated nucleic acid encoding at least one isolated mammalian anti-IL-12 antibody that binds to the same region of a IL-12 protein as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.

5. A prokaryotic or eukaryotic host cell comprising an isolated nucleic acid according to any one of claims 1 to 4.

6. At least one isolated mammalian anti-IL-12 antibody, comprising at least one variable region comprising SEQ ID NO:7 or SEQ ID NO:8.

7. At least one isolated mammalian anti-IL-12 antibody, comprising either (i) all of the heavy chain complementarity determining regions (CDR) amino acid sequences of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

8. At least one isolated mammalian anti-IL-12 antibody, comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.

9. At least one isolated mammalian anti-IL-12 antibody that binds to the same region of a IL-12 protein as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.

10. A composition comprising at least one isolated mammalian anti-IL-12 antibody according to any one of claims 6 to 9 and at least one pharmaceutically acceptable carrier or diluent.

11. The antibody of any one of claims 6 to 9 or the composition of claim 10 for use in diagnosing or treating a IL-12 related condition in a cell, tissue, organ or animal.

12. Any invention described herein.
FIGURE 1A

FIGURE 1B
**FIG. 3**

Donor 1411  Donor 1457

- β-actin
- IFNγ

1. Control
2. IL-2
3. IL-12
4. IL-2+IL-12
5. IL-2+IL-12+C340
6. IL-2+IL-12+C338 (isotype control for C340)
7. IL-2+IL-12+8.6.2
8. IL-2+IL-12+8.A.1 (isotype control for 8.6.2)
FIGURE 6
FIG. 7A

Mean Fluorescence Intensity Index:
(Percent > Unstimulated Control)

Conditions

- IL-12 + IL-2
- Stim. + C340 mAb

N=7
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