USE OF B CELL EXPANSION AGENTS IN GENERATING ANTIBODIES

FIELD OF THE INVENTION

This invention relates to the generation of antibodies. More particularly, the present invention is directed to a method of generating and enhanced antibody response and antibodies generated from such method, including specified portions or variants, specific for at least one protein or fragment thereof, as well as anti-idiotype antibodies, and nucleic acids encoding the antibodies, complementary nucleic acids, vectors, host cells, and methods of making and using thereof, including therapeutic formulations, administration and devices.

BACKGROUND OF THE INVENTION

The use of monoclonal antibodies (mAbs) as therapeutic reagents has become an effective approach for the treatment of various diseases. In addition, mAbs can represent a powerful tool to gain a better understanding of the immunopathogenesis of various diseases.

A standard method for the generation of mAbs consists of fusing myeloma cells with lymph node cells or splenocytes harvested from immunized BALB/c mice (Köhler and Milstein, *Nature 256*, 495-497 (1975); Köhler and Milstein, *Eur. J. Immunol. 6*, 511-519 (1976)). BALB/c mice represent the host of choice for raising mAbs since they are readily available and, when sensitized with foreign T-dependent antigens, the immune response in these mice is characterized by a polarization of T-cell derived cytokine production toward a Th2-like phenotype (reviewed in Reiner and Locksley, *Ann. Rev. Immunol. 13,* 151-177 (1995)). This Th2-like response is accompanied by the generation of high levels of antigen-specific IgG1 antibodies (Finkelman *et al., Ann. Rev. Immunol. 8*, 303-333 (1990)), which correlates with an increase in the frequency of antigen-specific B-cell clones and an increase in the number of hybrids following B-cell fusion.

The generation of a mAb by the method of Kohler and Milstein is dependent upon the success of a complex biological process coupled with the success of in vitro techniques to harvest and immortalize the antigen specific B cell of interest. Nevertheless, some antigens produce only low or undetectable antibody titers in

BALB/c mice making it difficult or impossible to generate hybrids following B-cell fusion. Therefore, in order to improve antigen-specific mAbs generation using B cells harvested from Th2-biased mice, there is a need to increase the frequency of antigen-specific B cell clones.

Furthermore, environmental factors such as stress can affect the generation of a proper humoral response. Animals subjected to stress at the time of contact with antigen generate reduced antibody responses through either direct downregulation by corticosteroids binding lymphocyte surfaces or through the activation of suppressive factors (Borysenko and Borysenko, Gen Hosp Psychiatry 4:59-67, 1982; Gross and Siegel, J Anim Sci, 66:2091-2094, 1988).

CD40 is a cell surface receptor that is expressed on the surface of all mature B cells, most mature B-cell malignancies, and some early B-cell acute lymphocytic leukemias, but is not expressed on plasma cells (Clark, *Tissue Antigens* 35: 33-36 (1990)). It is also expressed on monocytes, dendritic cells, endothelial cells, and epithelial cells (van Kooten and Banchereau, *J. Leuko. Biol.* 67: 2-17 (2000)).

CD40 has been found to mediate a broad variety of immune and inflammatory responses (Schonbeck and Libby, *Cell Molec. Life Sci.* 58: 4-43 (2001)). CD40 ligand, also known as CD154, is found primarily on T cells (Gauchat et al., *FEBS Lett.* 315: 259-266 (1993)). Activation of CD40 on B cells by CD40 ligand causes B cell proliferation, differentiation, immunoglobulin isotype switching, germinal center formation, and stimulation of the humoral memory response (Kawabe et al., *Immunity* 1: 167-178 (1994); Castigli et al., *Proc. Nat. Acad. Sci. USA* 91: 12135-12139 (1994)).

Crosslinking of CD40 by anti-CD40 monoclonal antibodies mediates B cell proliferation, adhesion, and differentiation (DiSanto et al., *Nature* 361: 541-543 (1993); Hollenbaugh et al., *EMBO J.* 11: 4313-4321 (1992)). Anti-CD40 agonist antibodies have also been used to activate and amplify human resting B lymphocytes. This has resulted in increased cell numbers available for the generation of human hybridomas or B cell clones (Niedbala and Stott, *Hybridoma*, 17: 299-304 (1998); Lagerkvist et al., Biotechniques, 18: 862-869 (1995)). In addition, in combination with IL-4, anti-CD40 antibodies induces homotypic adhesion, proliferation and differentiation of tonsillar B-lymphocytes into Ig-

producing cells (Bjorck et al., 1998).

Thus, a need exists for improved methods of antibody generation that can increase the frequency of antigen-specific B cell clones in rodents, such as BALB/c mice.

SUMMARY OF THE INVENTION

The present invention provides a method of generating antibodies comprising immunizing an animal capable of producing antibodies with a B cell expansion agent along with the target antigen. The target antigen may be a T cell dependent antigen or a T cell independent antigen.

In one aspect of the invention, the B cell expansion agent is a CD40 agonist and the use of a CD40 agonist, such as an anti-CD40 antibody agonist or a portion of an anti-CD40 antibody, increases the number of antigen specific B cells, for example, in a rodent being immunized. The B cell expansion agent used in the present invention may also be BAFF (BLyS), IL-6, APRIL, CD40L (CD154), and anti-IgM/IL4 co-stimulation.

The B cell expansion agent of the invention can be used along with a second agent that is used for targeting and/or B cell differentiation. An exemplary targeting agent is CD21. Exemplary B cell differentiation agents are unfolded protein response (UPR) pathway components and various B cell specific transcription factors.

In the method of the present invention, the B cell expansion agent can be administered in protein form, in the form of DNA encoding the B cell expansion agent, or combinations of them. In addition, the B cell expansion agent (in protein or DNA form) can be coupled to or administered along with a small molecule. For example, the B cell expansion agent can target the B cell surface and the small molecule can enhance the antibody response.

In another aspect of the invention, after a rodent is immunized with an antigen and B cell expansion agent, antigen-specific antibodies are isolated. Antibody producing cells can be obtained from the peripheral blood or, preferably, the spleen or lymph nodes, of humans or other suitable animals, e.g., rodents, that have been immunized with the antigen of interest and B cell expansion agent. 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).

The present invention further comprises isolated mammalian, including, without limitation, human, antibodies that have been generated using a B cell expansion agent, immunoglobulins, fragments, cleavage products and other specified portions and variants thereof, as well as antibody compositions, anti-idiotype antibodies, encoding or complementary nucleic acids, vectors, host cells, compositions, combinations, formulations, devices, transgenic animals, transgenic plants, and methods of making and using them.

The present invention also provides at least one composition comprising (a) a nucleic acid encoding an isolated antibody generated using a B cell expansion agent and/or antibody as described herein; and (b) a suitable and/or pharmaceutically acceptable carrier or diluent.

The present invention further provides at least one antibody generated using a B cell expansion agent composition or method, for administering a therapeutically effective amount to modulate or treat at least one disease or condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as described herein.

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 antibody generated using a B cell expansion agent, according to the present invention.

The present invention further provides at least one antibody generated using a B cell expansion agent composition or method, for diagnosing at least one disease or condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as described herein.

The present invention also provides at least one composition, device and/or

method of delivery for diagnosing at least one disease or condition, according to the present invention.

Also provided is a medical device, comprising at least one isolated antibody generated using a B cell expansion agent, wherein the device is suitable for contacting or administering the at least one antibody generated using a B cell expansion agent, anti-idiotypic antibody, nucleic acid molecule, compound, protein, and/or composition.

Also provided is an article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container comprising a solution or a lyophilized form of at least one antibody generated using a B cell expansion agent. The article of manufacture can optionally have the container as a component of a delivery device or system.

The present invention further provides any invention described herein.

DETAILED DESCRIPTION OF THE INVENTION

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as though fully set forth.

The term "antibodies" as used herein and in the claims means polyclonal, monoclonal or anti-idiotypic antibodies or fragments thereof, including, without limitation, any 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 (e.g., without limitation, single chain antibody, single domain antibody, mimetibody, minibody, etc.), or at least one portion of a receptor or binding protein, which can be incorporated into an antibody of the present invention. Such antibody optionally further affects a specific ligand, such as, but not limited to, where such antibody modulates, decreases, increases, antagonizes, agonizes, mitigates, alleviates, blocks, inhibits, abrogates and/or interferes with at least one activity or binding, or with receptor activity or binding, *in vitro, in* situ and/or in *vivo*. As a non-limiting

example, an antibody generated using a B cell expansion agent, specified portion or variant of the present invention can bind at least one target antigen, or specified portions, variants or domains thereof. A suitable antibody generated using a B cell expansion agent, specified portion, or variant can also optionally affect at least one of activity or function, such as but not limited to, RNA, DNA or protein synthesis, release, receptor signaling, membrane cleavage, activity, production and/or synthesis.

The term "antibody" is further intended to encompass antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain antibodies and fragments thereof. Functional fragments include antigen-binding fragments that bind to a mammalian protein. For example, antibody fragments capable of binding to protein or portions thereof, including, but not limited to, Fab (e.g., by papain digestion), Fab' (e.g., by pepsin digestion and partial reduction) and $F(ab')_2$ (e.g., by pepsin digestion), facb (e.g., by plasmin digestion), pFc' (e.g., by pepsin or plasmin digestion), Fd (e.g., by pepsin digestion, partial reduction and reaggregation), Fv or scFv (e.g., by molecular biology techniques) fragments, are encompassed by the invention (see, e.g., Colligan, Immunology, supra).

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 CH_1 domain and/or hinge region of the heavy chain. The various portions of antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques.

The term "human antibody," as used herein, is intended to include antibodies having variable and constant regions derived from or closely matching human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin

sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). Thus, as used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C_L, C_H domains (e.g., C_H1, C_H2, C_H3), hinge, (V_L, V_H)) is substantially similar to a human germline antibody. Human antibodies have been classified into groupings based on their amino acid sequence similarities, see e.g., http:// people.cryst.bbk.ac.uk/~ubcg07s/. Thus, using a sequence similarity search, an antibody with similar linear sequence can be chosen as a template to create "humanized antibodies."

"Humanization" (also called Reshaping or CDR-grafting) is now a well-established technique for reducing the immunogenicity of monoclonal antibodies (mAbs) from xenogeneic sources (commonly rodent) and for improving the effector functions (ADCC, complement activation, C1q binding). The engineered mAb is engineered using the techniques of molecular biology, however, simple CDR-grafting of the rodent complementarity-determining regions (CDRs) into human frameworks often results in loss of binding affinity and/or specificity of the original mAb. In order to humanize an antibody, the design of the humanized antibody includes variations such as conservative amino acid substitutions in residues of the CDRs, and back substitution of residues from the rodent mAb into the human framework regions (back mutations). The positions can be discerned or identified by sequence comparison for structural analysis or by analysis of a homology model of the variable regions' 3D structure. The process of affinity maturation has most recently used phage libraries to vary the amino acids at chosen positions. Similarly, many approaches have been used to choose the most appropriate human frameworks in which to graft the rodent CDRs. As the datasets of known parameters for antibody structures increases, so does the sophistication and refinement of these techniques. Consensus or germline sequences from a single antibody or fragments of the framework sequences within each light or heavy chain variable region from several different human mAbs can be used. Another approach to humanization is to modify only surface residues of the rodent sequence with the most common residues found in human mAbs and has been termed "resurfacing" or "veneering." Known human Ig sequences are disclosed, e.g., www. ncbi.nlm.nih.gov/entrez/query.fcgi;

www. ncbi.nih.gov/igblast; www. atcc.org/phage/hdb.html; www. kabatdatabase.com/top.html; www. antibodyresource.com/onlinecomp.html; www. appliedbiosystems.com; www. biodesign.com; antibody.bath.ac.uk; www. unizh.ch; www. cryst.bbk.ac.uk/~ubcg07s; Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference. Often, the human or humanized antibody is substantially non-immunogenic in humans.

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, and family specific antibodies. Further, chimeric antibodies can 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 or single domain 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.

The term "antigen" as used herein and in the claims means any molecule that has the ability to generate antibodies either directly or indirectly. Included within the definition of "antigen" is a protein-encoding nucleic acid.

The present invention provides methods for generating antibodies in rodents. In particular, the methods are useful for generating antibodies in rodents, such as mice having a BALB/c background.

Antibodies of the Present Invention – Production and Generation

At least one antibody generated using a B cell expansion agent can be optionally produced by a cell line, a mixed cell line, an immortalized cell or clonal

population of immortalized cells, as well known in the art. See, e.g., Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, NY (1989); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001).

Antibodies generated using a B cell expansion agent or fragments thereof can be raised against an appropriate immunogenic antigen and/or a portion thereof (including synthetic molecules, such as synthetic peptides). Other specific or general antibodies, including, without limitation, mammalian antibodies, can be similarly raised. Preparation of immunogenic antigens, and monoclonal antibody production can be performed using any suitable technique.

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, L243, 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, NAMALWA, NEURO 2A, or the like, or heteromylomas, 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, either as 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, hnRNA, mRNA, tRNA, 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.

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, for example, mice, rats, and other mammals. 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 that produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).

Methods for engineering or humanizing non-human or human antibodies can also be used and are well known in the art. An antibody to be humanized or engineered initially may have one or more amino acid residues from a source that is non-human, e.g., but not limited to, mouse, rat, rabbit, non-human primate or other mammal. These non-human amino acid residues are replaced by residues that 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. ncbi.nih.gov/igblast; www. atcc.org/phage/hdb.html; www. mrc-cpe.cam.ac.uk/ALIGNMENTS.php; www. kabatdatabase.com/top.html; ftp. ncbi.nih.gov/repository/kabat; www. sciquest.com; www. abcam.com; www. antibodyresource.com/onlinecomp.html; www. public.iastate.edu/~pedro/research_tools.html; www. whfreeman.com/immunology/CH05/kuby05.htm; www. hhmi.org/grants/lectures/1996/vlab; www. path.cam.ac.uk/~mrc7/mikeimages.html; mcb.harvard.edu/BioLinks/Immunology.html; www. immunologylink.com; pathbox. wustl.edu/~hcenter/index.html; www. appliedbiosystems.com; www. nal.usda.gov/awic/pubs/antibody; www. m.ehime-u.ac.jp/~yasuhito/Elisa.html; www. biodesign.com; www. cancerresearchuk.org; www. biotech.ufl.edu; www. isac-net.org; baserv. uci.kun.nl/~jraats/links1.html; www. recab.uni-hd.de/immuno.bme.nwu.edu; www. mrc-cpe.cam.ac.uk; www. ibt.unam.mx/vir/V_mice.html; http://www. bioinf.org.uk/abs; antibody.bath.ac.uk; www. unizh.ch; www. cryst.bbk.ac.uk/~ubcg07s; www.

nimr.mrc.ac.uk/CC/ccaewg/ccaewg.html; www.
path.cam.ac.uk/~mrc7/humanisation/TAHHP.html; www.
ibt.unam.mx/vir/structure/stat_aim.html; www.
biosci.missouri.edu/smithgp/index.html; www. jerini.de; 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. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Accordingly, part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions may be replaced with human or other amino acids.

Antibodies can also optionally be humanized or engineered or human antibodies engineered with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, humanized (or human) antibodies can be optionally prepared by a process of analysis of the parental sequences and various conceptual humanized and engineered products using three-dimensional models of the parental, engineered, 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, framework (FR) 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 addition, the antibodies generated using a B cell expansion agent may comprise a human germline light chain framework. In particular embodiments, the light chain germline sequence is selected from human VK sequences including, but

not limited to, A1, A10, A11, A14, A17, A18, A19, A2, A20, A23, A26, A27, A3, A30, A5, A7, B2, B3, L1, L10, L11, L12, L14, L15, L16, L18, L19, L2, L20, L22, L23, L24, L25, L4/18a, L5, L6, L8, L9, O1, O11, O12, O14, O18, O2, O4, and O8. In certain embodiments, this light chain human germline framework is selected from V1-11, V1-13, V1-16, V1-17, V1-18, V1-19, V1-2, V1-20, V1-22, V1-3, V1-4, V1-5, V1-7, V1-9, V2-1, V2-11, V2-13, V2-14, V2-15, V2-17, V2-19, V2-6, V2-7, V2-8, V3-2, V3-3, V3-4, V4-1, V4-2, V4-3, V4-4, V4-6, V5-1, V5-2, V5-4, and V5-6. See PCT WO 2005/005604 for a description of the different germline sequences.

In other embodiments, the antibody generated using a B cell expansion agent may comprise a human germline heavy chain framework. In particular embodiments, this heavy chain human germline framework is selected from VH1-18, VH1-2, VH1-24, VH1-3, VH1-45, VH1-46, VH1-58, VH1-69, VH1-8, VH2-26, VH2-5, VH2-70, VH3-11, VH3-13, VH3-15, VH3-16, VH3-20, VH3-21, VH3-23, VH3-30, VH3-33, VH3-35, VH3-38, VH3-43, VH3-48, VH3-49, VH3-53, VH3-64, VH3-66, VH3-7, VH3-72, VH3-73, VH3-74, VH3-9, VH4-28, VH4-31, VH4-34, VH4-39, VH4-4, VH4-59, VH4-61, VH5-51, VH6-1, and VH7-81. See PCT WO 2005/005604 for a description of the different germline sequences.

In particular embodiments, the light chain variable region and/or heavy chain variable region comprises a framework region or at least a portion of a framework region (e.g., containing 2 or 3 subregions, such as FR2 and FR3). In certain embodiments, at least FRL1, FRL2, FRL3, or FRL4 is fully human. In other embodiments, at least FRH1, FRH2, FRH3, or FRH4 is fully human. In some embodiments, at least FRL1, FRL2, FRL3, or FRL4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework (readily available at the sources of known human Ig sequences described above). In other embodiments, at least FRH1, FRH2, FRH3, or FRH4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework. In preferred embodiments, the framework region is a human framework region.

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 332:323

(1988); Verhoeyen 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. 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993), US patent Nos: 5723323, 5976862, 5824514, 5817483, 5814476, 5763192, 5723323, 5,766886, 5714352, 6204023, 6180370, 5693762, 5530101, 5585089, 5225539; 4816567, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, each entirely incorporated herein by reference, including references cited therein.

In certain embodiments, the antibody comprises an altered (e.g., mutated) Fc region. For example, in some embodiments, the Fc region has been altered to reduce or enhance the effector functions of the antibody. In some embodiments, the Fc region is an isotype selected from IgM, IgA, IgG, IgE, or other isotype.

Alternatively or additionally, it may be useful to combine amino acid modifications with one or more further amino acid modifications that alter C1q binding and/or the complement dependent cytotoxicity (CDC) function of the Fc region of an antibody generated using a B cell expansion agent or similar binding polypeptide. The binding polypeptide of particular interest may be one that binds to C1q and displays complement dependent cytotoxicity. Polypeptides with pre-existing C1q binding activity, optionally further having the ability to mediate CDC, may be modified such that one or both of these activities are enhanced. Amino acid modifications that alter C1q and/or modify its complement dependent cytotoxicity function are described, for example, in WO/0042072, which is hereby incorporated by reference.

As disclosed above, an Fc region of the antibody of the present invention can be provided with altered effector function, e.g., by modifying C1q binding and/or FcγR binding and thereby changing CDC activity and/or ADCC activity. "Effector functions" are responsible for activating or diminishing a biological activity (e.g., in a subject). Examples of effector functions include, but are not limited to: C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. Such effector functions may

require the Fc region to be combined with a binding domain (e.g., an antibody variable domain) and can be assessed using various assays (e.g., Fc binding assays, ADCC assays, CDC assays, etc.).

For example, one can generate a variant Fc region of the antibody with improved C1q binding and improved FcγRIII binding (e.g., having both improved ADCC activity and improved CDC activity). Alternatively, if it is desired that effector function be reduced or ablated, a variant Fc region can be engineered with reduced CDC activity and/or reduced ADCC activity. In other embodiments, only one of these activities may be increased, and, optionally, also the other activity reduced (e.g., to generate an Fc region variant with improved ADCC activity, but reduced CDC activity and vice versa).

Fc mutations can also be introduced or engineered to alter their interaction with the neonatal Fc receptor (FcRn) and improve their pharmacokinetic properties. A collection of human Fc variants with improved binding to the FcRn have been described (Shields et al., (2001). High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR, J. Biol. Chem. 276:6591-6604).

Another type of amino acid substitution serves to alter the glycosylation pattern of the Fc region of the antibody. Glycosylation of an Fc region is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. The recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain peptide sequences are asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline. Thus, the presence of either of these peptide sequences in a polypeptide creates a potential glycosylation site.

The glycosylation pattern may be altered, for example, by deleting one or more glycosylation site(s) found in the polypeptide, and/or adding one or more glycosylation site(s) that are not present in the polypeptide. Addition of glycosylation sites to the Fc region of an antibody is conveniently accomplished by

altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). An exemplary glycosylation variant has an amino acid substitution of residue Asn 297 of the heavy chain. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original polypeptide (for O-linked glycosylation sites). Additionally, a change of Asn 297 to Ala can remove one of the glycosylation sites.

In certain embodiments, the antibody of the present invention is expressed in cells that express beta (1,4)-N-acetylglucosaminyltransferase III (GnT III), such that GnT III adds GlcNAc to the antibody. Methods for producing antibodies in such a fashion are provided in WO/9954342, WO/03011878, patent publication 20030003097A1, and Umana et al., Nature Biotechnology, 17:176-180, Feb. 1999. An antibody can 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 an 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 98/24893, Lonberg *et al.* WO 98/24884, Lonberg *et al.* WO 97/13852, Lonberg *et al.* WO 94/25585, Kucherlapate *et al.* WO 96/34096, Kucherlapate *et al.* EP 0463 151 B1, Kucherlapate *et al.* EP 0710 719 A1, Surani *et al.* US. Pat. No. 5,545,807, Bruggemann *et al.* WO 90/04036, Bruggemann *et al.* EP 0438 474 B1, Lonberg *et al.* EP 0814 259 A2, Lonberg *et al.* GB 2 272 440 A, Lonberg *et al. Nature* 368:856-859 (1994), Taylor *et al., Int. Immunol.* 6(4)579-591 (1994), Green *et al, Nature Genetics* 7:13-21 (1994), Mendez *et al., Nature Genetics* 15:146-156 (1997), Taylor *et al., Nucleic Acids Research* 20(23):6287-6295 (1992), Tuaillon *et al., Proc Natl Acad Sci USA* 90(8)3720-3724 (1993), Lonberg *et al., Int Rev Immunol* 13(1):65-93 (1995) and Fishwald *et al., Nat Biotechnol* 14(7):845-851 (1996), which are each entirely incorporated herein by reference). Generally, these

mice comprise at least one transgene comprising DNA from at least one human immunoglobulin locus that is functionally rearranged, or which can undergo functional rearrangement. The endogenous immunoglobulin loci in such mice can be disrupted or deleted to eliminate the capacity of the animal to produce antibodies encoded by endogenous genes.

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, 91/18980, 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, 5656730, 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*.

Antibodies of the present invention can also be prepared using at least one antibody encoding nucleic acid to provide transgenic animals or mammals, such as goats, cows, horses, sheep, rabbits 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 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 proteins 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. Microbol. 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 references 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.

The antibodies of the invention can bind the target multi-subunit protein with a wide range of affinities (K_D). In a preferred embodiment, at least one mAb of the present invention can optionally bind the target multi-subunit protein with high affinity. For example, a human or other mAb can bind the target multi-subunit protein with a K_D 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}, 10^{-14}, 10^{-15} or any range or value therein, as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art.

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., K_D, K_{on}, K_{off}) are preferably made with standardized solutions of antibody and antigen, and a standardized buffer, such as the buffer described herein.

B Cell Expansion Agents

In one embodiment of the present invention, expansion of B cell numbers following immunization and boosts enhances the immune response to antigens that produce low titers of antibodies in mammals. In a specific embodiment in rodents, this method of the invention is useful in the generation of antigen-specific IgG mAbs. The antibodies generated by the method of the invention are useful as therapeutic agents, diagnostic agents or research reagents.

In this embodiment of the invention, the rodent is immunized with an antigen by techniques well known to those skilled in the art. The antigen can be a protein or nucleic acid and a T cell dependent antigen or a T cell independent antigen (including lipids and carbohydrates). T cell independent antigens include bacterial polysaccharides, polymeric proteins and lipopolysaccharides (LPS) and can directly stimulate naive B cells to produce strong antibody responses (generally IgM) in the absence of direct T cell helper functions. After necessary boosts, a B cell expansion agent is administered to the rodent to generate a higher frequency of antigen-specific B cell clones in Th2-biased hosts.

A B cell expansion agent useful in the method of the invention is an anti-CD-40 agonist, such as an anti-CD40 antibody agonist or a portion of an anti-CD40 antibody, that increases the number of antigen specific B cells, for example, in a rodent being immunized. The B cell expansion agent used in the present invention may also be BAFF (BLyS), IL-6, APRIL, CD40L (CD154), and anti-IgM/IL4 co-stimulation. The B cell expansion agent can be used with a second agent that can be

used as a targeting agent (moiety) and/or a B cell differentiation agent. CD21 can be used as a targeting agent.

Furthermore, unfolded protein response (UPR) pathway components may be used as B cell differentiation agents along with the B cell expansion agents of the invention. The UPR pathway has been demonstrated to be vital for driving B cell differentiaiton to plasmablasts/ plasma cells. By coupling a B cell expansion agent with a more specific UPR targeting of B cell differentiation during the immunization strategy, the number of antigen specific plasmablasts prior to fusion may be significantly increased. UPRs include BiP, XBP, CHOP, IRE1, PERK, ATF4, ATF6, eIF2alpha, GRP78, GRP94, calreticulin, chaperones, and variants having similar activity. Among preferred UPRs is XBP-1. Other B cell specific transcription factors (e.g., BLIMP-1) can also be used as B cell differentiation agents.

In the case of CD21, CD21 is present on the surface of B cells and follicular dendritic cells (FDCs), however, it functions differently in each environment. On B cells, CD21 transduces a signal that amplifies proliferation induced by the B-cell receptor and prevents apoptosis along with participating in ligand internalization. In contrast, on FDCs CD21 tethers complement coated pathogens (including HIV) to the cell surface. Coupling a protein that would activate Xbp-1 (such as ATF6 or IRE-1) to a CD21 binding-ligand, can expand antibody sysnthesis *in vivo*.

An exemplary anti-CD40 agonist is an anti-CD40 antibody or antibody fragment, such as a monoclonal anti-mouse CD40 antibody raised against a recombinant extracellular domain of mouse CD40. One of ordinary skill in the art could readily determine the amounts of anti-CD40 antibody to administer. For example, about 50 μg to about 100 μg of the anti-CD40 mAb (clone 1C10, Catalog No. MAB440, R&D Systems, Minneapolis, MN) administered about 3 days prior to lymphocyte harvest can be used to enhance the overall yield of antigen-reactive B lymphocytes from these mice.

Clonal populations of immortalized B cells are prepared by techniques known to the skilled artisan. Antigen-specific mAbs can be identified from clonal populations by screening for binding and/or biological activity toward the antigen of

interest by using peptide display libraries or other techniques known to those skilled in the art.

In another aspect, the invention relates to 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 pyrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

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 di-carboxylic 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 (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrolidone. 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_{5000} 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-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

Fatty acids and fatty acid esters suitable for modifying antibodies of the invention can be saturated or can contain one or more units of 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), octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic 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, preferably, one to about six, carbon atoms.

The modified 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, acrylolyl, 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 phosphorimide 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 C_1-C_{12} group 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, $-(CH_2)_3-$, $-NH-(CH_2)_6-NH-$, $-(CH_2)_2-NH-$ and $-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-O-CH-NH-$. Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (e.g., mono-Boc-ethylenediamine, 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 an 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 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).

Anti-Idiotype Antibodies to Antibody Compositions

In addition to monoclonal antibodies generated through use of the B cell expansion agent, 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 the antibody or a CDR containing region thereof. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody.

The present invention also provides at least one antibody composition comprising at least one, at least two, at least three, at least four, at least five, at least six or more 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 with various percentage identity, of the antibody amino acid sequences, or specified fragments, domains or variants thereof. Composition percentages are by weight, volume, concentration, molarity, or molality as liquid or dry solutions, mixtures, suspension, emulsions, particles, powder, or colloids, as known in the art or as described herein.

Antibody Compositions Comprising Further Therapeutically Active Ingredients

The antibody compositions of the invention can optionally further comprise an effective amount of at least one compound or protein selected from at least one of an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a

gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug or the like. Such drugs are well known in the art, including formulations, indications, dosing and administration for each presented herein (see, e.g., Nursing 2001 Handbook of Drugs, 21st edition, Springhouse Corp., Springhouse, PA, 2001; Health Professional's Drug Guide 2001, ed., Shannon, Wilson, Stang, Prentice-Hall, Inc, Upper Saddle River, NJ; Pharmcotherapy Handbook, Wells et al., ed., Appleton & Lange, Stamford, CT, each entirely incorporated herein by reference).

The anti-infective drug can be at least one selected from amebicides or at least one antiprotozoals, anthelmintics, antifungals, antimalarials, antituberculotics or at least one antileprotics, aminoglycosides, penicillins, cephalosporins, tetracyclines, sulfonamides, fluoroquinolones, antivirals, macrolide anti-infectives, and miscellaneous anti-infectives. The CV drug can be at least one selected from inotropics, antiarrhythmics, antianginals, antihypertensives, antilipemics, and miscellaneous cardiovascular drugs. The CNS drug can be at least one selected from nonnarcotic analgesics or at least one selected from antipyretics, nonsteroidal anti-inflammatory drugs, narcotic or at least one opiod analgesics, sedative-hypnotics, anticonvulsants, antidepressants, antianxiety drugs, antipsychotics, central nervous system stimulants, antiparkinsonians, and miscellaneous central nervous system drugs. The ANS drug can be at least one selected from cholinergics (parasympathomimetics), anticholinergics, adrenergics (sympathomimetics), adrenergic blockers (sympatholytics), skeletal muscle relaxants, and neuromuscular blockers. The respiratory tract drug can be at least one selected from antihistamines, bronchodilators, expectorants or at least one antitussive, and miscellaneous respiratory drugs. The GI tract drug can be at least one selected from antacids or at least one adsorbent or at least one antiflatulent, digestive enzyme or at least one gallstone solubilizer, antidiarrheals, laxatives, antiemetics, and antiulcer drugs. The hormonal drug can be at least one selected from corticosteroids, androgens or at least one anabolic steroid, estrogen or at least one progestin, gonadotropin, antidiabetic drug or at least one glucagon, thyroid hormone, thyroid hormone antagonist, pituitary hormone, and parathyroid-like drug. The drug for fluid and

electrolyte balance can be at least one selected from diuretics, electrolytes or at least one replacement solution, acidifier or at least one alkalinizer. The hematologic drug can be at least one selected from hematinics, anticoagulants, blood derivatives, and thrombolytic enzymes. The antineoplastics can be at least one selected from alkylating drugs, antimetabolites, antibiotic antineoplastics, antineoplastics that alter hormone balance, and miscellaneous antineoplastics. The immunomodulation drug can be at least one selected from immunosuppressants, vaccines or at least one toxoid, antitoxin or at least one antivenin, immune serum, and biological response modifier. The ophthalmic, otic, and nasal drugs can be at least one selected from ophthalmic anti-infectives, ophthalmic anti-inflammatories, miotics, mydriatics, ophthalmic vasoconstrictors, miscellaneous ophthalmics, otics, and nasal drugs. The topical drug can be at least one selected from local anti-infectives, scabicides or at least one pediculicide or topical corticosteroid. The nutritional drug can be at least one selected from vitamins, minerals, or calorics. See, e.g., contents *of Nursing 2001 Drug Handbook, supra*.

The at least one amebicide or antiprotozoal can be at least one selected from atovaquone, chloroquine hydrochloride, chloroquine phosphate, metronidazole, metronidazole hydrochloride, and pentamidine isethionate. The at least one anthelmintic can be at least one selected from mebendazole, pyrantel pamoate, and thiabendazole. The at least one antifungal can be at least one selected from amphotericin B, amphotericin B cholesteryl sulfate complex, amphotericin B lipid complex, amphotericin B liposomal, fluconazole, flucytosine, griseofulvin microsize, griseofulvin ultramicrosize, itraconazole, ketoconazole, nystatin, and terbinafine hydrochloride. The at least one antimalarial can be at least one selected from chloroquine hydrochloride, chloroquine phosphate, doxycycline, hydroxychloroquine sulfate, mefloquine hydrochloride, primaquine phosphate, pyrimethamine, and pyrimethamine with sulfadoxine. The at least one antituberculotic or antileprotic can be at least one selected from clofazimine, cycloserine, dapsone, ethambutol hydrochloride, isoniazid, pyrazinamide, rifabutin, rifampin, rifapentine, and streptomycin sulfate. The at least one aminoglycoside can be at least one selected from amikacin sulfate, gentamicin sulfate, neomycin sulfate, streptomycin sulfate, and tobramycin sulfate. The at least one penicillin can be at

least one selected from amoxcillin/clavulanate potassium, amoxicillin trihydrate, ampicillin, ampicillin sodium, ampicillin trihydrate, ampicillin sodium/sulbactam sodium, cloxacillin sodium, dicloxacillin sodium, mezlocillin sodium, nafcillin sodium, oxacillin sodium, penicillin G benzathine, penicillin G potassium, penicillin G procaine, penicillin G sodium, penicillin V potassium, piperacillin sodium, piperacillin sodium/tazobactam sodium, ticarcillin disodium, and ticarcillin disodium/clavulanate potassium. The at least one cephalosporin can be at least one selected from cefaclor, cefadroxil, cefazolin sodium, cefdinir, cefepime hydrochloride, cefixime, cefmetazole sodium, cefonicid sodium, cefoperazone sodium, cefotaxime sodium, cefotetan disodium, cefoxitin sodium, cefpodoxime proxetil, cefprozil, ceftazidime, ceftibuten, ceftizoxime sodium, ceftriaxone sodium, cefuroxime axetil, cefuroxime sodium, cephalexin hydrochloride, cephalexin monohydrate, cephradine, and loracarbef. The at least one tetracycline can be at least one selected from demeclocycline hydrochloride, doxycycline calcium, doxycycline hyclate, doxycycline hydrochloride, doxycycline monohydrate, minocycline hydrochloride, and tetracycline hydrochloride. The at least one sulfonamide can be at least one selected from co-trimoxazole, sulfadiazine, sulfamethoxazole, sulfisoxazole, and sulfisoxazole acetyl. The at least one fluoroquinolone can be at least one selected from alatrofloxacin mesylate, ciprofloxacin, enoxacin, levofloxacin, lomefloxacin hydrochloride, nalidixic acid, norfloxacin, ofloxacin, sparfloxacin, and trovafloxacin mesylate. The at least one fluoroquinolone can be at least one selected from alatrofloxacin mesylate, ciprofloxacin, enoxacin, levofloxacin, lomefloxacin hydrochloride, nalidixic acid, norfloxacin, ofloxacin, sparfloxacin, and trovafloxacin mesylate. The at least one antiviral can be at least one selected from abacavir sulfate, acyclovir sodium, amantadine hydrochloride, amprenavir, cidofovir, delavirdine mesylate, didanosine, efavirenz, famciclovir, fomivirsen sodium, foscarnet sodium, ganciclovir, indinavir sulfate, lamivudine, lamivudine/zidovudine, nelfinavir mesylate, nevirapine, oseltamivir phosphate, ribavirin, rimantadine hydrochloride, ritonavir, saquinavir, saquinavir mesylate, stavudine, valacyclovir hydrochloride, zalcitabine, zanamivir, and zidovudine. The at least one macroline anti-infective can be at least one selected from azithromycin, clarithromycin, dirithromycin, erythromycin base,

erythromycin estolate, erythromycin ethylsuccinate, erythromycin lactobionate, and erythromycin stearate. The at least one miscellaneous anti-infective can be at least one selected from aztreonam, bacitracin, chloramphenicol sodium sucinate, clindamycin hydrochloride, clindamycin palmitate hydrochloride, clindamycin phosphate, imipenem and cilastatin sodium, meropenem, nitrofurantoin macrocrystals, nitrofurantoin microcrystals, quinupristin/dalfopristin, spectinomycin hydrochloride, trimethoprim, and vancomycin hydrochloride. (See, e.g., pp. 24-214 of *Nursing 2001 Drug Handbook*.)

The at least one inotropic can be at least one selected from amrinone lactate, digoxin, and milrinone lactate. The at least one antiarrhythmic can be at least one selected from adenosine, amiodarone hydrochloride, atropine sulfate, bretylium tosylate, diltiazem hydrochloride, disopyramide, disopyramide phosphate, esmolol hydrochloride, flecainide acetate, ibutilide fumarate, lidocaine hydrochloride, mexiletine hydrochloride, moricizine hydrochloride, phenytoin, phenytoin sodium, procainamide hydrochloride, propafenone hydrochloride, propranolol hydrochloride, quinidine bisulfate, quinidine gluconate, quinidine polygalacturonate, quinidine sulfate, sotalol, tocainide hydrochloride, and verapamil hydrochloride. The at least one antianginal can be at least one selected from amlodipidine besylate, amyl nitrite, bepridil hydrochloride, diltiazem hydrochloride, isosorbide dinitrate, isosorbide mononitrate, nadolol, nicardipine hydrochloride, nifedipine, nitroglycerin, propranolol hydrochloride, verapamil, and verapamil hydrochloride. The at least one antihypertensive can be at least one selected from acebutolol hydrochloride, amlodipine besylate, atenolol, benazepril hydrochloride, betaxolol hydrochloride, bisoprolol fumarate, candesartan cilexetil, captopril, carteolol hydrochloride, carvedilol, clonidine, clonidine hydrochloride, diazoxide, diltiazem hydrochloride, doxazosin mesylate, enalaprilat, enalapril maleate, eprosartan mesylate, felodipine, fenoldopam mesylate, fosinopril sodium, guanabenz acetate, guanadrel sulfate, guanfacine hydrochloride, hydralazine hydrochloride, irbesartan, isradipine, labetalol hydrchloride, lisinopril, losartan potassium, methyldopa, methyldopate hydrochloride, metoprolol succinate, metoprolol tartrate, minoxidil, moexipril hydrochloride, nadolol, nicardipine hydrochloride, nifedipine, nisoldipine, nitroprusside sodium, penbutolol sulfate, perindopril erbumine, phentolamine

mesylate, pindolol, prazosin hydrochloride, propranolol hydrochloride, quinapril hydrochloride, ramipril, telmisartan, terazosin hydrochloride, timolol maleate, trandolapril, valsartan, and verapamil hydrochloride. The at least one antilipemic can be at least one selected from atorvastatin calcium, cerivastatin sodium, cholestyramine, colestipol hydrochloride, fenofibrate (micronized), fluvastatin sodium, gemfibrozil, lovastatin, niacin, pravastatin sodium, and simvastatin. The at least one miscellaneous CV drug can be at least one selected from abciximab, alprostadil, arbutamine hydrochloride, cilostazol, clopidogrel bisulfate, dipyridamole, eptifibatide, midodrine hydrochloride, pentoxifylline, ticlopidine hydrochloride, and tirofiban hydrochloride. (See, e.g., pp. 215-336 of *Nursing 2001 Drug Handbook*.)

The at least one nonnarcotic analgesic or antipyretic can be at least one selected from acetaminophen, aspirin, choline magnesium trisalicylate, diflunisal, and magnesium salicylate. The at least one nonsteroidal anti-inflammatory drug can be at least one selected from celecoxib, diclofenac potassium, diclofenac sodium, etodolac, fenoprofen calcium, flurbiprofen, ibuprofen, indomethacin, indomethacin sodium trihydrate, ketoprofen, ketorolac tromethamine, nabumetone, naproxen, naproxen sodium, oxaprozin, piroxicam, rofecoxib, and sulindac. The at least one narcotic or opiod analgesic can be at least one selected from alfentanil hydrochloride, buprenorphine hydrochloride, butorphanol tartrate, codeine phosphate, codeine sulfate, fentanyl citrate, fentanyl transdermal system, fentanyl transmucosal, hydromorphone hydrochloride, meperidine hydrochloride, methadone hydrochloride, morphine hydrochloride, morphine sulfate, morphine tartrate, nalbuphine hydrochloride, oxycodone hydrochloride, oxycodone pectinate, oxymorphone hydrochloride, pentazocine hydrochloride, pentazocine hydrochloride and naloxone hydrochloride, pentazocine lactate, propoxyphene hydrochloride, propoxyphene napsylate, remifentanil hydrochloride, sufentanil citrate, and tramadol hydrochloride. The at least one sedative-hypnotic can be at least one selected from chloral hydrate, estazolam, flurazepam hydrochloride, pentobarbital, pentobarbital sodium, phenobarbital sodium, secobarbital sodium, temazepam, triazolam, zaleplon, and zolpidem tartrate. The at least one anticonvulsant can be at least one selected from acetazolamide sodium, carbamazepine, clonazepam, clorazepate

dipotassium, diazepam, divalproex sodium, ethosuximde, fosphenytoin sodium, gabapentin, lamotrigine, magnesium sulfate, phenobarbital, phenobarbital sodium, phenytoin, phenytoin sodium, phenytoin sodium (extended), primidone, tiagabine hydrochloride, topiramate, valproate sodium, and valproic acid. The at least one antidepressant can be at least one selected from amitriptyline hydrochloride, amitriptyline pamoate, amoxapine, bupropion hydrochloride, citalopram hydrobromide, clomipramine hydrochloride, desipramine hydrochloride, doxepin hydrochloride, fluoxetine hydrochloride, imipramine hydrochloride, imipramine pamoate, mirtazapine, nefazodone hydrochloride, nortriptyline hydrochloride, paroxetine hydrochloride, phenelzine sulfate, sertraline hydrochloride, tranylcypromine sulfate, trimipramine maleate, and venlafaxine hydrochloride. The at least one antianxiety drug can be at least one selected from alprazolam, buspirone hydrochloride, chlordiazepoxide, chlordiazepoxide hydrochloride, clorazepate dipotassium, diazepam, doxepin hydrochloride, hydroxyzine embonate, hydroxyzine hydrochloride, hydroxyzine pamoate, lorazepam, mephrobamate, midazolam hydrochloride, and oxazepam. The at least one antipsychotic drug can be at least one selected from chlorpromazine hydrochloride, clozapine, fluphenazine decanoate, fluephenazine enanthate, fluphenazine hydrochloride, haloperidol, haloperidol decanoate, haloperidol lactate, loxapine hydrochloride, loxapine succinate, mesoridazine besylate, molindone hydrochloride, olanzapine, perphenazine, pimozide, prochlorperazine, quetiapine fumarate, risperidone, thioridazine hydrochloride, thiothixene, thiothixene hydrochloride, and trifluoperazine hydrochloride. The at least one central nervous system stimulant can be at least one selected from amphetamine sulfate, caffeine, dextroamphetamine sulfate, doxapram hydrochloride, methamphetamine hydrochloride, methylphenidate hydrochloride, modafinil, pemoline, and phentermine hydrochloride. The at least one antiparkinsonian can be at least one selected from amantadine hydrochloride, benztropine mesylate, biperiden hydrochloride, biperiden lactate, bromocriptine mesylate, carbidopa-levodopa, entacapone, levodopa, pergolide mesylate, pramipexole dihydrochloride, ropinirole hydrochloride, selegiline hydrochloride, tolcapone, and trihexyphenidyl hydrochloride. The at least one miscellaneous central nervous system drug can be at least one selected from bupropion

hydrochloride, donepezil hydrochloride, droperidol, fluvoxamine maleate, lithium carbonate, lithium citrate, naratriptan hydrochloride, nicotine polacrilex, nicotine transdermal system, propofol, rizatriptan benzoate, sibutramine hydrochloride monohydrate, sumatriptan succinate, tacrine hydrochloride, and zolmitriptan. (See, e.g., pp. 337-530 of *Nursing 2001 Drug Handbook*.)

The at least one cholinergic (e.g., parasymathomimetic) can be at least one selected from bethanechol chloride, edrophonium chloride, neostigmine bromide, neostigmine methylsulfate, physostigmine salicylate, and pyridostigmine bromide. The at least one anticholinergic can be at least one selected from atropine sulfate, dicyclomine hydrochloride, glycopyrrolate, hyoscyamine, hyoscyamine sulfate, propantheline bromide, scopolamine, scopolamine butylbromide, and scopolamine hydrobromide. The at least one adrenergic (sympathomimetics) can be at least one selected from dobutamine hydrochloride, dopamine hydrochloride, metaraminol bitartrate, norepinephrine bitartrate, phenylephrine hydrochloride, pseudoephedrine hydrochloride, and pseudoephedrine sulfate. The at least one adrenergic blocker (sympatholytic) can be at least one selected from dihydroergotamine mesylate, ergotamine tartrate, methysergide maleate, and propranolol hydrochloride. The at least one skeletal muscle relaxant can be at least one selected from baclofen, carisoprodol, chlorzoxazone, cyclobenzaprine hydrochloride, dantrolene sodium, methocarbamol, and tizanidine hydrochloride. The at least one neuromuscular blocker can be at least one selected from atracurium besylate, cisatracurium besylate, doxacurium chloride, mivacurium chloride, pancuronium bromide, pipecuronium bromide, rapacuronium bromide, rocuronium bromide, succinylcholine chloride, tubocurarine chloride, and vecuronium bromide. (See, e.g., pp. 531-84 of *Nursing 2001 Drug Handbook*.)

The at least one antihistamine can be at least one selected from brompheniramine maleate, cetirizine hydrochloride, chlorpheniramine maleate, clemastine fumarate, cyproheptadine hydrochloride, diphenhydramine hydrochloride, fexofenadine hydrochloride, loratadine, promethazine hydrochloride, promethazine theoclate, and triprolidine hydrochloride. The at least one bronchodilator can be at least one selected from albuterol, albuterol sulfate, aminophylline, atropine sulfate, ephedrine sulfate, epinephrine, epinephrine

bitartrate, epinephrine hydrochloride, ipratropium bromide, isoproterenol, isoproterenol hydrochloride, isoproterenol sulfate, levalbuterol hydrochloride, metaproterenol sulfate, oxtriphylline, pirbuterol acetate, salmeterol xinafoate, terbutaline sulfate, and theophylline. The at least one expectorant or antitussive can be at least one selected from benzonatate, codeine phosphate, codeine sulfate, dextramethorphan hydrobromide, diphenhydramine hydrochloride, guaifenesin, and hydromorphone hydrochloride. The at least one miscellaneous respiratory drug can be at least one selected from acetylcysteine, beclomethasone dipropionate, beractant, budesonide, calfactant, cromolyn sodium, dornase alfa, epoprostenol sodium, flunisolide, fluticasone propionate, montelukast sodium, nedocromil sodium, palivizumab, triamcinolone acetonide, zafirlukast, and zileuton. (See, e.g., pp. 585-642 of *Nursing 2001 Drug Handbook.*)

The at least one antacid, adsorbent, or antiflatulent can be at least one selected from aluminum carbonate, aluminum hydroxide, calcium carbonate, magaldrate, magnesium hydroxide, magnesium oxide, simethicone, and sodium bicarbonate. The at least one digestive enzyme or gallstone solubilizer can be at least one selected from pancreatin, pancrelipase, and ursodiol. The at least one antidiarrheal can be at least one selected from attapulgite, bismuth subsalicylate, calcium polycarbophil, diphenoxylate hydrochloride and atropine sulfate, loperamide, octreotide acetate, opium tincture, and opium tincure (camphorated). The at least one laxative can be at least one selected from bisocodyl, calcium polycarbophil, cascara sagrada, cascara sagrada aromatic fluidextract, cascara sagrada fluidextract, castor oil, docusate calcium, docusate sodium, glycerin, lactulose, magnesium citrate, magnesium hydroxide, magnesium sulfate, methylcellulose, mineral oil, polyethylene glycol or electrolyte solution, psyllium, senna, and sodium phosphates. The at least one antiemetic can be at least one selected from chlorpromazine hydrochloride, dimenhydrinate, dolasetron mesylate, dronabinol, granisetron hydrochloride, meclizine hydrochloride, metoclopramide hydrochloride, ondansetron hydrochloride, perphenazine, prochlorperazine, prochlorperazine edisylate, prochlorperazine maleate, promethazine hydrochloride, scopolamine, thiethylperazine maleate, and trimethobenzamide hydrochloride. The at least one antiulcer drug can be at least one selected from cimetidine, cimetidine

hydrochloride, famotidine, lansoprazole, misoprostol, nizatidine, omeprazole, rabeprozole sodium, rantidine bismuth citrate, ranitidine hydrochloride, and sucralfate. (See, e.g., pp. 643-95 of *Nursing 2001 Drug Handbook*.)

The at least one corticosteroid can be at least one selected from betamethasone, betamethasone acetate or betamethasone sodium phosphate, betamethasone sodium phosphate, cortisone acetate, dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, fludrocortisone acetate, hydrocortisone, hydrocortisone acetate, hydrocortisone cypionate, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, prednisolone, prednisolone acetate, prednisolone sodium phosphate, prednisolone tebutate, prednisone, triamcinolone, triamcinolone acetonide, and triamcinolone diacetate. The at least one androgen or anabolic steroid can be at least one selected from danazol, fluoxymesterone, methyltestosterone, nandrolone decanoate, nandrolone phenpropionate, testosterone, testosterone cypionate, testosterone enanthate, testosterone propionate, and testosterone transdermal system. The at least one estrogen or progestin can be at least one selected from esterified estrogens, estradiol, estradiol cypionate, estradiol/norethindrone acetate transdermal system, estradiol valerate, estrogens (conjugated), estropipate, ethinyl estradiol, ethinyl estradiol and desogestrel, ethinyl estradiol and ethynodiol diacetate, ethinyl estradiol and desogestrel, ethinyl estradiol and ethynodiol diacetate, ethinyl estradiol and levonorgestrel, ethinyl estradiol and norethindrone, ethinyl estradiol and norethindrone acetate, ethinyl estradiol and norgestimate, ethinyl estradiol and norgestrel, ethinyl estradiol and norethindrone and acetate and ferrous fumarate, levonorgestrel, medroxyprogesterone acetate, mestranol and norethindron, norethindrone, norethindrone acetate, norgestrel, and progesterone. The at least one gonadroptropin can be at least one selected from ganirelix acetate, gonadoreline acetate, histrelin acetate, and menotropins. The at least one antidiabetic or glucaon can be at least one selected from acarbose, chlorpropamide, glimepiride, glipizide, glucagon, glyburide, insulins, metformin hydrochloride, miglitol, pioglitazone hydrochloride, repaglinide, rosiglitazone maleate, and troglitazone. The at least one thyroid hormone can be at least one selected from levothyroxine sodium, liothyronine sodium, liotrix, and thyroid. The

at least one thyroid hormone antagonist can be at least one selected from methimazole, potassium iodide, potassium iodide (saturated solution), propylthiouracil, radioactive iodine (sodium iodide ^{131}I), and strong iodine solution. The at least one pituitary hormone can be at least one selected from corticotropin, cosyntropin, desmophressin acetate, leuprolide acetate, repository corticotropin, somatrem, somatropin, and vasopressin. The at least one parathyroid-like drug can be at least one selected from calcifediol, calcitonin (human), calcitonin (salmon), calcitriol, dihydrotachysterol, and etidronate disodium. (See, e.g., pp. 696-796 of *Nursing 2001 Drug Handbook*.)

The at least one diuretic can be at least one selected from acetazolamide, acetazolamide sodium, amiloride hydrochloride, bumetanide, chlorthalidone, ethacrynate sodium, ethacrynic acid, furosemide, hydrochlorothiazide, indapamide, mannitol, metolazone, spironolactone, torsemide, triamterene, and urea. The at least one electrolyte or replacement solution can be at least one selected from calcium acetate, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, calcium lactate, calcium phosphate (dibasic), calcium phosphate (tribasic), dextran (high-molecular-weight), dextran (low-molecular-weight), hetastarch, magnesium chloride, magnesium sulfate, potassium acetate, potassium bicarbonate, potassium chloride, potassium gluconate, Ringer's injection, Ringer's injection (lactated), and sodium chloride. The at least one acidifier or alkalinizer can be at least one selected from sodium bicarbonate, sodium lactate, and tromethamine. (See, e.g., pp. 797-833 of *Nursing 2001 Drug Handbook*.)

The at least one hematinic can be at least one selected from ferrous fumarate, ferrous gluconate, ferrous sulfate, ferrous sulfate (dried), iron dextran, iron sorbitol, polysaccharide-iron complex, and sodium ferric gluconate complex. The at least one anticoagulant can be at least one selected from ardeparin sodium, dalteparin sodium, danaparoid sodium, enoxaparin sodium, heparin calcium, heparin sodium, and warfarin sodium. The at least one blood derivative can be at least one selected from albumin 5%, albumin 25%, antihemophilic factor, anti-inhibitor coagulant complex, antithrombin III (human), factor IX (human), factor IX complex, and plasma protein fractions. The at least one thrombolytic enzyme can be at least one

selected from alteplase, anistreplase, reteplase (recombinant), streptokinase, and urokinase. (See, e.g., pp. 834-66 of *Nursing 2001 Drug Handbook.*)

The at least one alkylating drug can be at least one selected from busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, ifosfamide, lomustine, mechlorethamine hydrochloride, melphalan, melphalan hydrochloride, streptozocin, temozolomide, and thiotepa. The at least one antimetabolite can be at least one selected from capecitabine, cladribine, cytarabine, floxuridine, fludarabine phosphate, fluorouracil, hydroxyurea, mercaptopurine, methotrexate, methotrexate sodium, and thioguanine. The at least one antibiotic antineoplastic can be at least one selected from bleomycin sulfate, dactinomycin, daunorubicin citrate liposomal, daunorubicin hydrochloride, doxorubicin hydrochloride, doxorubicin hydrochloride liposomal, epirubicin hydrochloride, idarubicin hydrochloride, mitomycin, pentostatin, plicamycin, and valrubicin. The at least one antineoplastic that alters hormone balance can be at least one selected from anastrozole, bicalutamide, estramustine phosphate sodium, exemestane, flutamide, goserelin acetate, letrozole, leuprolide acetate, megestrol acetate, nilutamide, tamoxifen citrate, testolactone, and toremifene citrate. The at least one miscellaneous antineoplastic can be at least one selected from asparaginase, bacillus Calmette-Guerin (BCG) (live intravesical), dacarbazine, docetaxel, etoposide, etoposide phosphate, gemcitabine hydrochloride, irinotecan hydrochloride, mitotane, mitoxantrone hydrochloride, paclitaxel, pegaspargase, porfimer sodium, procarbazine hydrochloride, rituximab, teniposide, topotecan hydrochloride, trastuzumab, tretinoin, vinblastine sulfate, vincristine sulfate, and vinorelbine tartrate. (See, e.g., pp. 867-963 of *Nursing 2001 Drug Handbook.*)

The at least one immunosuppressant can be at least one selected from azathioprine, basiliximab, cyclosporine, daclizumab, lymphocyte immune globulin, muromonab-CD3, mycophenolate mofetil, mycophenolate mofetil hydrochloride, sirolimus, and tacrolimus. The at least one vaccine or toxoid can be at least one selected from BCG vaccine, cholera vaccine, diphtheria and tetanus toxoids (adsorbed), diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed, diphtheria and tetanus toxoids and whole-cell pertussis vaccine, *Haemophilius* b conjugate vaccines, hepatitis A vaccine (inactivated), hepatisis B vaccine

(recombinant), influenza virus vaccine 1999-2000 trivalent types A & B (purified surface antigen), influenza virus vaccine 1999-2000 trivalent types A & B (subvirion or purified subvirion), influenza virus vaccine 1999-2000 trivalent types A & B (whole virion), Japanese encephalitis virus vaccine (inactivated), Lyme disease vaccine (recombinant OspA), measles and mumps and rubella virus vaccine (live), measles and mumps and rubella virus vaccine (live attenuated), measles virus vaccine (live attenuated), meningococcal polysaccharide vaccine, mumps virus vaccine (live), plague vaccine, pneumococcal vaccine (polyvalent), poliovirus vaccine (inactivated), poliovirus vaccine (live, oral, trivalent), rabies vaccine (adsorbed), rabies vaccine (human diploid cell), rubella and mumps virus vaccine (live), rubella virus vaccine (live, attenuated), tetanus toxoid (adsorbed), tetanus toxoid (fluid), typhoid vaccine (oral), typhoid vaccine (parenteral), typhoid Vi polysaccharide vaccine, varicella virus vaccine, and yellow fever vaccine. The at least one antitoxin or antivenin can be at least one selected from black widow spider antivenin, Crotalidae antivenom (polyvalent), diphtheria antitoxin (equine), amd *Micrurus fulvius* antivenin. The at least one immune serum can be at least one selected from cytomegalovirus immune globulin (intraveneous), hepatitis B immune globulin (human), immune globulin intramuscular, immune globulin intravenous, rabies immune globulin (human), respiratory syncytial virus immune globulin intravenous (human), Rh_0(D) immune globulin (human), Rh_0(D) immune globulin intravenous (human), tetanus immune globulin (human), and varicella-zoster immune globulin. The at least one biological response modifier can be at least one selected from aldesleukin, epoetin alfa, filgrastim, glatiramer acetate for injection, interferon alfacon-1, interferon alfa-2a (recombinant), interferon alfa-2b (recombinant), interferon beta-1a, interferon beta-1b (recombinant), interferon gamma-1b, levamisole hydrochloride, oprelvekin, and sargramostim. (See, e.g., pp. 964-1040 of *Nursing 2001 Drug Handbook.*)

The at least one ophthalmic anti-infective can be selected form bacitracin, chloramphenicol, ciprofloxacin hydrochloride, erythromycin, gentamicin sulfate, ofloxacin 0.3%, polymyxin B sulfate, sulfacetamide sodium 10%, sulfacetamide sodium 15%, sulfacetamide sodium 30%, tobramycin, and vidarabine. The at least one ophthalmic anti-inflammatory can be at least one selected from dexamethasone,

dexamethasone sodium phosphate, diclofenac sodium 0.1%, fluorometholone, flurbiprofen sodium, ketorolac tromethamine, prednisolone acetate (suspension) and prednisolone sodium phosphate (solution). The at least one miotic can be at least one selected from acetylocholine chloride, carbachol (intraocular), carbachol (topical), echothiophate iodide, pilocarpine, pilocarpine hydrochloride, and pilocarpine nitrate. The at least one mydriatic can be at least one selected from atropine sulfate, cyclopentolate hydrochloride, epinephrine hydrochloride, epinephryl borate, homatropine hydrobromide, phenylephrine hydrochloride, scopolamine hydrobromide, and tropicamide. The at least one ophthalmic vasoconstrictor can be at least one selected from naphazoline hydrochloride, oxymetazoline hydrochloride, and tetrahydrozoline hydrochloride. The at least one miscellaneous ophthalmic can be at least one selected from apraclonidine hydrochloride, betaxolol hydrochloride, brimonidine tartrate, carteolol hydrochloride, dipivefrin hydrochloride, dorzolamide hydrochloride, emedastine difumarate, fluorescein sodium, ketotifen fumarate, latanoprost, levobunolol hydrochloride, metipranolol hydrochloride, sodium chloride (hypertonic), and timolol maleate. The at least one otic can be at least one selected from boric acid, carbamide peroxide, chloramphenicol, and triethanolamine polypeptide oleate-condensate. The at least one nasal drug can be at least one selected from beclomethasone dipropionate, budesonide, ephedrine sulfate, epinephrine hydrochloride, flunisolide, fluticasone propionate, naphazoline hydrochloride, oxymetazoline hydrochloride, phenylephrine hydrochloride, tetrahydrozoline hydrochloride, triamcinolone acetonide, and xylometazoline hydrochloride. (See, e.g., pp. 1041-97 of *Nursing 2001 Drug Handbook.*)

The at least one local anti-infective can be at least one selected from acyclovir, amphotericin B, azelaic acid cream, bacitracin, butoconazole nitrate, clindamycin phosphate, clotrimazole, econazole nitrate, erythromycin, gentamicin sulfate, ketoconazole, mafenide acetate, metronidazole (topical), miconazole nitrate, mupirocin, naftifine hydrochloride, neomycin sulfate, nitrofurazone, nystatin, silver sulfadiazine, terbinafine hydrochloride, terconazole, tetracycline hydrochloride, tioconazole, and tolnaftate. The at least one scabicide or pediculicide can be at least one selected from crotamiton, lindane, permethrin, and pyrethrins. The at least one

topical corticosteroid can be at least one selected from betamethasone dipropionate, betamethasone valerate, clobetasol propionate, desonide, desoximetasone, dexamethasone, dexamethasone sodium phosphate, diflorasone diacetate, fluocinolone acetonide, fluocinonide, flurandrenolide, fluticasone propionate, halcionide, hydrocortisone, hydrocortisone acetate, hydrocortisone butyrate, hydrocorisone valerate, mometasone furoate, and triamcinolone acetonide. (See, e.g., pp. 1098-1136 of *Nursing 2001 Drug Handbook.*)

The at least one vitamin or mineral can be at least one selected from vitamin A, vitamin B complex, cyanocobalamin, folic acid, hydroxocobalamin, leucovorin calcium, niacin, niacinamide, pyridoxine hydrochloride, riboflavin, thiamine hydrochloride, vitamin C, vitamin D, cholecalciferol, ergocalciferol, vitamin D analogue, doxercalciferol, paricalcitol, vitamin E, vitamin K analogue, phytonadione, sodium fluoride, sodium fluoride (topical), trace elements, chromium, copper, iodine, manganese, selenium, and zinc. The at least one caloric can be at least one selected from amino acid infusions (crystalline), amino acid infusions in dextrose, amino acid infusions with electrolytes, amino acid infusions with electrolytes in dextrose, amino acid infusions for hepatic failure, amino acid infusions for high metabolic stress, amino acid infusions for renal failure, dextrose, fat emulsions, and medium-chain triglycerides. (See, e.g., pp. 1137-63 of *Nursing 2001 Drug Handbook.*)

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 antibody contacted or administered 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 chemical or protein antagonist, TNF monoclonal or polyclonal antibody or fragment, a soluble TNF receptor (e.g., p55, p70 or p85) or fragment, fusion polypeptides thereof, or a small molecule TNF antagonist, e.g., TNF binding protein I or II (TBP-1 or TBP-II), nerelimonmab, infliximab, etanercept, CDP-571, CDP-870, afelimomab, lenercept, and the like), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide,

sulfasalzine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anethetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a flurorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteriod, 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 antiulcer, a laxative, an anticoagulant, an erythropoietin (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 cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, 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. Non-limiting examples of such cytokines include, but are not limted to, any of IL-1 to IL-28 (e.g., IL-1, IL-2, etc.). 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.

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 pathological 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 0157: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 dificile, Clostridium botulinum*), *Camphlobacter* species (e.g., *Camphlobacter jejuni, Camphlobacter fetus*), *Heliobacter* species, (e.g., *Heliobacter pylori*), *Aeromonas* species (e.g., *Aeromonas sobria, Aeromonas hydrophila, Aeromonas caviae*), *Pleisomonas shigelloides, Yersina enterocolitica, Vibrios* species (e.g., *Vibrios cholerae, Vibrios parahemolyticus*), *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.

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, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed., *Remington's Pharmaceutical*

Sciences, 18th Edition, Mack Publishing Co. (Easton, PA) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the antibody, fragment or variant composition as well known in the art or as described herein.

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 alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin, such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

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, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

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, carbonic 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, 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., polysorbates, 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 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. Preferrred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents. An exemplary carrier molecule is the mucopolysaccharide, hyaluronic acid, which may be useful for intraarticular delivery.

Formulations

As noted above, the invention provides for stable formulations, which preferably comprise a phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one 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 nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, polymers, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as about 0.0015%, or any range, value, or fraction therein. Non-limiting examples include, no preservative, about 0.1-2% m-cresol (e.g., 0.2, 0.3. 0.4, 0.5, 0.9, 1.0%), about 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), about 0.001-0.5% thimerosal (e.g., 0.005, 0.01), about 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 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 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 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 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.

The range of at least one antibody in the product of the present invention includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0 μg/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

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.

Other excipients, e.g., isotonicity agents, buffers, antioxidants, and 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 a pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably, sodium phosphate, particularly, phosphate buffered saline (PBS).

Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), 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® polyls, 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.

The formulations of the present invention can be prepared by a process which comprises mixing at least one 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 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 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.

The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one 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.

The present claimed articles of manufacture are useful for administration over a period ranging from immediate 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°C to about 40°C and retain the biological 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.

The solutions of at least one antibody of 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.

The claimed products can be provided to patients as clear solutions or as dual

vials comprising a vial of lyophilized at least one 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.

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 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 and/or patients.

Recognized devices comprising single vial systems include pen-injector devices for delivery of a solution, such as BD Pens, BD Autojector®, Humaject®, NovoPen®, B-D®Pen, AutoPen®, and OptiPen®, GenotropinPen®, Genotronorm Pen®, Humatro Pen®, Reco-Pen®, Roferon Pen®, Biojector®, Iject®, J-tip Needle-Free Injector®, Intraject®, Medi-Ject®, e.g., as made or developed by Becton Dickensen (Franklin Lakes, NJ, www. bectondickenson.com), Disetronic (Burgdorf, Switzerland, www. disetronic.com; Bioject, Portland, Oregon (www. bioject.com); National Medical Products, Weston Medical (Peterborough, UK, www. weston-medical.com), Medi-Ject Corp (Minneapolis, MN, www. mediject.com), and similary suitable devices. Recognized devices comprising a dual vial system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for delivery of the reconstituted solution, such as the HumatroPen®. Examples of other devices suitable include pre-filled syringes, auto-injectors, needle free injectors and needle free IV infusion sets.

The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product can be used. The packaging material of the present invention provides instructions to the patient to reconstitute the at least one antibody in the aqueous diluent to form a solution and to use the solution over a

period of 2-24 hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution can be used over a period of 2-24 hours or greater. The presently claimed products are useful for human pharmaceutical product use.

The formulations of the present invention can be prepared by a process that comprises mixing at least one antibody and a selected buffer, preferably, a phosphate buffer containing saline or a chosen salt. Mixing the at least one antibody and buffer 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 antibody in water or buffer is combined with the desired buffering agent in water in quantities sufficient to provide the protein and 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.

The claimed stable or preserved formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one antibody that is reconstituted with a second vial containing a preservative or buffer and excipients 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 provides a more convenient treatment regimen than currently available.

Other formulations or methods of stablizing the antibody may result in other than a clear solution of lyophilized powder comprising the antibody. Among non-clear solutions are formulations comprising particulate suspensions, said particulates being a composition containing the antibody in a structure of variable dimension and known variously as a microsphere, microparticle, nanoparticle, nanosphere, or liposome. Such relatively homogenous, essentially spherical, particulate formulations containing an active agent can be formed by contacting an aqueous phase containing the active agent and a polymer and a nonaqueous phase followed by evaporation of the nonaqueous phase to cause the coalescence of particles from

the aqueous phase as taught in U.S. 4,589,330. Porous microparticles can be prepared using a first phase containing active agent and a polymer dispersed in a continuous solvent and removing said solvent from the suspension by freeze-drying or dilution-extraction-precipitation as taught in U.S. 4,818,542. Preferred polymers for such preparations are natural or synthetic copolymers or polymers selected from the group consisting of gleatin agar, starch, arabinogalactan, albumin, collagen, polyglycolic acid, polylactic aced, glycolide-L(-) lactide poly(episilon-caprolactone, poly(epsilon-caprolactone-CO-lactic acid), poly(epsilon-caprolactone-CO-glycolic acid), poly(ß-hydroxy butyric acid), polyethylene oxide, polyethylene, poly(alkyl-2-cyanoacrylate), poly(hydroxyethyl methacrylate), polyamides, poly(amino acids), poly(2-hydroxyethyl DL-aspartamide), poly(ester urea), poly(L-phenylalanine/ethylene glycol/1,6-diisocyanatohexane) and poly(methyl methacrylate). Particularly preferred polymers are polyesters, such as polyglycolic acid, polylactic aced, glycolide-L(-) lactide poly(episilon-caprolactone, poly(epsilon-caprolactone-CO-lactic acid), and poly(epsilon-caprolactone-CO-glycolic acid. Solvents useful for dissolving the polymer and/or the active include: water, hexafluoroisopropanol, methylenechloride, tetrahydrofuran, hexane, benzene, or hexafluoroacetone sesquihydrate. The process of dispersing the active containing phase with a second phase may include pressure forcing said first phase through an orifice in a nozzle to affect droplet formation.

Dry powder formulations may result from processes other than lyophilization, such as by spray drying or solvent extraction by evaporation or by precipitation of a crystalline composition followed by one or more steps to remove aqueous or nonaqueous solvent. Preparation of a spray-dried antibody preparation is taught in U.S. 6,019,968. The antibody-based dry powder compositions may be produced by spray drying solutions or slurries of the antibody and, optionally, excipients, in a solvent under conditions to provide a respirable dry powder. Solvents may include polar compounds, such as water and ethanol, which may be readily dried. Antibody stability may be enhanced by performing the spray drying procedures in the absence of oxygen, such as under a nitrogen blanket or by using nitrogen as the drying gas. Another relatively dry formulation is a dispersion of a plurality of perforated microstructures dispersed in a suspension medium that

typically comprises a hydrofluoroalkane propellant as taught in WO 9916419. The stabilized dispersions may be administered to the lung of a patient using a metered dose inhaler. Equipment useful in the commercial manufacture of spray dried medicaments are manufactured by Buchi Ltd. or Niro Corp.

At least one antibody in either the stable or preserved formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a variety of delivery methods including SC or IM injection; transdermal, pulmonary, transmucosal, implant, osmotic pump, cartridge, micro pump, or other means appreciated by the skilled artisan, as well-known in the art.

Therapeutic Applications

The present invention also provides a method for modulating or treating at least one antigen-related disease, in a cell, tissue, organ, animal, or patient, as known in the art or as described herein, using at least one antibody of the present invention, e.g., administering or contacting the cell, tissue, organ, animal, or patient with a therapeutic effective amount of antibody. The present invention also provides a method for modulating or treating at least one antigen related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of obesity, an immune related disease, a cardiovascular disease, an infectious disease, a malignant disease or a neurologic disease.

The present invention also provides a method for modulating or treating at least one antigen related 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, osteolysis, aseptic loosening of orthopedic implants, inflammatory bowel disease, ulcerative colitis, systemic lupus erythematosus, antiphospholipid syndrome, iridocyclitis/uveitis/optic neuritis, idiopathic pulmonary fibrosis, systemic vasculitis/wegener's granulomatosis, sarcoidosis, orchitis/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, hypersensitity 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 translplant 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, allograft rejection, anti-receptor hypersensitivity reactions, Graves disease, Raynaud's disease, type B insulin-resistant diabetes, asthma, myasthenia gravis, antibody-meditated cytotoxicity, type III hypersensitivity reactions, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes syndrome, antiphospholipid syndrome, pemphigus, scleroderma, mixed connective tissue disease, idiopathic Addison's disease, diabetes mellitus, chronic active hepatitis, primary billiary cirrhosis, vitiligo, vasculitis, post-MI cardiotomy syndrome, type IV hypersensitivity, contact dermatitis, hypersensitivity pneumonitis, allograft rejection, granulomas due to intracellular organisms, drug sensitivity, metabolic/idiopathic, Wilson's disease, hemachromatosis, alpha-1-antitrypsin deficiency, diabetic retinopathy, hashimoto's thyroiditis, osteoporosis, hypothalamic-pituitary-adrenal axis evaluation, primary biliary cirrhosis, thyroiditis, encephalomyelitis, cachexia, cystic fibrosis, neonatal chronic lung disease, chronic obstructive pulmonary disease (COPD), familial hematophagocytic lymphohistiocytosis, dermatologic conditions, psoriasis, alopecia, nephrotic syndrome, nephritis, glomerular nephritis, acute renal failure, hemodialysis, uremia, toxicity, preeclampsia, okt3 therapy, anti-cd3 therapy, cytokine therapy, chemotherapy, radiation therapy (e.g., including but not limited to,

asthenia, anemia, cachexia, and the like), chronic salicylate intoxication, and the like. See, e.g., the Merck Manual, 12th-17th Editions, Merck & Company, Rahway, NJ (1972, 1977, 1982, 1987, 1992, 1999), Pharmacotherapy Handbook, Wells et al., eds., Second Edition, Appleton and Lange, Stamford, Conn. (1998, 2000), each entirely incorporated by reference.

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, at least one of cardiac stun syndrome, myocardial infarction, congestive heart failure, stroke, ischemic stroke, hemorrhage, acute coronary syndrome, arteriosclerosis, atherosclerosis, restenosis, diabetic ateriosclerotic disease, hypertension, arterial hypertension, renovascular hypertension, syncope, shock, syphilis of the cardiovascular system, heart failure, cor pulmonale, primary pulmonary hypertension, cardiac arrhythmias, atrial ectopic beats, atrial flutter, atrial fibrillation (sustained or paroxysmal), post perfusion syndrome, cardiopulmonary bypass inflammation response, chaotic or multifocal atrial tachycardia, regular narrow QRS tachycardia, specific arrythmias, ventricular fibrillation, His bundle arrythmias, atrioventricular block, bundle branch block, myocardial ischemic disorders, coronary artery disease, angina pectoris, myocardial infarction, cardiomyopathy, dilated congestive cardiomyopathy, restrictive cardiomyopathy, valvular heart diseases, endocarditis, pericardial disease, cardiac tumors, aordic and peripheral aneuryisms, aortic dissection, inflammation of the aorta, occlusion of the abdominal aorta and its branches, peripheral vascular disorders, occlusive arterial disorders, peripheral atherlosclerotic disease, thromboangitis obliterans, functional peripheral arterial disorders, Raynaud's phenomenon and disease, acrocyanosis, erythromelalgia, venous diseases, venous thrombosis, varicose veins, arteriovenous fistula, lymphederma, lipedema, unstable angina, reperfusion injury, post pump syndrome, ischemia-reperfusion injury, and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

The present invention also provides a method for modulating or treating at

least one antigen related infectious disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: acute or chronic bacterial infection, acute and chronic parasitic or infectious processes, including bacterial, viral and fungal infections, HIV infection/HIV neuropathy, meningitis, hepatitis (e.g., A, B or C, or the like), septic arthritis, peritonitis, pneumonia, epiglottitis, e. coli 0157:h7, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, malaria, dengue hemorrhagic fever, leishmaniasis, leprosy, toxic shock syndrome, streptococcal myositis, gas gangrene, mycobacterium tuberculosis, mycobacterium avium intracellulare, pneumocystis carinii pneumonia, pelvic inflammatory disease, orchitis/epidydimitis, legionella, lyme disease, influenza a, epstein-barr virus, viral-associated hemaphagocytic syndrome, viral encephalitis/aseptic meningitis, and the like.

The present invention also provides a method for modulating or treating at least one antigen related malignant disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), acute myelogenous leukemia, chromic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodyplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignamt lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, Kaposi's sarcoma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, solid tumors, bladder cancer, breast cancer, colorectal cancer, endometiral cancer, head cancer, neck cancer, hereditary nonpolyposis cancer, Hodgkin's lymphoma, liver cancer, lung cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, testicular cancer, adenocarcinomas, sarcomas, malignant melanoma, hemangioma, metastatic disease, cancer related bone resorption, cancer related bone pain, and the like.

The present invention also provides a method for modulating or treating at least one antigen related neurologic disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: neurodegenerative diseases, multiple

sclerosis, migraine headache, AIDS dementia complex, demyelinating diseases, such as multiple sclerosis and acute transverse myelitis; extrapyramidal and cerebellar disorders, such as lesions of the corticospinal system; disorders of the basal ganglia; hyperkinetic movement disorders, such as Huntington's Chorea and senile chorea; drug-induced movement disorders, such as those induced by drugs which block CNS dopamine receptors; hypokinetic movement disorders, such as Parkinson's disease; Progressive supranucleo Palsy; structural lesions of the cerebellum; spinocerebellar degenerations, such as spinal ataxia, Friedreich's ataxia, cerebellar cortical degenerations, multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and Machado-Joseph); systemic disorders (Refsum's disease, abetalipoprotemia, ataxia, telangiectasia, and mitochondrial multi-system disorder); demyelinating core disorders, such as multiple sclerosis, acute transverse myelitis; and disorders of the motor unit, such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); Alzheimer's disease; Down's Syndrome in middle age; Diffuse Lewy body disease; Senile Dementia of Lewy body type; Wernicke-Korsakoff syndrome; chronic alcoholism; Creutzfeldt-Jakob disease; Subacute sclerosing panencephalitis, Hallerrorden-Spatz disease; Dementia pugilistica; neurotraumatic injury (e.g., spinal cord injury, brain injury, concussion, repetitive concussion); pain; inflammatory pain; autism; depression; stroke; cognitive disorders; epilepsy; and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one TNF antibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. See, e.g., the Merck Manual, 16th Edition, Merck & Company, Rahway, NJ (1992).

The present invention also provides a method for modulating or treating at least one antigen related wound, trauma or tissue injury or related chronic condition, in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: bodily injury or a trauma associated with oral surgery including periodontal surgery, tooth extraction(s), endodontic treatment, insertion of tooth implants, application and use of tooth prosthesis; or wherein the wound is selected from the

group consisting of aseptic wounds, contused wounds, incised wounds, lacerated wounds, non-penetrating wounds, open wounds, penetrating wounds, perforating wounds, puncture wounds, septic wounds, infarctions and subcutaneous wounds; or wherein the wound is selected from the group consisting of ischemic ulcers, pressure sores, fistulae, severe bites, thermal burns and donor site wounds; or wherein the wound is an aphthous wound, a traumatic wound or a herpes associated wound.

Wounds and/or ulcers are normally found protruding from the skin or on a mucosal surface or as a result of an infarction in an organ ("stroke"). A wound may be a result of a soft tissue defect or a lesion or of an underlying condition. In the present context, the term "skin" relates to the outermost surface of the body of an animal, including a human, and embraces intact or almost intact skin as well as an injured skin surface. The term "mucosa" relates to undamaged or damaged mucosa of an animal, such as a human, and may be the oral, buccal, aural, nasal, lung, eye, gastrointestinal, vaginal, or rectal mucosa.

In the present context, the term "wound" denotes a bodily injury with disruption of the normal integrity of tissue structures. The term is also intended to encompass the terms "sore," "lesion," "necrosis," and "ulcer." Normally, the term "sore" is a popular term for almost any lesion of the skin or mucous membranes and the term "ulcer" is a local defect, or excavation, of the surface of an organ or tissue, which is produced by the sloughing of necrotic tissue. Lesion generally relates to any tissue defect. Necrosis is related to dead tissue resulting from infection, injury, inflammation or infarctions.

The term "wound" used in the present context denotes any wound (see below for a classification of wounds) and at any particular stage in the healing process, including the stage before any healing has initiated or even before a specific wound like a surgical incision is made (prophylactic treatment). Examples of wounds which can be prevented and/or treated in accordance with the present invention are, e.g., aseptic wounds, contused wounds, incised wounds, lacerated wounds, non-penetrating wounds (i.e., wounds in which there is no disruption of the skin but there is injury to underlying structures), open wounds, penetrating wounds, perforating wounds, puncture wounds, septic wounds, subcutaneous wounds, etc. Examples of sores are bed sores, canker sores, chrome sores, cold sores, pressure sores, etc.

Examples of ulcers are, e.g., a peptic ulcer, duodenal ulcer, gastric ulcer, gouty ulcer, diabetic ulcer, hypertensive ischemic ulcer, stasis ulcer, ulcus cruris (venous ulcer), sublingual ulcer, submucous ulcer, symptomatic ulcer, trophic ulcer, tropical ulcer, and veneral ulcer, e.g., caused by gonorrhoea (including urethritis, endocervicitis and proctitis). Conditions related to wounds or sores which may be successfully treated according to the invention are burns, anthrax, tetanus, gas gangrene, scarlatina, erysipelas, sycosis barbae, folliculitis, impetigo contagiosa, or impetigo bullosa, etc. There is often a certain overlap between the use of the terms "wound" and "ulcer" and "wound" and "sore" and, furthermore, the terms are often used at random. Therefore, as mentioned above, in the present context the term "wound" encompasses the terms "ulcer," "lesion," "sore" and "infarction," and the terms are indiscriminately used unless otherwise indicated.

The kinds of wounds to be treated according to the invention include also (i) general wounds, such as, e.g., surgical, traumatic, infectious, ischemic, thermal, chemical and bullous wounds; (ii) wounds specific for the oral cavity, such as, e.g., post-extraction wounds, endodontic wounds especially in connection with treatment of cysts and abscesses, ulcers and lesions of bacterial, viral or autoimmunological origin, mechanical, chemical, thermal, infectious and lichenoid wounds; herpes ulcers, stomatitis aphthosa, acute necrotising ulcerative gingivitis and burning mouth syndrome are specific examples; and (iii) wounds on the skin, such as, e.g., neoplasm, burns (e.g. chemical, thermal), lesions (bacterial, viral, autoimmunological), bites and surgical incisions. Another way of classifying wounds is as (i) small tissue loss due to surgical incisions, minor abrasions and minor bites, or as (ii) significant tissue loss. The latter group includes ischemic ulcers, pressure sores, fistulae, lacerations, severe bites, thermal burns and donor site wounds (in soft and hard tissues) and infarctions.

Other wounds that are of importance in connection with the present invention are wounds like ischemic ulcers, pressure sores, fistulae, severe bites, thermal burns and donor site wounds. Ischemic ulcers and pressure sores are wounds which normally only heal very slowly and especially in such cases, an improved and more rapid healing process is of course of great importance for the patient. Furthermore, the costs involved in the treatment of patients suffering from

such wounds are markedly reduced when the healing is improved and takes place more rapidly.

Donor site wounds are wounds which, e.g., occur in connection with removal of hard tissue from one part of the body to another part of the body, e.g., in connection with transplantation. The wounds resulting from such operations are very painful and an improved healing is therefore most valuable. The term "skin" is used in a very broad sense embracing the epidermal layer of the skin and — in those cases where the skin surface is more or less injured — also the dermal layer of the skin. Apart from the stratum corneum, the epidermal layer of the skin is the outer (epithelial) layer and the deeper connective tissue layer of the skin is called the dermis.

The present invention also provides a method for modulating or treating psoriasis, psoriatic arthritis, Crohn's disease, multiple sclerosis, and optic neuritis, among the other diseases listed above as antigen related, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of immune related disease, cardiovascular disease, infectious, malignant and/or neurologic disease. Such a method can optionally comprise administering an effective amount of at least one composition or pharmaceutical composition comprising at least one antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

Any method of the present invention can comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one 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 diseases or disorders, wherein the administering of said at least one antibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one TNF antagonist (e.g., but not limited to, a TNF chemical or protein antagonist, TNF monoclonal or polyclonal antibody or fragment, a soluble TNF receptor (e.g., p55, p70 or p85) or fragment, fusion polypeptides thereof, or a small molecule TNF antagonist, e.g., TNF binding protein I or II (TBP-1 or TBP-II), nerelimonmab, infliximab, etanercept (Enbrel™), adalimulab

(Humira™), CDP-571, CDP-870, afelimomab, lenercept, and the like), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalzine), 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., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a flurorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteriod, 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 antiulcer, a laxative, an anticoagulant, an erythropoietin (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 cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, 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. 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); Nursing 2001 Handbook of Drugs, 21st edition, Springhouse Corp., Springhouse, PA, 2001; Health Professional's Drug Guide 2001, ed., Shannon, Wilson, Stang, Prentice-Hall, Inc, Upper Saddle River, NJ.each of which references are entirely incorporated herein by reference.

Therapeutic Treatments

Any method of the present invention can comprise a method for treating an antigen mediated disorder, comprising administering an effective amount of a composition or pharmaceutical composition comprising at least one 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 diseases or disorders, wherein the administering of said at least one antibody, specified portion or variant thereof, further comprises administering before, concurrently, and/or after, at least one selected from an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug or the like, 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, sulfasalzine), 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., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a flurorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteriod, 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 antiulcer, a laxative, an anticoagulant, an erythropoietin (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 cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, 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. Such drugs are well known in the art, including formulations, indications, dosing and administration for each

presented herein (see, e.g., Nursing 2001 Handbook of Drugs, 21st edition, Springhouse Corp., Springhouse, PA, 2001; Health Professional's Drug Guide 2001, ed., Shannon, Wilson, Stang, Prentice-Hall, Inc, Upper Saddle River, NJ; Pharmcotherapy Handbook, Wells et al., ed., Appleton & Lange, Stamford, CT, each entirely incorporated herein by reference).

Typically, treatment of pathologic conditions is effected by administering an effective amount or dosage of at least one antibody composition that total, on average, a range from at least about 0.01 to 500 milligrams of at least one antibody per kilogram of patient per dose, and, preferably, from at least about 0.1 to 100 milligrams antibody/kilogram of patient per single or multiple administration, depending upon the specific activity of the active agent contained in the composition. Alternatively, the effective serum concentration can comprise 0.1-5000 □g/ml serum concentration per single or multiple adminstration. Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to provide for repeated administration, *i.e.*, repeated individual administrations of a particular monitored or metered dose, where the individual administrations are repeated until the desired daily dose or effect is achieved.

Preferred doses can optionally include about 0.1-99 and/or 100-500 mg/kg/administration, or any range, value or fraction thereof, or to achieve a serum concentration of about 0.1-5000 μg/ml serum concentration per single or multiple administration, or any range, value or fraction thereof. A preferred dosage range for the antibody of the present invention is from about 1 mg/kg, up to about 3, about 6 or about 12 mg/kg of body weight of the patient.

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 about 0.1 to 100 mg/kg or any range, value or fraction thereof per day, on at least one of day 1-40, or, alternatively or additionally, at least one of week 1-52, or, alternatively or additionally, at least one of 1-20 years, or any combination thereof, using single, infusion or repeated doses.

Dosage forms (composition) suitable for internal administration generally contain from about 0.001 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-99.999% by weight based on the total weight of the composition.

For parenteral administration, the antibody can be formulated as a solution, suspension, emulsion, particle, powder, 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 about 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.

Alternative Administration

Many known and developed modes can be used according to the present invention for administering pharmaceutically effective amounts of at least one 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. Antibodies of the present invention can be delivered in a carrier, as a solution, emulsion, colloid, or suspension, or as a dry powder, using any of a variety of devices and methods

suitable for administration by inhalation or other modes described here within or known in the art.

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, a sterile injectable solution or suspension in a solvent. As the usable 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 semisynthtetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needle-less 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.

Alternative Delivery

The invention further relates to the administration of at least one antibody by parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. At least one antibody composition can be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) or any other administration particularly in the form of liquid solutions or suspensions; for use in vaginal or

rectal administration particularly in semisolid forms, such as, but not limited to, creams and suppositories; for buccal, or sublingual administration, such as, but not limited to, in the form of tablets or capsules; or intranasally, such as, but not limited to, the form of powders, nasal drops or aerosols or certain agents; or transdermally, such as not limited to a gel, ointment, lotion, suspension or patch delivery system with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In "Drug Permeation Enhancement;" Hsieh, D. S., Eds., pp. 59-90 (Marcel Dekker, Inc. New York 1994, entirely incorporated herein by reference), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways, such as electroporation, or to increase the mobility of charged drugs through the skin, such as iontophoresis, or application of ultrasound, such as sonophoresis (U.S. Pat. Nos. 4,309,989 and 4,767,402) (the above publications and patents being entirely incorporated herein by reference).

Pulmonary/Nasal Administration

For pulmonary administration, preferably, at least one 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 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 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 Inhale, 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 antibody is delivered by a dry powder inhaler or a sprayer. There are 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 advantageously reliable, reproducible, and accurate. The inhalation device can optionally deliver small dry particles, e.g., less than about 10 μm, preferably about 1-5 μm, for good respirability.

Administration of Antibody Compositions as a Spray

A spray including antibody composition can be produced by forcing a suspension or solution of at least one antibody through a nozzle under pressure. 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 antibody composition delivered by a sprayer 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 antibody composition suitable for use with a sprayer typically include antibody composition in an aqueous solution at a concentration of about 0.1 mg to about 100 mg of at least one antibody composition per ml of solution or mg/gm, or any range, value, or fraction therein. 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, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating antibody compositions include albumin, protamine, or the like. Typical carbohydrates useful in formulating antibody compositions include sucrose, mannitol, lactose, trehalose, glucose, or the like. The antibody composition formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the antibody composition 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 antibodies, or specified portions or variants, can also be included in the formulation.

Administration of Antibody Compositions by a Nebulizer

Antibody compositions of the invention 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 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, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of antibody composition either directly or through a coupling fluid, creating an aerosol including the antibody composition. Advantageously, particles of antibody composition 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 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 antibody per ml of solution. 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 at least one antibody composition, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating antibody compositions include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one antibody include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one antibody formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one 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, and polyoxyethylene sorbital fatty acid esters. Amounts will generally range between about 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 Antibody Compositions by a Metered Dose Inhaler

In a metered dose inhaler (MDI), a propellant, at least one 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 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 antibody for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one 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 (hydrofluroalkane-134a), HFA-227 (hydrofluroalkane-227), or the like. Preferably, the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one 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 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 antibody composition via devices not described herein.

Oral Formulations and Administration

Formulations for oral administration rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants, such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene 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 (DFF) and trasylol) to inhibit enzymatic degradation. Formulations for delivery of hydrophilic agents including proteins and antibodies and a combination of at least two surfactants intended for oral, buccal, mucosal, nasal, pulmonary, vaginal transmembrane, or rectal administration are taught in U.S. 6,309,663. 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, .alpha.-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 and used to deliver biologically active agents orally are known in the art.

Mucosal Formulations and Administration

A formulation for orally administering a bioactive agent encapsulated in one or more biocompatible polymer or copolymer excipients, preferably, a biodegradable polymer or copolymer, affording microcapsules which due to the proper size of the resultant microcapsules results in the agent reaching and being taken up by the folliculi lymphatic aggregati, otherwise known as the "Peyer's patch," or "GALT" of the animal without loss of effectiveness due to the agent having passed through the gastrointestinal tract. Similar folliculi lymphatic aggregati can be found in the bronchei tubes (BALT) and the large intestine. The above-described tissues are referred to in general as mucosally associated lymphoreticular tissues (MALT). For absorption through mucosal surfaces, compositions and methods of administering at least 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 mucoadhesion of the emulsion particles (U.S. Pat. No. 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, pregelinatined starch, and the like (U.S. Pat. No. 5,849,695).

Transdermal Formulations and Administration

For transdermal administration, the at least one 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 polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers, such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U.S. Pat. No. 5,814,599).

Prolonged Administration and Formulations

It can be 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 encapsulation 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 liquid liposomes, are known in the literature (U.S. Pat. No. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

Having generally described the invention, the same will be more readily understood by reference to the following example, which is provided by way of illustration and is not intended as limiting.

EXAMPLE: Enhancement of Antigen-specific mAbs generation by anti-CD40 Treatment in BALB/c mice

BALB/c mice (8 to 12 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Antibodies were generated in two BALB/c mouse treatment groups against IL-23. Briefly, mice were immunized intraperitoneally with protein emulsified in Freund's adjuvant. Boost injections were given biweekly over the course of several weeks. Specific serum titer responses determined that each animal developed a measurable immune response to the cytokine antigen. Three days prior to lymphocyte harvest, mice in group A received a subcutaneous injection of 100 μg anti-murine CD40 monoclonal antibody (clone 1C10, Catalog No. MAB440, R&D Systems, Minneapolis, MN). Mice in group B did not receive any treatment.

The harvested lymphocytes were fused with murine myeloma cells and the fusions were screened by ELISA to assess the number of reactive hybrids. Table 1 shows that there is a significant increase (1321%, p=0.001) in the generation of reactive hybrids in Balb/c mice immunized with cytokine protein and primed with anti-CD40 agonist Mab when compared to the mice without anti-CD40 agonist mAb priming.

Table 1.

Group	Anti-CD40 Agonist Mab Boost	Reactive Hybrids Identified	Geometric Mean of Reactive Hybrids (n)
A	Yes	32	40.2 (n=10)
	Yes	41	
	Yes	47	
	Yes	36	
	Yes	49	
	Yes	48	
	Yes	8	
	Yes	143	
	Yes	68	
	Yes	27	
B	No	4	2.8 (n=2)
	No	2	

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

CLAIMS

1. A method for generating an antibody in a host animal comprising the steps of:

immunizing the host animal with a target antigen;

administering a B cell expansion agent to the host animal; and

isolating a target antigen-specific antibody.

2. The method of claim 1, wherein the host animal is a rodent.

3. The method of claim 2, wherein the rodent is a mouse.

4. The method of claim 3, wherein the mouse is a BALB/c mouse.

5. The method of claim 2, wherein the rodent is a rat.

6. The method of claim 1, wherein the target antigen is a T cell antigen or a T cell independent antigen.

7. The method of any one of claims 1-6, wherein the B cell expansion agent is at least one member selected from the group consisting of an anti-CD40 agonist, BAFF (BLyS), IL-6, APRIL, CD40L (CD154), and anti-IgM/IL4 co-stimulation.

8. The method of claim 7, wherein the B cell expansion agent is administered in the form of a protein, a DNA molecule encoding a protein, or a combination of protein and DNA molecule encoding a protein.

9. The method of claim 7, wherein the anti-CD40 agonist is an anti-CD40 antibody acting as an anti-CD40 agonist.

10. The method of claim 9, wherein the anti-CD40 antibody is a rat anti-mouse CD40 antibody.

11. The method of claim 9, wherein the anti-CD40 antibody is administered in an amount of about 50 μg to about 100 μg per dose.

12. The method of claim 7, further comprising administering the B cell expansion agent along with a second agent.

13. The method of claim 12, wherein the second agent is at least one of a targeting agent and a B cell differentiation agent.

14. The method of claim 13, wherein the targeting agent is CD21 and the B cell differentiation agent is at least one member selected from the group consisting of unfolded protein response (UPR) pathway components and B cell specific

transcription factors.

15. The method of claim 14, wherein the unfolded protein response (UPR) pathway components are selected from the group consisting of BiP, XBP, CHOP, IRE1, PERK, ATF4, ATF6, eIF2alpha, GRP78, GRP94, calreticulin, chaperones, and variants.

16. An antibody produced by any of the methods of claims 1-15.

17. The antibody of claim 16, wherein said antibody binds the target antigen with at least one affinity selected from at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M, and at least 10^{-12} M, at least 10^{-13} M, at least 10^{-14} M, and at least 10^{-15} M, as determined by surface plasmon resonance or the Kinexa method.

18. An antibody that competitively binds to the target antigen with the antibody according to claims 16 or 17.

19. An antibody according to any of claims 16-18, wherein said antibody substantially modulates an activity of the target antigen.

20. An antibody according to any of claims 16-19, wherein said antibody is an anti-IL-23 antibody.

21. The antibody of claim 20, wherein said antibody binds to the p19 subunit of the IL-23 protein.

22. A composition comprising an antibody according to any of claims 16-21 and at least one pharmaceutically acceptable carrier or diluent.

23. A composition according to claim 22, further comprising at least one compound or polypeptide selected from a detectable label or reporter, a TNF antagonist, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an opthalmic, otic or nasal drug, a topical drug, a nutritional drug, a cytokine, and a cytokine antagonist.

24. An anti-idiotype antibody or fragment that specifically binds an antibody according to any of claims 16-21.

25. A method for diagnosing or treating a disease or condition related to the target antigen in a cell, tissue, organ or animal, comprising contacting or

administering a composition comprising an effective amount of an antibody according to any of claims 16-21, with, or to, said cell, tissue, organ or animal.

26. The method according to claim 25, wherein said effective amount is about 0.001-50 mg/kilogram of said cells, tissue, organ or animal.

27. The method according to claim 25, wherein said contacting or said administering is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intraarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

28. The method according to claim 25, further comprising administering, prior, concurrently or after said contacting or administering, at least one composition comprising an effective amount of at least one compound or polypeptide selected from a detectable label or reporter, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug, a cytokine, and a cytokine antagonist.

29. A medical device, comprising an antibody according to any of claims 16-21, wherein said device is suitable for contacting or administering said antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

30. An article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container comprising a solution or a lyophilized form of an antibody according to any of claims 16-21.

31. The article of manufacture of claim 30, wherein said container is a component of a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery device or system.

32. A method for producing an antibody according to any of claims 16-21, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts said antibody.

33. A method for increasing a B-cell response of a host animal being immunized with a target antigen, comprising administering a B cell expansion agent to the host animal prior to, at the same time as, and/or subsequent to immunizing with the target antigen.

34. The method of claim 33, further comprising, after the administering step, isolating a target antigen-specific antibody.

35. The method of claim 33, wherein the number of B lymphocytes generated specific to the target antigen is increased.