

ANTIBODIES TO LYMPHOTOXIN-ALPHA

5 This non-provisional application claims the benefit of priority of US Provisional Application Ser. No. 60/829,257 filed on 12 October 2006, and of US Provisional Application Ser. No. 60/938, 999 filed on 18 May 2007, which are incorporated by reference
10 in their entirety.

FIELD OF THE INVENTION

The present invention concerns antibodies and their uses to treat autoimmune disorders. More particularly, the present invention concerns antibodies that bind to lymphotoxin- α and may also block binding of the lymphotoxin- α ligand to one or more
15 receptors.

BACKGROUND OF THE INVENTION

TNF and LT

Autoimmune diseases remain clinically important diseases in humans. As the name implies, autoimmune diseases wreak their havoc through the body's own immune system.
20 While the pathological mechanisms differ among individual types of autoimmune diseases, one general mechanism involves the binding of certain antibodies (referred to herein as self-reactive antibodies or autoantibodies) present. Physicians and scientists have identified more than 70 clinically distinct autoimmune diseases, including rheumatoid arthritis (RA), multiple sclerosis (MS), vasculitis, immune-mediated diabetes, and lupus such as systemic lupus
25 erythematosus (SLE). While many autoimmune diseases are rare – affecting fewer than 200,000 individuals – collectively, these diseases afflict millions of Americans, an estimated five percent of the population, with women disproportionately affected by most diseases. The chronic nature of these diseases leads to an immense social and financial burden.

Tumor Necrosis Factor (TNF)-related proteins are recognized in the art as a large
30 family of proteins having a variety of activities ranging from host defense to immune regulation to apoptosis. TNF was first identified as a serum-derived factor that was cytotoxic for several transformed cell lines *in vitro* and caused necrosis of certain tumors *in vivo*. A similar factor in the superfamily was identified and referred to as lymphotoxin ("LT"). Due to observed similarities between TNF and LT in the early 1980's, it was proposed that TNF
35 and LT be referred to as TNF- α and TNF- β , respectively. Scientific literature thus makes

reference to both nomenclatures. As used in the present application, the term "TNF" refers to TNF- α . Later research revealed two forms of lymphotoxin, referred to as LT α and LT β . US 2005-0129614 describes another polypeptide member of the TNF ligand super-family based on structural and biological similarities, designated TL-5.

5 Members of the TNF family of proteins exist in membrane-bound forms that act locally through cell-cell contact, or as secreted proteins. A family of TNF-related receptors react with these proteins and trigger a variety of signalling pathways including cell death or apoptosis, cell proliferation, tissue differentiation, and proinflammatory responses. TNF- α by itself has been implicated in inflammatory diseases, autoimmune diseases, viral, bacterial,
10 and parasitic infections, malignancies, and/or neurodegenerative diseases and is a useful target for specific biological therapy in diseases such as RA and Crohn's disease.

Cloning of the TNF and LT α proteins and further characterization of their respective biological activities reveal that the proteins differ in many aspects. Aggarwal *et al.*, *Cytokines and Lipocortins in Inflammation and Differentiation*, Wiley-Liss, Inc. 1990, pp.
15 375-384. For instance, LT α is a secreted, soluble protein of approximately 20 kDa (25 kDa if N- and O-glycosylated). TNF, in contrast, has no site for glycosylation and is synthesized with an apparent transmembrane domain that results in the original protein transcript being cell associated. Proteolysis of the cell-associated TNF protein results in the release of the soluble form of the protein having a molecular weight of approximately 19 kDa. TNF is
20 produced primarily by activated macrophages, whereas LT is produced by activated lymphocytes. Wong *et al.*, *Tumor Necrosis Factors: The Molecules and their Emerging Role in Medicine*, Beutler, B., ed., Raven Press (1991), pp. 473-484. The sequences encoding TNF and LT α also differ. TNF and LT α share only approximately 32% amino acid sequence identity. Regarding the different biological activities of TNF and LT α , TNF increases
25 production of endothelial-cell interleukin-1 ("IL-1"), whereas LT α has little effect thereon. Further, TNF induces production of macrophage-colony-stimulating factor from macrophages, whereas LT α has no effect thereon. These and other biological activities are discussed in Aggarwal, *Tumor Necrosis Factors: Structure, Function and Mechanism of Action*, Aggarwal and Viced, eds. (1992), pp. 61-78.

30 TNF and LT α are described further in the review articles by Spriggs, "Tumor Necrosis Factor: Basic Principles and Preclinical Studies," *Biologic Therapy of Cancer*, DeVita *et al.*, eds., J.B. Lippincott Company (1991) Ch. 16, pp. 354-377; Ruddle, *Current Opinion in Immunology*, 4:327-332 (1992); Wong *et al.*, "Tumor Necrosis Factor," *Human*

Monocytes, Academic Press (1989), pp. 195-215; and Paul *et al.*, *Ann. Rev. Immunol.*, 6:407-438 (1988).

In non-tumor cells, TNF and TNF-related cytokines are active in a variety of immune responses. Both TNF and LT α ligands bind to and activate TNF receptors (p55 or p60 and p75 or p80; herein called "TNF-R").

Cell-surface LT complexes have been characterized in CD4⁺ T cell hybridoma cells (II-23.D7), which express high levels of LT (Browning *et al.*, *J. Immunol.*, 147: 1230-1237 (1991); Androlewicz *et al.*, *J. Biol. Chem.*, 267: 2542-2547 (1992)). The expression and biological roles of LT β -R, LT subunits, and surface LT complexes are reviewed in Ware *et al.*, "The ligands and receptors of the lymphotoxin system", in *Pathways for Cytolysis, Current Topics Microbiol. Immunol.*, Springer-Verlag, pp. 175-218 (1995).

LT α expression is induced and LT α secreted primarily by activated T and B lymphocytes and natural killer (NK) cells. Among the T helper cell subclasses, LT α appears to be produced by Th1 but not Th2 cells. LT α has also been detected in melanocytes. LT β (also called p33) has been identified on the surface of T lymphocytes, T cell lines, B cell lines and lymphokine-activated killer (LAK) cells. Studies have shown that LT β is not functional in the absence of LT α .

LT α exists either as a homotrimer (LT α 3) or a heterotrimer with LT β . These heterotrimers contain either two subunits of LT α and one subunit of LT β (LT α 2 β 1), or one subunit of LT α and two of LT β (LT α 1 β 2).

The only known cell-surface receptors for the LT α homotrimer are the two TNF receptors, p55 and p75. However, the LT α β heterotrimer, LT α 1 β 2, does not bind to the TNF receptors and instead binds to a member of the TNF receptor superfamily, lymphotoxin β receptor (referred to herein as LT β -R). The heterotrimeric form LT α 2 β 1 likely binds TNF receptors.

LT β -R has a well-described role both in the development of the immune system and in the functional maintenance of a number of cells in the immune system, including follicular dendritic cells and a number of stromal cell types (Matsumoto *et al.*, *Immunol. Rev.* 156:137 (1997)). Known ligands to the LT β -R include not only LT α 1 β 2, but also a second ligand called LIGHT (Mauri *et al.*, *Immunity* 8:21 (1998)). Activation of LT β -R has been shown to induce the apoptotic death of certain cancer cell lines *in vivo* (US 6,312,691). Humanized antibodies to LT β -R and methods of use thereof are provided in US 2004-0058394 and stated as being useful for treating or reducing the advancement, severity, or effects of neoplasia in

humans. Further, EP 1585547 (WO 2004/058183) (LePage and Gill) discloses combination therapies that include a composition that activates LT β -R signaling in combination with one or more other chemotherapeutic agents, as well as therapeutic methods and screening methods for identifying agents that in combination with a LT β -R agonist agent have an additive effect on tumor inhibition.

LT is important for lymphoneogenesis, as evident from knockout mice. See Futterer *et al. Immunity*, 9 (1): 59-70 (1998), showing that mice deficient in LT β -R lacked lymph nodes and Peyer's patches and also showing impaired antibody affinity maturation. Rennert *et al., Immunity*, 9 (1): 71-9 (1998) reported that an agonist monoclonal antibody against LT β -R restored the ability to make lymph nodes in LT α knockout mice. See also Wu *et al., J. Immunology*, 166 (3): 1684-9 (2001) and Endres *et al., J. Exp. Med.*, 189 (1): 159-68 (1999); Dohi *et al., J. Immunology*, 167 (5): 2781-90 (2001); and Matsumoto *et al., J. Immunology*, 163 (3): 1584-91 (1999). Korner *et al. Eur. J. Immun.*, 27 (10): 2600-9 (1997) reported that mice lacking both TNF and LT showed retarded B-cell maturation and serum immunoglobulin deficiencies, whereas mice lacking only TNF showed no such deficiencies.

In addition, LT is important for inflammation. LT α is overexpressed in the pancreas of RIP.LT α transgenic mice, which have shown inflammation, increased chemokine expression, and a lymphoid-like structure, and in which overexpression of LT β alone has demonstrated no additional inflammation. Further, LT α -deficient mice exhibit impaired TNF- α production, and defective splenic architecture and function are restored when such mice are crossed to TNF-transgene (Kollias, *J. Exp. Med.*, 188:745 (1998); Chaplin, *Ann Rev Imm* 17:399 (1999)), and decreased TNF levels are restored after pathogenic challenge (Eugster, *Eur. J.Immun.* 31:1935 (2001)).

When TNF- α or LT α_3 interacts with the TNF receptors TNFRI and/or TNFRII, the result is proinflammatory responses and/or apoptosis. When LT $\alpha_1\beta_2$ interacts with the receptor LT β -R, the result is lymphoneogenesis and induction of chemokines and adhesion molecules. Autoimmune diseases are associated with lymphoneogenesis and inflammatory responses, and there is increased LT expression in patients with autoimmune disease, including MS, inflammatory bowel disease (IBD), and RA (Weyand *et al., Curr. Opin. Rheumatol.*, 15: 259-266 (2003); Selmaj *et al., J. Clin.Invest.*, 87: 949-954 (1991); Matusiewicz *et al., J. Neuroimm.*, 66: 115-123 (1996); Powell *et al., International Immunology*, 2 (6): 539-44 (1990); Zipp *et al., Annals of Neurology*, 38/5: 723-730 (1995); Voskuhl *et al., Autoimmunity* 15 (2): 137-43 (1993); Selmaj *et al., J. Immunology*, 147: 1522-

29 (1991); Agyekum *et al.*, *Journal Pathology*, 199 (1): 115-21 (2003); and Takemura *et al.*, *J. Immunol.*, 167: 1072 (2001)).

As to MS specifically, serum LT α correlates with lesions/disease burden in MS (Kraus *et al.*, *Acta Neurologica Scandinavica*, 105 (4): 300-8 (2002)). LT α is involved in
5 demyelination but not remyelination in an *in vivo* cuprizone model, whereas TNF- α is required for both (Plant *et al.*, *Glia* 49:1-14 (2005)).

As to RA specifically, levels of human LT α 3 and TNF- α in RA patients are elevated over those of normal donors (Stepien, *Eur Cytokine Net* 9: 145 (1998)). The roles of LT α in
10 RA include: serum LT α is present in some RA patients, increased LT α protein is present in synovium, the LT pathway is associated with ectopic lymphoneogenesis in synovium, and there is increased LT β -R expression on fibroblast-like synoviocytes in RA patients. In addition, a case report discloses that neutralizing LT α 3 is beneficial for an infliximab-resistant RA patient (Buch *et al.*, *Ann. Rheum.Dis.*, 63: 1344-46 (2004)). Also, Han *et al.*, *Arthrit. Rheumat.*, 52: 3202-3209 (2005) describes that blockading the LT pathway
15 exacerbates autoimmune arthritis by enhancing the Th1 response.

Preclinical efficacy for prevention and treatment with LT β R-Ig in collagen-induced arthritis (CIA) is shown in Fava *et al.*, *J. Immunology*, 171 (1): 115-26 (2003). Further, LT α -deficient mice are resistant to experimental autoimmune encephalomyelitis (EAE) (Suen *et al.*, *J. Exp. Med.*, 186: 1233-40 (1997); Sean Riminton *et al.*, *J. Exp. Med.*, 187 (9): 1517-28
20 (1998)). There is also published efficacy of LT β R-Ig in EAE (Gommerman *et al.*, *J. Clin. Invest.*, 112 (5): 755-67 (2003)). Also, LT β R-Ig disrupts lymphogenesis in mice. Mackay *et al.*, *Europ. J. Immunol.*, 27 (8): 2033-42 (1997)). Further, administration of LT β R-Ig decreases insulin-dependent diabetes mellitus (IDDM) in non-obese diabetic mice (Wu *et al.*, *J. Exp. Med.*, 193 (11): 1327-32 (2001)). The role of LT in lymphogenesis in
25 non-human primates was investigated by Gommerman *et al.*, *J. Clin. Invest.*, 110 (9): 1359-69 (2002) using LT β R-Ig. Further, LT α -deficient mice are less susceptible to *M. bovis* BCG than TNF- α -deficient mice. Eugster *et al.*, *Europ. J. Immunol.*, 31: 1935 (2001).

LT has also been used to treat cancer. See US 5,747,023.

Antibodies

30 Antibodies are proteins that exhibit binding specificity to a specific antigen. Native antibodies are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages

varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are responsible for the binding specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of the variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β -sheet structure. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)).

The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies or immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.* IgG1, IgG2, IgG3, and IgG4; IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. Of the human immunoglobulin classes, only human IgG1, IgG2, IgG3, and IgM are known to activate complement, and human IgG1 and IgG3 mediate antibody-dependent cell-mediated cytotoxicity (ADCC) more effectively than IgG2 and IgG4.

Two main approaches have been used to develop therapeutic compounds that inhibit TNF- α . The first, exemplified by infliximab, a chimeric monoclonal antibody to TNF- α also

known as REMICADE[®], is to neutralize TNF- α action by using a specific monoclonal antibody of high affinity and potency to prevent binding of TNF- α to its receptors. The second, exemplified by etanercept, also known as ENBREL[®], is to inhibit TNF- α by using a TNF receptor-based molecule that functions as a "decoy" to reduce the binding of TNF- α to its natural receptors. Although both types of molecules can prevent the binding of TNF α to its receptors, receptor-based inhibitors such as etanercept will also prevent receptor binding of LT α .

In the patent literature, antibodies to cachectin (TNF), disclosed by EP 212489, are reported as useful in diagnostic immunoassays and in therapy of shock in bacterial infections. EP 218868 discloses monoclonal antibodies to human TNF and their uses. EP 288,088 discloses anti-TNF antibodies, and their utility in immunoassay diagnosis of pathologies, in particular Kawasaki's pathology and bacterial infection. Anti-TNF antibodies are also described, for example, in EP 1585477 (Giles-Komar *et al.*); US 2006-0147452; US 2006-0222646; and US 2006-0121037. A method of treating RA by using a single-domain antibody polypeptide construct that antagonizes human TNF α 's binding to a receptor is described in US 2005-0271663. Methods of treating RA using anti-TNF receptor fusion proteins are described in US 2005-0260201. Methods for treating a TNF-mediated disease using a composition comprising methotrexate and an anti-TNF antibody, including RA, Crohn's disease, and acute and chronic immune diseases associated with transplantation, are described in EP 1593393. Antibodies that bind to TNF-R include those described in US 7,057,022. Novel proteins with TNF-alpha antagonist activity are described in US 7,056,695. US 2006-0147448 discloses treatment of immunological renal disorders by LT pathway inhibitors.

LT β and LT β /LT α complexes and LT β -R and antibodies thereto as well as LT β blocking agents are described, for example, in WO 1992/00329; WO 1994/13808; US 5,661,004; US 5,795,964; EP 0954333; US 5,670,149; US 5,925,351; US 2005-0037003; US 6,403,087; US 6,669,941; US 6,312,691; US 7,001,598; US 7,030,080; US 2006-0222644; and US 2005-0163747. The application US 2006-0134102 discloses LT β -R agents in combination with chemotherapeutic agents. Antibodies to the LT β -R are described in US 2005-0281811. See also WO 2006/114284 regarding use of LT β -R antibodies to prevent and/or treat obesity and obesity-related disorders. Humanized antibodies specific for the LT β -R alone or in combination with chemotherapeutic agent(s) in therapeutic methods are disclosed in EP 1539793. LT α and antibodies thereto are described, for example, in US

4,959,457; 5,824,509; 4,920,196; and 5,683,688; US 2005-0266004 (WO 2005/067477) and US 5,188,969 (EP 347728B1). See also US 2002-0039580; WO 2004/039329; and US 2002-0197254 regarding LT β or LT β -R technology. Multivalent antibodies (that bind the TNF receptor superfamily) are described, for example, in US 2002-0004587 and US 2006-0025576.

There is a continuing need in the art to produce antibodies, in particular, therapeutic antibodies having improved function, such as anti-LT α antibodies or fragments thereof that block LT α , since LT α has more interactions with the various receptors than TNF- α or LT β alone.

SUMMARY

The disclosure is in part based on the identification of a variety of antagonists of the LT biological pathway, which is a biological/cellular process presenting as an important therapeutic target. The disclosure provides compositions and methods based on interfering with LT α activation, including but not limited to interfering with LT α binding to various receptors.

Accordingly, the disclosure is as claimed.

In one aspect, there is provided an isolated anti-lymphotoxin- α (LT α) antibody wherein the antibody comprises at least one complementarity-determining region (CDR) sequence selected from the group consisting of:

- (a) a CDR-L1 sequence comprising amino acids A1-A11, wherein A1-A11 is KASQAVSSAVA (SEQ ID NO:1) or RASQAVSSAVA (SEQ ID NO:2), or comprising amino acids A1-A17, wherein A1-A17 is KSSQSLLYSTXQKXFLA (SEQ ID NO:3) or KSSQSLLYSAXQKXFLA (SEQ ID NO:4) or KSSQSLLYSTXQKXALA (SEQ ID NO:6), where X is any amino acid;
- (b) a CDR-L2 sequence comprising amino acids B1-B7, wherein B1-B7 is SASHRYT (SEQ ID NO:7) or WASTRDS (SEQ ID NO:8);
- (c) a CDR-L3 sequence comprising amino acids C1-C9, wherein C1-C9 is QQHYSTPWT (SEQ ID NO:9) or QEXYSTPWT (SEQ ID NO:11) or QQYYSYPRT (SEQ ID NO:13) or QQYASYPRT (SEQ ID NO:14) or QQYYAYPRT (SEQ ID NO:15), where X is any amino acid;
- (d) a CDR-H1 sequence comprising amino acids D1-D10, wherein D1-D10 is GYTFTSYVIH (SEQ ID NO:16) or GYTFSSYWIE (SEQ ID NO:17);

(e) a CDR-H2 sequence comprising amino acids E1-E17, wherein E1-E17 is YXXPYXDGTXYXEKFKG (SEQ ID NO:18) or EISPGSGSTXYXEEFKG (SEQ ID NO:19) or YXXPYXAGTXYXEKFKG (SEQ ID NO:101) or EIXPGSGSTTYXEKFKG (SEQ ID NO:110), wherein X is any amino acid; and

(f) a CDR-H3 sequence comprising amino acids F1-F9, wherein F1-F9 is PTMLPWFAY (SEQ ID NO:20), or comprising amino acids F1-F5, wherein F1-F5 is GYHGY (SEQ ID NO:21) or GYHGA (SEQ ID NO:22);

wherein the antibody:

(i) binds to lymphotoxin- α_3 trimer (LT α_3) and blocks the interaction of LT α_3 with tumor necrosis factor receptor-I (TNFRI) and tumor necrosis factor receptor-II (TNFRII); and

(ii) blocks the interaction of LT $\alpha\beta$ with lymphotoxin-beta receptor (LT β -R).

In one embodiment, the disclosure provides an isolated anti-lymphotoxin- α (LT α) antibody comprising at least one complementarity-determining region (CDR) sequence selected from the group consisting of:

(a) a CDR-L1 sequence comprising amino acids A1-A11, wherein A1-A11 is KASQAVSSAVA (SEQ ID NO:1) or RASQAVSSAVA (SEQ ID NO:2), or comprising amino acids A1-A17, wherein A1-A17 is KSSQSLLYSTXQKXFLA (SEQ ID NO:3) or KSSQSLLYSAXQKXFLA (SEQ ID NO:4) or KSSQSLLYSTXQKXALA (SEQ ID NO:6), where X is any amino acid (chimeric 2C8 or humanized 2C8.v2/2C8.vX or chimeric 3F12/humanized 3F12.v5/humanized 3F12.v14, or 3F12 clone 14 or 17, respectively);

(b) a CDR-L2 sequence comprising amino acids B1-B7, wherein B1-B7 is SASHRYT (SEQ ID NO:7) or WASTRDS (SEQ ID NO:8) (chimeric 2C8/humanized 2C8.v2/humanized 2C8.vX or chimeric 3F12/humanized 3F12.v5/ humanized 3F12.v14, respectively);

(c) a CDR-L3 sequence comprising amino acids C1-C9, wherein C1-C9 is QQHYSTPWT (SEQ ID NO:9) or QEXYSTPWT (SEQ ID NO:11) or QQYYSYPRT (SEQ ID NO:13) or QQYASYPRT (SEQ ID NO:14) or QQYYAYPRT (SEQ ID NO:15), where X is any amino acid (chimeric 2C8/humanized 2C8.v2 or 2C8 clone G7 or chimeric

3F12/humanized 3F12.v5/ humanized 3F12.v14 or 3F12 clone 20 or 3F12 clone 21, respectively);

(d) a CDR-H1 sequence comprising amino acids D1-D10, wherein D1-D10 is GYTFTSYVIH (SEQ ID NO:16) or GYTFSSYWIE (SEQ ID NO:17) (chimeric 2C8/humanized 2C8.v2/humanized 2C8.vX or chimeric 3F12/humanized 3F12.v5/ humanized 3F12.v14, respectively);

(e) a CDR-H2 sequence comprising amino acids E1-E17, wherein E1-E17 is YXXPYXDGTXYXEKFKG (SEQ ID NO:18) or EISPGSGSTXYXEEFKG (SEQ ID NO:19) or YXXPYXAGTXYXEKFKG (SEQ ID NO:101) or EIXPGSGSTIYXEKFKG (SEQ ID NO:110), wherein X is any amino acid (chimeric 2C8/humanized 2C8.v2 or chimeric 3F12/humanized 3F12.v5, or humanized 2C8.vX, or humanized 3F12.v14, respectively); and

(f) a CDR-H3 sequence comprising amino acids F1-F9, wherein F1-F9 is PTMLPWFAY (SEQ ID NO:20), or comprising amino acids F1-F5, wherein F1-F5 is GYHGY (SEQ ID NO:21) or GYHGA (SEQ ID NO:22) (chimeric 2C8/humanized 2C8.v2/humanized 2C8.vX or chimeric 3F12/humanized 3F12.v5/ humanized 3F12.v14 or 3F12 clone 12, respectively).

Preferably, SEQ ID NO:3 is KSSQSLLYSTAQKXFLA (SEQ ID NO:5) (3F12 clone 15). In another preferred embodiment, SEQ ID NO:11 is QESYSTPWT (SEQ ID NO:10) (2C8 clone A8/humanized 2C8.vX) or QEVYSTPWT (SEQ ID NO:12) (2C8 clone H6).

In a further preferred embodiment, the CDR-L1 sequence is SEQ ID NO:2 or 3 or 4 or 6.

In another preferred embodiment, the antibody comprises either (i) all of the CDR-L1 to CDR-L3 amino acid sequences of SEQ ID NOS:1 or 2 and 7 and 9, or of SEQ ID NOS:1 or 2 and 7 or 8 and 11, or of SEQ ID NOS:3, 8, and 13, or of SEQ ID NOS:4, 5, or 6, 8, and 13, or of SEQ ID NOS:3, 8, and 14 or 15, or of SEQ ID NOS:4, 5, or 6, 8, and 14 or 15; or (ii) all of the CDR-H1 to CDR-H3 amino acid sequences of SEQ ID NOS:16, 18, and 20, or all of SEQ ID NOS:16, 101, and 20, or all of SEQ ID NOS:17, 19, and 21 or 22, or all of SEQ ID NOS:17, 110, and 21.

In a still further preferred embodiment, the antibody comprises either all of SEQ ID NOS:1 or 2 and 7 and 9, or all of SEQ ID NOS:16, 18, and 20, or all of SEQ ID NOS:16, 101, and 20. In an alternative embodiment, the antibody comprises either all of SEQ ID NOS:3, 8, and 13, or all of SEQ ID NOS:17, 19, and 21 or 22. In an alternative embodiment, the antibody

comprises either all of SEQ ID NOS:4, 8, and 14, or all of SEQ ID NOS:17, 110, and 21. In other embodiments, the antibody comprises (i) all of the CDR-L1 to CDR-L3 amino acid sequences of SEQ ID NOS:1 or 2, 7 and 9, or of SEQ ID NOS:1 or 2 and 7 or 8 and 11, or of SEQ ID NOS:3, 8, and 13, or of SEQ ID NOS:4, 5, or 6, 8, and 13, or of SEQ ID NOS:3, 8, and 14 or 15, or of SEQ ID NOS:4, 5, or 6, 8, and 14 or 15; and (ii) all of the CDR-H1 to CDR-H3 amino acid sequences of SEQ ID NOS:16, 18, and 20, or of SEQ ID NOS:16, 101, and 20, or of SEQ ID NOS:17, 19, and 21 or 22, or of SEQ ID NOS:17, 110, and 21.

In addition, the antibodies herein are preferably chimeric or humanized, most preferably humanized. A humanized antibody of the disclosure may comprise one or more suitable human and/or human consensus non-CDR (e.g., framework) sequences in its heavy- and/or light-chain variable domains, provided the antibody exhibits the desired biological characteristics (e.g., a desired binding affinity).. Preferably, at least a portion of such humanized antibody framework sequence is a human consensus framework sequence.

In some embodiments, one or more additional modifications are present within the human and/or human consensus non-CDR sequences. In one embodiment, the heavy-chain variable domain of an antibody of the disclosure comprises at least a portion of (preferably all of) a human consensus framework sequence, which in one embodiment is the subgroup III consensus framework sequence. In one embodiment, an antibody of the disclosure comprises a variant subgroup III consensus framework sequence modified at at least one amino acid position. For example, in one embodiment, a variant subgroup III consensus framework sequence may comprise a substitution at one or more of positions 71, 73, and/or 78. In one embodiment, said substitution is R71A, N73T, and/or N78A, in any combination thereof, preferably all three. In another embodiment, these antibodies comprise or further comprise at least a portion of (preferably all of) a human κ light-chain consensus framework sequence. In a preferred embodiment, an antibody of the disclosure comprises at least a portion of (preferably all of) a human κ subgroup I framework consensus sequence.

The amino acid position/boundary delineating a CDR of an antibody can vary, depending on the context and the various definitions known in the art (as described below). Some positions within a variable domain may be viewed as hybrid hypervariable positions in that these positions can be deemed to be within a CDR under one set of criteria while being deemed to be outside a CDR under a different set of criteria. One or more of these positions can also be found in extended CDRs (as further defined below). The disclosure provides antibodies comprising modifications in these hybrid hypervariable positions. In one

embodiment, these hybrid hypervariable positions include one or more of positions 26-30, 33-35B, 47-49, 57-65, 93, 94, and 102 in a heavy-chain variable domain. In one embodiment, these hybrid hypervariable positions include one or more of positions 24-29, 35-36, 46-49, 56, and 97 in a light-chain variable domain. In one embodiment, an antibody of the disclosure comprises a variant human subgroup consensus framework sequence modified at one or more hybrid hypervariable positions.

In another preferred embodiment, the antibody binds to LT α 3 and blocks the interaction of LT α 3 with tumor necrosis factor receptor-1 (TNFR1) and tumor necrosis factor receptor-2 (TNFR2). Preferably, it binds also to one or more LT $\alpha\beta$ complexes and especially on the cell surface. Preferably, it also blocks the function of one or more LT $\alpha\beta$ complexes. Also, it preferably decreases levels of inflammatory cytokines associated with rheumatoid arthritis in an *in vitro* arthritis assay such as an *in vitro* collagen-induced arthritis assay or an *in vitro* antibody-induced arthritis assay.

In some embodiments, such as the murine S5H3 antibody, the antibody does not block the interaction of LT $\alpha\beta$ with LT β -R. In other embodiments, the antibody does block the interaction of LT $\alpha\beta$ with LT β -R. Preferably, the antibody modulates LT $\alpha\beta$ -expressing cells. In other embodiments, the anti-LT α antibody substantially neutralizes at least one activity of at least one LT α protein. Preferably, the antibody herein targets any cell expressing LT α , and more preferably depletes LT α -positive or -secreting cells.

The preferred antibody binds LT α with an affinity of at least about 10^{-12} M (picomolar levels), and more preferably at least about 10^{-13} M. Also preferred is an IgG antibody, more preferably human IgG. Human IgG encompasses any of the human IgG isotypes of IgG1, IgG2, IgG3, and IgG4. Murine IgG encompasses the isotypes of IgG1, 2a, 2b, and 3. More preferably, the murine IgG is IgG2a and the human IgG is IgG1. In other preferred embodiments of the human IgG, the VH and VL sequences provided are joined to human IgG1 constant region.

In another embodiment, the disclosure provides an anti-LT α antibody having a light-chain variable domain comprising SEQ ID NO:23 or 24, or a heavy-chain variable domain comprising SEQ ID NO:25 or 26, or having light-chain and heavy-chain variable domains comprising both SEQ ID NOS:23 and 25, or comprising both SEQ ID NOS:24 and 26.

In a still further embodiment, the disclosure provides an anti-LT α antibody having a light-chain variable domain comprising SEQ ID NO:27 or 28, or a heavy-chain variable domain comprising SEQ ID NO:29 or 30 or 31, or having light-chain and heavy-chain

variable domains comprising both SEQ ID NOS:27 and 29, or comprising both SEQ ID NOS:27 and 30, or comprising both SEQ ID NOS:27 and 31, or comprising both SEQ ID NOS:28 and 30, or comprising both SEQ ID NOS:28 and 31.

In a still further embodiment, the disclosure provides an anti-LT α antibody having a light-chain variable domain comprising SEQ ID NO:102, or a heavy-chain variable domain comprising SEQ ID NO:103, or having light-chain and heavy-chain variable domains comprising both SEQ ID NOS:102 and 103.

In a still further embodiment, the disclosure provides an anti-LT α antibody having a light-chain variable domain comprising SEQ ID NO:108, or a heavy-chain variable domain comprising SEQ ID NO:109, or having light-chain and heavy-chain variable domains comprising both SEQ ID NOS:108 and 109.

In other preferred embodiments, the antibody has an Fc region. In one embodiment, such Fc region is a wild-type (or native-sequence) Fc region. In another embodiment, the antibody further comprises one or more amino acid substitutions in its Fc region that result in the polypeptide exhibiting at least one of the following properties: increased Fc γ R binding, increased antibody-dependent cell-mediated cytotoxicity (ADCC), increased complement-dependent cytotoxicity (CDC), decreased CDC, increased ADCC and CDC function, increased ADCC but decreased CDC function, increased FcRn binding, and increased serum half life, as compared to an antibody having a native-sequence Fc region. More preferably, the antibody further comprises one or more amino acid substitutions in its Fc region that result in it having increased ADCC function as compared to the same antibody having a native-sequence Fc region.

In a particularly preferred embodiment, the antibody has amino acid substitutions in its Fc region at any one or any combination of positions that are 268D, or 298A, or 326D, or 333A, or 334A, or 298A together with 333A, or 298A together with 334A, or 239D together with 332E, or 239D together with 298A and 332E, or 239D together with 268D and 298A and 332E, or 239D together with 268D and 298A and 326A and 332A, or 239D together with 268D and 298A and 326A and 332E, or 239D together with 268D and 283L and 298A and 332E, or 239D together with 268D and 283L and 298A and 326A and 332E, or 239D together with 330L and 332E and 272Y and 254T and 256E, or 250Q together with 428L, or 265A, or 297A, wherein the 265A substitution is in the absence of 297A and the 297A substitution is in the absence of 265A. In one particular embodiment, the Fc region has from one to three such amino acid substitutions, for example, substitutions at positions 298, 333,

and 334, and more preferably the combination of 298A, 333A, and 334A. The letter after the number in each of these designations represents the changed amino acid at that position.

Such anti-LT α antibodies effect varying degrees of disruption of the LT α /LT β signaling pathway. For example, in one embodiment, the disclosure provides an anti-LT α antibody (preferably humanized) wherein the monovalent affinity of the antibody to human LT α (e.g., affinity of the antibody as a Fab fragment to human LT α) is about the same as or greater than that of a murine antibody (e.g., affinity of the murine antibody as a Fab fragment to human LT α) produced by a hybridoma cell line deposited under American Type Culture Collection Accession Number (ATCC) PTA-7538 (hybridoma murine Lymphotoxin alpha2 beta1 s5H3.2.2). The monovalent affinity is preferably expressed as a K_d value and/or is measured by optical biosensor that uses surface plasmon resonance (SPR) (BIAcore® technology) or radioimmunoassay.

Further antibodies herein include those with any of the properties above having reduced fucose relative to the amount of fucose on the same antibody produced in a wild-type Chinese hamster ovary cell. More preferred are those antibodies having no fucose.

In one aspect, there is provided an antibody composition comprising the antibodies described herein having an Fc region, wherein about 20-100% of the antibodies in the composition comprise a mature core carbohydrate structure in the Fc region that lacks a fucose. Preferably, such composition comprises antibodies having an Fc region that has been altered to change one or more of the ADCC, CDC, or pharmacokinetic properties of the antibody compared to a wild-type IgG Fc sequence by substituting an amino acid selected from the group consisting of A, D, E, L, Q, T, and Y at any one or any combination of positions of the Fc region selected from the group consisting of: 238, 239, 246, 248, 249, 250, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 297, 298, 301, 303, 305, 307, 309, 312, 314, 315, 320, 322, 324, 326, 327, 329, 330, 331, 332, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 428, 430, 434, 435, 437, 438 and 439.

The above-described antibody composition is more preferably one wherein the antibody further comprises an Fc substitution that is 268D or 326D or 333A together with 334A, or 298A together with 333A, or 298A together with 334A, or 239D together with 332E, or 239D together with 298A and 332E, or 239D together with 268D and 298A and 332E, or 239D together with 268D and 298A and 326A and 332A, or 239D together with 268D and 298A and 326A and 332E, or 239D together with 268D and 283L and 298A and

332E, or 239D together with 268D and 283L and 298A and 326A and 332E, or 239D together with 330L and 332E, wherein the letter after the number in each of these designations represents the changed amino acid at that position.

The above-described antibody composition is additionally preferably one wherein the antibody binds an FcγRIII. The composition is preferably one wherein the antibody has ADCC activity in the presence of human effector cells or has increased ADCC activity in the presence of human effector cells compared to the otherwise same antibody comprising a human wild-type IgG1Fc. The composition is also preferably one wherein the antibody binds the FcγRIII with better affinity, or mediates ADCC more effectively, than a glycoprotein with a mature core carbohydrate structure including fucose attached to the Fc region of the glycoprotein. In addition, the composition is preferably one wherein the antibody has been produced by a Chinese hamster ovary (CHO) cell, preferably a Lec13 cell. The composition is also preferably one wherein the antibody has been produced by a mammalian cell lacking a fucosyltransferase gene, more preferably the *FUT8* gene.

In one embodiment, the above-described composition is one wherein the antibody is free of bisecting N-acetylglucosamine (GlcNAc) attached to the mature core carbohydrate structure. In an alternative embodiment, the composition is one wherein the antibody has bisecting GlcNAc attached to the mature core carbohydrate structure.

In another embodiment, the above-described composition is one wherein the antibody has one or more galactose residues attached to the mature core carbohydrate structure. In an alternative embodiment, the composition is one wherein the antibody is free of one or more galactose residues attached to the mature core carbohydrate structure.

In a further embodiment, the above-described composition is one wherein the antibody has one or more sialic acid residues attached to the mature core carbohydrate structure. In an alternative embodiment, the composition is one wherein the antibody is free of one or more sialic acid residues attached to the mature core carbohydrate structure.

The above-described composition preferably comprises at least about 2% afucosylated antibodies, more preferably at least about 4% afucosylated antibodies, still more preferably at least about 10% afucosylated antibodies, even more preferably at least about 19% afucosylated antibodies, and most preferably about 100% afucosylated antibodies.

Also included herein is an anti-idiotypic antibody that specifically binds any of the antibodies herein.

A therapeutic agent for use in a host subject preferably elicits little to no immunogenic response against the agent in said subject. In one embodiment, the disclosure provides a chimeric or humanized antibody that elicits and/or is expected to elicit a human anti-mouse antibody response (HAMA) at a substantially reduced level compared to a murine antibody in a host subject. In another example, the disclosure provides a chimeric or humanized antibody that elicits and/or is expected to elicit minimal or no human anti-mouse antibody response (HAMA). In one example, an antibody of the disclosure elicits an anti-mouse antibody response that is at or below a clinically acceptable maximum level.

Antibodies of the disclosure can be used to modulate one or more aspects of $LT\alpha$ -associated effects, including but not limited to $LT\alpha$ receptor activation, downstream molecular signaling, cell proliferation, cell migration, cell survival, cell morphogenesis, and angiogenesis. These effects can be modulated by any biologically relevant mechanism, including disruption of ligand (e.g., $LT\alpha$), binding to and blocking the $LT\alpha_3$ receptor or one or both of the heterodimeric receptors, as well as receptor phosphorylation, and/or receptor multimerization.

In another embodiment, the disclosure provides a method of inhibiting $LT\alpha$ -activated cell proliferation, said method comprising contacting a cell or tissue with an effective amount of one or more of the antibodies of the disclosure.

In one aspect, there is provided an *ex vivo* method of inhibiting lymphotoxin- α -activated cell proliferation, said method comprising contacting a cell or tissue with an effective amount of the antibody as defined above.

In a preferred embodiment, such $LT\alpha$ -activated cell proliferation is due to an autoimmune disorder, more preferably RA, MS, Sjogren's syndrome, lupus, myasthenia gravis, or IBD, or cancer, especially breast cancer, lung cancer, prostate cancer, a lymphoma, or leukemia. In another preferred embodiment, the method further comprises contacting the cell or tissue with a second medicament, wherein the first medicament is one or more of the antibodies herein. In a still further preferred embodiment, the cell or tissue is a mammalian cell or tissue, more preferably human, and still more preferably the method is an *in vivo* method. In such preferred *in vivo* method, preferably, the subject having the cell or tissue, most preferably a human subject, is administered the effective amount of the antibodies herein, such as by intravenous or subcutaneous administration.

In one aspect, there is provided an isolated anti-lymphotoxin- α (LT α) antibody as defined above for use in a method of treating an autoimmune disorder in a subject.

In one aspect, there is provided the use of an isolated anti-lymphotoxin- α (LT α) antibody as defined above in the manufacture of a medicament for treating an autoimmune disorder in a subject.

In another embodiment, the disclosure provides a method of treating an autoimmune disorder in a subject comprising administering to the subject an effective amount of the antibody of the disclosure. Preferably, the autoimmune disorder is selected from the group consisting of rheumatoid arthritis (RA), lupus, Wegener's disease, inflammatory bowel disease (IBD), idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic

purpura (TTP), autoimmune thrombocytopenia, multiple sclerosis (MS), psoriasis, IgA nephropathy, IgM polyneuropathies, myasthenia gravis, vasculitis, diabetes mellitus, Reynaud's syndrome, Sjögren's syndrome, glomerulonephritis, Hashimoto's thyroiditis, Graves' disease, helicobacter-pylori gastritis, and chronic hepatitis C.

Perferably, the autoimmune disorder is RA, MS, Sjögren's syndrome, lupus, myasthenia gravis, or IBD, more preferably the autoimmune disorder is RA, IBD, lupus, or MS. More preferably, the lupus is systemic lupus erythematosus or lupus nephritis, and the IBD is Crohn's disease or ulcerative colitis.

In other embodiments, the antibody is a naked antibody. In a further embodiment, the antibody is conjugated with another molecule, such as a cytotoxic agent.

In another embodiment, the method is such that the antibody is administered intravenously. In an alternative embodiment, the antibody is administered subcutaneously.

In another embodiment, the subject has RA and the antibody induces a major clinical response in the subject.

In a further embodiment, the subject has an abnormal level of one or more regulatory cytokines, anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), infiltrating CD20 cells, anti-double stranded DNA (dsDNA) antibodies, anti-Sm antibodies, anti-nuclear ribonucleoprotein antibodies, anti-phospholipid antibodies, anti-ribosomal P antibodies, anti-Ro/SS-A antibodies, anti-Ro antibodies, anti-La antibodies, antibodies directed against Sjögren's-associated antigen A or B (SS-A or SS-B), antibodies directed against centromere protein B (CENP B) or centromere protein C (CENP C), autoantibodies to ICA69, anti-Smith antigen (Sm) antibodies, anti-nuclear ribonucleoprotein antibodies, anti-ribosomal P antibodies, autoantibodies staining the nuclear or perinuclear zone of neutrophils (pANCA), anti-Saccharomyces cerevisiae antibodies, cross-reactive antibodies to GM1 ganglioside or GQ1b ganglioside, anti-acetylcholine receptor (AChR), anti-AChR subtype, or anti-muscle specific tyrosine kinase (MuSK) antibodies, serum anti-endothelial cell antibodies, IgG or anti-desmoglein (Dsg) antibodies, anti-centromere, anti-topoisomerase-1 (Scl-70), anti-RNA polymerase or anti-U3-ribonucleoprotein (U3-RNP) antibodies, anti-glomerular basement membrane (GBM) antibodies, anti-glomerular basement membrane (GBM) antibodies, anti-mitochondrial (AMA) or anti-mitochondrial M2 antibodies, anti-thyroid peroxidase (TPO), anti-thyroglobin (TG) or anti-thyroid stimulating hormone receptor (TSHR) antibodies, anti-nucleic (AN), anti-actin (AA) or anti-smooth

muscle antigen (ASM) antibodies, IgA anti-endomysial, IgA anti-tissue transglutaminase, IgA anti-gliadin or IgG anti-gliadin antibodies, anti-CYP21A2, anti-CYP11A1 or anti-CYP17 antibodies, anti-ribonucleoprotein (RNP), or myositis-specific antibodies, anti-myelin associated glycoprotein (MAG) antibodies, anti-hepatitis C virus (HCV) antibodies, anti-GM1 ganglioside, anti-sulfate-3-glycuronyl paragloboside (SGPG), or IgM anti-glycoconjugate antibodies, IgM anti-ganglioside antibody, anti-thyroid peroxidase (TPO), anti-thyroglobin (TG) or anti-thyroid stimulating hormone receptor (TSHR) antibodies, anti-myelin basic protein or anti-myelin oligodendrocytic glycoprotein antibodies, IgM rheumatoid factor antibodies directed against the Fc portion of IgG, anti-Factor VIII antibodies, or a combination thereof.

In another preferred embodiment of this method, the antibody is administered no more than about once every other week, more preferably about once a month, to the subject.

In another embodiment, a second medicament, wherein the antibody is a first medicament, is administered to the subject in an effective amount to treat the subject in the methods above. Such second medicament is preferably an immunosuppressive agent, an antagonist that binds a B-cell surface marker, a BAFF antagonist, a disease-modifying anti-rheumatic drug (DMARD), an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, or a combination thereof. Additionally, it may be a hyaluronidase glycoprotein as an active delivery vehicle. Most preferably it is a DMARD or methotrexate.

In another embodiment, the subject has never been previously treated with a medicament for the disorder. In a further embodiment, the subject has never been previously treated with a TNF antagonist.

In an alternative embodiment, the subject has been previously treated with a medicament for the disorder, preferably with a TNF antagonist (such as an anti-TNF antibody or a TNF receptor-Ig such as etanercept) or a DMARD.

In another embodiment, the disclosure provides a method of treating RA in a subject comprising administering to the subject an effective amount of an antibody of this disclosure.

In this method, preferably the antibody induces a major clinical response in the subject. More preferably, the subject has been treated with a medicament for the disorder, preferably with a TNF antagonist (such as an anti-TNF antibody or a TNF receptor-Ig such as etanercept) or a DMARD. In another preferred embodiment of this method, the antibody is

administered no more than about once every other week, more preferably about once a month, to the subject.

In another embodiment of this RA treatment method, a second medicament, wherein the antibody is a first medicament, is administered to the subject being treated for RA in an effective amount to treat the subject. Such second medicament is preferably an immunosuppressive agent, an antagonist that binds a B-cell surface marker, a BAFF antagonist, a disease-modifying anti-rheumatic drug (DMARD), an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, or a combination thereof. Additionally, it may be a hyaluronidase glycoprotein as an active delivery vehicle. Most preferably it is a DMARD or methotrexate. Preferably, the antibody used for treating RA is administered subcutaneously or intravenously. Further it may be a naked antibody or conjugated, *e.g.*, to a cytotoxic agent. In another preferred embodiment of this method, the antibody is administered no more than about once every other week, more preferably about once a month, to the subject.

The disclosure also provides a composition comprising the antibody of any of the preceding embodiments and a carrier, such as a pharmaceutically acceptable carrier.

In one aspect, there is provided a composition comprising the antibody as defined above and a carrier.

Another embodiment of the disclosure is an isolated nucleic acid encoding an antibody of any one of the preceding embodiments. Expression vectors comprising such nucleic acid, and those encoding the antibodies of the disclosure, are also provided. Also provided is a host cell comprising a nucleic acid encoding an antibody of the disclosure. Any of a variety of host cells can be used. In one embodiment, the host cell is a prokaryotic cell, for example, *E. coli*. In another embodiment, the host cell is a eukaryotic cell, for example a yeast cell or mammalian cell such as a Chinese Hamster Ovary (CHO) cell.

In another embodiment, the disclosure provides methods for making an antibody of the disclosure. For example, the disclosure provides a method of making or producing an anti-LT α antibody (which, as defined herein includes full length and fragments thereof, provided they contain an Fc region), said method comprising culturing a suitable host cell comprising a nucleic acid encoding an antibody of the disclosure (preferably comprising a recombinant vector of the disclosure encoding said antibody (or fragment thereof)), under conditions to produce the antibody, and recovering said antibody. The antibody may be recovered from the host cell or host cell culture. In a preferred embodiment, the antibody is a naked antibody. In another preferred embodiment, the antibody is conjugated with another molecule, the other molecule preferably being a cytotoxic agent.

Still another embodiment of the disclosure is an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody of any of the preceding embodiments and a package insert indicating that the composition can be used to treat the indication the antibody is intended for, such as an autoimmune disorder. A second medicament, as noted above, may be added to such article, for example, in a separate container, in addition to the antibody, which is the first medicament.

In a further aspect, there is provided a hybridoma deposited at the American Type Culture Collection (ATCC) on April 19, 2006 under Deposit No. PTA-7538. In another aspect, there is provided an antibody that is secreted by a hybridoma as defined above.

If the autoimmune disease is RA specifically, one embodiment is a method wherein the patient has never been previously administered a medicament for the RA. An alternative embodiment is a method wherein the patient has been previously administered at least one medicament for the RA, more preferably wherein the patient was not responsive to at least one medicament that was previously administered. The previously administered medicament or medicaments to which the patient may be non-responsive include an immunosuppressive agent, cytokine antagonist, integrin antagonist, corticosteroid, analgesic, DMARD, NSAID, or CD20 antagonist, and more preferably an immunosuppressive agent, cytokine antagonist, integrin antagonist, corticosteroid, DMARD, NSAID, or CD20 antagonist, such as a CD20 antibody, which is preferably not rituximab or a humanized 2H7. Most preferably, such previously administered medicament(s) are a DMARD or a TNF inhibitor such as anti-TNF-alpha antibody or TNF-Ig.

In another preferred embodiment of the disclosure for using the antibodies herein to treat an autoimmune disease such as RA, the patient has exhibited an inadequate response to one or more TNF inhibitors or to one or more DMARDs. More preferably, the RA is early RA or incipient RA.

In another embodiment, a method is provided wherein at least about three months after the administration of the antibody herein to a patient with RA or joint damage, an imaging test is given that measures a reduction in bone or soft tissue joint damage as compared to baseline prior to the administration, and the amount of the antibody administered is effective in achieving a reduction in the joint damage. In a preferred embodiment, the test measures a total modified Sharp score. In a preferred embodiment, the method further comprises an additional administration to the patient of the antibody herein in an amount effective to

achieve a continued or maintained reduction in joint damage as compared to the effect of a prior administration of the antibody. In a further embodiment, the antibody herein is additionally administered to the patient even if there is no clinical improvement in the patient at the time of the testing after a prior administration. The clinical improvement is preferably determined by assessing the number of tender or swollen joints, conducting a global clinical assessment of the patient, assessing erythrocyte sedimentation rate, assessing the amount of C-reactive protein level, or using composite measures of disease activity.

Preferred second medicaments for treatment of RA and joint damage include an immunosuppressive agent, a DMARD, a pain-control agent, an integrin antagonist, a NSAID, a cytokine antagonist, a bisphosphonate, an antagonist to a B-cell surface marker such as, for example, BR3-Fc, BR3 antibody, CD20 antibody, CD40 antibody, CD22 antibody, CD23 antibody, or a combination thereof. If the second medicament is a DMARD, preferably it is selected from the group consisting of auranofin, chloroquine, D-penicillamine, injectable gold, oral gold, hydroxychloroquine, sulfasalazine, myocrisin, and methotrexate. If the second medicament is a NSAID, preferably it is selected from the group consisting of: fenbufen, naprosyn, diclofenac, etodolac, indomethacin, aspirin and ibuprofen. If the second medicament is an immunosuppressive agent, it is preferably selected from the group consisting of etanercept, infliximab, adalimumab, leflunomide, anakinra, azathioprine, and cyclophosphamide. Other groups of preferred second medicaments include anti- α 4, etanercept, infliximab, etanercept, adalimumab, kinaret, efalizumab, osteoprotegerin (OPG), anti-receptor activator of NF κ B ligand (anti-RANKL), anti-receptor activator of NF κ B-Fc (RANK-Fc), pamidronate, alendronate, actonel, zoledronate, clodronate, methotrexate, azulfidine, hydroxychloroquine, doxycycline, leflunomide, sulfasalazine (SSZ), prednisolone, interleukin-1 receptor antagonist, prednisone, or methylprednisolone. Another preferred group of second medicaments includes infliximab, an infliximab/methotrexate (MTX) combination, MTX, etanercept, a corticosteroid, cyclosporin A, azathioprine, auranofin, hydroxychloroquine (HCQ), combination of prednisolone, MTX, and SSZ, combinations of MTX, SSZ, and HCQ, the combination of cyclophosphamide, azathioprine, and HCQ, and the combination of adalimumab with MTX. More preferably, the corticosteroid is prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone. In another preferred embodiment, the second medicament is MTX, which is more preferably administered perorally or parenterally.

In another embodiment, the treatment methods herein further comprise re-treating the patient by re-administering an effective amount of the antibody herein to the patient. Preferably, the re-treatment is commenced at at least about 24 weeks after the first administration of the antibody. In another embodiment, a second re-treatment is commenced, and more preferably wherein the second re-treatment is commenced at at least about 24 weeks after the second administration of the antibody. In another embodiment, where the autoimmune disease is RA, joint damage has been reduced after the re-treatment. In an alternative embodiment, no clinical improvement is observed in the patient at the time of the testing after the re-treatment, especially wherein the clinical improvement is determined by assessing the number of tender or swollen joints, conducting a global clinical assessment of the patient, assessing erythrocyte sedimentation rate, assessing the amount of C-reactive protein level, or using composite measures of disease activity.

In another embodiment, the disclosure herein provides a method for advertising an antibody herein or a pharmaceutically acceptable composition thereof comprising promoting, to a target audience, the use of the antibody or pharmaceutical composition thereof for treating a patient or patient population exhibiting an autoimmune disease such as RA, MS, lupus, or an IBD.

In another embodiment, the disclosure provides an article of manufacture comprising, packaged together, a pharmaceutical composition comprising an antibody herein and a pharmaceutically acceptable carrier and a label stating that the antibody or pharmaceutical composition is indicated for treating patients with an autoimmune disease such as RA, MS, lupus, or an IBD. In a preferred embodiment, the article further comprises a container comprising a second medicament, wherein the antibody herein is a first medicament, further comprising instructions on the package insert for treating the patient with an effective amount of the second medicament, which is preferably methotrexate.

In a still further embodiment, the disclosure provides a method for packaging an antibody herein or a pharmaceutical composition of the antibody comprising combining in a package the antibody or pharmaceutical composition and a label stating that the antibody or pharmaceutical composition is indicated for treating patients exhibiting an autoimmune disease such as RA, MS, lupus, or an IBD.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A depicts the full-length sequences for the 2C8 chimera light and heavy chains (SEQ ID NOS:32 and 33, respectively).

5 FIG. 1B depicts the full-length sequences for the 3F12 chimera light and heavy chains (SEQ ID NOS:34 and 35, respectively), as well as the 3F12 chimera heavy-chain variable region (SEQ ID NO:31).

FIG. 1C depicts the sequence of the light-chain variable region for an improved 3F12 chimera (referred to as chimera2 in the examples below) (3F12.2D3) (SEQ ID NO:36) and the
10 sequence of the light-chain constant region of 3F12.2D3 (SEQ ID NO:37). The bold type of the residues indicates those identified from various chemical/enzymatic cleavages.

FIG. 1D depicts the sequence of the heavy-chain variable region for an improved 3F12 chimera (3F12.2D3) (SEQ ID NO:38) and the sequence of the heavy-chain constant region of 3F12.2D3 (SEQ ID NO:39). The bold type of the residues indicates those
15 identified from various chemical/enzymatic cleavages, and the asterisk indicates a glycosylation site.

FIG. 2A depicts alignment of sequences of the variable light chain for the following: chimeric 2C8 antibody (SEQ ID NO:23), humanized 2C8.v2 (SEQ ID NO: 24), and human kappa subgroup I consensus sequence (SEQ ID NO:40). The CDR sequences of each are
20 shown in brackets, and asterisks are used to show differences among the aligned sequence amino acid residues.

FIG. 2B depicts alignment of sequences of the variable heavy chain for the following: chimeric 2C8 antibody (SEQ ID NO:25), humanized 2C8.v2 (SEQ ID NO:26), and human subgroup III consensus sequence (SEQ ID NO:41). The CDR sequences of each are shown
25 in brackets, and asterisks are used to show differences among the aligned sequence amino acid residues.

FIG. 2C depicts the light- and heavy-chain sequences for versions 2C8.v7 and 2C8.v12 (SEQ ID NOS:42-45).

FIG. 3A depicts alignment of sequences of the variable light chain for the following: chimeric 3F12 antibody (SEQ ID NO:27), humanized 3F12.v5 (SEQ ID NO:28), and human
30 kappa subgroup I consensus sequence (SEQ ID NO:40). The CDR sequences of each are shown in brackets, and asterisks are used to show differences among the aligned sequence amino acid residues.

FIG. 3B depicts alignment of sequences of the variable heavy chain for the following: original chimeric 3F12 antibody (not 3F12.2D3) (SEQ ID NO:29), humanized 3F12.v5 (SEQ ID NO:30), and human subgroup III consensus sequence (SEQ ID NO:41). The CDR sequences of each are shown in brackets, and asterisks are used to show differences among the aligned sequence amino acid residues.

FIG. 4A and 4B show that hamster-murine chimeric anti-LT α antibody S5H3 inhibits antibody-induced arthritis (AIA). Figure 4A shows the results of preventative use of the anti-LT α antibody versus a Fc DANA mutation (anti-LT.Fcmutant) and a TNFRII.Fc mutant (a TNFR.Ig immunoadhesin) as well as a control isotype (anti-ragweed IgG2a monoclonal antibody). Figure 4B shows that the anti-LT α antibody inhibited development of arthritis in an AIA model when administered therapeutically, versus control isotype and TNFRII.Fc mutant.

FIG. 5A and 5B show that hamster-murine chimeric anti-LT α antibody S5H3 inhibits collagen-induced arthritis (CIA). Figure 5A shows that S5H3 inhibited arthritis in a CIA-preventative model, versus a Fc DANA mutation (anti-LT.Fc mutant) as well as a control isotype (anti-ragweed IgG2a monoclonal antibody) and was comparable to a TNFRII.Fc mutant. Figure 5B shows that the S5H3 anti-LT α antibody inhibited development of arthritis in a CIA model when administered therapeutically, versus control isotype, and was comparable to the TNFRII.Fc mutant.

FIG. 6 shows that hamster-murine chimeric anti-LT α antibody S5H3 delays onset and severity of EAE disease in MBP-TCR transgenic mice, versus a control isotype (anti-gp120 IgG1 monoclonal antibody).

FIG. 7A, 7B, and 7C show that treatment with hamster-murine chimeric anti-LT α antibody S5H3 does not affect the T-cell-dependent anti-TNP IgM, IgG1, and IgG2a responses, respectively, as compared to isotype control (anti-ragweed), anti-LT.Fc mutant, and CTLA4.Fc (a positive control).

FIG. 8 shows that treatment with hamster-murine chimeric anti-LT α antibody S5H3 does not affect T-cell-independent antibody (IgM, IgG1, IgG2a, and IgG3) responses relative to isotype control (anti-ragweed) and TNFRII.Fc mutant.

FIG. 9 shows that anti-LT α chimeric monoclonal antibody 2C8 prevents GVHD in human SCID mice as compared to the DANA anti-LT α 2C8 Fc mutant and the isotype control (trastuzumab, human IgG1). CTLA4.Fc mutant was also used as a positive control.

FIG. 10A shows that chimeric anti-LT α antibodies 2C8 and 3F12 bind to human LT α 3.

FIG. 10B shows that hamster-murine chimeric anti-LT α antibody S5H3 as well as anti-LT.Fc mutant bind to murine LT α 3.

5 FIG. 11A shows through enzyme-linked immunosorbent assay (ELISA) results that hamster-murine chimeric anti-LT α S5H3 antibody binds to murine LT α 1 β 2 complex, as does the anti-LT.Fc mutant.

FIG. 11B shows FACS assay results for binding of chimeric anti-LT α antibodies 3F12 and 2C8 to the LT α on the surface of human cells complexed with LT β . They are
10 compared to LT β -receptor.huIgG1 and isotype control huIgG1 (trastuzumab).

FIG. 11C shows FACS assay results for binding of hamster-murine chimeric anti-LT α antibody S5H3 to the LT α on the surface of murine cells complexed with LT β . It is compared to LT β -receptor murine IgG2a, anti-LT S5H3 Fc mutant, and isotype control (anti-IL122 muIgG2a).

15 FIG. 12A and 12B show that chimeric anti-LT α antibodies 2C8 and 3F12 bind to human Th1 and Th2 cells, respectively, and show binding results of LTbR.Fc, TNFR1I.Fc, and isotype control for comparison.

FIG. 12C-12E show that anti-LT α humanized antibody 2C8.vX does not bind to resting cells (FIG. 12C), but does bind to human Th1 and Th17 cells (FIG. 12D and E,
20 respectively), with a comparison of its binding with that of isotype control.

FIG. 13A shows unactivated T cells treated with LTbR.Fc (solid line), chimeric anti-LT α 3F12 antibody (dashed line), and isotype control (shaded area).

FIG. 13B shows anti-CD3- and anti-CD28-activated CD4 T cells treated with the reagents of Fig. 13A.

25 FIG. 13C shows anti-CD3- and anti-CD28-activated CD8 T cells treated with the reagents of Fig. 13A.

FIG. 13D shows IL15-activated CD56 NK cells treated with the reagents of Fig. 13A.

FIG. 13E shows IgM- and CD40L-activated CD19 B cells treated with the reagents of Fig. 13A.

30 FIG. 14A shows that chimeric anti-LT α antibodies 2C8 and 3F12 functionally block human LT α 3 versus the isotype control (trastuzumab).

FIG. 14B shows that hamster-murine chimeric anti-LT α antibody S5H3 and the anti-LT.Fc mutant functionally block murine LT α 3 versus the isotype control (trastuzumab).

FIG. 14C shows that chimeric anti-LT α antibodies 2C8 and 3F12 block LT α 3-induced IL-8 in A549 cells versus the isotype control (trastuzumab).

FIG. 14D shows that chimeric anti-LT α antibodies 2C8 and 3F12, as well as TNFRILFc mutant, block LT α 3-induced ICAM expression on HUVEC versus isotype control (trastuzumab). The response of unstimulated cells is also shown.

FIG. 14E shows that chimeric anti-LT α antibody 2C8 blocks LT α 3-induced NF κ B activation, versus the isotype control. The response of unstimulated cells is also shown.

FIG. 15A shows that chimeric anti-LT α antibody 2C8 functionally blocks LT α 1 β 2-induced NF κ B activation versus the isotype control. The response of unstimulated cells is also shown.

FIG. 15B, 15C, and 15D show that chimeric anti-LT α antibody 2C8 blocks, respectively, LT α 3-, LT α 2 β 1-, and LT α 1 β 2-induced cytotoxicity.

FIG. 15E shows that chimeric anti-LT α antibodies 2C8 and 3F12 block LT α 1 β 2-induced ICAM expression on HUVEC, versus the isotype control. The response of unstimulated cells is also shown.

FIG. 16 shows that chimeric anti-LT α antibody 2C8 can kill LT α β -expressing cells, versus anti-LT α 2C8 Fc mutant.

FIG. 17A-H show that chimeric anti-LT α antibody 2C8 blocks cytokine and chemokine secretion in HUVEC cells, with Figures 17A-17D respectively showing KC/IL-8, RANTES, IP10, and IL-6 with LT α 1 β 2, and Figures 17E-17H respectively showing KC/IL-8, RANTES, IP10, and IL-6 with LT α 3 versus control.

FIG. 17I-P show that hamster-murine chimeric anti-LT α antibody S5H3 blocks cytokine and chemokine secretion in 3T3 cells, with Figures 17I-17L respectively showing KC, RANTES, IP10, and IL-6 with LT α 1 β 2, and Figures 17M-17P respectively showing KC, RANTES, IP10, and IL-6 with LT α 3 versus control.

FIG. 18A depicts alignment of sequences of the variable light chain for the following: chimeric 2C8 antibody (SEQ ID NO:23), humanized 2C8.vX (SEQ ID NO:102), and human kappa subgroup I consensus sequence (SEQ ID NO:40). The CDR sequences of each are shown in brackets, and asterisks are used to show differences among the aligned sequence amino acid residues.

FIG. 18B depicts alignment of sequences of the variable heavy chain for the following: chimeric 2C8 antibody (SEQ ID NO:25), humanized 2C8.vX (SEQ ID NO:103), and human subgroup III consensus sequence (SEQ ID NO:41). The CDR sequences of each

are shown in brackets, and asterisks are used to show differences among the aligned sequence amino acid residues.

FIG. 19A and 19B show, respectively, ELISAs of LT α 3 and LT α 1 β 2 binding of 2C8.vX antibodies in comparison to TNFR1L.Fc/isotype control.

5 FIG. 20A and 20B show, respectively, blocking of human LT α 3 and LT α 1 β 2 by 2C8.vX antibodies in comparison to isotype control/TNFR1L.Fc using an assay for testing blocking of human LT-induced cytotoxicity.

FIG. 21 provides a comparison of how various molecules blocked LT α 3 in a functional assay showing inhibition of ICAM1 upregulation by LT α 3 on HUVEC cells.
10 Specifically, isotype, 2C8.vX and TNFR1L.Fc were compared.

FIG. 22 provides a comparison of how various molecules blocked LT α 1 β 2 in a functional assay showing LT α 1 β 2 blocking using 293-LT β R cells. Specifically, isotype and 2C8.vX were compared along with unstimulated cells.

FIG. 23 shows ADCC activity of 2C8.vX using a protocol involving 293-LT α 1 β 2
15 cells. 2C8.vX was compared with the 2C8.vX DANA mutant (Fc mutant) and isotype control.

FIG. 24 shows GVHD survival results with 2C8.vX and CTLA4-Fc, as compared to controls (2C8.vX DANA Fc mutant and isotype control), in a human SCID model.

FIG. 25A-D show that the 2C8.vX antibody depletes LT α 1 β 2-expressing T and B
20 cells in the human SCID GVHD model. The results in FIG. 25A show total positive human cells at day 2 for isotype control, 2C8.vX, and 2C8.vX.DANA mutant. FIG. 25B-D show the results of gating using the CD4, CD8, and CD19 antibodies, respectively.

FIG. 26A depicts alignment of sequences of the variable light chain for the following: chimeric 3F12 antibody (SEQ ID NO:27), humanized 3F12.v14 (SEQ ID NO:108), and
25 human kappa subgroup I consensus sequence (SEQ ID NO:40). The CDR sequences of each are shown in brackets, and asterisks are used to show differences among the aligned sequence amino acid residues.

FIG. 26B depicts alignment of sequences of the variable heavy chain for the following: chimeric 3F12 antibody (SEQ ID NO:29), humanized 3F12.v14 (SEQ ID
30 NO:109), and human subgroup III consensus sequence (SEQ ID NO:41). The CDR sequences of each are shown in brackets, and asterisks are used to show differences among the aligned sequence amino acid residues.

FIG. 27 shows ADCC activity of 2C8.vX and its afucosylated counterpart, wherein AF = afucosylated antibody and WT = regular CHO cell-line production antibody.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

General Techniques

5 The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook *et al.*, 1989); *Oligonucleotide Synthesis* (M. J. Gait, ed., 1984); *Animal Cell Culture* (R. I. Freshney, ed., 1987); *Methods in Enzymology* (Academic Press, Inc.); *Current Protocols in Molecular Biology* (F. M. Ausubel *et al.*, eds., 1987, and periodic updates); *PCR: The Polymerase Chain Reaction*, (Mullis *et al.*, ed., 1994); *A Practical Guide to Molecular Cloning* (Perbal Bernard V., 1988); *Phage Display: A Laboratory Manual* (Barbas *et al.*, 2001).

Definitions

15 "Lymphotoxin- α or "LT α " is defined herein as a biologically active polypeptide having the amino acid sequence shown in FIG. 2A of US Pat. No. 5,824,509. LT α is defined to specifically exclude human TNF α or its natural animal analogues (Pennica *et al.*, *Nature* 312:20/27 : 724-729 (1984) and Aggarwal *et al.*, *J. Biol. Chem.* 260: 2345-2354 (1985)). LT α is defined to specifically exclude human LT β as defined, for example, in US 5,661,004.

20 "Lymphotoxin- α 3 trimer" or "LT α 3" refers to a homotrimer of LT α monomers. This homotrimer is anchored to the cell surface by the LT β , transmembrane and cytoplasmic domains.

"Lymphotoxin- $\alpha\beta$ " or "LT $\alpha\beta$ " or "LT $\alpha\beta$ complex" refers to a heterotrimer of LT α with LT β . These heterotrimers contain either two subunits of LT α and one subunit of LT β (LT α 2 β 1), or one subunit of LT α and two of LT β (LT α 1 β 2).

"Tumor necrosis factor receptor-I" or "TNFRI" and "tumor necrosis factor receptor-II" or "TNFRII" refer to cell-surface TNF receptors for the LT α 3 homotrimer, also known as p55 and p75, respectively.

30 "Lymphotoxin- β receptor" or "LT β -R" refers to the receptor to which the LT $\alpha\beta$ heterotrimers bind.

"Regulatory cytokines" are cytokines the abnormal levels of which indicate the presence of an autoimmune disorder in a patient. Such cytokines include, for example, interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-14, IL-

15, IL-18, IL-23, IL-24, IL-25, IL-26, BLyS/April, TGF- α , TGF- β , interferon- α (IFN- α), IFN- β , IFN- γ , MIP-1, MIF, MCP-1, M-CSF or G-CSF, a lymphotoxin, LIGHT, 4-1BB ligand, CD27 ligand, CD30 ligand, CD40 ligand, Fas ligand, GITR ligand, OX40 ligand, RANK ligand, THANK, TRAIL, TWEAK and VEG1. This group includes TNF family
5 members, which include but are not limited to, TNF- α , LTs such as LT α , LT β , and LIGHT. For a review of the TNF superfamily, see MacEwan, *Br. J. Pharmacology* 135: 855-875 (2002). Preferably, the regulatory cytokine is an IL such as IL-1b or IL-6 and/or a TNF family member.

"Inflammatory cytokines associated with rheumatoid arthritis" refer to IL-6, IL-1b,
10 and TNF α , associated with RA pathology, which can be inhibited systemically and or in the joints in an *in vitro* collagen-induced arthritis assay.

"LT $\alpha\beta$ -expressing cells" are cells that express or secrete the LT $\alpha\beta$ heterotrimers.

The expression "modulates LT $\alpha\beta$ -expressing cells" refers to depleting or altering proteins made by the cells such as cytokines, chemokines, or growth factors, with the cells
15 including, for example, monocytes, dendritic cells, T cells, and B cells.

The terms "antibody" and "immunoglobulin" are used interchangeably in the broadest sense and include monoclonal antibodies (*e.g.*, full-length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, and multispecific antibodies (*e.g.*, bispecific antibodies so long as they exhibit the desired biological activity), and may also include
20 certain antibody fragments (as described in greater detail herein). An antibody can be chimeric, human, humanized, and/or affinity matured.

"Antibody fragments" comprise only a portion of an intact antibody, wherein the portion preferably retains at least one, more preferably most or all, of the functions normally associated with that portion when present in an intact antibody. In one embodiment, an
25 antibody fragment comprises an antigen-binding site of the intact antibody and thus retains the ability to bind antigen. In another embodiment, an antibody fragment, for example one that comprises the Fc region, retains at least one of the biological functions normally associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half-life modulation, ADCC function, and complement binding. In one
30 embodiment, an antibody fragment is a monovalent antibody that has an *in vivo* half life substantially similar to an intact antibody. For example, such an antibody fragment may comprise an antigen binding arm linked to an Fc sequence capable of conferring *in vivo* stability to the fragment. In one embodiment, an antibody of the invention is a one-armed

antibody as described in WO 2005/063816, for example, an antibody comprising Fc mutations constituting "knobs" and "holes". For example, a hole mutation can be one or more of T366A, L368A and/or Y407V in an Fc polypeptide, and a cavity mutation can be T366W. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; 5 diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

For the purposes herein, an "intact antibody" is one comprising heavy and light variable domains as well as an Fc region.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a 10 population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), 15 each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present invention may be prepared by the hybridoma 20 methodology first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made using recombinant DNA methods in, e.g., bacterial, non-animal eukaryotic, animal, or plant cells (see, e.g., US 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in, e.g., Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991).

25 The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or 30 belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (US 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Methods of making chimeric antibodies are known in the art.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are

5 human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are found neither

10 in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a

15 human immunoglobulin sequence, although the FR regions may include one or more amino acid substitutions that improve binding affinity. The number of these amino acid substitutions in the FR is typically no more than six in the H chain, and no more than three in the L chain. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further

20 details, see Jones *et al.*, *Nature*, 321:522-525 (1986); Reichmann *et al.*, *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992). See also Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem.Soc.Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994). The humanized antibody includes a PRIMATIZED[®] antibody wherein the

25 antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with the antigen of interest. Methods of making humanized antibodies are known in the art.

A "human antibody" is one that possesses an amino acid sequence that corresponds to that of an antibody produced by a human and/or has been made using any of the techniques

30 for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can also be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et*

al., *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991).

5 An "affinity-matured" antibody is an antibody with one or more alterations in one or more CDRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. Marks *et al.* *Bio/Technology* 10:779-783 (1992) describes affinity maturation by VH and VL domain
10 shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas *et al.*, *Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al.*, *Gene* 169:147-155 (1995); Yelton *et al.*, *J. Immunol.* 155:1994-2004 (1995); Jackson *et al.*, *J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al.*, *J. Mol. Biol.* 226:889-896 (1992).

An "antigen" is a predetermined antigen to which an antibody can selectively bind.
15 The target antigen may be polypeptide, carbohydrate, nucleic acid, lipid, hapten, or another naturally occurring or synthetic compound. Preferably, the target antigen is a polypeptide. An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework, or from a human consensus framework. An acceptor human framework "derived
20 from" a human immunoglobulin framework or human consensus framework may comprise the same amino acid sequence thereof, or may contain pre-existing amino acid sequence changes. Where pre-existing amino acid changes are present, preferably no more than five, and more preferably four or less, and still more preferably three or less, pre-existing amino acid changes are present. Where pre-existing amino acid changes are present in a VH,
25 preferably those changes are only at three, two, or one of positions 71, 73, and 78; for instance, the histidine residues at those positions may be alanine residues. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

A "human consensus framework" is a framework that represents the most commonly occurring amino acid residue in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Typically, this subgroup of sequences is a subgroup as in Kabat *et al.*, *supra*. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat *et al.*, *supra*. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat *et al.*, *supra*.

A "VH subgroup III consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable heavy subgroup III of Kabat *et al.*, *supra*. In one embodiment, the VH subgroup III consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences:
 EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:46)-H1-WVRQAPGKGGLEWVG
 (SEQ ID NO:47)-H2-RFTISRDNKNTLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:48)-
 H3-WGQGTLVTVSS (SEQ ID NO:49), where N is any amino acid residue.

In another embodiment, the VH subgroup III consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences:

EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:46)-H1-
 WVRQAPGKGGLEWVG (SEQ ID NO:47)-H2-
 RATFSADNSKNTAYLQMNSLRAEDTAVYYCAD (SEQ ID NO:50)-H3-
 WGQGTLVTVSS (SEQ ID NO:49), where N is any amino acid residue.

A "VL subgroup I consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable light kappa subgroup I of Kabat *et al.*, *supra*. In one embodiment, the VL subgroup I consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences:
 DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:51)-L1-WYQQKPGKAPKLQIY (SEQ ID
 NO:52)-L2-GVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO: 53)-L3-
 FGQGTKVEIKR (SEQ ID NO:54).

In another embodiment, the VL subgroup I consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences:
 DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:51)-L1-WYQQKPGKAPKLLIY (SEQ ID
 NO:55)-L2-GVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:53)-L3-
 FGQGTKVEIKR (SEQ ID NO:54).

The term “complementarity-determining region”, or “CDR”, when used herein refers to the regions of an antibody variable domain that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of CDR delineations are in use and are encompassed herein. The Kabat CDRs are based on sequence variability and are the most commonly used (Kabat *et al.*, *supra*). Chothia refers instead to the location of the structural loops (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The “contact” hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions are noted below.

Loop	Kabat	AbM	Chothia	Contact
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L1	L24-L34	L24-L34	L24-L32	L30-L36
L2	L50-L56	L50-L56	L50-L56	L46-L55
L3	L89-L97	L89-L97	L89-L97	L89-L96
H1	H31-H35B	H26-H32B	H26-H32	H30-H35B
(Kabat Numbering)				
H1	H31-H35	H26-H35	H26-H32	H30-H35
(Chothia Numbering)				
H2	H50-H65	H50-H58	H52-H56	H47-H58
H3	H95-H102	H95-H102	H95-H102	H93-H101

CDRs may comprise “extended CDRs” as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2), and 89-97 (L3) in the VL, and 26-35 (H1), 50-65 or 49-65 (H2), and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat *et al.*, *supra*, for each of these definitions. Throughout the present specification and claims, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat *et al.*, *supra*. The “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody.

An “unmodified human framework” is a human framework that has the same amino acid sequence as the acceptor human framework, *e.g.* lacking human to non-human amino acid substitution(s) in the acceptor human framework.

An "altered CDR" for the purposes herein is a CDR comprising one or more (*e.g.* one to about 16) amino acid substitution(s) therein.

An "un-modified CDR" for the purposes herein is a CDR having the same amino acid sequence as a non-human antibody from which it was derived, *i.e.* one lacking one or more amino acid substitutions.

"Framework" or "FR" residues are those variable domain residues other than the CDR residues as herein defined.

A "blocking" antibody or an "antagonist" antibody is one that inhibits or reduces biological activity of the antigen it binds. Preferred blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

An "agonist antibody", as used herein, is an antibody that mimics at least one of the functional activities of a polypeptide of interest.

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably to more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The term "variable-domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat", and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, *supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, *etc* according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given

antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat-numbered sequence.

A "parent polypeptide" is a polypeptide comprising an amino acid sequence that lacks one or more of the Fc-region modifications disclosed herein and that differs in effector function compared to a polypeptide variant as herein disclosed. The parent polypeptide may comprise a native-sequence Fc region or an Fc region with pre-existing amino acid sequence modifications (such as additions, deletions, and/or substitutions).

The term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native-sequence Fc region or a variant Fc region.

Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3. The last residue, lysine, in the heavy chain of IgG1 can, but does not have to, be present as the terminal residue in the Fc in the mature protein.

The "CH2 domain" of a human IgG Fc region (also referred to as "Cy2" domain) usually extends from about amino acid 231 to about amino acid 340. The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It has been speculated that the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, *Molec. Immunol.* 22:161-206 (1985).

The "CH3 domain" comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (*i.e.* from about amino acid residue 341 to about amino acid residue 447 of an IgG)

A "functional Fc region" possesses an "effector function" of a native-sequence Fc region. Exemplary "effector functions" include C1q binding; CDC; Fc receptor binding; ADCC; phagocytosis; down regulation of cell- surface receptors (*e.g.* LT receptor), *etc.* Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.* an antibody variable domain) and can be assessed using various assays as herein disclosed, for example.

A "native-sequence Fc region" or "wild-type Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native-

sequence human Fc regions are known in the art and include a native-sequence human IgG1 Fc region (non-A and A allotypes); native-sequence human IgG2 Fc region; native-sequence human IgG3 Fc region; and native-sequence human IgG4 Fc region, as well as naturally occurring allelic variants thereof.

5 A "variant Fc region" comprises an amino acid sequence that differs from that of a native-sequence Fc region by virtue of at least one "amino acid modification" as herein defined. Preferably, the variant Fc region has at least one amino acid substitution compared to a native-sequence Fc region or to the Fc region of a parent polypeptide, *e.g.* from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native-sequence Fc region or in the Fc region of the parent polypeptide.
10 The variant Fc region herein will preferably possess at least about 80% homology with a native-sequence Fc region and/or with an Fc region of a parent polypeptide, more preferably at least about 90% homology therewith, and most preferably at least about 95% homology therewith.

15 The term "Fc region-containing polypeptide" refers to a polypeptide, such as an antibody or immunoadhesin (see definitions herein), which comprises an Fc region.

The terms "Fc receptor" and "FcR" are used to describe a receptor that binds to the Fc region of an IgG antibody. The preferred FcR is a native-sequence human FcR. In one embodiment, the FcR is a FcγR that includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors.
20 FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (see
25 review in Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). The term includes allotypes, such as FcγRIIIA allotypes: FcγRIIIA-Phe158, FcγRIIIA-Val158, FcγRIIA-R131, and/or FcγRIIA-H131. FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.*,
30 126:330-341 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *Eur. J. Immunol.* 24:2429-2434 (1994)).

“Antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.* Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies “arm” the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII, and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.*, 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMCs) and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes *et al.*, *PNAS (USA)* 95:652-656 (1998).

“Human effector cells” are leukocytes that express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes that mediate ADCC include PBMCs, NK cells, monocytes, cytotoxic T cells, and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source thereof, *e.g.* from blood or PBMCs as described herein.

“Complement-dependent cytotoxicity” or “CDC” refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) that are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.* as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996), may be performed.

A polypeptide with a variant IgG Fc having “altered” FcR binding affinity or ADCC activity is one that has either enhanced or diminished FcR binding activity (FcγR or FcRn) and/or ADCC activity compared to a parent polypeptide or to a polypeptide comprising a native-sequence Fc region. The variant Fc that “exhibits increased binding” to an FcR binds at least one FcR with better affinity than the parent polypeptide. The improvement in binding compared to a parent polypeptide may be about three-fold, preferably about 5-, 10-, 25-, 50-, 60-, 100-, 150-, 200-, and up to 500-fold, or about 25% to 1000% improvement in binding.

The polypeptide variant that "exhibits decreased binding" to an FcR binds at least one FcR with less affinity than a parent polypeptide. The decrease in binding compared to a parent polypeptide may be about 40% or more decrease in binding. Such Fc variants that display decreased binding to an FcR may possess little or no appreciable binding to an FcR, *e.g.*,
5 about 0-20% binding to the FcR compared to a native-sequence IgG Fc region.

The polypeptide having a variant Fc that binds an FcR with "better affinity" or "higher affinity" than a polypeptide or parent polypeptide having wild-type or native-sequence IgG Fc is one that binds any one or more of the above identified FcRs with substantially better binding affinity than the parent polypeptide with native-sequence Fc,
10 when the amounts of polypeptide with variant Fc and parent polypeptide in the binding assay are essentially the same. For example, the variant Fc polypeptide with improved FcR binding affinity may display from about two-fold to about 300-fold, preferably, from about three-fold to about 170-fold, improvement in FcR binding affinity compared to the parent polypeptide, where FcR binding affinity is determined as known in the art.

The polypeptide comprising a variant Fc region that "exhibits increased ADCC" or mediates ADCC in the presence of human effector cells more effectively than a polypeptide having wild-type IgG Fc is one that *in vitro* or *in vivo* is substantially more effective at mediating ADCC, when the amounts of polypeptide with variant Fc region and the polypeptide with wild-type Fc region used in the assay are essentially the same. Generally,
20 such variants will be identified using the *in vitro* ADCC assay as herein disclosed, but other assays or methods for determining ADCC activity, *e.g.* in an animal model, *etc.*, are contemplated. The preferred variant is from about five-fold to about 100-fold, more preferably from about 25- to about 50-fold, more effective at mediating ADCC than the wild-type Fc.

25 An "amino acid modification" refers to a change in the amino acid sequence of a predetermined amino acid sequence. Exemplary modifications include an amino acid substitution, insertion, and/or deletion. The preferred amino acid modification herein is a substitution.

An "amino acid modification at" a specified position, *e.g.* of the Fc region, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid
30 residue adjacent to the specified residue. By insertion "adjacent to" a specified residue is meant insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue.

An "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a predetermined amino acid sequence with another different "replacement" amino acid residue. The replacement residue or residues may be "naturally occurring amino acid residues" (*i.e.*, encoded by the genetic code) and selected from the group consisting of: alanine (Ala); arginine (Arg); asparagine (Asn); aspartic acid (Asp); cysteine (Cys); glutamine (Gln); glutamic acid (Glu); glycine (Gly); histidine (His); isoleucine (Ile); leucine (Leu); lysine (Lys); methionine (Met); phenylalanine (Phe); proline (Pro); serine (Ser); threonine (Thr); tryptophan (Trp); tyrosine (Tyr); and valine (Val). Preferably, the replacement residue is not cysteine. Substitution with one or more non-naturally occurring amino acid residues is also encompassed by the definition of an amino acid substitution herein. A "non-naturally occurring amino acid residue" refers to a residue, other than those naturally occurring amino acid residues listed above, which is able to covalently bind adjacent amino acid residues(s) in a polypeptide chain. Examples of non-naturally occurring amino acid residues include norleucine, ornithine, norvaline, homoserine, and other amino acid residue analogues such as those described in Ellman *et al.*, *Meth. Enzym.* 202:301-336 (1991). For generation of such non-naturally occurring amino acid residues, the procedures of Noren *et al.*, *Science* 244:182 (1989) and Ellman *et al.*, *supra*, can be used. Briefly, these procedures involve chemically activating a suppressor tRNA with a non-naturally occurring amino acid residue followed by *in vitro* transcription and translation of the RNA.

The term "conservative" amino acid substitution as used within this invention is meant to refer to amino acid substitutions that substitute functionally equivalent amino acids. Conservative amino acid changes result in silent changes in the amino acid sequence of the resulting polypeptide. For example, one or more amino acids of a similar polarity act as functional equivalents and result in a silent alteration within the amino acid sequence of the polypeptide. In general, substitutions within a group may be considered conservative with respect to structure and function. However, the skilled artisan will recognize that the role of a particular residue is determined by its context within the three-dimensional structure of the molecule in which it occurs. For example, Cys residues may occur in the oxidized (disulfide) form, which is less polar than the reduced (thiol) form. The long aliphatic portion of the Arg side chain may constitute a critical feature of its structural or functional role, and this may be best conserved by substitution of a nonpolar, rather than another basic, residue. Also, it will be recognized that side chains containing aromatic groups (Trp, Tyr, and Phe) can participate in ionic-aromatic or "cation-pi" interactions. In these cases, substitution of one of these side

chains with a member of the acidic or uncharged polar group may be conservative with respect to structure and function. Residues such as Pro, Gly, and Cys (disulfide form) can have direct effects on the main-chain conformation, and often may not be substituted without structural distortions.

5 An "amino acid insertion" refers to the incorporation of at least one amino acid into a predetermined amino acid sequence. While the insertion will usually consist of the insertion of one or two amino acid residues, the present application contemplates larger "peptide insertions", e.g., insertion of about three to about five or even up to about ten amino acid residues. The inserted residue(s) may be naturally occurring or non-naturally occurring as
10 disclosed above.

An "amino acid deletion" refers to the removal of at least one amino acid residue from a predetermined amino acid sequence.

Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New
15 York (1975)):

- (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)
- (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)
- (3) acidic: Asp (D), Glu (E)
- (4) basic: Lys (K), Arg (R), His(H)

20 Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- 25 (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

The "hinge region" is generally defined as stretching from Glu216 to Pro230 of human IgG1 (Burton, *Molec. Immunol.* 22:161-206 (1985)). Hinge regions of other IgG
30 isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions.

The "lower hinge region" of an Fc region is normally defined as the stretch of residues immediately C-terminal to the hinge region, i.e., residues 233 to 239 of the Fc region.

"C1q" is a polypeptide that includes a binding site for the Fc region of an immunoglobulin. C1q together with two serine proteases, C1r and C1s, forms the complex C1, the first component of the CDC pathway. Human C1q can be purchased commercially from, *e.g.* Quidel, San Diego, CA.

5 The term "binding domain" refers to the region of a polypeptide that binds to another molecule. In the case of an FcR, the binding domain can comprise a portion of a polypeptide chain thereof (*e.g.*, the α chain thereof) that is responsible for binding an Fc region. One useful binding domain is the extracellular domain of an FcR α chain.

10 "Functional fragments" of the antibodies of the invention comprise a portion of an intact antibody, generally including the antigen-binding or variable region of the intact antibody or the Fc region of an antibody that retains FcR binding capability. Examples of antibody fragments include linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments.

15 As used herein, the term "immunoadhesin" designates antibody-like molecules that combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity that is other than the antigen-recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant-domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant-domain sequence in the immunoadhesin can be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD, or IgM. For example, useful immunoadhesins as second medicaments herein include polypeptides that comprise the B-lymphocyte stimulator (BLyS) binding portions of a BLyS receptor without the transmembrane or cytoplasmic sequences of the BLyS receptor. In one embodiment, the extracellular domain of BR3 (BLyS receptor 3), TACI (transmembrane activator and calcium-modulator and cyclophilin ligand interactor), or BCMA (B-cell maturation antigen) is fused to a constant domain of an immunoglobulin sequence.

30 A "fusion protein" and a "fusion polypeptide" refer to a polypeptide having two portions covalently linked together, where each of the portions is a polypeptide having a different property. The property may be a biological property, such as activity *in vitro* or *in vivo*. The property may also be a simple chemical or physical property, such as binding to a

target molecule, catalysis of a reaction, *etc.* The two portions may be linked directly by a single peptide bond or through a peptide linker containing one or more amino acid residues. Generally, the two portions and the linker will be in reading frame with each other.

“Binding affinity” generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative embodiments are described in the following.

An “on-rate” or “rate of association” or “association rate” or “ k_{on} ” according to this invention can be determined with the surface plasmon resonance technique using a BIACORE®-2000 or a BIACORE® - 3000 (BIAcore, Inc., Piscataway, NJ) optical biosensor at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore Inc.) are activated with *N*-ethyl-*N*’-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier’s instructions. Antigen is diluted with 10mM sodium acetate, pH 4.8, into 5ug/ml (~0.2 μ M) before injection at a flow rate of 5 μ l/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of 1M ethanolamine to block unreacted groups, for kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in phosphate-buffered saline (PBS) with 0.05% TWEEN™ 20 (PBST) at 25°C at a flow rate of approximately 25 μ l/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2™) by simultaneous fitting the association and dissociation sensorgram. The equilibrium dissociation constant (K_d) was calculated as the ratio k_{off}/k_{on} . See, *e.g.*, Chen *et al.*, *J. Mol Biol* 293:865-881 (1999). However, if the on-rate exceeds $10^6 \text{ M}^{-1} \text{ S}^{-1}$ by the surface plasmon resonance assay above, then the on-rate is preferably determined by using a fluorescent quenching technique that measures the increase or decrease in

fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCOTM spectrophotometer (ThermoSpectronic) with a stirred cuvette.

The “Kd” or “Kd value” according to this invention is, in one embodiment, measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of the antibody and antigen molecule as described by the following assay that measures solution binding affinity of Fabs for antigen by equilibrating Fab with a minimal concentration of (¹²⁵I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (Chen *et al.*, *J. Mol Biol* 293:865-881 (1999)). To establish conditions for the assay, microtiter plates (Dynex) are coated overnight with 5 µg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [¹²⁵I]-antigen are mixed with serial dilutions of a Fab of interest (consistent with assessment of an anti-VEGF antibody, Fab-12, in Presta *et al.*, *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, 65 hours) to insure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature for one hour. The solution is then removed and the plate washed eight times with 0.1% TWEENTM-20 in PBS. When the plates have dried, 150 µl/well of scintillant (MicroScint-20; Packard) is added, and the plates are counted on a TOPCOUNTTM gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

The phrase “substantially similar” or “substantially the same,” as used herein, denotes a sufficiently high degree of similarity between two numeric values (generally one associated with an antibody of the invention and the other associated with a reference/comparator antibody) such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values). The difference between said two values is preferably less than about 50%, more preferably less than about 40%, still more preferably less than about 30%, even more preferably less than about 20%, and most

preferably less than about 10% as a function of the value for the reference/comparator antibody.

The phrase "substantially reduced," or "substantially different," as used herein, denotes a sufficiently high degree of difference between two numeric values (generally one associated with an antibody of the invention and the other associated with a reference/comparator antibody) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (*e.g.*, K_d values, HAMA response). The difference between said two values is preferably greater than about 10%, more preferably greater than about 20%, still more preferably greater than about 30%, even more preferably greater than about 40%, and most preferably greater than about 50% as a function of the value for the reference/comparator antibody.

"Percent (%) amino acid sequence identity" and "homology" with respect to a peptide or polypeptide sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, or MEGALIGNTM (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, authored by Genentech, Inc. The source code of ALIGN-2 has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given

amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

5 100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of
10 amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described herein using the ALIGN-2 computer program.

A "disorder" is any condition that would benefit from treatment with an antibody or
15 method of the invention, regardless of mechanism, but including inhibiting or blocking the action of $LT\alpha3$ or $LT\alpha\beta$ and/or by depleting $LT\alpha$ -positive cells. This condition includes, but is not limited to, a medical condition or illness mediated by or related to elevated expression or activity, or abnormal activation, of $LT\alpha3$ and/or $LT\alpha\beta$ by any cell. This includes chronic and acute disorders such as those pathological conditions that predispose the mammal to the
20 disorder in question.

An "autoimmune disorder" herein is a disease or disorder arising from and directed against an individual's own tissues or organs or a co-segregate or manifestation thereof or resulting condition therefrom. In many of these autoimmune and inflammatory disorders, a number of clinical and laboratory markers may exist, including, but not limited to,
25 hypergammaglobulinemia, high levels of autoantibodies, antigen-antibody complex deposits in tissues, benefit from corticosteroid or immunosuppressive treatments, and lymphoid cell aggregates in affected tissues. Without being limited to any one theory regarding B-cell mediated autoimmune disorder, it is believed that B cells demonstrate a pathogenic effect in human autoimmune diseases through a multitude of mechanistic pathways, including
30 autoantibody production, immune complex formation, dendritic and T-cell activation, cytokine synthesis, direct chemokine release, and providing a nidus for ectopic neolymphogenesis. Each of these pathways may participate to different degrees in the pathology of autoimmune diseases.

As used herein, an "autoimmune disorder" can be an organ-specific disease (*i.e.*, the immune response is specifically directed against an organ system such as the endocrine system, the hematopoietic system, the skin, the cardiopulmonary system, the gastrointestinal and liver systems, the renal system, the thyroid, the ears, the neuromuscular system, the central nervous system, *etc.*) or a systemic disease that can affect multiple organ systems (for example, systemic lupus erythematosus (SLE), rheumatoid arthritis, polymyositis, *etc.*). Preferred such diseases include autoimmune rheumatologic disorders (such as, for example, rheumatoid arthritis, Sjögren's syndrome, scleroderma, lupus such as SLE and lupus nephritis, polymyositis/dermatomyositis, cryoglobulinemia, anti-phospholipid antibody syndrome, and psoriatic arthritis), autoimmune gastrointestinal and liver disorders (such as, for example, inflammatory bowel diseases (e.g., ulcerative colitis and Crohn's disease), autoimmune gastritis and pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and celiac disease), vasculitis (such as, for example, ANCA-negative vasculitis and ANCA-associated vasculitis, including Churg-Strauss vasculitis, Wegener's granulomatosis, and microscopic polyangiitis), autoimmune neurological disorders (such as, for example, multiple sclerosis (MS), opsoclonus myoclonus syndrome, myasthenia gravis, neuromyelitis optica, Parkinson's disease, Alzheimer's disease, and autoimmune polyneuropathies), renal disorders (such as, for example, glomerulonephritis, Goodpasture's syndrome, and Berger's disease), autoimmune dermatologic disorders (such as, for example, psoriasis, urticaria, hives, pemphigus vulgaris, bullous pemphigoid, and cutaneous lupus erythematosus), hematologic disorders (such as, for example, thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, and autoimmune hemolytic anemia), atherosclerosis, uveitis, autoimmune hearing diseases (such as, for example, inner ear disease and hearing loss), Behcet's disease, Raynaud's syndrome, organ transplant, and autoimmune endocrine disorders (such as, for example, diabetic-related autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), Addison's disease, and autoimmune thyroid disease (*e.g.*, Graves' disease and thyroiditis)). More preferred such diseases include, for example, RA, IBD, including Crohn's disease and ulcerative colitis, ANCA-associated vasculitis, lupus, MS, Sjögren's syndrome, Graves' disease, IDDM, pernicious anemia, thyroiditis, and glomerulonephritis. Still more preferred are RA, IBD, lupus, and MS, and more preferred RA and IBD, and most preferred RA.

Specific examples of other autoimmune disorders as defined herein, which in some cases encompass those listed above, include, but are not limited to, arthritis (acute and

chronic, rheumatoid arthritis including juvenile-onset rheumatoid arthritis and stages such as rheumatoid synovitis, gout or gouty arthritis, acute immunological arthritis, chronic inflammatory arthritis, degenerative arthritis, type II collagen-induced arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, Still's disease, vertebral arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, menopausal arthritis, estrogen-depletion arthritis, and ankylosing spondylitis/rheumatoid spondylitis), autoimmune lymphoproliferative disease, inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, guttate psoriasis, pustular psoriasis, and psoriasis of the nails, atopy including atopic diseases such as hay fever and Job's syndrome, dermatitis including contact dermatitis, chronic contact dermatitis, exfoliative dermatitis, allergic dermatitis, allergic contact dermatitis, hives, dermatitis herpetiformis, nummular dermatitis, seborrheic dermatitis, non-specific dermatitis, primary irritant contact dermatitis, and atopic dermatitis, x-linked hyper IgM syndrome, allergic intraocular inflammatory diseases, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, myositis, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma (including systemic scleroderma), sclerosis such as systemic sclerosis, multiple sclerosis (MS) such as spino-optical MS, primary progressive MS (PPMS), and relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, ataxic sclerosis, neuromyelitis optica (NMO), inflammatory bowel disease (IBD) (for example, Crohn's disease, autoimmune-mediated gastrointestinal diseases, gastrointestinal inflammation, colitis such as ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, and transmural colitis, and autoimmune inflammatory bowel disease), bowel inflammation, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, respiratory distress syndrome, including adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, graft-versus-host disease, angioedema such as hereditary angioedema, cranial nerve damage as in meningitis, herpes gestationis, pemphigoid gestationis, pruritis scroti, autoimmune premature ovarian failure, sudden hearing loss due to an autoimmune condition, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis such as Rasmussen's encephalitis and limbic and/or brainstem encephalitis, uveitis, such as anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior

uveitis, or autoimmune uveitis, glomerulonephritis (GN) with and without nephrotic syndrome such as chronic or acute glomerulonephritis such as primary GN, immune-mediated GN, membranous GN (membranous nephropathy), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN (RPGN), proliferative nephritis, autoimmune polyglandular endocrine failure, balanitis including balanitis circumscripta plasmacellularis, balanoposthitis, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiform, granuloma annulare, lichen nitidus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, lichen planus, lamellar ichthyosis, epidermolytic hyperkeratosis, premalignant keratosis, pyoderma gangrenosum, allergic conditions and responses, food allergies, drug allergies, insect allergies, rare allergic disorders such as mastocytosis, allergic reaction, eczema including allergic or atopic eczema, asteatotic eczema, dyshidrotic eczema, and vesicular palmoplantar eczema, asthma such as asthma bronchiale, bronchial asthma, and auto-immune asthma, conditions involving infiltration of T cells and chronic inflammatory responses, immune reactions against foreign antigens such as fetal A-B-O blood groups during pregnancy, chronic pulmonary inflammatory disease, autoimmune myocarditis, leukocyte adhesion deficiency, lupus, including lupus nephritis, lupus cerebritis, pediatric lupus, non-renal lupus, extra-renal lupus, discoid lupus and discoid lupus erythematosus, alopecia lupus, SLE, such as cutaneous SLE or subacute cutaneous SLE, neonatal lupus syndrome (NLE), and lupus erythematosus disseminatus, juvenile onset (Type I) diabetes mellitus, including pediatric IDDM, adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, diabetic retinopathy, diabetic nephropathy, diabetic colitis, diabetic large-artery disorder, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including lymphomatoid granulomatosis, agranulocytosis, vasculitides (including large-vessel vasculitis such as polymyalgia rheumatica and giant-cell (Takayasu's) arteritis, medium-vessel vasculitis such as Kawasaki's disease and polyarteritis nodosa/periarteritis nodosa, immunovascularitis, CNS vasculitis, cutaneous vasculitis, hypersensitivity vasculitis, necrotizing vasculitis such as fibrinoid necrotizing vasculitis and systemic necrotizing vasculitis, ANCA-negative vasculitis, and ANCA-associated vasculitis such as Churg-Strauss syndrome (CSS), Wegener's granulomatosis, and microscopic polyangiitis), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic

anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia(s), cytopenias such as pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, Alzheimer's disease, Parkinson's disease, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex-mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, motoneuritis, allergic neuritis, Behçet's disease/syndrome, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjögren's syndrome, Stevens-Johnson syndrome, pemphigoid or pemphigus such as pemphigoid bullous, cicatricial (mucous membrane) pemphigoid, skin pemphigoid, pemphigus vulgaris, paraneoplastic pemphigus, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, and pemphigus erythematosus, epidermolysis bullosa acquisita, ocular inflammation, preferably allergic ocular inflammation such as allergic conjunctivitis, linear IgA bullous disease, autoimmune-induced conjunctival inflammation, autoimmune polyendocrinopathies, Reiter's disease or syndrome, thermal injury due to an autoimmune condition, preeclampsia, an immune complex disorder such as immune complex nephritis, antibody-mediated nephritis, neuroinflammatory disorders, polyneuropathies, chronic neuropathy such as IgM polyneuropathies or IgM-mediated neuropathy, thrombocytopenia (as developed by myocardial infarction patients, for example), including thrombotic thrombocytopenic purpura (TTP), post-transfusion purpura (PTP), heparin-induced thrombocytopenia, and autoimmune or immune-mediated thrombocytopenia including, for example, idiopathic thrombocytopenic purpura (ITP) including chronic or acute ITP, scleritis such as idiopathic cerato-scleritis, episcleritis, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune endocrine diseases including thyroiditis such as autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis), or subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, Grave's eye disease (ophthalmopathy or thyroid-associated ophthalmopathy), polyglandular syndromes such as autoimmune polyglandular syndromes, for example, type I (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis such as allergic encephalomyelitis or encephalomyelitis allergica and experimental allergic

encephalomyelitis (EAE), myasthenia gravis such as thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant-cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, pneumonitis such as lymphoid interstitial pneumonitis (LIP), bronchiolitis obliterans (non-transplant) vs. NSIP, Guillain-Barré syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, acute febrile neutrophilic dermatosis, subcorneal pustular dermatosis, transient acantholytic dermatosis, cirrhosis such as primary biliary cirrhosis and pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac or Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia such as mixed cryoglobulinemia, amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AIED), autoimmune hearing loss, polychondritis such as refractory or relapsed or relapsing polychondritis, pulmonary alveolar proteinosis, keratitis such as Cogan's syndrome/nonsyphilitic interstitial keratitis, Bell's palsy, Sweet's disease/syndrome, rosacea autoimmune, zoster-associated pain, amyloidosis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis (e.g., benign monoclonal gammopathy and monoclonal gammopathy of undetermined significance, MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal or segmental or focal segmental glomerulosclerosis (FSGS), endocrine ophthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases and chronic inflammatory demyelinating polyneuropathy, Dressler's syndrome, alopecia areata, alopecia totalis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, e.g., due to anti-spermatozoan antibodies, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial

lung disease, transfusion reaction, leprosy, malaria, parasitic diseases such as leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, fibrosing mediastinitis, pulmonary fibrosis, idiopathic

5 pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis (acute or chronic), or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, SCID, acquired immune deficiency syndrome (AIDS), echovirus infection, sepsis (systemic

10 inflammatory response syndrome (SIRS)), endotoxemia, pancreatitis, thyroxicosis, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis obliterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant-cell polymyalgia, chronic hypersensitivity

15 pneumonitis, conjunctivitis, such as vernal catarrh, keratoconjunctivitis sicca, and epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, transplant organ reperfusion, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway/pulmonary disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders (cerebral vascular insufficiency) such

20 as arteriosclerotic encephalopathy and arteriosclerotic retinopathy, aspermiogenesis, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensorineural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis

25 regionalis, leucopenia, mononucleosis infectiosa, transverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia sympathica (sympathetic ophthalmitis), neonatal ophthalmitis, optic neuritis, orchitis granulomatosa, pancreatitis, polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyroiditis, acquired splenic atrophy, non-malignant thymoma, lymphofollicular thymitis, vitiligo, toxic-shock syndrome, food poisoning,

30 conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease,

autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic
 gastritis, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulinitis,
 polyendocrine failure, autoimmune polyglandular syndromes, including polyglandular
 syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), cardiomyopathy such as
 5 dilated cardiomyopathy, epidermolysis bullosa acquisita (EBA), hemochromatosis,
 myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent
 sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, allergic
 sinusitis, an eosinophil-related disorder such as eosinophilia, pulmonary infiltration
 eosinophilia, eosinophilia-myalgia syndrome, Löffler's syndrome, chronic eosinophilic
 10 pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma,
 or granulomas containing eosinophils, anaphylaxis, spondyloarthropathies, seronegative
 spondyloarthritis, polyendocrine autoimmune disease, sclerosing cholangitis, sclera,
 episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient
 hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia
 15 syndrome, angiectasis, autoimmune disorders associated with collagen disease, rheumatism
 such as chronic arthrorheumatism, lymphadenitis, reduction in blood pressure response,
 vascular dysfunction, tissue injury, cardiovascular ischemia, hyperalgesia, renal ischemia,
 cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity
 disorders, glomerulonephritides, reperfusion injury, ischemic re-perfusion disorder,
 20 reperfusion injury of myocardial or other tissues, lymphomatous tracheobronchitis,
 inflammatory dermatoses, dermatoses with acute inflammatory components, multiple organ
 failure, bullous diseases, renal cortical necrosis, acute purulent meningitis or other central
 nervous system inflammatory disorders, ocular and orbital inflammatory disorders,
 granulocyte transfusion-associated syndromes, cytokine-induced toxicity, narcolepsy, acute
 25 serious inflammation, chronic intractable inflammation, pyelitis, endarterial hyperplasia,
 peptic ulcer, valvulitis, and endometriosis.

As used herein, "rheumatoid arthritis" or "RA" refers to a recognized disease state that
 may be diagnosed according to the 2000 revised American Rheumatoid Association criteria
 for the classification of RA, or any similar criteria, and includes active, early, and incipient
 30 RA, as defined below. Physiological indicators of RA include symmetric joint swelling,
 which is characteristic though not invariable in rheumatoid arthritis. Fusiform swelling of the
 proximal interphalangeal (PIP) joints of the hands as well as metacarpophalangeal (MCP),
 wrists, elbows, knees, ankles, and metatarsophalangeal (MTP) joints are commonly affected

and swelling is easily detected. Pain on passive motion is the most sensitive test for joint inflammation, and inflammation and structural deformity often limit the range of motion for the affected joint. Typical visible changes include ulnar deviation of the fingers at the MCP joints, hyperextension, or hyperflexion of the MCP and PIP joints, flexion contractures of the elbows, and subluxation of the carpal bones and toes. The subject with RA may be resistant to DMARDs, in that the DMARDs are not effective or fully effective in treating symptoms. Further candidates for therapy according to this invention include those who have experienced an inadequate response to previous or current treatment with TNF inhibitors such as etanercept, infliximab, and/or adalimumab because of toxicity or inadequate efficacy (for example, etanercept for 3 months at 25 mg twice a week or at least 4 infusions of infliximab at 3 mg/kg).

A patient with "active rheumatoid arthritis" means a patient with active and not latent symptoms of RA. Subjects with "early active rheumatoid arthritis" are those subjects with active RA diagnosed for at least eight weeks but no longer than four years, according to the revised 1987 ACR criteria for the classification of RA. Subjects with "early rheumatoid arthritis" are those subjects with RA diagnosed for at least eight weeks but no longer than four years, according to the revised 1987 ACR criteria for classification of RA. Early RA includes, for example, juvenile-onset RA, juvenile idiopathic arthritis (JIA), or juvenile RA (JRA).

Patients with "incipient RA" have early polyarthritis that does not fully meet ACR criteria for a diagnosis of RA, but is associated with the presence of RA-specific prognostic biomarkers such as anti-CCP and shared epitope. They include patients with positive anti-CCP antibodies who present with polyarthritis, but do not yet have a diagnosis of RA, and are at high risk for going on to develop *bona fide* ACR criteria RA (95% probability).

"Joint damage" is used in the broadest sense and refers to damage or partial or complete destruction to any part of one or more joints, including the connective tissue and cartilage, where damage includes structural and/or functional damage of any cause, and may or may not cause joint pain/arthritis. It includes, without limitation, joint damage associated with or resulting from inflammatory joint disease as well as non-inflammatory joint disease. This damage may be caused by any condition, such as an autoimmune disease, especially arthritis, and most especially RA. Exemplary such conditions include acute and chronic arthritis, RA including juvenile-onset RA, juvenile idiopathic arthritis (JIA), or juvenile RA (JRA), and stages such as rheumatoid synovitis, gout or gouty arthritis, acute immunological

arthritis, chronic inflammatory arthritis, degenerative arthritis, type II collagen-induced arthritis, infectious arthritis, septic arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, Still's disease, vertebral arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, menopausal arthritis, estrogen-depletion arthritis, and ankylosing spondylitis/rheumatoid spondylitis), rheumatic autoimmune disease other than RA, and significant systemic involvement secondary to RA (including but not limited to vasculitis, pulmonary fibrosis or Felty's syndrome). For purposes herein, joints are points of contact between elements of a skeleton (of a vertebrate such as an animal) with the parts that surround and support it and include, but are not limited to, for example, hips, joints between the vertebrae of the spine, joints between the spine and pelvis (sacroiliac joints), joints where the tendons and ligaments attach to bones, joints between the ribs and spine, shoulders, knees, feet, elbows, hands, fingers, ankles, and toes, but especially joints in the hands and feet.

As used herein, "treatment" refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishing of any direct or indirect pathological consequences of the disease, prevention of metastasis, decreasing of the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, the antibodies of the invention are used to delay development of a disease or disorder. A subject is successfully "treated" for example, for an autoimmune disorder if, after receiving a therapeutic amount of an antibody of the invention according to the methods of the present invention, the subject shows observable and/or measurable reduction in or absence of one or more signs and symptoms of the particular disease.

In one preferred embodiment of successful treatment, the antibody induces a major clinical response in a subject with RA. For purposes herein, a "major clinical response" is defined as achieving an American College of Rheumatology 70 response (ACR 70) for six consecutive months. ACR response scores are categorized as ACR 20, ACR 50, and ACR 70, with ACR 70 being the highest level of sign and symptom control in this evaluation system. ACR response scores measure improvement in RA disease activity, including joint swelling and tenderness, pain, level of disability, and overall patient and physician assessment. An

example of a different type of antibody that induces a major clinical response as recognized by the FDA and as defined herein is etanercept (ENBREL®).

An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

5 A "therapeutically effective amount" of a medicament herein may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicament, *e.g.*, antibody, to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the drug in question, *e.g.*, antibody, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, 10 to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or 15 prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.* At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³² and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small-molecule toxins or enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof.

20 A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, 25 triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); 30 bryostatin; callistatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; CDP323, an oral alpha-4 integrin

inhibitor; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, *e.g.*, Nicolaou *et al.*, *Angew. Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores); other antibiotics such as aclacinomycin, actinomycin, authramycin, azaserine, bleomycin, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycin, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®), and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycin, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, and trimetrexate; purine analogs such as fludarabine, 6-mercaptapurine, thiamiprine, and thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; anti-adrenals such as aminoglutethimide, mitotane, trilostane; and folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C");

thiotepa; taxoids, *e.g.*, paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and doxorubicin (DOXIL®); chlorambucil; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin and carboplatin; vinorelbine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovorin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

Also included in this definition are anti-hormonal agents that act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and are often in the form of systemic or whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene (EVISTA®), droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (FARESTON®); anti-progesterones; estrogen receptor down-regulators (ERDs); estrogen receptor antagonists such as fulvestrant (FASLODEX®); agents that function to suppress or shut down the ovaries, for example, leutinizing hormone-releasing hormone (LHRH) agonists such as leuprolide acetate (LUPRON® and ELIGARD®), goserelin acetate, busserelin acetate and triptorelin; anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate (MEGASE®), exemestane (AROMASIN®), formestane, fadrozole, vorozole (RIVISOR®), letrozole (FEMARA®), and anastrozole (ARIMIDEX®). In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in

signaling pathways implicated in adherent cell proliferation, such as, for example, PKC-
alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as
THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine,
LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1 inhibitor (*e.g.*,
5 LURTOTECAN®); rmrh (*e.g.*, ABARELIX®); lapatinib ditosylate (an ErbB-2 and EGFR
dual tyrosine kinase small-molecule inhibitor also known as GW572016); and
pharmaceutically acceptable salts, acids, or derivatives of any of the above.

The term "cytokine" is a generic term for proteins released by one cell population that
act on another cell as intercellular mediators. Examples of such cytokines are lymphokines,
10 monokines, interleukins (ILs) such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-
9, IL-11, IL-12, and IL-15, including PROLEUKIN® rIL-2, a TNF such as TNF- α or TNF- β ,
and other polypeptide factors including leukocyte-inhibitory factor (LIF) and kit ligand (KL).
As used herein, the term cytokine includes proteins from natural sources or from recombinant
cell culture and biologically active equivalents of the native-sequence cytokines, including
15 synthetically produced small-molecule entities and pharmaceutically acceptable derivatives
and salts thereof. A "cytokine antagonist" is a molecule that inhibits or antagonizes such
cytokines by any mechanism, including, for example, antibodies to the cytokine, antibodies to
the cytokine receptor, and immunoadhesins.

For purposes herein, "tumor necrosis factor alpha" or "TNF-alpha" or "TNF α " refers
20 to a human TNF-alpha molecule comprising the amino acid sequence as described in Pennica
et al., *Nature*, 312:721 (1984) or Aggarwal *et al.*, *J. Biol. Chem.*, 260:2345 (1985).

A "TNF antagonist" or "TNF inhibitor" is defined herein as a molecule that decreases,
blocks, inhibits, abrogates, or otherwise interferes with TNF α activity *in vitro*, *in situ*, and/or
preferably *in vivo*. Such an agent inhibits, to some extent, a biological function of TNF-
25 alpha, generally through binding to TNF-alpha and neutralizing its activity. A suitable TNF
antagonist can also decrease block, abrogate, interfere, prevent and/or inhibit TNF RNA,
DNA, or protein synthesis, TNF α release, TNF α receptor signaling, membrane TNF α
cleavage, TNF α activity, and TNF α production and/or synthesis. Such TNF antagonists
include, but are not limited to, anti-TNF α antibodies, antigen-binding fragments thereof,
30 specified mutants or domains thereof that bind specifically to TNF α that, upon binding to
TNF α , destroy or deplete cells expressing the TNF α in a mammal and/or interfere with one
or more functions of those cells, a soluble TNF receptor (*e.g.*, p55, p70 or p85) or fragment,
fusion polypeptides thereof, a small-molecule TNF antagonist, *e.g.*, TNF binding protein I or

II (TBP-I or TBP-II), nerelimonmab, CDP-571, infliximab (REMICADE®), etanercept (ENBREL®), adalimumab (HUMIRA™), CDP-571, CDP-870, afelimomab, lenercept, and the like), antigen-binding fragments thereof, and receptor molecules that bind specifically to TNF α , compounds that prevent and/or inhibit TNF α synthesis, TNF α release, or its action on target cells, such as thalidomide, tenidap, phosphodiesterase inhibitors (*e.g.*, pentoxifylline and rolipram), A2b adenosine receptor agonists, and A2b adenosine receptor enhancers, compounds that prevent and/or inhibit TNF α receptor signaling, such as mitogen-activated protein (MAP) kinase inhibitors, compounds that block and/or inhibit membrane TNF α cleavage, such as metalloproteinase inhibitors, compounds that block and/or inhibit TNF α activity, such as angiotensin-converting enzyme (ACE) inhibitors (*e.g.*, captopril), and compounds that block and/or inhibit TNF α production and/or synthesis, such as MAP kinase inhibitors. The preferred antagonist comprises an antibody or an immunoadhesin. Examples of TNF antagonists specifically contemplated herein are etanercept (ENBREL®), infliximab (REMICADE®), and adalimumab (HUMIRA™).

The term "hormone" refers to polypeptide hormones, which are generally secreted by glandular organs with ducts. Included among the hormones are, for example, growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; estradiol; hormone-replacement therapy; androgens such as calusterone, dromostanolone propionate, epitioestanol, mepitioestane, or testolactone; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); prolactin, placental lactogen, mouse gonadotropin-associated peptide, gonadotropin-releasing hormone; inhibin; activin; mullerian-inhibiting substance; and thrombopoietin. As used herein, the term hormone includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native-sequence hormone, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "growth factor" refers to proteins that promote growth, and include, for example, hepatic growth factor; fibroblast growth factor; vascular endothelial growth factor; nerve growth factors such as NGF- β ; platelet-derived growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- α , - β , and - γ ; and colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF), granulocyte-macrophage-CSF

(GM-CSF), and granulocyte-CSF (G-CSF). As used herein, the term growth factor includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native-sequence growth factor, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

5 The term "integrin" refers to a receptor protein that allows cells both to bind to and to respond to the extracellular matrix and is involved in a variety of cellular functions such as wound healing, cell differentiation, homing of tumor cells, and apoptosis. They are part of a large family of cell adhesion receptors that are involved in cell-extracellular matrix and cell-cell interactions. Functional integrins consist of two transmembrane glycoprotein subunits,
10 called alpha and beta, that are non-covalently bound. The alpha subunits all share some homology to each other, as do the beta subunits. The receptors always contain one alpha chain and one beta chain. Examples include Alpha6beta1, Alpha3beta1, Alpha7beta1, LFA-1, *etc.* As used herein, the term "integrin" includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native-sequence integrin,
15 including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

 An "integrin antagonist" is a molecule that inhibits or antagonizes such integrins by any mechanism, including, for example, antibodies to the integrin. Examples of "integrin antagonists or antibodies" herein include an LFA-1 antibody, such as efalizumab
20 (RAPTIVA[®]) commercially available from Genentech, or other CD11/11a and CD18 antibodies, or an alpha 4 integrin antibody such as natalizumab (ANTEGREN[®]) available from Biogen, or diazacyclic phenylalanine derivatives (WO 2003/89410), phenylalanine derivatives (WO 2003/70709, WO 2002/28830, WO 2002/16329 and WO 2003/53926), phenylpropionic acid derivatives (WO 2003/10135), enamine derivatives (WO 2001/79173),
25 propanoic acid derivatives (WO 2000/37444), alkanoic acid derivatives (WO 2000/32575), substituted phenyl derivatives (US 6,677,339 and 6,348,463), aromatic amine derivatives (US 6,369,229), ADAM disintegrin domain polypeptides (US 2002/0042368), antibodies to alphavbeta3 integrin (EP 633945), aza-bridged bicyclic amino acid derivatives (WO 2002/02556), *etc.*

30 "Corticosteroid" refers to any one of several synthetic or naturally occurring substances with the general chemical structure of steroids that mimic or augment the effects of the naturally occurring corticosteroids. Examples of synthetic corticosteroids include prednisone, prednisolone (including methylprednisolone, such as SOLU-MEDROL[®])

methylprednisolone sodium succinate), dexamethasone or dexamethasone triamcinolone, hydrocortisone, and betamethasone. The preferred corticosteroids herein are prednisone, methylprednisolone, hydrocortisone, or dexamethasone.

The term "immunosuppressive agent" as used herein for adjunct therapy refers to substances that act to suppress or mask the immune system of the mammal being treated herein. This would include substances that suppress cytokine production, down-regulate or suppress self-antigen expression, or mask the MHC antigens. Examples of such agents include 2-amino-6-aryl-5-substituted pyrimidines (see US 4,665,077); non-steroidal anti-inflammatory drugs (NSAIDs); ganciclovir, tacrolimus, glucocorticoids such as cortisol or aldosterone, anti-inflammatory agents such as a cyclooxygenase inhibitor, a 5-lipoxygenase inhibitor, or a leukotriene receptor antagonist; purine antagonists such as azathioprine or mycophenolate mofetil (MMF); trocade (Ro32-355); a peripheral sigma receptor antagonist such as ISR-31747; alkylating agents such as cyclophosphamide; bromocryptine; danazol; dapson; glutaraldehyde (which masks the MHC antigens, as described in US 4,120,649); anti-idiotypic antibodies for MHC antigens and MHC fragments; cyclosporin A; steroids such as corticosteroids or glucocorticosteroids or glucocorticoid analogs, *e.g.*, prednisone, methylprednisolone, including SOLU-MEDROL[®] methylprednisolone sodium succinate, rimexolone, and dexamethasone; dihydrofolate reductase inhibitors such as methotrexate (oral or subcutaneous); anti-malarial agents such as chloroquine and hydroxychloroquine; sulfasalazine; leflunomide; cytokine release inhibitors such as SB-210396 and SB-217969 monoclonal antibodies and a MHC II antagonist such as ZD2315; a PG1 receptor antagonist such as ZD4953; a VLA4 adhesion blocker such as ZD7349; anti-cytokine or anti-cytokine receptor antibodies including anti-interferon-alpha, -beta, or -gamma antibodies, anti-TNF-alpha antibodies (infliximab (REMICADE[®]) or adalimumab), anti-TNF-alpha immunoadhesin (etanercept), anti-TNF-beta antibodies, interleukin-1 (IL-1) blockers such as recombinant HuIL-1Ra and IL-1B inhibitor, anti-interleukin-2 (IL-2) antibodies and anti-IL-2 receptor antibodies; IL-2 fusion toxin; anti-L3T4 antibodies; leflunomide; heterologous anti-lymphocyte globulin; OPC-14597; NISV (immune response modifier); an essential fatty acid such as gammalinolenic acid or eicosapentaenoic acid; CD-4 blockers and pan-T antibodies, preferably anti-CD3 or anti-CD4/CD4a antibodies; co-stimulatory modifier (*e.g.*, CTLA4-Fc fusion, also known as ABATACEPTTM); anti-interleukin-6 (IL-6) receptor antibodies and antagonists; anti-LFA-1 antibodies, including anti-CD11a and anti-CD18 antibodies; soluble peptide containing a LFA-3-binding domain (WO 1990/08187); streptokinase; IL-10;

transforming growth factor-beta (TGF-beta); streptodornase; RNA or DNA from the host; FK506; RS-61443; enlimomab; CDP-855; PNP inhibitor; CH-3298; GW353430; 4162W94, chlorambucil; deoxyspergualin; rapamycin; T-cell receptor (Cohen *et al.*, US 5,114,721); T-cell receptor fragments (Offner *et al.*, *Science*, 251: 430-432 (1991); WO 1990/11294; Janeway, *Nature*, 341: 482-483 (1989); and WO 91/01133); BAFF antagonists such as BAFF antibodies and BR3 antibodies; zTNF4 antagonists (Mackay and Mackay, *Trends Immunol.*, 23:113-5 (2002)); biologic agents that interfere with T cell helper signals, such as anti-CD40 receptor or anti-CD40 ligand (CD154), including blocking antibodies to CD40-CD40 ligand (e.g., Durie *et al.*, *Science*, 261: 1328-30 (1993); Mohan *et al.*, *J. Immunol.*, 154: 1470-80 (1995)) and CTLA4-Ig (Finck *et al.*, *Science*, 265: 1225-7 (1994)); and T-cell receptor antibodies (EP 340,109) such as T10B9. Some preferred immunosuppressive agents herein include cyclophosphamide, chlorambucil, azathioprine, leflunomide, MMF, or methotrexate.

Examples of "disease-modifying anti-rheumatic drugs" or "DMARDs" include chloroquine, hydroxycloquine, mycristin, auranofin, sulfasalazine, methotrexate, leflunomide, etanercept, infliximab (plus oral and subcutaneous methotrexate), azathioprine, D-penicillamine, gold salts (oral), gold salts (intramuscular), minocycline, cyclosporine including cyclosporine A and topical cyclosporine, *staphylococcal* protein A (Goodyear and Silverman, *J. Exp. Med.*, 197, (9), p1125-39 (2003)), including salts and derivatives thereof, etc.

Examples of "non-steroidal anti-inflammatory drugs" or "NSAIDs" include aspirin, acetylsalicylic acid, ibuprofen and ibuprofen retard, fenoprofen, piroxicam, flurbiprofen, naproxen, ketoprofen, naproxen, tenoxicam, benorylate, diclofenac, naproxen, nabumetone, indomethacin, ketoprofen, mefenamic acid, diclofenac, fenbufen, azapropazone, acetaminophen, tiaprofenic acid, indomethacin, sulindac, tolmetin, phenylbutazone, diclofenac and diclofenac retard, cyclooxygenase (COX)-2 inhibitors such as GR 253035, MK966, celecoxib (CELEBREX®; 4-(5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl), benzenesulfonamide and valdecoxib (BEXTRA®), and meloxicam (MOBIC®), including salts and derivatives thereof, etc. Preferably, they are aspirin, naproxen, ibuprofen, indomethacin, or tolmetin. Such NSAIDs are optionally used with an analgesic such as codine, tramadol, and/or dihydrocodine or narcotic such as morphine.

A "B cell" is a lymphocyte that matures within the bone marrow, and includes a naïve B cell, memory B cell, or effector B cell (plasma cells). The B cell herein may be a normal or non-malignant B cell.

The "CD20" antigen, or "CD20," is an about 35-kDa, non-glycosylated phosphoprotein found on the surface of greater than 90% of B cells from peripheral blood or lymphoid organs. CD20 is present on both normal B cells as well as malignant B cells, but is not expressed on stem cells. Other names for CD20 in the literature include "B-lymphocyte-restricted antigen" and "Bp35". The CD20 antigen is described in Clark *et al.*, *Proc. Natl. Acad. Sci. (USA)* 82:1766 (1985), for example. The preferred CD20 is non-human primate or human CD20, most preferably human CD20.

The "CD22" antigen, or "CD22," also known as BL-CAM or Lyb8, is a type 1 integral membrane glycoprotein with molecular weight of about 130 (reduced) to 140kD (unreduced). It is expressed in both the cytoplasm and cell membrane of B-lymphocytes. CD22 antigen appears early in B-cell lymphocyte differentiation at approximately the same stage as the CD19 antigen. Unlike other B-cell markers, CD22 membrane expression is limited to the late differentiation stages comprised between mature B cells (CD22+) and plasma cells (CD22-). The CD22 antigen is described, for example, in Wilson *et al.*, *J. Exp. Med.* 173:137 (1991) and Wilson *et al.*, *J. Immunol.* 150:5013 (1993).

An "antagonist to a B-cell surface marker" is a molecule that, upon binding to a B-cell surface marker, destroys or depletes B cells in a mammal and/or interferes with one or more B-cell functions, *e.g.* by reducing or preventing a humoral response elicited by the B cell. The antagonist preferably is able to deplete B cells (*i.e.* reduce circulating B-cell levels) in a mammal treated therewith. Such depletion may be achieved via various mechanisms such as ADCC and/or CDC, inhibition of B-cell proliferation and/or induction of B-cell death (*e.g.* via apoptosis). Antagonists included within the scope of the present invention include antibodies, synthetic or native-sequence peptides, immunoadhesins, and small-molecule antagonists that bind to a B-cell surface marker, optionally conjugated with or fused to another molecule. The preferred antagonist comprises an antibody or immunoadhesin. It includes BLyS antagonists such as immunoadhesins, and is preferably lumiliximab (anti-CD23), CD20, CD22, or BR3 antibody or BLyS immunoadhesin. The BLyS immunoadhesin preferably is selected from the group consisting of BR3 immunoadhesin comprising the extracellular domain of BR3, TACI immunoadhesin comprising the extracellular domain of TACI, and BCMA immunoadhesin comprising the extracellular domain of BCMA. The most preferred BR3 immunoadhesin is hBR3-Fc of SEQ ID NO:2 of WO 2005/00351 and US 2005/0095243. See also US 2005/0163775 and WO 2006/068867. Another preferred BLyS antagonist is an anti-BLyS antibody, more preferably wherein the anti-BLyS antibody binds

BLyS within a region of BLyS comprising residues 162-275, or an anti-BR3 antibody, more preferably wherein the anti-BR3 antibody binds BR3 in a region comprising residues 23-38 of human BR3.

Examples of CD20 antibodies include: "C2B8," which is now called "rituximab" ("RITUXAN®") (US 5,736,137) and fragments thereof that retain the ability to bind CD20; the yttrium-[90]-labelled 2B8 murine antibody designated "Y2B8" or "Ibritumomab Tiuxetan" (ZEVALIN®) commercially available from IDEC Pharmaceuticals, Inc. (US 5,736,137; 2B8 deposited with ATCC under accession no. HB11388 on June 22, 1993); murine IgG2a "B1," also called "Tositumomab," optionally labelled with ¹³¹I to generate the "131I-B1" or "iodine I131 tositumomab" antibody (BEXXAR™) commercially available from Corixa (see, also, US 5,595,721); murine monoclonal antibody "1F5" (Press *et al. Blood* 69(2):584-591 (1987) and variants thereof including "framework patched" or humanized 1F5 (WO 2003/002607, Leung, S.; ATCC deposit HB-96450); murine 2H7 and chimeric 2H7 antibody (US 5,677,180); a humanized 2H7 (WO 2004/056312 (Lowman *et al.*) and as set forth below); HUMAX-CD20™ fully human, high-affinity antibody targeted at the CD20 molecule in the cell membrane of B-cells (Genmab, Denmark; see, for example, Glennie and van de Winkel, *Drug Discovery Today* 8: 503-510 (2003) and Cragg *et al., Blood* 101: 1045-1052 (2003)); the human monoclonal antibodies set forth in WO 2004/035607 (Teeling *et al.*); AME-133™ antibodies (Applied Molecular Evolution); GA101 (GlycArt; US 2005/0123546); A20 antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, hA20, respectively) (US 2003/0219433, Immunomedics); and monoclonal antibodies L27, G28-2, 93-1B3, B-C1 or NU-B2 available from the International Leukocyte Typing Workshop (Valentine *et al.*, In: *Leukocyte Typing III* (McMichael, Ed., p. 440, Oxford University Press (1987)). The preferred CD20 antibodies herein are chimeric, humanized, or human CD20 antibodies, more preferably rituximab, a humanized 2H7, chimeric or humanized A20 antibody (Immunomedics), and HUMAX-CD20™ human CD20 antibody (Genmab).

Purely for the purposes herein and unless indicated otherwise, a "humanized 2H7" refers to a humanized CD20 antibody, or an antigen-binding fragment thereof, wherein the antibody is effective to deplete primate B cells *in vivo*. The antibody includes those set forth in US 2006/0062787 and the figures thereof, and including those antibodies the sequences of which are provided in US 2006/0188495. See also US 2006/0034835 and US 2006/0024300. In a summary of various preferred embodiments of the invention, the V region of variants

based on 2H7 version 16 as disclosed in US 2006/0062787 will have the amino acid sequences of v16 except at the positions of amino acid substitutions that are indicated in the table below. Unless otherwise indicated, the 2H7 variants will have the same L chain as that of v16.

2H7 version	Heavy chain (V _H) changes	Light chain (V _L) changes	Fc changes
16			-
A	-	-	S298A, E333A, K334A
B	N100A	M32L	
C	N100A	M32L	S298A, E333A, K334A
D	D56A, N100A	S92A	
E	D56A, N100A	M32L, S92A	S298A, E333A, K334A
F	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L
G	D56A, N100A	M32L, S92A	S298A, K334A, K322A
H	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A
I	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A, N434W
J	-	-	K334L

5

The preferred humanized 2H7 is an intact antibody or antibody fragment having the sequence of version 16, or any of the versions shown above.

"BAFF antagonists" herein are any molecules that block the activity of BAFF or BR3. They include immunoadhesins comprising a portion of BR3, TACI, or BCMA that binds BAFF, or variants thereof that bind BAFF. In other embodiments, the BAFF antagonist is a BAFF antibody. A "BAFF antibody" is an antibody that binds BAFF, and preferably binds BAFF within a region of human BAFF comprising residues 162-275 of human BAFF. In another embodiment, the BAFF antagonist is BR3 antibody. A "BR3 antibody" is an antibody that binds BR3, and is preferably one that binds BR3 within a region of human BR3 comprising residues 23-38 of human BR3. The sequences of human BAFF and human BR3 are found, for example, in US 2006/0062787. Other examples of BAFF-binding polypeptides or BAFF antibodies can be found in, *e.g.*, WO 2002/092620, WO 2003/014294, Gordon *et al.*, *Biochemistry* 42(20):5977-5983 (2003), Kelley *et al.*, *J. Biol. Chem.*, 279(16):16727-16735 (2004), WO 1998/18921, WO 2001/12812, WO 2000/68378 and WO 2000/40716.

20

"Chronic" administration refers to administration of the medicament(s) in a

continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH-buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low-molecular-weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEENTM, polyethylene glycol (PEG), and PLURONICSTM.

The term "mammal" refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal herein is human.

A "package insert" refers to instructions customarily included in commercial packages of medicaments that contain information about the indications, usage, dosage, administration, contraindications, other medicaments to be combined with the packaged product, and/or warnings concerning the use of such medicaments, *etc.*

A "kit" is any manufacture (*e.g.*, a package or container) comprising at least one reagent, *e.g.*, a medicament for treatment of an autoimmune disease such as RA, lupus, MS, or IBD. The manufacture is preferably promoted, distributed, or sold as a unit for performing the methods of the present invention.

A "target audience" is a group of people or an institution to whom or to which a particular medicament is being promoted or intended to be promoted, as by marketing or advertising, especially for particular uses, treatments, or indications, such as individual patients, patient populations, readers of newspapers, medical literature, and magazines, television or internet viewers, radio or internet listeners, physicians, drug companies, *etc.*

A "medicament" is an active drug to treat the disorder in question or its symptoms or side effects.

The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field.

Modes for Carrying Out the Invention

In one embodiment, the invention contemplates an isolated LT α antibody comprising at least one CDR sequence selected from the group consisting of:

(a) a CDR-L1 sequence comprising amino acids A1-A11, wherein A1-A11 is KASQAVSSAVA (SEQ ID NO:1) or RASQAVSSAVA (SEQ ID NO:2), or comprising amino acids A1-A17, wherein A1-A17 is KSSQSLLYSTXQKXFLA (SEQ ID NO:3) or KSSQSLLYSAXQKXFLA (SEQ ID NO:4) or KSSQSLLYSTXQKXALA (SEQ ID NO:6), where X is any amino acid (chimeric 2C8 or humanized 2C8.v2/2C8.vX or chimeric 3F12/humanized 3F12.v5/humanized 3F12.v14, or 3F12 clone 14 or 17, respectively);

(b) a CDR-L2 sequence comprising amino acids B1-B7, wherein B1-B7 is SASHRYT (SEQ ID NO:7) or WASTRDS (SEQ ID NO:8) (chimeric 2C8/humanized 2C8.v2/humanized 2C8.vX or chimeric 3F12/humanized 3F12.v5/ humanized 3F12.v14, respectively);

(c) a CDR-L3 sequence comprising amino acids C1-C9, wherein C1-C9 is QQHYSTPWT (SEQ ID NO:9) or QEXYSTPWT (SEQ ID NO:11) or QQYYSPRT (SEQ ID NO:13) or QQYASYPRT (SEQ ID NO:14) or QQYYAYPRT (SEQ ID NO:15), where X is any amino acid (chimeric 2C8/humanized 2C8.v2 or 2C8 clone G7 or chimeric 3F12/humanized 3F12.v5/ humanized 3F12.v14 or 3F12 clone 20 or 3F12 clone 21, respectively);

(d) a CDR-H1 sequence comprising amino acids D1-D10, wherein D1-D10 is GYTFTSYVIH (SEQ ID NO:16) or GYTFSSYWIE (SEQ ID NO:17) (chimeric 2C8/humanized 2C8.v2/humanized 2C8.vX or chimeric 3F12/humanized 3F12.v5/ humanized 3F12.v14, respectively);

(e) a CDR-H2 sequence comprising amino acids E1-E17, wherein E1-E17 is YXXPYXDGTXYXEKFKG (SEQ ID NO:18) or EISPGSGSTXYXEEFKG (SEQ ID NO:19) or YXXPYXAGTXYXEKFKG (SEQ ID NO:101) or EIXPGSGSTIYXEKFKG (SEQ ID NO:110), wherein X is any amino acid (chimeric 2C8/humanized 2C8.v2 or chimeric 3F12/humanized 3F12.v5, or humanized 2C8.vX, or humanized 3F12.v14, respectively); and

(f) a CDR-H3 sequence comprising amino acids F1-F9, wherein F1-F9 is PTMLPWFAY (SEQ ID NO:20), or comprising amino acids F1-F5, wherein F1-F5 is

GYHGY (SEQ ID NO:21) or GYHGA (SEQ ID NO:22) (chimeric 2C8/humanized 2C8.v2/humanized 2C8.vX or chimeric 3F12/humanized 3F12.v5/ humanized 3F12.v14 or 3F12 clone 12, respectively).

In one preferred embodiment, SEQ ID NO:3 is KSSQSLLYSTAQKXFLA (SEQ ID NO:5) (3F12 clone 15). In a further preferred embodiment, SEQ ID NO:11 is QESYSTPWT (SEQ ID NO:10) (2C8 clone A8/humanized 2C8.vX) or QEVYSTPWT (SEQ ID NO:12) (2C8 clone H6).

In another preferred embodiment, the CDR-L1 sequence is SEQ ID NO:2 or 3 or 4 or 6.

In another preferred aspect, the antibody comprises either (i) all of the CDR-L1 to CDR-L3 amino acid sequences of SEQ ID NOS:1 or 2 and 7 and 9, or of SEQ ID NOS:1 or 2 and 7 or 8 and 11, or of SEQ ID NOS:3, 8, and 13, or of SEQ ID NOS:4, 5, or 6, 8, and 13, or of SEQ ID NOS:3, 8, and 14 or 15, or of SEQ ID NOS:4, 5, or 6, 8, and 14 or 15; or (ii) all of the CDR-H1 to CDR-H3 amino acid sequences of SEQ ID NOS:16, 18, and 20, or all of SEQ ID NOS:16, 101, and 20, or all of SEQ ID NOS:17, 19, and 21 or 22, or all of SEQ ID NOS:17, 110, and 21.

In another aspect, the antibody comprises either all of SEQ ID NOS:1 or 2 and 7 and 9, or all of SEQ ID NOS:16, 18, and 20, or all of SEQ ID NOS:16, 101, and 20. In an alternative embodiment, the antibody comprises either all of SEQ ID NOS:3, 8, and 13, or all of SEQ ID NOS:17, 19, and 21 or 22. In an alternative embodiment, the antibody comprises either all of SEQ ID NOS:4, 8, and 14, or all of SEQ ID NOS:17, 110, and 21. In other embodiments, the antibody comprises (i) all of the CDR-L1 to CDR-L3 amino acid sequences of SEQ ID NOS:1 or 2, 7 and 9, or of SEQ ID NOS:1 or 2 and 7 or 8 and 11, or of SEQ ID NOS:3, 8, and 13, or of SEQ ID NOS:4, 5, or 6, 8, and 13, or of SEQ ID NOS:3, 8, and 14 or 15, or of SEQ ID NOS:4, 5, or 6, 8, and 14 or 15; and (ii) all of the CDR-H1 to CDR-H3 amino acid sequences of SEQ ID NOS:16, 18, and 20, or of SEQ ID NOS:16, 101, and 20, or of SEQ ID NOS:17, 19, and 21 or 22, or of SEQ ID NOS:17, 110, and 21.

One preferred aspect is an anti- LT α antibody having a light-chain variable domain comprising SEQ ID NO:23 or 24, or a heavy-chain variable domain comprising SEQ ID NO:25 or 26, or having light-chain and heavy-chain variable domains comprising both SEQ ID NOS:23 and 25 or both SEQ ID NOS:24 and 26. Further preferred is an anti-LT α antibody having a light-chain variable domain comprising SEQ ID NO:27 or 28, or a heavy-chain variable domain comprising SEQ ID NO:29, 30, or 31, or having light-chain and

heavy-chain variable domains comprising both SEQ ID NOS:27 and 29, or both SEQ ID NOS:27 and 30, or both SEQ ID NOS:27 and 31, or both SEQ ID NOS:28 and 30, or both SEQ ID NOS:28 and 31. In a still further aspect, the invention provides an anti-LT α antibody having a light-chain variable domain comprising SEQ ID NO:102, or a heavy-chain variable domain comprising SEQ ID NO:103, or having light-chain and heavy-chain variable domains comprising both SEQ ID NOS:102 and 103. In a still further aspect, the invention provides an anti-LT α antibody having a light-chain variable domain comprising SEQ ID NO:108, or a heavy-chain variable domain comprising SEQ ID NO:109, or having light-chain and heavy-chain variable domains comprising both SEQ ID NOS:108 and 109.

Antibodies in other embodiments comprise a human κ subgroup 1 consensus framework sequence, and/or they comprise a heavy-chain human subgroup III consensus framework sequence, wherein the framework sequence preferably comprises a substitution at position 71, 73, and/or 78. Such substitutions are preferably R71A, N73T, or N78A, or any combination thereof, most preferably all three.

The antibodies of this invention preferably bind to LT α 3 and block the interaction of LT α 3 with TNFRI and TNFRII. Preferably, they also bind to LT $\alpha\beta$ complex and especially on the cell surface. Preferably, they also block LT $\alpha\beta$ function, and/or preferably contain an Fc region, and/or decrease levels of inflammatory cytokines associated with RA in an *in vitro* collagen-induced arthritis assay or an *in vitro* antibody-induced arthritis assay.

Further preferred antibodies block the interaction of LT $\alpha\beta$ with LT β -R and/or modulate LT $\alpha\beta$ -expressing cells. In other preferred embodiments, the anti-LT α antibody herein substantially neutralizes at least one activity of at least one LT α protein. Preferably, the antibody herein targets any cell expressing LT α , and preferably depletes LT α -positive or -secreting cells.

Preferred antibodies herein bind LT α with an affinity of at least about 10^{-12} M, more preferably at least about 10^{-13} M. The antibodies also preferably are of the IgG isotype, such as IgG1, IgG2a, IgG2b, or IgG3, more preferably human IgG, and most preferably IgG1 or IgG2a.

Another preferred antibody has a monovalent affinity to human LT α that is about the same as or greater than the monovalent affinity to human LT α of a murine antibody produced by a hybridoma cell line deposited under American Type Culture Collection Accession Number PTA-7538 (hybridoma murine Lymphotoxin alpha2 beta1 s5H3.2.2).

As is well established in the art, binding affinity of a ligand to its receptor can be determined using any of a variety of assays, and expressed in terms of a variety of quantitative values. Accordingly, in one embodiment, the binding affinity is expressed as K_d values and reflects intrinsic binding affinity (*e.g.*, with minimized avidity effects). Generally and preferably, binding affinity is measured *in vitro*, whether in a cell-free or cell-associated setting. Fold difference in binding affinity can be quantified in terms of the ratio of the monovalent binding affinity value of a humanized antibody (*e.g.*, in Fab form) and the monovalent binding affinity value of a reference/comparator antibody (*e.g.*, in Fab form) (*e.g.*, a murine antibody having donor CDR sequences), wherein the binding affinity values are determined under similar assay conditions.

Thus, in one embodiment, the fold difference in binding affinity is determined as the ratio of the K_d values of the humanized antibody in Fab form and said reference/comparator Fab antibody. For example, in one embodiment, if an antibody of the invention (A) has an affinity that is “three-fold lower” than the affinity of a reference antibody (M), then if the K_d value for A is 3x, the K_d value of M would be 1x, and the ratio of K_d of A to K_d of M would be 3:1. Conversely, in one embodiment, if an antibody of the invention (C) has an affinity that is “three-fold greater” than the affinity of a reference antibody (R), then if the K_d value for C is 1x, the K_d value of R would be 3x, and the ratio of K_d of C to K_d of R would be 1:3. Any assays known in the art, including those described herein, can be used to obtain binding affinity measurements, including, for example, an optical biosensor that uses SPR (BIAcore® technology), RIA, and ELISA. Preferably, the measurement is by optical biosensor or radioimmunoassay.

The antibodies herein are preferably chimeric or humanized, more preferably humanized, and still more preferably antibodies wherein at least a portion of their framework sequence is a human consensus framework sequence.

Another embodiment herein is an anti-idiotypic antibody that specifically binds the antibody herein.

Monoclonal antibodies

Monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (US 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as described above to elicit lymphocytes that produce or are capable

of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium which medium preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells (also referred to as fusion partner). For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred fusion partner myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 and derivatives, *e.g.*, X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); and Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as RIA or ELISA.

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for

example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal, *e.g.*, by i.p. injection of the cells into mice.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (*e.g.*, using protein A or protein G-SEPHAROSETM medium) or ion-exchange chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, *etc.*

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high-affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA that encodes the antibody may be modified to produce chimeric or fusion antibody polypeptides, for example, by substituting human heavy-chain and light-chain constant-domain (C_H and C_L) sequences for the homologous murine sequences (US 4,816,567 and Morrison, *et al.*, *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by fusing the immunoglobulin coding sequence with all or part of the coding sequence for a non-immunoglobulin polypeptide (heterologous polypeptide). The non-immunoglobulin

polypeptide sequences can substitute for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

5 *Humanized antibodies*

The present invention encompasses humanized antibodies. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al. Nature* 321:522-525 (1986); Riechmann *et al. Nature* 332:323-327 (1988); Verhoeven *et al. Science* 239:1534-1536 (1988)), by substituting CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (US 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the rodent is then accepted as the human framework for the humanized antibody (Sims *et al., J. Immunol.* 151:2296 (1993); Chothia *et al., J. Mol. Biol.* 196:901 (1987). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al., Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al. J. Immunol.*, 151:2623 (1993).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly

available and are familiar to those skilled in the art. Computer programs are available that 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, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

The humanized antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody may be a full-length antibody, such as a full-length IgG1 antibody.

Human antibodies and phage display methodology

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggemann *et al.*, *Year in Immuno.*, 7:33 (1993); US 5,545,806, 5,569,825, 5,591,669 (all of GenPharm); and 5,545,807; and WO 1997/17852.

Alternatively, phage-display technology (McCafferty *et al.*, *Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody

exhibiting those properties. Thus, the phage mimics some of the properties of the LT α cell. Phage display can be performed in a variety of formats, reviewed in, e.g., Johnson and Chiswell, *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991) or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). See, also, US 5,565,332 and 5,573,905.

As discussed above, human antibodies may also be generated by *in vitro* activated B cells (see US 5,567,610 and 5,229,275).

Antibody fragments

In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al.*, *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')₂ fragment with increased *in vivo* half-life comprising a salvage receptor binding epitope residues are described in U.S. Patent No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See, e.g., WO 1993/16185; U.S. 5,571,894; and U.S. 5,587,458. Fv and sFv are the only species with intact combining sites that are devoid of constant regions; thus, they are suitable for reduced nonspecific binding during *in vivo* use. sFv fusion proteins may

be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an sFv. The antibody fragment may also be a "linear antibody", *e.g.*, as described in U.S. 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

5 *Bispecific antibodies*

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the LT α protein. Other such antibodies may combine a LT α binding site with a binding site for another protein. Alternatively, an anti-LT α arm may be combined with an arm that binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD3), or Fc receptors for IgG (Fc γ R), such as Fc γ RI (CD64), Fc γ RII (CD32) and Fc γ RIII (CD16), or NKG2D or other NK-cell-activating ligand, so as to focus and localize cellular defense mechanisms to the LT α -expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells that express LT α . These antibodies possess a LT α -binding arm and an arm that binds the cytotoxic agent (*e.g.*, saporin, anti-interferon- α , vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full-length antibodies or antibody fragments (*e.g.*, F(ab')₂ bispecific antibodies).

WO 1996/16673 describes a bispecific anti-ErbB2/anti-Fc γ RIII antibody and US 5,837,234 discloses a bispecific anti-ErbB2/anti-Fc γ RI antibody. A bispecific anti-ErbB2/Fc α antibody is shown in WO 1998/02463. US 5,821,337 teaches a bispecific anti-ErbB2/anti-CD3 antibody.

Methods for making bispecific antibodies are known in the art. Traditional production of full-length bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 1993/08829, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant-

domain sequences. Preferably, the fusion is with an Ig heavy-chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. It is preferred to have the first heavy-chain constant region (C_H1) containing the site necessary for light-chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant effect on the yield of the desired chain combination.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy-chain/light-chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 1994/04690. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

In another approach (US 5,731,168), the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers that are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to

unwanted cells (US 4,676,980), and for treatment of HIV infection (WO 1991/00360, WO 1992/20373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed, for example, in US 4,676,980, along with a number of cross-linking techniques.

5 Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science*, 229: 81 (1985) describes a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize
10 vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of
15 enzymes.

 Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.*, 175: 217-225 (1992) describes the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to
20 directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

 Various techniques for making and isolating bispecific antibody fragments directly
25 from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody
30 heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Holliger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a V_H connected to a V_L by a linker that is too short to

allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.*, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, *J. Immunol.* 147: 60 (1991).

Multivalent Antibodies

A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen-binding sites (*e.g.*, tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen-binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen-binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise $VD1-(X1)_n-VD2-(X2)_n-Fc$, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable-domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light-chain variable-domain polypeptides. The light-chain variable-domain polypeptides contemplated herein comprise a light-chain variable-domain and, optionally, further comprise a CL domain.

Vectors, Host Cells, and Recombinant Methods

Selection and transformation of host cells

Suitable host cells for cloning or expressing the recombinant monoclonal antibodies,

immunoadhesins, and other polypeptide antagonists described herein are prokaryote, yeast, or higher eukaryotic cells. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g.,
5 *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable.

10 These examples are illustrative rather than limiting.

Full-length antibody, antibody fragments, and antibody fusion proteins can be produced in bacteria, in particular when glycosylation and Fc effector functions are not needed, such as when the therapeutic antibody is conjugated to a cytotoxic agent (e.g., a toxin) and the immunoconjugate by itself shows effectiveness in tumor cell destruction. Full-
15 length antibodies have greater half life in circulation. Production in *E. coli* is faster and more cost efficient. For expression of antibody fragments and polypeptides in bacteria, see, e.g., US 5,648,237; 5,789,199; and 5,840,523, which describe translation- initiation region (TIR) and signal sequences for optimizing expression and secretion. After expression, the antibody is isolated from the *E. coli* cell paste in a soluble fraction and can be purified through, e.g., a
20 protein A or G column depending on the isotype. Final purification can be carried out similar to the process for purifying antibody expressed e.g., in CHO cells. For general monoclonal antibody production, see also US 7,011,974.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding, such as LT α -antibody-encoding,
25 vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, e.g., *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum*
30 (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g.,

Neurospora, *Penicillium*, *Tolyopocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

Suitable host cells for the expression of, e.g., glycosylated LT α -binding antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (e.g., 293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, e.g., ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)), including, but not limited to CHO K1, CHO pro3⁻, CHO DG44, CHO DUXB11, Lec13, B-Ly1, and CHO DP12 cells, preferably a CHO DUX (DHFR-) or subclone thereof (herein called "CHO DUX"); C127 cells, mouse L cells; Ltk⁻ cells; mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse myeloma cells; NS0; hybridoma cells such as mouse hybridoma cells; COS cells; mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with expression or cloning vectors for production of the LT α -binding antibody herein, and cultured in conventional nutrient media modified as

appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Culturing the host cells

The host cells used to produce an antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), US 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 1990/03430; WO 1987/00195; or US Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCINTM drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

In one particular aspect, a suitable medium contains a basal medium component such as a DMEM/HAM F-12 based formulation (for composition of DMEM and HAM F12 media and especially serum-free media, see culture media formulations in the *American Type Culture Collection Catalogue of Cell Lines and Hybridomas*, Sixth Edition, 1988, pages 346-349) (the formulations of medium as described in US 5,122,469 may be appropriate) with suitably modified, if necessary, concentrations of some components such as amino acids, salts, sugar, and vitamins, and optionally containing glycine, hypoxanthine, and thymidine; recombinant human insulin, hydrolyzed peptone, such as PROTEASE PEPTONE 2 and 3TM, PRIMATONE HSTM or PRIMATONE RLTM (Difco, USA; Sheffield, England), or the equivalent; a cell-protective agent, such as PLURONIC F68TM or the equivalent PLURONICTM polyol; GENTAMYCINTM antibiotic; and trace elements. Preferably the cell culture media is serum free.

In one aspect of the invention, antibody production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for

production of recombinant proteins. Large-scale fermentations have at least about 1000 liters of capacity, preferably about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose (the preferred carbon/energy source). Small-scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about one liter to about 100 liters.

In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, *e.g.*, an OD₅₅₀ of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

To improve the production yield and quality of the polypeptides of the invention, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of the secreted antibody polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl *cis,trans*-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen *et al.*, *J Bio Chem* 274:19601-19605 (1999); US 6,083,715 and 6,027,888; Bothmann and Pluckthun, *J. Biol. Chem.* 275:17100-17105 (2000); Ramm and Pluckthun, *J. Biol. Chem.* 275:17106-17113 (2000); and Arie *et al.*, *Mol. Microbiol.* 39:199-210 (2001).

To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present invention. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some *E. coli* protease-deficient strains are available and described in, for example, US 5,264,365; 5,508,192; and Hara *et al.*, *Microbial Drug Resistance*, 2:63-72 (1996).

In one embodiment, *E. coli* strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins are used as host cells in the expression system herein.

Purification of antibody

When using recombinant techniques, the antibody can be produced intracellularly, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an AMICONTM or MILLIPORE PELLICONTM ultrafiltration unit. A protease inhibitor such as phenylmethylsulphonylfluoride (PMSF) may be included in any of the foregoing steps to inhibit proteolysis, and antibiotics may be included to prevent the growth of adventitious contaminants.

In one embodiment, the antibody protein produced herein is further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: hydroxylapatite chromatography, chromatography on heparin SEPHAROSETM, gel electrophoresis, dialysis, fractionation on immunoaffinity columns, ethanol precipitation, reverse-phase HPLC, chromatography on silica, chromatography on an anion- or cation-exchange resin (such as DEAE or a polyaspartic acid column), chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, SEPHADEXTM G-75 resin. Affinity chromatography is a preferred purification technique. For analytical-scale purification, smaller volumes are passed through columns and used; for preparative- or commercial-scale purification to produce quantities of antibody useful in therapeutic applications, larger volumes are employed. The skilled artisan will understand which scale should be used for which application. Preferably, preparative scale is employed for this invention.

The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human $\gamma 1$, $\gamma 2$, or $\gamma 4$ heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human $\gamma 3$ (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled-pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody

comprises a C_H3 domain, the BAKERBOND ABX™ resin (J. T. Baker, Phillipsburg, NJ) is useful for purification.

For Protein A chromatography, the solid phase to which Protein A is immobilized is preferably a column comprising a glass or silica surface, more preferably a controlled pore glass column or a silicic acid column. In some applications, the column has been coated with a reagent, such as glycerol, to prevent nonspecific adherence of contaminants. The solid phase is then washed to remove contaminants non-specifically bound to the solid phase. Finally, the antibody of interest is recovered from the solid phase by elution.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic-interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0 to 0.25M salt).

Generating variant antibodies exhibiting reduced or absence of HAMA response

Reduction or elimination of a HAMA response is a significant aspect of clinical development of suitable therapeutic agents. See, e.g., Khazzaeli *et al.*, *J. Natl. Cancer Inst.*, 80:937 (1988); Jaffers *et al.*, *Transplantation*, 41:572 (1986); Shawler *et al.*, *J. Immunol.*, 135:1530 (1985); Sears *et al.*, *J. Biol. Response Mod.*, 3:138 (1984); Miller *et al.*, *Blood*, 62:988 (1983); Hakimi *et al.*, *J. Immunol.*, 147:1352 (1991); Reichmann *et al.*, *Nature*, 332:323 (1988); and Junghans *et al.*, *Cancer Res.*, 50:1495 (1990). In some embodiments herein, the invention provides antibodies that are humanized such that HAMA response is reduced or eliminated. Variants of these antibodies can further be obtained using routine methods known in the art, some of which are further described below.

For example, an amino acid sequence from an antibody as described herein can serve as a starting (parent) sequence for diversification of the framework and/or CDR sequence(s). A selected framework sequence to which a starting CDR sequence is linked is referred to herein as an acceptor human framework. While the acceptor human frameworks may be from, or derived from, a human immunoglobulin (the VL and/or VH regions thereof), preferably the acceptor human frameworks are from, or derived from, a human consensus framework sequence, as such frameworks have been demonstrated to have minimal, or no, immunogenicity in human patients.

Where the acceptor is derived from a human immunoglobulin, one may optionally select a human framework sequence based on its homology to the donor framework sequence by aligning the donor framework sequence with various human framework sequences in a collection of human framework sequences and selecting the most homologous framework sequence as the acceptor.

In one embodiment, human consensus frameworks herein are from, or derived from, VH subgroup III and/or VL kappa subgroup I consensus framework sequences.

Thus, the VH acceptor human framework may comprise one, two, three, or all of the following framework sequences:

- FR1 comprising EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:46),
- FR2 comprising WVRQAPGKGLEWVG (SEQ ID NO:47),
- FR3 comprising FR3 comprises RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:48), or RATFSADNSKNTAYLQMNSLRAEDTAVYYCAD (SEQ ID NO:50), and
- FR4 comprising WGQGTLVTVSS (SEQ ID NO:49).

The VL acceptor human framework may comprise one, two, three, or all of the following framework sequences:

- FR1 comprising DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:51),
- FR2 comprising WYQQKPGKAPKLIY (SEQ ID NO:52), or
- WYQQKPGKAPKLLIY (SEQ ID NO:55),
- FR3 comprising GVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:53), and
- FR4 comprising FGQGTKVEIKR (SEQ ID NO: 54).

While the acceptor may be identical in sequence to the human framework sequence selected, whether that be from a human immunoglobulin or a human consensus framework, the present invention contemplates that the acceptor sequence may comprise pre-existing amino acid substitutions relative to the human immunoglobulin sequence or human consensus framework sequence. These pre-existing substitutions are preferably minimal, usually only four, three, two, or one amino acid difference relative to the human immunoglobulin sequence or consensus framework sequence.

CDR residues of the non-human antibody are incorporated into the VL and/or VH acceptor human frameworks. For example, one may incorporate residues corresponding to the Kabat CDR residues, the Chothia hypervariable loop residues, the AbM residues, and/or the contact residues. Optionally, the extended CDR residues as follows are incorporated: 24-

34 (L1), 50-56 (L2) and 89-97 (L3), 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3).

"Incorporation" of CDR residues can be achieved in various ways, *e.g.*, nucleic acid encoding the desired amino acid sequence can be generated by mutating nucleic acid encoding the mouse variable domain sequence so that the framework residues thereof are changed to acceptor human framework residues, or by mutating nucleic acid encoding the human variable domain sequence so that the CDR residues are changed to non-human residues, or by synthesizing nucleic acid encoding the desired sequence, *etc.*

CDR-grafted variants may be generated by Kunkel mutagenesis of nucleic acid encoding the human acceptor sequences, using a separate oligonucleotide for each CDR. Kunkel *et al.*, *Methods Enzymol.* 154:367-382 (1987). Appropriate changes can be introduced within the framework and/or CDR, using routine techniques, to correct and re-establish proper CDR-antigen interactions.

Phage(mid) display (also referred to herein as phage display in some contexts) can be used as a convenient and fast method for generating and screening many different potential variant antibodies in a library generated by sequence randomization. However, other methods for making and screening altered antibodies are available to the skilled person.

Phage(mid) display technology has provided a powerful tool for generating and selecting novel proteins that bind to a ligand, such as an antigen. Using the techniques of phage(mid) display allows the generation of large libraries of protein variants that can be rapidly sorted for those sequences that bind to a target molecule with high affinity. Nucleic acids encoding variant polypeptides are generally fused to a nucleic acid sequence encoding a viral coat protein, such as the gene III protein or the gene VIII protein. Monovalent phagemid display systems where the nucleic acid sequence encoding the protein or polypeptide is fused to a nucleic acid sequence encoding a portion of the gene III protein have been developed. (Bass, *Proteins*, 8:309 (1990); Lowman and Wells, *Methods: A Companion to Methods in Enzymology*, 3:205 (1991)). In a monovalent phagemid display system, the gene fusion is expressed at low levels and wild-type gene III proteins are also expressed so that infectivity of the particles is retained. Methods of generating peptide libraries and screening those libraries have been disclosed in many patents (*e.g.*, US 5,723,286; 5,432,018; 5,580,717; 5,427,908; and 5,498,530).

Libraries of antibodies have been prepared in a number of ways including by altering a single gene by inserting random DNA sequences or cloning a family of related genes.

Methods for displaying antibodies or antigen-binding fragments using phage(mid) display are described in US 5,750,373; 5,733,743; 5,837,242; 5,969,108; 6,172,197; 5,580,717; and 5,658,727. The library is then screened for expression of antibodies or antigen-binding proteins with the desired characteristics.

5 The sequence of oligonucleotides includes one or more of the designed codon sets for the CDR residues to be altered. A codon set is a set of different nucleotide triplet sequences used to encode desired variant amino acids. Codon sets can be represented using symbols to designate particular nucleotides or equimolar mixtures of nucleotides as shown below according to the IUB code.

10 IUB CODES

G Guanine

A Adenine

T Thymine

C Cytosine

15 R (A or G)

Y (C or T)

M (A or C)

K (G or T)

S (C or G)

20 W (A or T)

H (A or C or T)

B (C or G or T)

V (A or C or G)

D (A or G or T) H

25 N (A or C or G or T)

For example, in the codon set DVK, D can be nucleotides A or G or T; V can be A or G or C; and K can be G or T. This codon set can present 18 different codons and can encode amino acids Ala, Trp, Tyr, Lys, Thr, Asn, Lys, Ser, Arg, Asp, Glu, Gly, and Cys.

Oligonucleotide or primer sets can be synthesized using standard methods. A set of
30 oligonucleotides can be synthesized, for example, by solid-phase synthesis, containing sequences that represent all possible combinations of nucleotide triplets provided by the codon set and that will encode the desired group of amino acids. Synthesis of oligonucleotides with selected nucleotide "degeneracy" at certain positions is well known in

that art. Such sets of nucleotides having certain codon sets can be synthesized using commercial nucleic acid synthesizers (available from, for example, Applied Biosystems, Foster City, CA), or can be obtained commercially (for example, from Life Technologies, Rockville, MD). Therefore, a set of oligonucleotides synthesized having a particular codon set will typically include a plurality of oligonucleotides with different sequences, the differences established by the codon set within the overall sequence. Oligonucleotides, as used herein, have sequences that allow for hybridization to a variable-domain nucleic acid template and also can include restriction enzyme sites for cloning purposes.

In one method, nucleic acid sequences encoding variant amino acids can be created by oligonucleotide-mediated mutagenesis. This technique is well known in the art as described by Zoller *et al. Nucleic Acids Res.* 10:6487-6504(1987). Briefly, nucleic acid sequences encoding variant amino acids are created by hybridizing an oligonucleotide set encoding the desired codon sets to a DNA template, where the template is the single-stranded form of the plasmid containing a variable-region nucleic acid template sequence. After hybridization, DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will contain the codon sets as provided by the oligonucleotide set.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation(s). This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that in Crea *et al., Proc. Nat'l. Acad. Sci. USA*, 75:5765 (1978).

The DNA template is generated by those vectors that are derived from bacteriophage M13 vectors (the commercially available M13mp18 and M13mp19 vectors are suitable), or that contain a single-stranded phage origin of replication as described by Viera *et al., Meth. Enzymol.*, 153:3 (1987). Thus, the DNA to be mutated can be inserted into one of these vectors to generate single-stranded template. Production of the single-stranded template is described in sections 4.21-4.41 of Sambrook *et al., supra*.

To alter the native DNA sequence, the oligonucleotide is hybridized to the single stranded template under suitable hybridization conditions. A DNA-polymerizing enzyme, for example, T7 DNA polymerase or the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer

for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of gene 1, and the other strand (the original template) encodes the native, unaltered sequence of gene 1. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as *E. coli* JM101. After growing the cells, they are plated
5 onto agarose plates and screened using the oligonucleotide primer radiolabeled with a ^{32}P phosphate to identify the bacterial colonies that contain the mutated DNA.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single-stranded oligonucleotide is annealed to the single-
10 stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTTP), is combined with a modified thiodeoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham). This mixture is added to the template-oligonucleotide complex. Upon
addition of DNA polymerase to this mixture, a strand of DNA identical to the template except
15 for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion. After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction
20 is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell.

As indicated previously, the sequence of the oligonucleotide set is of sufficient length
25 to hybridize to the template nucleic acid and may also, but does not necessarily, contain restriction sites. The DNA template can be generated by those vectors that are either derived from bacteriophage M13 vectors or vectors that contain a single-stranded phage origin of replication as described by Viera *et al. Meth. Enzymol.*, 153:3 (1987). Thus, the DNA that is to be mutated must be inserted into one of these vectors in order to generate single-stranded
30 template. Production of the single-stranded template is described in sections 4.21-4.41 of Sambrook *et al., supra*.

In another method, a library can be generated by providing upstream and downstream oligonucleotide sets, each set having a plurality of oligonucleotides with different sequences.

These sequences are established by the codon sets provided within the sequence of the oligonucleotides. The upstream and downstream oligonucleotide sets, along with a variable-domain template nucleic acid sequence, can be used in a polymerase chain reaction (PCR) to generate a "library" of PCR products. The PCR products can be referred to as "nucleic acid cassettes", as they can be fused with other related or unrelated nucleic acid sequences, for example, viral coat proteins and dimerization domains, using established molecular biology techniques.

The sequence of the PCR primers includes one or more of the designed codon sets for the solvent-accessible and highly diverse positions in a CDR. As described above, a codon set is a set of different nucleotide triplet sequences used to encode desired variant amino acids.

Antibody selectants that meet the desired criteria, as selected through appropriate screening/selection steps, can be isolated and cloned using standard recombinant techniques.

Activity Assays

The antibodies of the present invention can be characterized for their physical/chemical properties and biological functions by various assays known in the art.

The purified immunoglobulins can be further characterized by a series of assays including, but not limited to, N-terminal sequencing, amino-acid analysis, non-denaturing size-exclusion high-pressure liquid chromatography (HPLC), mass spectrometry, ion-exchange chromatography, and papain digestion.

In certain embodiments of the invention, the antibodies produced herein are analyzed for their biological activity. In some embodiments, the antibodies herein are tested for their antigen-binding activity. The antigen-binding assays known in the art and useful herein include, without limitation, any direct or competitive-binding assays using techniques such as Western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, and protein A immunoassays.

In one embodiment, the present invention contemplates an altered antibody that possesses some but not all effector functions, which make it a desired candidate for many applications in which the half life of the antibody *in vivo* is important, yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In certain embodiments, the Fc activities of the produced immunoglobulin are measured to ensure that only the desired properties are maintained. *In vitro* and/or *in vivo* cytotoxicity assays can be

conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). An example of an *in vitro* assay to assess ADCC activity of a molecule of interest is described in US 5,500,362 or 5,821,337. Useful effector cells for such assays include PBMCs and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro *et al., J. Immunol. Methods* 202:163 (1996), may be performed. FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art.

Antibody Variants

In some embodiments, amino-acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced into the subject antibody amino acid sequence at the time that the sequence is made.

A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is called "alanine-scanning mutagenesis" as described by Cunningham and Wells, *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by

introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed immunoglobulins are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide that increases the serum half-life of the antibody.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the CDRs, but FR alterations are also contemplated. Conservative substitutions are shown in Table A under the heading of "preferred substitutions." If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table A, or as further described below in reference to amino acid classes, may be introduced and the products screened.

TABLE A

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu

Original Residue	Exemplary Substitutions	Preferred Substitutions
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (as described, for example, in A. L. Lehninger, *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) acidic: Asp (D), Glu (E)

(4) basic: Lys (K), Arg (R), His(H)

Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

One type of substitutional variant involves substituting one or more CDR residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several CDR sites (*e.g.*, 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibodies thus generated are displayed from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.*, binding affinity) as herein disclosed. For location of candidate CDR sites for modification, alanine-scanning mutagenesis can be performed to identify CDR residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

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

The antibodies of the present invention preferably have the native-sequence Fc region. However, it may be desirable to introduce one or more amino acid modifications into an Fc region thereof, generating a Fc-region variant. The Fc-region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.*, a substitution) at one or more amino acid positions including that of a hinge cysteine. These antibodies would nonetheless retain substantially the same characteristics required for therapeutic utility as compared to their wild-type counterpart.

Such amino acid substitutions may, *e.g.*, improve or reduce other Fc function or further improve the same Fc function, increase antigen-binding affinity, increase stability, alter glycosylation, or include allotypic variants. The antibodies may further comprise one or more amino acid substitutions in the Fc region that result in the antibody exhibiting one or more of the properties selected from increased FcγR binding, increased ADCC, increased CDC, decreased CDC, increased ADCC and CDC function, increased ADCC but decreased CDC function (*e.g.*, to minimize infusion reaction), increased FcRn binding, and increased serum half life, as compared to the same antibodies that have the wild-type Fc region. These activities can be measured by the methods described herein. For example, see WO 1999/51642. See also Duncan and Winter, *Nature* 322:738-40 (1988); US 5,648,260; US 5,624,821; and WO 1994/29351 concerning other examples of Fc region variants.

For additional amino acid alterations that improve Fc function, see, *e.g.*, US 6,737,056. Any of the antibodies of the present invention may further comprise at least one amino acid substitution in the Fc region that decreases CDC activity, for example, comprising at least the substitution K322A. See US 6,528,624 (Idusogie *et al.*).

In another preferred embodiment, the antibody has amino acid substitutions at any one or any combination of positions that are 268D, or 298A, or 326D, or 333A, or 334A, or 298A together with 333A, or 298A together with 334A, or 239D together with 332E, or 239D together with 298A and 332E, or 239D together with 268D and 298A and 332E, or 239D together with 268D and 298A and 326A and 332A, or 239D together with 268D and 298A and 326A and 332E, or 239D together with 268D and 283L and 298A and 332E, or 239D together with 268D and 283L and 298A and 326A and 332E, or 239D together with 330L and 332E and 272Y and 254T and 256E, or 250Q together with 428L, or 265A, or 297A, wherein the 265A substitution is in the absence of 297A and the 297A substitution is in the absence of 265A. The letter after the number in each of these designations represents the changed amino acid at that position.

Mutations that improve ADCC and CDC include substitutions at one to three positions of the Fc region, including positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues), especially S298A together with E333A and K334A (S298A/E333A/K334A, or synonymously a combination of 298A, 333A, and 334A), also referred to herein as the triple Ala mutant. K334L increases binding to CD16. K322A results in reduced CDC activity; K326A or K326W enhances CDC activity. D265A results in reduced ADCC activity.

Stability variants are variants that show improved stability with respect to, *e.g.*, oxidation and deamidation.

A further type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. Such altering includes deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody. Glycosylation variants that increase ADCC function are described, *e.g.*, in WO 2003/035835. See also US 2006/0067930.

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

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

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US 2003/0157108 (Presta). See also US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in, *e.g.*, WO 2003/011878, Jean-Mairet *et al.* and US 6,602,684 (Umana *et al.*). Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported, for example, in WO 1997/30087 (Patel *et al.*). See, also, WO 1998/58964 (Raju) and WO 1999/22764 (Raju) concerning antibodies with altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 (Umana *et al.*); US 2004/0072290 (Umana *et al.*); US 2003/0175884 (Umana *et al.*); WO 2005/044859 (Umana *et al.*); and US 2007/0111281 (Sondermann *et al.*) on antigen-binding molecules

with modified glycosylation, including antibodies with an Fc region containing N-linked oligosaccharides; and US 2007/0010009 (Kanda *et al.*)

One preferred glycosylation antibody variant herein comprises an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose, which may improve ADCC function. Specifically, antibodies are contemplated herein that have reduced fucose relative to the amount of fucose on the same antibody produced in a wild-type CHO cell. That is, they are characterized by having a lower amount of fucose than they would otherwise have if produced by native CHO cells. Preferably the antibody is one wherein less than about 10% of the N-linked glycans thereon comprise fucose, more preferably wherein less than about 5% of the N-linked glycans thereon comprise fucose, and most preferably, wherein none of the N-linked glycans thereon comprise fucose, *i.e.*, wherein the antibody is completely without fucose, or has no fucose.

Such "defucosylated" or "fucose-deficient" antibodies may be produced, for example, by culturing the antibodies in a cell line such as that disclosed in, for example, US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; US 2006/0063254; US 2006/0064781; US 2006/0078990; US 2006/0078991; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); and Yamane-Ohnuki *et al., Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US 2003/0157108 A1 (Presta) and WO 2004/056312 A1 (Adams *et al.*, especially at Example 11) and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*- knockout CHO cells (Yamane-Ohnuki *et al., Biotech. Bioeng.* 87: 614 (2004)). See also Kanda *et al., Biotechnol. Bioeng.*, 94: 680-8 (2006). US 2007/0048300 (Biogen-IDEC) discloses a method of producing aglycosylated Fc-containing polypeptides, such as antibodies, having desired effector function, as well as aglycosylated antibodies produced according to the method and methods of using such antibodies as therapeutics, all being applicable herein. Additionally, US 7,262,039 relates to a polypeptide having an alpha-1,3-fucosyltransferase activity, including a method for producing a fucose-containing sugar chain using the polypeptide.

See also US 2006/024304 (Gerngross *et al.*); US Patent No. 7,029,872 (Gerngross); US 2004/018590 (Gerngross *et al.*); US 2006/034828 (Gerngross *et al.*); US 2006/034830

(Gerngross *et al.*); US 2006/029604 (Gerngross *et al.*); WO 2006/014679 (Gerngross *et al.*); WO 2006/014683 (Gerngross *et al.*); WO 2006/014685 (Gerngross *et al.*); WO 2006/014725 (Gerngross *et al.*); and WO 2006/014726 (Gerngross *et al.*) on recombinant glycoproteins and glycosylation variants that are applicable herein.

5 In another embodiment, the invention provides an antibody composition comprising the antibodies described herein having an Fc region, wherein about 20-100% of the antibodies in the composition comprise a mature core carbohydrate structure in the Fc region that lacks a fucose. Preferably, such composition comprises antibodies having an Fc region that has been altered to change one or more of the antibody-dependent cell-mediated
10 cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), or pharmacokinetic properties of the antibody compared to a wild-type IgG Fc sequence by substituting an amino acid selected from the group consisting of A, D, E, L, Q, T, and Y at any one or any combination of positions of the Fc region selected from the group consisting of: 238, 239, 246, 248, 249, 250, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280,
15 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 297, 298, 301, 303, 305, 307, 309, 312, 314, 315, 320, 322, 324, 326, 327, 329, 330, 331, 332, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 428, 430, 434, 435, 437, 438, and 439.

The composition is more preferably one wherein the antibody further comprises an Fc substitution that is 268D or 326D or 333A together with 334A, or 298A together with 333A,
20 or 298A together with 334A, or 239D together with 332E, or 239D together with 298A and 332E, or 239D together with 268D and 298A and 332E, or 239D together with 268D and 298A and 326A and 332A, or 239D together with 268D and 298A and 326A and 332E, or 239D together with 268D and 283L and 298A and 332E, or 239D together with 268D and 283L and 298A and 326A and 332E, or 239D together with 330L and 332E, wherein the
25 letter after the number in each of these designations represents the changed amino acid at that position.

The composition is additionally preferably one wherein the antibody binds an FcγRIII. The composition further is preferably such that the antibody has ADCC activity in the presence of human effector cells or has increased ADCC activity in the presence of
30 human effector cells compared to the otherwise same antibody comprising a human wild-type IgG1Fc region.

The composition is also preferably one wherein the antibody binds the FcγRIII with better affinity, or mediates ADCC more effectively, than a glycoprotein with a mature core

carbohydrate structure including fucose attached to the Fc region of the glycoprotein. In addition, the composition is preferably one wherein the antibody has been produced by a CHO cell, preferably a Lec13 cell. The composition is also preferably one wherein the antibody has been produced by a mammalian cell lacking a fucosyltransferase gene, more preferably the *FUT8* gene.

In one aspect, the composition is one wherein the antibody is free of bisecting N-acetylglucosamine (GlcNAc) attached to the mature core carbohydrate structure. Alternatively, the composition is such that the antibody has bisecting GlcNAc attached to the mature core carbohydrate structure.

In another aspect, the composition is one wherein the antibody has one or more galactose residues attached to the mature core carbohydrate structure. Alternatively, the composition is such that the antibody is free of one or more galactose residues attached to the mature core carbohydrate structure.

In a further aspect, the composition is one wherein the antibody has one or more sialic acid residues attached to the mature core carbohydrate structure. Alternatively, the composition is such that the antibody is free of one or more sialic acid residues attached to the mature core carbohydrate structure.

This composition preferably comprises at least about 2% afucosylated antibodies. The composition more preferably comprises at least about 4% afucosylated antibodies. The composition still more preferably comprises at least about 10% afucosylated antibodies. The composition even more preferably comprises at least about 19% afucosylated antibodies. The composition most preferably comprises about 100% afucosylated antibodies.

Immunoconjugates

The invention also pertains to immunoconjugates, or antibody-drug conjugates (ADC), comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, a drug, a growth-inhibitory agent, a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, *e.g.*, drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos, *Anticancer Research* 19:605-614 (1999); Niculescu-Duvaz and Springer, *Adv. Drug Del. Rev.* 26:151-172 (1997); and US 4,975,278) allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of

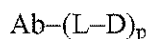
these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin *et al.*, *Lancet*, 603-05 (1986); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in *Monoclonal Antibodies '84: Biological And Clinical Applications*, A. Pinchera *et al.* (eds), pp. 475-506 (1985)). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland *et al.*, *Cancer Immunol. Immunother.*, 21:183-87 (1986)). Drugs used in these methods include daunomycin, doxorubicin, methotrexate, and vindesine. Toxins used in antibody-toxin conjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small-molecule toxins such as geldanamycin (Mandler *et al.*, *J. Nat. Cancer Inst.*, 92(19):1573-1581 (2000); Mandler *et al.*, *Bioorganic & Med. Chem. Letters*, 10:1025-1028 (2000); and Mandler *et al.*, *Bioconjugate Chem.*, 13: 786-791 (2002)), maytansinoids (EP 1391213 and Liu *et al.*, *Proc. Natl. Acad. Sci. USA*, 93: 8618-8623 (1996)), and calicheamicin (Lode *et al.*, *Cancer Res.*, 58:2928 (1998); and Hinman *et al.*, *Cancer Res.*, 53:3336-3342 (1993)). Without being limited to any one theory, the toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition. Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re . Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and

bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, *Science*, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, for example, WO 1994/11026.

Conjugates of an antibody and one or more small-molecule toxins, such as a calicheamicin, maytansinoid, trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein.

In the ADCs of the invention, an antibody (Ab) is conjugated to one or more drug moieties (D), *e.g.*, about 1 to about 20 drug moieties per antibody, through a linker (L). The ADC of Formula I may be prepared by several routes, employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent, to form Ab-L, via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with the nucleophilic group of an antibody.



Formula I

Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side-chain amine groups, *e.g.*, lysine, (iii) side-chain thiol groups, *e.g.*, cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; and (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, *i.e.*, cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol). Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through the reaction of lysines with 2-iminothiolane (Traut's reagent) resulting in conversion of an amine into a thiol.

Antibody-drug conjugates of the invention may also be produced by modification of the antibody to introduce electrophilic moieties, which can react with nucleophilic substituents on the linker reagent or drug. The sugars of glycosylated antibodies may be

oxidized, *e.g.*, with periodate oxidizing reagents, to form aldehyde or ketone groups that may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, *e.g.*, by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield
5 carbonyl (aldehyde and ketone) groups in the protein that can react with appropriate groups on the drug (Domen *et al.*, *J. Chromatog.*, 510: 293-302 (1990)). In another embodiment, proteins containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan
10 and Stroh, *Bioconjugate Chem.*, 3:138-146 (1992) and US 5,362,852). Such aldehyde can be reacted with a drug moiety or linker nucleophile.

Likewise, nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on
15 linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; and (iii) aldehydes, ketones, carboxyl, and maleimide groups.

Alternatively, a fusion protein comprising the antibody and cytotoxic agent may be made, *e.g.*, by recombinant techniques or peptide synthesis. The length of DNA may
20 comprise respective regions encoding the two portions of the conjugate either adjacent one another or separated by a region encoding a linker peptide that does not destroy the desired properties of the conjugate.

In yet another embodiment, the antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is
25 administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) that is conjugated to a cytotoxic agent (*e.g.*, a radionucleotide).

The ADCs herein are optionally prepared with cross-linker reagents, such as, for example, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB,
30 SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate), which are commercially available (*e.g.*, from Pierce Biotechnology, Inc., Rockford, IL., U.S.A). See pages 467-498, 2003-2004 Applications Handbook and Catalog.

Antibody Derivatives

The antibodies of the present invention can be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. Preferably, the moieties suitable for derivatization of the antibody are water-soluble polymers. Non-limiting examples of water-soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, *etc.*

Pharmaceutical Formulations

Therapeutic formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients, or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low-molecular-weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates

including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose, or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

5 A further formulation and delivery method herein involves that described, for example, in WO 2004/078140, including the ENHANZE™ drug delivery technology (Halozyme Inc.). This technology is based on a recombinant human hyaluronidase (rHuPH20). rHuPH20 is a recombinant form of the naturally occurring human enzyme approved by the FDA that temporarily clears space in the matrix of tissues such as skin. That
10 is, the enzyme has the ability to break down hyaluronic acid (HA), the space-filling "gel"-like substance that is a major component of tissues throughout the body. This clearing activity is expected to allow rHuPH20 to improve drug delivery by enhancing the entry of therapeutic molecules through the subcutaneous space. Hence, when combined or co-formulated with certain injectable drugs, this technology can act as a "molecular machete" to facilitate the
15 penetration and dispersion of these drugs by temporarily opening flow channels under the skin. Molecules as large as 200 nanometers may pass freely through the perforated extracellular matrix, which recovers its normal density within approximately 24 hours, leading to a drug delivery platform that does not permanently alter the architecture of the skin.

20 Hence, the present invention includes a method of delivering an antibody herein to a tissue containing excess amounts of glycosaminoglycan, comprising administering a hyaluronidase glycoprotein (sHASEGP) (this protein comprising a neutral active soluble hyaluronidase polypeptide and at least one N-linked sugar moiety, wherein the N-linked sugar moiety is covalently attached to an asparagine residue of the polypeptide) to the tissue
25 in an amount sufficient to degrade glycosaminoglycans sufficiently to open channels less than about 500 nanometers in diameter; and administering the antibody to the tissue comprising the degraded glycosaminoglycans.

 In another embodiment, the invention includes a method for increasing the diffusion of an antibody herein that is administered to a subject comprising administering to the subject
30 a sHASEGP polypeptide in an amount sufficient to open or to form channels smaller than the diameter of the antibody and administering the antibody, whereby the diffusion of the therapeutic substance is increased. The sHASEGP and antibody may be administered

separately or simultaneously in one formulation, and consecutively in either order or at the same time.

Exemplary anti-LT α antibody formulations are described in WO 1998/56418, which include a liquid multidose formulation comprising the anti-LT α antibody at 40 mg/mL, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2-8°C. Another anti-LT α formulation of interest comprises 10 mg/mL antibody in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7mg/mL POLYSORBATE 80TM surfactant, and Sterile Water for Injection, pH 6.5. Yet another aqueous pharmaceutical formulation comprises 10-30 mM sodium acetate from about pH 4.8 to about pH 5.5, preferably at pH 5.5, POLYSORBATETM as a surfactant in an amount of about 0.01-0.1% v/v, trehalose at an amount of about 2-10% w/v, and benzyl alcohol as a preservative (US 6,171,586). Lyophilized formulations adapted for subcutaneous administration are described, for example, in WO 1997/04801 and US 6,267,958 (Andya *et al.*). Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

Crystallized forms of the antibody are also contemplated. See, for example, US 2002/0136719 (Shenoy *et al.*).

The formulation herein may also contain more than one active compound (a second medicament as noted above) as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a cytotoxic agent, chemotherapeutic agent, cytokine antagonist, integrin antagonist, or immunosuppressive agent (*e.g.*, one that acts on T cells, such as cyclosporin or an antibody that binds T cells, *e.g.*, one that binds LFA-1). The type and effective amounts of such second medicaments depend, for example, on the amount of antibody present in the formulation, the type of disease or disorder or treatment, the clinical parameters of the subjects, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein or from about 1 to 99% of the heretofore employed dosages. The preferred such medicaments are noted above.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate)

microcapsules, respectively, in colloidal drug-delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules), or in macroemulsions. Such techniques are disclosed, for example, in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

5 Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (US 10 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

15 The formulations to be used for *in-vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Uses

20 An antibody of the present invention may be used in, for example, *in vitro*, *ex vivo*, and *in vivo* therapeutic methods. Antibodies of the invention can be used as an antagonist to partially or fully block the specific LT α activity *in vitro*, *ex vivo*, and/or *in vivo*. Moreover, at least some of the antibodies of the invention can neutralize antigen activity from other species. Accordingly, the antibodies of the invention can be used to inhibit a specific antigen activity, *e.g.*, in a cell culture containing the antigen, in human subjects, or in other mammalian subjects having the antigen with which an antibody of the invention cross-reacts (*e.g.*, chimpanzee, baboon, marmoset, cynomolgus, rhesus, pig, or mouse). In one 25 embodiment, the antibody of the invention can be used for inhibiting antigen activities by contacting the antibody with the antigen such that antigen activity is inhibited. Preferably, the antigen is a human protein molecule.

30 In one embodiment, an antibody of the invention can be used in a method for inhibiting an antigen in a subject suffering from a disorder in which the antigen activity is detrimental, comprising administering to the subject an antibody of the invention such that the antigen activity in the subject is inhibited. Preferably, the antigen is a human protein molecule and the subject is a human subject. Alternatively, the subject can be a mammal expressing the antigen to which an antibody of the invention binds. Still further, the subject

can be a mammal into which the antigen has been introduced (*e.g.*, by administration of the antigen or by expression of an antigen transgene). An antibody of the invention can be administered to a human subject for therapeutic purposes. Moreover, an antibody of the invention can be administered to a non-human mammal expressing an antigen with which the immunoglobulin cross-reacts (*e.g.*, a primate, pig, or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (*e.g.*, testing of dosages and time courses of administration). The antibodies of the invention can be used to treat, inhibit, delay progression of, prevent/delay recurrence of, ameliorate, or prevent autoimmune diseases, disorders, or conditions as defined herein.

In one aspect, a blocking antibody of the invention is specific to a ligand antigen, and inhibits the antigen activity by blocking or interfering with the ligand-receptor interaction involving the ligand antigen, thereby inhibiting the corresponding signal pathway and other molecular or cellular events. The invention also features receptor-specific antibodies that do not necessarily prevent ligand binding but interfere with receptor activation, thereby inhibiting any responses that would normally be initiated by the ligand binding. The invention also encompasses antibodies that either preferably or exclusively bind to ligand-receptor complexes. An antibody of the invention can also act as an agonist of a particular antigen receptor, thereby potentiating, enhancing, or activating either all or partial activities of the ligand-mediated receptor activation.

The antibody may be a naked antibody or alternatively is conjugated with another molecule, such as a cytotoxic agent. The antibody is preferably administered intravenously or subcutaneously, most preferably subcutaneously.

In one embodiment, the subject has never been previously treated with drug(s), such as immunosuppressive agent(s), to treat the disorder, and in a particular embodiment has never been previously treated with a TNF antagonist. In an alternative embodiment, the subject has been previously treated with drug(s) to treat the disorder, including with a TNF antagonist.

In a still further aspect, the patient has relapsed with the disorder. In an alternative embodiment, the patient has not relapsed with the disorder.

In another aspect, the antibody herein is the only medicament administered to the subject to treat the disorder. In an alternative aspect, the antibody herein is one of the medicaments used to treat the disorder.

In a further aspect, the subject only has RA as an autoimmune disorder. Alternatively, the subject only has MS as an autoimmune disorder. Still alternatively, the subject only has lupus, or ANCA-associated vasculitis, or Sjögren's syndrome as an autoimmune disorder. In all cases autoimmune disorder is defined above.

5 In a still further embodiment, the subject has an abnormal level of one or more regulatory cytokines, anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), infiltrating CD20 cells, anti-double stranded DNA (dsDNA) antibodies, anti-Sm antibodies, anti-nuclear ribonucleoprotein antibodies, anti-phospholipid antibodies, 10 anti-ribosomal P antibodies, anti-Ro/SS-A antibodies, anti-Ro antibodies, anti-La antibodies, antibodies directed against Sjögren's-associated antigen A or B (SS-A or SS-B), antibodies directed against centromere protein B (CENP B) or centromere protein C (CENP C), autoantibodies to ICA69, anti-Smith antigen (Sm) antibodies, anti-nuclear ribonucleoprotein antibodies, anti-ribosomal P antibodies, autoantibodies staining the nuclear or perinuclear 15 zone of neutrophils (pANCA), anti-Saccharomyces cerevisiae antibodies, cross-reactive antibodies to GM1 ganglioside or GQ1b ganglioside, anti-acetylcholine receptor (AChR), anti-AChR subtype, or anti-muscle specific tyrosine kinase (MuSK) antibodies, serum anti-endothelial cell antibodies, IgG or anti-desmoglein (Dsg) antibodies, anti-centromere, anti-topoisomerase-1 (Scl-70), anti-RNA polymerase or anti-U3-ribonucleoprotein (U3-RNP) 20 antibodies, anti-glomerular basement membrane (GBM) antibodies, anti-glomerular basement membrane (GBM) antibodies, anti-mitochondrial (AMA) or anti-mitochondrial M2 antibodies, anti-thyroid peroxidase (TPO), anti-thyroglobin (TG) or anti-thyroid stimulating hormone receptor (TSHR) antibodies, anti-nucleic (AN), anti-actin (AA) or anti-smooth muscle antigen (ASM) antibodies, IgA anti-endomysial, IgA anti-tissue transglutaminase, 25 IgA anti-gliadin or IgG anti-gliadin antibodies, anti-CYP21A2, anti-CYP11A1 or anti-CYP17 antibodies, anti-ribonucleoprotein (RNP), or myositis-specific antibodies, anti-myelin associated glycoprotein (MAG) antibodies, anti-hepatitis C virus (HCV) antibodies, anti-GM1 ganglioside, anti-sulfate-3-glycuronyl paragloboside (SGPG), or IgM anti-glycoconjugate antibodies, IgM anti-ganglioside antibody, anti-thyroid peroxidase (TPO), 30 anti-thyroglobin (TG) or anti-thyroid stimulating hormone receptor (TSHR) antibodies, anti-myelin basic protein or anti-myelin oligodendrocytic glycoprotein antibodies, IgM rheumatoid factor antibodies directed against the Fc portion of IgG, anti-Factor VIII antibodies, or a combination thereof.

The parameters for assessing efficacy or success of treatment of an autoimmune disorder (which includes an autoimmune-related disease) will be known to the physician of skill in the appropriate disease. Generally, the physician of skill will look for reduction in the signs and symptoms of the specific disease. The following are by way of examples.

5 In one embodiment, the methods and compositions of the invention are useful to treat RA. RA is characterized by inflammation of multiple joints, cartilage loss, and bone erosion that leads to joint destruction and ultimately reduced joint function. Additionally, since RA is a systemic disease, it can have effects in other tissues such as the lungs, eyes, and bone marrow.

10 If the subject has RA, which is a preferred indication herein, preferably the antibodies herein induce a major clinical response in the subject.

The antibodies herein can be used as first-line therapy in patients with early RA (*i.e.*, methotrexate (MTX) naive), or in combination with, *e.g.*, MTX or cyclophosphamide.

Alternatively, the antibodies can be used in treatment as second-line therapy for patients who were, for example, refractory to DMARD and/or TNF inhibitor, and/or MTX, in combination with, *e.g.*, MTX. The LT α -binding antibodies can also be administered in combination with B-cell mobilizing agents such as integrin antibodies that mobilize B cells into the bloodstream for more effective killing. The LT α -binding antibodies are useful to prevent and control joint damage, delay structural damage, decrease pain associated with inflammation in RA, and generally reduce the signs and symptoms in moderate-to-severe RA. The RA patient can be treated with the LT α antibody prior to, after, or together with treatment with other drugs used in treating RA (see combination therapy described herein). Patients who had previously failed DMARDs and/or had an inadequate response to MTX alone are, in one embodiment, treated with a LT α -binding antibody. In another embodiment, such patients are administered humanized LT α -binding antibody plus cyclophosphamide or LT α -binding antibody plus MTX. Most preferably, the antibodies herein are administered with MTX for treatment of RA, and not with high-dose steroids.

One method of evaluating treatment efficacy in RA is based on American College of Rheumatology (ACR) criteria, which are used to measure the percentage of improvement in tender and swollen joints, among other things. The RA patient can be scored at, for example, ACR 20 (20 percent improvement) compared with no antibody treatment (*e.g.*, baseline before treatment) or treatment with placebo. Other ways of evaluating the efficacy of antibody treatment include X-ray scoring such as the Sharp X-ray score used to score

structural damage such as bone erosion and joint space narrowing. Patients can also be evaluated for the prevention of or improvement in disability based on Health Assessment Questionnaire (HAQ) score, AIMS score, or SF-36 at time periods during or after treatment. The ACR 20 criteria may include 20% improvement in both tender (painful) joint count and swollen joint count plus a 20% improvement in at least three of five additional measures:

1. patient's pain assessment by visual analog scale (VAS),
2. patient's global assessment of disease activity (VAS),
3. physician's global assessment of disease activity (VAS),
4. patient's self-assessed disability measured by the Health Assessment Questionnaire, and
5. acute phase reactants, CRP or ESR.

The ACR 50 and 70 are defined analogously. Preferably, the patient is administered an amount of an LT α -binding antibody of the invention effective to achieve at least a score of ACR 20, preferably at least ACR 30, more preferably at least ACR 50, even more preferably at least ACR 70, and most preferably at least ACR 75.

Psoriatic arthritis has unique and distinct radiographic features. For psoriatic arthritis, joint erosion and joint space narrowing can be evaluated by the Sharp score as well. The antibodies disclosed herein can be used to prevent the joint damage as well as reduce disease signs and symptoms of the disorder.

Yet another aspect of the invention is a method of treating lupus, including systemic lupus erythematosus (SLE) and lupus nephritis, by administering to the subject having such disease an effective amount of an antibody of the invention. For example, SLEDAI scores provide a numerical quantitation of disease activity. The SLEDAI is a weighted index of 24 clinical and laboratory parameters known to correlate with disease activity, with a numerical range of 0-103. See Gescuk and Davis, *Current Opinion in Rheumatology*, 14: 515-521 (2002). Antibodies to double-stranded DNA are believed to cause renal flares and other manifestations of lupus. Patients undergoing antibody treatment can be monitored for time to renal flare, which is defined as a significant, reproducible increase in serum creatinine, urine protein, or blood in the urine. Alternatively or in addition, patients can be monitored for levels of antinuclear antibodies and antibodies to double-stranded DNA. Treatments for SLE include high-dose corticosteroids and/or cyclophosphamide (HDCC).

Spondyloarthropathies are a group of disorders of the joints, including ankylosing spondylitis, psoriatic arthritis, and Crohn's disease. Treatment success can be determined by validated patient and physician global assessment measuring tools.

Various medications are used to treat psoriasis; treatment differs directly in relation to disease severity. Patients with a more mild form of psoriasis typically utilize topical treatments, such as topical steroids, anthralin, calcipotriene, clobetasol, and tazarotene, to manage the disease, while patients with moderate and severe psoriasis are more likely to employ systemic (methotrexate, retinoids, cyclosporine, PUVA, and UVB) therapies. Tars are also used. These therapies are disadvantageous due to safety concerns, time-consuming regimens, and/or inconvenient processes of treatment. Furthermore, some require expensive equipment and dedicated space in the office setting. Such systemic medications can produce serious side effects, including hypertension, hyperlipidemia, bone-marrow suppression, liver disease, kidney disease, and gastrointestinal upset. Also, the use of phototherapy can increase the incidence of skin cancers. In addition to the inconvenience and discomfort associated with the use of topical therapies, phototherapy and such systemic treatments also require cycling patients on and off therapy and monitoring lifetime exposure due to their side effects.

Treatment efficacy for psoriasis is assessed by monitoring changes in clinical signs and symptoms of the disease, including Physician's Global Assessment (PGA) changes, Psoriasis Area and Severity Index (PASI) scores, and Psoriasis Symptom Assessment (PSA), compared with the baseline condition. The patient can be measured periodically throughout treatment on the Visual Analog Scale (VAS) used to indicate the degree of itching experienced at specific time points.

Assays

Ligand/receptor binding studies may be carried out in any known assay method, such as competitive-binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Cell-based assays and animal models can be used to understand the interaction between the ligands and receptors identified herein and the development and pathogenesis of the conditions and diseases referred to herein.

In one approach, mammalian cells may be transfected with the ligands or receptors described herein, and the ability of the antibody herein to inhibit binding or activity is analyzed. Suitable cells can be transfected with the desired gene, and monitored for activity.

Such transfected cell lines can then be used to test the ability of antibody, for example, to modulate LT $\alpha\beta$ complex expression of the cells.

In addition, primary cultures derived from transgenic animals can be used in the cell-based assays. Techniques to derive continuous cell lines from transgenic animals are well known in the art. See, *e.g.*, Small *et al.*, *Mol. Cell. Biol.*, 5:642-648 (1985).

One suitable cell-based assay is the addition of epitope-tagged ligand (*e.g.*, LT α) to cells that have or express the respective receptor, and the analysis of binding (in the presence or absence of prospective antibodies) by FACS staining with anti-tag antibody. In another assay, the ability of the antibody herein to inhibit the expression of LT $\alpha\beta$ complex on cells expressing such complex is assayed. For example, suitable expressing cell lines are cultured in the presence or absence of prospective antibodies and the modulation of LT $\alpha\beta$ complex expression can be measured by ^3H -thymidine incorporation or cell number.

The results of the cell-based *in vitro* assays can be further verified using *in vivo* animal models. Many animal models can be used to test the efficacy of the antibodies identified herein in relation to, for instance, immune-related disease. The *in vivo* nature of such models makes them particularly predictive of responses in human patients. Animal models of immune-related diseases include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, *e.g.*, murine models. Such models can be generated by introducing cells into syngeneic mice using standard techniques, *e.g.* subcutaneous injection, tail-vein injection, spleen implantation, intraperitoneal implantation, and implantation under the renal capsule.

Graft-versus-host disease is an example of a disease for which an animal model has been designed. Graft-versus-host disease occurs when immunocompetent cells are transplanted into immunosuppressed or tolerant patients. The donor cells recognize and respond to host antigens. The response can vary from life-threatening severe inflammation to mild cases of diarrhea and weight loss. Graft-versus-host disease models provide a means of assessing T-cell reactivity against MHC antigens and minor transplant antigens. A suitable procedure is described in detail in *Current Protocols in Immunology*, Eds. Coligan *et al.*, (John Wiley & Sons, Inc., 1994), unit 4.3.

An animal model for skin allograft rejection tests the ability of T cells to mediate *in vivo* tissue destruction to measure their role in anti-viral immunity, and is described, for example, in *Current Protocols in Immunology, supra*, unit 4.4. Other transplant rejection models useful to test the antibodies herein include the allogeneic heart-transplant models

described by Tanabe *et al.*, *Transplantation*, 58:23 (1994) and Tinubu *et al.*, *J. Immunol.*, 4330-4338 (1994).

Animal models for delayed-type hypersensitivity provide an assay of cell-mediated immune function. Delayed-type hypersensitivity reactions are a T-cell-mediated *in vivo* immune response characterized by inflammation that does not reach a peak until after a period of time has elapsed after challenge with an antigen. These reactions also occur in tissue-specific autoimmune diseases such as MS and experimental autoimmune encephalomyelitis (EAE, a model for MS). A suitable, exemplary model is described in detail in *Current Protocols in Immunology*, *supra*, unit 4.5.

The collagen-induced arthritis (CIA) model is considered a suitable model for studying potential drugs or biologics active in human arthritis because of the many immunological and pathological similarities to human RA, the involvement of localized major histocompatibility, complete class-II-restricted T-helper lymphocyte activation, and the similarity of histological lesions. Features of this CIA model that are similar to that found in RA patients include: erosion of cartilage and bone at joint margins (as can be seen in radiographs), proliferative synovitis, and symmetrical involvement of small and medium-sized peripheral joints in the appendicular, but not the axial, skeleton. Jamieson *et al.*, *Invest. Radiol.* 20: 324-9 (1985). Furthermore, IL-1 and TNF- α appear to be involved in CIA as well as in RA. Joosten *et al.*, *J. Immunol.* 163: 5049-5055 (1999). TNF-neutralizing antibodies, and separately, TNFR.Fc, reduced the symptoms of RA in this model (Williams *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:9784-9788 (1992); Wooley *et al.*, *J. Immunol.*, 151: 6602-6607 (1993)).

In this model for RA, type II collagen is purified from bovine articular cartilage (Miller, *Biochemistry* 11:4903 (1972)) and used to immunized mice (Williams *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:2762 (1994)). Symptoms of arthritis include erythema and/or swelling of the limbs as well as erosions or defects in cartilage and bone as determined by histology. This widely used model is also described, for example, by Holmdahl *et al.*, *APMIS*, 97:575 (1989), in *Current Protocols in Immunology*, *supra*, units 15.5, and in Issekutz *et al.*, *Immunology*, 88:569 (1996).

A model of asthma has been described in which antigen-induced airway hyper-reactivity, pulmonary eosinophilia, and inflammation are induced by sensitizing an animal with ovalbumin and challenging the animal with the same protein delivered by aerosol. Animal models such as rodent and non-human primate models exhibit symptoms similar to

atopic asthma in humans upon challenge with aerosol antigens. Suitable procedures to test the antibodies herein for suitability in treating asthma include those described by Wolyniec *et al.*, *Am. J. Respir. Cell Mol. Biol.*, 18:777 (1998).

Additionally, the antibodies herein can be tested in the SCID/SCID mouse model for immune disorders. For example, as described by Schon *et al.*, *Nat. Med.*, 3:183 (1997), the mice demonstrate histopathologic skin lesions resembling psoriasis. Another suitable model is the human skin/SCID mouse chimera prepared as described by Nickoloff *et al.*, *Am. J. Path.*, 146:580 (1995).

Dosage

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with a second medicament as noted below) will depend, for example, on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The dosage is preferably efficacious for the treatment of that indication while minimizing toxicity and side effects.

The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 500 mg/kg (preferably about 0.1 mg/kg to 400 mg/kg) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 500 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 400 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg or 50 mg/kg or 100 mg/kg or 300 mg/kg or 400 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, *e.g.*, every week or every three weeks (*e.g.*, such that the patient receives from about two to about twenty, *e.g.*, about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses, may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 to 500 mg/kg, followed by a weekly maintenance dose of about 2 to 400 mg/kg of

the antibody. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

For the treatment of an autoimmune disorder, the therapeutically effective dosage will typically be in the range of about 50 mg/m² to about 3000 mg/m², preferably about 50 to 1500 mg/m², more preferably about 50-1000 mg/m². In one embodiment, the dosage range is about 125-700 mg/m². For treating RA, in one embodiment, the dosage range for the humanized antibody is about 50 mg/m² or 125 mg/m² (equivalent to about 200 mg/dose) to about 1000 mg/m², given in two doses, *e.g.*, the first dose of about 200 mg is administered on day one followed by a second dose of about 200 mg on day 15. In different embodiments, the dosage is about any one of 50 mg/dose, 80 mg/dose, 100 mg/dose, 125 mg/dose, 150 mg/dose, 200 mg/dose, 250 mg/dose, 275 mg/dose, 300 mg/dose, 325 mg/dose, 350 mg/dose, 375 mg/dose, 400 mg/dose, 425 mg/dose, 450 mg/dose, 475 mg/dose, 500 mg/dose, 525 mg/dose, 550 mg/dose, 575 mg/dose, or 600 mg/dose, or 700 mg/dose, or 800 mg/dose, or 900 mg/dose, or 1000 mg/dose, or 1500 mg/dose.

In treating disease, the LT α -binding antibodies of the invention can be administered to the patient chronically or intermittently, as determined by the physician of skill in the disease.

A patient administered a drug by intravenous infusion or subcutaneously may experience adverse events such as fever, chills, burning sensation, asthenia, and headache. To alleviate or minimize such adverse events, the patient may receive an initial conditioning dose(s) of the antibody followed by a therapeutic dose. The conditioning dose(s) will be lower than the therapeutic dose to condition the patient to tolerate higher dosages.

The antibodies herein may be administered at a frequency that is within the skill and judgment of the practicing physician, depending on various factors noted above, for example, the dosing amount. This frequency includes twice a week, three times a week, once a week, bi-weekly, or once a month. In a preferred aspect of this method, the antibody is administered no more than about once every other week, more preferably about once a month.

Route of administration

The antibodies used in the methods of the invention (as well as any second medicaments) are administered to a subject or patient, including a human patient, in accord with suitable methods, such as those known to medical practitioners, depending on many factors, including whether the dosing is acute or chronic. These routes include, for example, parenteral, intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period

of time, by subcutaneous, intramuscular, intra-arterial, intraperitoneal, intrapulmonary, intracerebrospinal, intra-articular, intrasynovial, intrathecal, intralesional, or inhalation routes (e.g., intranasal). Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antibody is suitably administered by pulse infusion, particularly with declining doses of the antibody. Preferred routes herein are intravenous or subcutaneous administration, most preferably subcutaneous.

In one embodiment, the antibody herein is administered by intravenous infusion, and more preferably with about 0.9 to 20% sodium chloride solution as an infusion vehicle.

Combination Therapy

In any of the methods herein, one may administer to the subject or patient along with the antibody herein an effective amount of a second medicament (where the antibody herein is a first medicament), which is another active agent that can treat the condition in the subject that requires treatment; for example, if the condition is an autoimmune disorder, the second medicament is a drug that can treat the autoimmune disorder, alone or with another active agent. For treatment of autoimmune disorders, the second medicament includes, for example, a chemotherapeutic agent, an immunosuppressive agent, an antagonist (such as an antibody) that binds a B-cell surface marker such as rituximab or humanized 2H7, a BAFF antagonist, a DMARD, a cytotoxic agent, an integrin antagonist such as a CD11 or CD18 antagonist including CD11a or CD18 antibodies, e.g., efalizumab (RAPTIVA®), a NSAID, a cytokine antagonist, such as a TNF antagonist, an anti-rheumatic agent, a muscle relaxant, a narcotic, an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunizing agent, an immunoglobulin, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine, a cytokine, cells for repressing B-cell autoantibody secretion as set forth in WO 2005/027841, a hyaluronidase glycoprotein as an active delivery vehicle as set forth in, for example, WO 2004/078140, or a combination thereof.

For treatment of proliferative diseases such as cancer, the second medicament would include, for example, chemotherapeutic agents, hormones, cytotoxic agents, and other biologics such as antibodies to HER-2, to VEGF, to B-cell surface antigens such as TAHO antigen (e.g., US 2006/0251662), CD20, and CD22, and to EGF/EGF-R.

Preferably, such second medicament for autoimmune diseases is an immunosuppressive agent, an antagonist that binds a B-cell surface marker (such as an

antibody, e.g., CD20 antibody or CD22 antibody), a BAFF antagonist, a DMARD, an integrin antagonist, a hyaluronidase glycoprotein (as an active delivery vehicle), a NSAID, a cytokine antagonist, more preferably a TNF (*e.g.*, TNF- α), CD11, or CD18 antagonist, or a combination thereof. More preferably, it is methotrexate, a TNF antagonist, an antagonist to a B-cell surface marker, or a DMARD.

The type of such second medicament depends on various factors, including the type of autoimmune disorder, the severity of the disease, the condition and age of the patient, the type and dose of first medicament employed, *etc.*

The antibodies herein can be administered concurrently, sequentially, or alternating with the second medicament or upon non-responsiveness with other therapy. Thus, the combined administration of a second medicament includes co-administration (concurrent administration), using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) medicaments simultaneously exert their biological activities. All these second medicaments may be used in combination with each other or by themselves with the first medicament, so that the expression "second medicament" as used herein does not mean it is the only medicament besides the first medicament, respectively. Thus, the second medicament need not be one medicament, but may constitute or comprise more than one such drug.

These second medicaments as set forth herein are generally used in the same dosages and with the same administration routes as the first medicaments, or from about 1 to 99% of the dosages of the first medicaments. If such second medicaments are used at all, preferably, they are used in lower amounts than if the first medicament were not present, especially in subsequent dosings beyond the initial dosing with the first medicament, so as to eliminate or reduce side effects caused thereby.

For the treatment of RA, for example, the patient can be treated with an antibody of the invention in conjunction with any one or more of the following drugs: integrin antagonists, DMARDs (*e.g.*, MTX), NSAIDs, cytokine inhibitors such as HUMIRATM (adalimumab; Abbott Laboratories), ARAVA® (leflunomide), REMICADE® (infliximab; Centocor Inc., of Malvern, Pa), ENBREL® (etanercept; Immunex, WA), corticosteroids, or COX-2 inhibitors. DMARDs commonly used in RA include hydroxychloroquine, sulfasalazine, methotrexate, leflunomide, etanercept, infliximab, azathioprine, D-penicillamine, Gold (oral), Gold (intramuscular), minocycline, cyclosporine, or

Staphylococcal protein A immunoadsorption. Adalimumab is a human monoclonal antibody that binds to TNF α . Infliximab is a chimeric monoclonal antibody that binds to TNF α . Etanercept is an "immunoadhesin" fusion protein consisting of the extracellular ligand binding portion of the human 75 kD (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of a human IgG1. For conventional treatment of RA, see, *e.g.*, American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines, *Arthritis & Rheumatism* 46(2): 328-346 (2002). In a specific embodiment, the RA patient is treated with an LT α antibody of the invention in conjunction with MTX. An exemplary dosage of MTX is about 7.5–25 mg/kg/wk. MTX can be administered orally and subcutaneously.

For the treatment of ankylosing spondylitis, psoriatic arthritis, and Crohn's disease, the patient can be treated with an antibody of the invention in conjunction with, for example, REMICADE® (infliximab; from Centocor Inc., Malvern, PA) or ENBREL® (etanercept; Amgen, CA).

Treatments for SLE include high-dose corticosteroids and/or cyclophosphamide (HDCC) in conjunction with the antibodies herein.

For the treatment of psoriasis, patients can be administered a LT α -binding antibody in conjunction with topical treatments, such as topical steroids, anthralin, calcipotriene, clobetasol, and tazarotene, or with MTX, retinoids, cyclosporine, PUVA and UVB therapies, and integrin antagonists such as anti-CD11a or anti-CD18 antibodies, including, *e.g.*, efalizumab (RAPTIVA®). In one embodiment, the psoriasis patient is treated with the LT α -binding antibody sequentially or concurrently with cyclosporine or with efalizumab.

In certain embodiments, an immunoconjugate comprising the antibody herein conjugated with a cytotoxic agent is administered to the patient. In some embodiments, the immunoconjugate and/or antigen to which it is bound is/are internalized by the cell, resulting in increased therapeutic efficacy of the immunoconjugate in killing the target cell to which it binds. In one embodiment, the cytotoxic agent targets or interferes with nucleic acid in the target cell. Examples of such cytotoxic agents include any of the chemotherapeutic agents noted herein (such as a maytansinoid or a calicheamicin), a radioactive isotope, a ribonuclease, or a DNA endonuclease.

Articles of Manufacture

In another embodiment of the invention, articles of manufacture containing materials useful for the treatment of the disorders described above are provided. In one aspect, the article of manufacture comprises (a) a container comprising the antibodies herein (preferably

the container comprises the antibody and a pharmaceutically acceptable carrier or diluent within the container); and (b) a package insert with instructions for treating the disorder in a patient.

In a preferred embodiment, the article of manufacture herein further comprises a container comprising a second medicament, wherein the antibody is a first medicament. This article further comprises instructions on the package insert for treating the patient with the second medicament, in an effective amount. The second medicament may be any of those set forth above, with an exemplary second medicament being an antagonist binding to a B-cell surface marker (*e.g.*, a CD20 antibody), a BAFF antagonist, an immunosuppressive agent, including MTX and corticosteroids, an integrin antagonist, a cytokine antagonist such as a TNF, CD11, or CD18 antagonist, or a combination thereof. The preferred second medicaments are a steroid, a cytokine antagonist, and/or an immunosuppressive agent.

In this aspect, the package insert is on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, *etc.* The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition that is effective for treating the disorder in question and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the antibody herein. The label or package insert indicates that the composition is used for treating the particular disorder in a patient or subject eligible for treatment with specific guidance regarding administration of the compositions to the patients, including dosing amounts and intervals of antibody and any other medicament being provided. Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contra-indications, and/or warnings concerning the use of such therapeutic products. The article of manufacture may further comprise an additional container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution, and/or dextrose solution. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

In a specific embodiment of the invention, an article of manufacture is provided comprising, packaged together, a pharmaceutical composition comprising an antibody herein and a pharmaceutically acceptable carrier and a label stating that the antibody or

pharmaceutical composition is indicated for treating patients with an autoimmune disease such as RA, MS, lupus, or IBD.

In a preferred embodiment the article of manufacture herein further comprises a container comprising a second medicament, wherein the antibody is a first medicament, and which article further comprises instructions on the package insert for treating the patient with the second medicament, in an effective amount. The second medicament may be any of those set forth above, with an exemplary second medicament being those set forth above, including, especially for RA treatment, an immunosuppressive agent, a corticosteroid, a DMARD, an integrin antagonist, a NSAID, a cytokine antagonist, a bisphosphonate, or a combination thereof, more preferably a DMARD, NSAID, cytokine antagonist, integrin antagonist, or immunosuppressive agent. Most preferably, the second medicament is methotrexate or a DMARD if the disease is RA.

In another aspect, the invention provides a method for packaging or manufacturing an antibody herein or a pharmaceutical composition thereof comprising combining in a package the antibody or pharmaceutical composition and a label stating that the antibody or pharmaceutical composition is indicated for treating patients with an autoimmune disease such as RA, MS, lupus, or IBD.

Methods of Advertising

The invention herein also encompasses a method for advertising an antibody herein or a pharmaceutically acceptable composition thereof comprising promoting, to a target audience, the use of the antibody or pharmaceutical composition thereof for treating a patient or patient population with an autoimmune disease such as RA, MS, lupus, or IBD.

Advertising is generally paid communication through a non-personal medium in which the sponsor is identified and the message is controlled. Advertising for purposes herein includes publicity, public relations, product placement, sponsorship, underwriting, and sales promotion. This term also includes sponsored informational public notices appearing in any of the print communications media designed to appeal to a mass audience to persuade, inform, promote, motivate, or otherwise modify behavior toward a favorable pattern of purchasing, supporting, or approving the invention herein.

The advertising and promotion of the treatment methods herein may be accomplished by any means. Examples of advertising media used to deliver these messages include television, radio, movies, magazines, newspapers, the internet, and billboards, including commercials, which are messages appearing in the broadcast media. Advertisements also

include those on the seats of grocery carts, on the walls of an airport walkway, and on the sides of buses, or heard in telephone hold messages or in-store PA systems, or anywhere a visual or audible communication can be placed, generally in public places. More specific examples of promotion or advertising means include television, radio, movies, the internet such as webcasts and webinars, interactive computer networks intended to reach simultaneous users, fixed or electronic billboards and other public signs, posters, traditional or electronic literature such as magazines and newspapers, other media outlets, presentations or individual contacts by, *e.g.*, e-mail, phone, instant message, postal, courier, mass, or carrier mail, in-person visits, *etc.*

The type of advertising used will depend on many factors, for example, on the nature of the target audience to be reached, *e.g.*, hospitals, insurance companies, clinics, doctors, nurses, and patients, as well as cost considerations and the relevant jurisdictional laws and regulations governing advertising of medicaments. The advertising may be individualized or customized based on user characterizations defined by service interaction and/or other data such as user demographics and geographical location.

The following are non-limiting examples of the methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLE 1: Preparation of anti-human and anti-murine LT α monoclonal antibodies and hamster-murine chimeras

Anti-human LT α monoclonal antibodies 2C8 and 3F12 were generated as follows: BALB/c mice (Charles River Laboratories, Wilmington, DE) were hyperimmunized by injection with 5 μ g/dose of purified recombinant human LT α expressed in *E. coli* (Genentech, Inc., South San Francisco, CA; Genentech Lot# 8360-2B; see also Spriggs, "Tumor Necrosis Factor: Basic Principles and Preclinical Studies," *Biologic Therapy of Cancer*, DeVita *et al.*, eds., J.B. Lippincott Company (1991) Ch. 16, pp. 354-377; Ruddle, *Current Opinion in Immunology*, 4:327-332 (1992); Wong *et al.*, "Tumor Necrosis Factor," *Human Monocytes*, Academic Press (1989), pp. 195-215; Aggarwal *et al.*, *Cytokines and Lipocortins in Inflammation and Differentiation*, Wiley-Liss, Inc. 1990, pp. 375-384; and Paul *et al.*, *Ann. Rev. Immunol.*, 6:407-438 (1988)) in DETOXTM adjuvant (RIBI ImmunoChem Research, Inc., Hamilton, MO). Specifically, 10 μ g LT α 3 was mixed with 50 μ L of the adjuvant and injected subcutaneously into the rear footpads of mice (strain

BALB/c) on days 1, 4, 15, 29, 41, 56, and 71. Serum was collected on Days 15 and 56 for detection of anti-LT α 3-specific antibodies by binding ELISA.

On day 75, the animals were sacrificed, spleens were harvested, and 3×10^7 cells were fused with 5×10^7 cells of the mouse myeloma line X63-Ag8.653 using 50% polyethylene glycol (PEG) 4000 by an established procedure (Oi and Herzenberg, in *Selected Methods in Cellular Immunology*, B. Mishel and S. Schiigi, eds., (W.J. Freeman Co., San Francisco, CA, 1980)). The fused cells were plated into 96-well microtiter plates at a density of 2×10^5 cells/well containing HAT medium for selection (Littlefield, *Science*, 145:709 (1964)). After 12 days, the supernatants were harvested and screened for antibody production by direct ELISA. The supernatants harvested from each hybridoma lineage were purified by affinity chromatography (Pharmacia fast protein liquid chromatography (FPLC); Pharmacia, Uppsala, Sweden). The purified antibody preparations were then sterile filtered (0.2- μ m pore size; Nalgene, Rochester NY) and stored at 4°C in phosphate-buffered saline (PBS).

Anti-murine LT α monoclonal antibody S5H3 was generated as follows: Armenian hamsters were immunized with recombinant mouse LT α 2 β 1 (R&D Systems Inc. (1008-LY)). The hamsters were immunized with 2 μ g of antigen by footpad for 12 injections, with an IP boost of 2 μ g of antigen once. The lymph nodes and spleen were fused by PEG treatment. Primary screening was done by ELISA on Immunogen- vs. Genentech-manufactured murine LT α -6His (murine LT α with six histidines attached at the C-terminal end). Detection was done with peroxidase-conjugated AFFINIPURETM goat anti-Armenian hamster IgG (H+L) (Jackson ImmunoResearch 127-035-160). Secondary screening was performed by FACS on irrelevant transfected HEK 293 cells vs. HEK 293 cells stably transfected with murine LT α β (under G418 selection). Detection was performed using R-phycoerythrin-conjugated AFFINIPURETM F(ab')₂ goat anti-Armenian hamster IgG (H+L) (Jackson ImmunoResearch 127-116-160). The hybridoma from which this antibody was derived was deposited with ATCC as Deposit No. PTA-7538 (hybridoma murine Lymphotoxin alpha2 beta1 s5H3.2.2).

All anti-murine and anti-human LT α antibodies generated in these experiments were selected for recognition of LT α 3. These were then screened for (a) blocking of binding of LT α 3 to TNFR1 and TNFR11 by ELISA, (b) binding of LT α β by ELISA and FACS of cells stably transfected with LT α and LT β , and (c) blocking of LT α β interaction with the LT β receptor by ELISA. Antibodies 3F12, 2C8, and S5H3 were selected because they all bound and neutralized LT α 3 and LT α β .

For use in the murine *in vivo* studies described below, the hamster anti-murine LT α hybridoma was cloned as a hamster/mouse chimera. Total RNA was extracted from S5H3 hybridoma cells using a RNEASY MINITM kit (Qiagen) and the manufacturer's suggested protocol. RT-PCR was accomplished using a Qiagen ONE-STEPTM kit, and primers were
5 designed to amplify the light- and heavy-chain variable domains and add restriction enzyme sites for cloning.

For construction of the light chain of this chimeric antibody S5H3, two sequential cloning steps were used. In the first step, RT-PCR, *Cla*I and *Asc*I sites were added, using primer set one, allowing cloning into a pRK-based vector simply to amplify DNA for
10 sequencing. After the DNA sequence of the light-chain variable domain was obtained, a round of PCR was performed, using this plasmid as template, in order to add *Eco*RV and *Kpn*I sites that are compatible with the expression vector.

For construction of the heavy chain of S5H3, likewise two cloning steps were used. Specifically, RT-PCR was used to amplify cDNA and add *Cla*I and *Asc*I sites for cloning into
15 a vector for sequencing. Subsequently, this plasmid was used as template for PCR to add *Bsi*WI and *Apa*I sites that are compatible with the expression vector.

For RT-PCR, the primers used were as follows:

S5H3 light-chain set one:

5' primer: GGA TCA TCG ATA CAR CTN GTV YTN CAN CAR TCN CC (SEQ ID
20 NO:56)
3' primer: GGT AAC GGC GCG CCG YTC AGA AGA TGG TGG RAA (SEQ ID NO:57)

S5H3 light-chain set two:

5' primer: GGA TCC GAT ATC CAG CTG GTA TTG ACC CAA TCT
25 (SEQ ID NO:58)
3' primer: GGA TCA GGT ACC GCT GCC AAA AAC ACA CGA CCC (SEQ ID NO:59)

S5H3 heavy chain set one:

5' primer: TGA TAA TCG ATG ARG TNC CAT TTR GTN GAR (SEQ ID NO:60)
30 3' primer: TAG TAA GGC GCG CCT GGT CAG GGA NCC NGA RTT CCA (SEQ ID
NO:61)

S5H3 heavy chain set two:

5' primer: TGA TCG CGT ACG CTG AGG TTC AAT TGG TTG AG (SEQ ID NO:62)

3' primer: TGA TCG TGG GCC CTT TGT TGT GGC TGA GGA GAC GG (SEQ ID

NO:63) wherein R = A or G, Y = C or T, and N = all four nucleic acids A, G, C, and T.

5 Purified PCR products for the light-chain variable domain were cloned into a pRK mammalian cell expression vector (pRK.LPG2.mukappa) containing the murine kappa constant domain. For the heavy chain, PCR products were cloned into a pRK mammalian cell expression vector (pRK.LPG10.muIgG2a) encoding the murine CH1, hinge, CH2, and CH3 domains of murine isotype IgG2a. These vectors are derivatives of previously described
10 vectors for expression of IgGs in 293 cells (Shields *et al.*, *J. Biol. Chem.*, 276:6591-6604 (2000) and Gorman *et al.*, *DNA Prot Eng Tech*, 2:3-10 (1990)). For S5H3 chimeric antibody expression, the plasmids for the light and heavy chains were transiently co-transfected into 293 cells (an adenovirus-transformed human embryonic kidney cell line (Graham *et al.*, *J. Gen. Virol.*, 36:59-74 (1977)) or CHO cells. The antibody protein was purified from cell-
15 culture supernatants by protein A affinity chromatography.

The LT α 3 binding ELISA was carried out as follows: Microtiter wells were coated with a 1 μ g/ml LT α 3 in 50 mM carbonate buffer solution (50 μ L/well) overnight. The unadsorbed solution was aspirated from the wells. Wells were blocked with 200 μ L PBS containing 5 mg/ml bovine serum albumin (PBS-BSA) for 2 hours. 50 μ L of test sample
20 appropriately diluted in PBS-BSA was added to each well, incubated for one hour, and washed with PBS containing 0.05% TWEEN-20TM surfactant. 100 μ L of horse radish peroxidase-labeled goat anti-mouse IgG in PBS-BSA buffer was added to each well and incubated for one hour. Each well was washed with PBS/0.05%TWEEN-20TM surfactant and then citrate phosphate buffer, pH 5, containing 0.1 mg o-phenylenediamine/ml (substrate
25 solution), and aqueous 30% H₂O₂ was added to each well. The wells were incubated for 30 minutes; then the reaction was stopped with 50 μ L 2.5M sulfuric acid H₂SO₄, and absorbance was read at 490 nm.

The LT α 3-blocking ELISA, the LT α β -binding ELISA and FACS assays, and the LT α β -blocking ELISA were carried out by procedures described further below.

EXAMPLE 2: Sequencing, humanization, and affinity maturation of anti-LT α antibody 3F12

1. *De novo* sequencing of anti-LT α 3F12 using LTQ-FTMS-MS/MS analysis and Edman degradation

5 Because the hybridoma cell line 3F12.2D3 was lost from the frozen cell line bank, anti-LT α monoclonal antibody protein, purified from ascites 3F12.2D3, was submitted for sequencing at a concentration of about 3.0 mg/mL in PBS. The antibody (Ab) was separated on 4-20% TRISTM HCl SDS-PAGE under reducing conditions and each resolved heavy chain (HC) and light chain (LC) was subjected to N-terminal sequencing (25-30 residues were
10 sequenced) to establish monoclonality. The HC was found to be blocked with a pyroglutamyl group that made the N-terminal amino acid inaccessible to Edman sequencing. The blocking group was subsequently removed using the pyroglutamate aminopeptidase enzyme (PGAP) and the HC chain subjected to sequencing again. Both the HC and LC were confirmed to be monoclonal. The Ab was then treated with various enzymatic and chemical
15 cleavages according to a strategy described in *Analytical Biochemistry*, 35:77-86 (2006). In brief, these consisted of the enzymes trypsin, endo Lys-C, Asp-N, and chemical treatments with CNBr and dilute acid (Asp/Pro cleavage).

Chemical cleavages generated a mixture of peptides that were sequenced on model 494 PROCISETM sequencers and identified using Genentech's SEQSORT program
20 (SEQSORT is a utility program designed to make the output produced by the programs in Pittsburgh Supercomputing Center's sequence analysis suite more manageable and readable). These chemical cleavages allowed identification of the constant region of the HC and LC by identity with antibodies in protein sequence databases. Peptides generated from the enzymatic digestions were collected from capillary RP-HPLC separations and the peptide
25 masses measured using MALDI-TOF analysis. Peptides with masses that did not match the constant regions were subjected to further analysis using Edman degradation or liquid chromatography-electrospray ionization tandem mass spectrometry (LC-MS/MS) on an LTQ-FT mass spectrometer. Sequences of the variable regions of the HC and LC were derived from peptides with overlapping sequences obtained by the various proteolytic cleavages.

30 For analysis of the peptides by LC-MS/MS a micro-capillary C18 reverse-phase chromatography column (0.15 x 150 mm i.d., 5 μ m, 300Å) was employed using a flow rate of 1 μ L/minute on an AGILENTTM 1100 series capillary LC system. Solvent A was applied, and a gradient from 2 to 80% solvent B was applied, solvent A consisting of 0.1% v/v formic

acid in water and solvent B consisting of 0.1% v/v formic acid in acetonitrile. The LC was coupled in-line with the LTQ-FT mass spectrometer, and peptides with m/z between 350 and 1800 were analyzed in data-dependent mode with the five most abundant species in each full mass range survey scan being selected for collision-induced dissociation (CID). The resulting CID spectra were screened with the search program MASCOT to find exact matches to database sequences, and potentially novel sequences were determined by manual *de novo* interpretation. Peptides that could not be completely sequenced or that contained the isobaric amino acid pairs of leucine/isoleucine and glutamine/lysine were analyzed further using Edman degradation.

A Fab portion of anti-LT α was obtained from papain digestion of the intact antibody followed by size-exclusion chromatography. Masses of both heavy-chain and light-chain components of the Fab were obtained by quadruple-TOF mass spectrometry after inter-chain disulfides were reduced. The experimental masses corresponded to those calculated based on the obtained sequences of the heavy chain and light chain to within 0.5 Da each.

Sequence of anti-LT α light chain for chimera 3F12.2D3:

DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSTNQKNFLAWYQQKPGQSPKLLIYWA
STRDSGVPDRFTGSGSGTDFTLTISVKAEDLAVYYCQQYYSPRTFGGGTKLEIKRA
DAAPTVISIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSEKQNGVLNSWTDQ
DSKDSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID

NO:64)

Sequence of anti-LT α heavy-chain variable region spanning into constant region for chimera 3F12.2D3:

QGQLQQSGAELMKPGASVKISCKATGYTFSSYWIEWVKQRPGHGLEWIGEISPGSGS
TNYNEEFKGGKATFTADKSSNTAYIQLSSLTSEDSAVYYCADGYHGYWGQGTTTLTV
SSAKTTTPPSVYPLAPGCGDTTGSSVTLGCLVKGYFPESVTVTWNSGSLSSSVHTFPAL
LQSGLYTMSSSVTVPSSTWPSQTVTCSVAHPASSTTVDDKKLEPSGPIST (SEQ ID

NO:65)

wherein N is any amino acid.

Figure 1C shows the light-chain variable region for this clone, as well as the light-chain constant region as filled in by homology with other antibodies, but not itself sequenced. Figure 1D shows the heavy-chain variable region for this clone, as well as the heavy-chain constant region as filled in by homology with other antibodies.

2. Synthesis of DNA encoding the derived amino acid sequence of chimeric 3F12

Using a pRK-based plasmid containing the gene for the light-chain of anti-TGF-beta monoclonal antibody 2G7 (WO 2005/97832) as a template, and the amino acid sequences derived above, several rounds of oligonucleotide-directed mutagenesis were performed to derive the gene for the light chain of chimeric antibody 3F12. Similarly, the heavy chain of monoclonal antibody 2G7 was the template for construction of the heavy chain of 3F12.

Both light- and heavy-chain templates contained the human constant domains, C kappa for the light chain, and CH1, hinge, CH2, and CH3 of IgG1 isotype, for the heavy chain. The oligonucleotides used in the synthesis are given below for the light and heavy chains.

Oligonucleotides for synthesis of chimeric light chain of 3F12:

CA1845

GCT CAT AGT GAC CTT TTC TCC AAC AGA CAC AGC CAG AGA TGA TGG CGA
CTG TGA CAT CAC GAT ATC TGA ATG TAC TCC (SEQ ID NO:66)

CA1846

GCT GTA AGT CCA GTC AAA GTC TTT TAT ACA GTA CCA ATC AGA AGA ACT
TCT TGG CCT GGT ACC AGC (SEQ ID NO:67)

CA1847

GCG ATC AGG GAC ACC AGA TTC CCT AGT GGA TGC CC (SEQ ID NO:68)

CA1848

GCC ACG TCT TCA GCT TTT ACA CTG CTG ATG G (SEQ ID NO:69)

CA1849

GGT CCC CCC TCC GAA CGT GCG CGG GTA GGA GTA GTA TTG CTG ACA GTA
ATA AAC TGC CAG GTC (SEQ ID NO:70)

Oligonucleotides for synthesis of chimeric heavy chain of 3F12:

CA1850

GCT GCA GCT GAC CTT CTG AAT GTA CTC C (SEQ ID NO:71)

CA1851

GCC TTG CAG GAG ATC TTC ACT GAA GCC CCA GGC TTC ATC AGC TCA GCT
CC (SEQ ID NO:72)

CA1852

CCC ACT CTA TCC AGT AAC TAG AGA AGG TGT ATC CAG TAG CCT TGC AGG
(SEQ ID NO:73)

CA1853

CCA CTT CCA GGA CTA ATC TCT CCA ATC CAC TCA AGG CCA TGT CCA GGC C
(SEQ ID NO:74)

CA1854

GCC CTT GAA CTC CTC ATT GTA ATT AGT ACT ACC ACT TCC AGG (SEQ ID NO:75)

5 CA1855

CCG CAG AGT CCT CAG ATG TGA TCA GGC TGC TGA GCT GGA TGT AGG CAG
TGT TGG AGG ATT TGT CTG CAG TGA ATG TTG CCT TGC C (SEQ ID NO:76)

10 CA1856

GGC TGA GGA GAC GGT GAC TGT GGT GCC TTG GCC CCA GTA GCC ATG GTA
CCC GTC TGC ACA GTA ATA GAC CTC (SEQ ID NO:77)

15 CA1871

CCA GAC TGC TGC AGC TGA CCT TGT GAA TGT ACT CCA GTT GC (SEQ ID NO:78)

Since the original 3F12 murine monoclonal antibody had the isotype of IgG2b, the murine constant domains for the light and heavy chains of this isotype were swapped for the human constant domains in the chimera, giving a fully murine antibody. For comparison, the murine IgG2a isotype was also constructed. DNA for the light and heavy chain of each variant was co-transfected into an adenovirus-transformed human embryonic kidney cell line, 293, for transient expression, and protein was purified from conditioned media using Protein A affinity chromatography. Binding to LT α in an ELISA format was compared between the original 3F12 monoclonal antibody from ascites, and the synthetic muIgG2a and muIgG2b variants.

Briefly, a NUNC MAXISORPTM plate was coated with two micrograms per ml of LT α (as described in Example 1 above) in 50 mM carbonate buffer, pH 9.6, overnight at 4°C, and then blocked with 0.5% BSA, 10 PPM PROCLINTM in PBS at room temperature for 1 hour. Serial dilutions of samples in PBS containing 0.5% BSA, 0.05% TWEENTM, 10 PPM PROCLINTM were incubated on the plates for 2 hours. After washing, bound antibody was detected with horseradish peroxidase-conjugated anti-human Fc (Jackson ImmunoResearch) using 3,3',5,5'-tetramethyl benzidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD) as substrate. Absorbance was read at 450 nm. Both the IgG2a- and IgG2b-cloned variants bound similarly to the 3F12 antibody purified from ascites, indicating that the correct sequence had been synthesized.

35 3. Humanization of 3F12

Using the amino acid sequence of the chimeric clone, the CDR residues of the light and heavy chain were identified (Kabat). Oligonucleotide-directed mutagenesis was used to swap these CDRs onto vectors containing the coding sequences of the light and heavy chains of humanized anti-TGF β 2G7 version 5 (WO 2005/97832), obtaining the CDRswap version of 3F12. The framework sequences thus derived are for the VL kappa subgroup I and VH subgroup III consensus sequences, respectively:

DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:51)-L1-WYQQKPGKAPKLLIY (SEQ ID NO:55)-L2-GVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:53)-L3-FGQGTKVEIKR (SEQ ID NO:54) and
EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:46)-H1-WVRQAPGKGLEWVG (SEQ ID NO:45)-H2-RATFSADNSKNTAYLQMNSLRAEDTAVYYCAD (SEQ ID NO:50)-H3-WGQGTLVTVSS (SEQ ID NO: 49).

After expression and purification as described above, binding of the CDRswap to LT α was compared to that of the chimera. Binding for the CDR swap was almost completely lost.

To restore binding of the humanized antibody, mutations were constructed using DNA from the CDRswap as template. Using a computer-generated model, these mutations were designed to change human framework residues to their murine counterparts at positions where the change might affect CDR conformation of the antibody-antigen surface. Mutants and their relative binding by ELISA as described above are shown in Table 1 below.

Changing isoleucine 69 to phenylalanine, to give v2, increased binding significantly, as did the change Leu78Ala, while the change Asn73Lys gave no improvement over the CDRswap. Combining Ile69Phe and Leu78Ala gave version 5, which binds LT α approximately 5-fold less well than the chimera in this ELISA assay format.

Two variants were made to move the framework sequence closer to the human consensus. Version 6, with the changes Lys24Arg and Ser25Ala, was equivalent in binding to version 5. However, an attempt to change the usual aspartic acid at position 94 to the consensus arginine (version 7) resulted in almost complete loss of binding.

TABLE 1

3F12 variant	Heavy chain (VH) substitutions	Light chain (VL) substitutions	Relative binding*
Chimera			1
CDRswap	(CDR swap)	(CDR swap)	no binding
v2	I69F	(CDR swap)	>1000x down
v3	N73K	(CDR swap)	>1000x down
v4	L78A	(CDR swap)	>500x down

v5	I68F, L78A	(CDR swap)	5 to 20 fold down
v6	(CDR swap)	K24R, S25A	5 to 20 fold down
v7	D94R	(CDR swap)	no binding

*These values are relative to the original chimera (with heavy chain SEQ ID NO:35 and heavy-chain variable region set forth in Figure 1B), which was discovered later to have an “extra” serine in the FR3 of the heavy chain (a serine after the L at position c in SEQ ID NO:29 in Figure 3B). This serine was taken out to make chimera2 (having the heavy-chain variable region of SEQ ID NO:38 in Figure 1D), and the ELISA binding was found to be the same as above.

All animal studies described below using the 3F12 chimera refer to the original chimera, and not chimera2 as described in the footnote above.

4. Affinity maturation of antibody 3F12

For identification of which residues and regions in the CDRs might be most important for binding LT α , and thus candidates for inclusion in phage-display libraries for affinity maturation, partial alanine scanning was performed, using v5 as single-strand template for oligonucleotide-directed mutagenesis.

As shown in Table 2, several residues were found which when mutated to alanine produced a loss or gain in binding to LT α .

TABLE 2

version #	Position	relative binding*
	L1	
13	Y31A	3.40
14	T33A	0.72
15	N34A	0.49
16	K36A	3.70
17	F38A	0.32
	L3	
18	Q89A	4.20
19	Y91A	2.30
20	Y92A	0.71
21	S93A	0.82
22	Y94A	14.30
23	R96A	18.20
	H2	
24	G53A	34.10
25	G55A	1.20

26	S56A	1.07
27	N58A	1.70
28	Y59A	2.73
29	N60A	2.31
30	E61A	1.78
31	E62A	1.93
	H3	
8	G95A	>100
9	Y99A	10.00
10	H100A	>100
11	G101A	
12	Y102A	0.81

*The ratio of the EC50 of the variant divided by the EC50 of version 5 in plate-based ELISA against LT α . Higher numbers mean weaker binding, numbers >1 mean binding is less than that of version 5.

Thus, versions 14, 15, 17, 20, 21, and 12 have positions where changes could be made to improve binding affinity, and it is expected that conservative substitutions (or even non-conservative in some instances as determined by the skilled artisan by routine testing) could be made at those respective positions to increase binding.

EXAMPLE 3: Preparation of chimeric antibody 2C8 and humanization

1. Preparation of chimeric antibody

The variable regions of the heavy and light chains of the murine anti-LT α antibody 2C8 that bound and neutralized LT α 3 and LT α 3 were cloned and formatted as a murine/human chimeric antibody essentially as described above in Example 1 for the cloning of S5H3.

For RT-PCR, the primers used were as follows:

2C8 light chain:

5' primer: GCC ATA GAT ATC GTR ATG CAN CAG TCT C (SEQ ID NO:79)

3' primer: TTT KAT YTC CAG CTT GGT ACC (SEQ ID NO:80)

2C8 heavy chain:

5' primer: GAT CGA CGT ACG CTG AGG TYC AGC TSC AGC TSC AGC AGT CTG G (SEQ ID NO:81)

3' primer: ACA GAT GGG CCC TTG GTG GAG GCT GMR GAG ACD GTG ASH RDR GT (SEQ ID NO:82).

wherein K = G or T, S = C or G, D = A or G or T, M = A or C, H = A or C or T, N is T, G, A, or C (any nucleic acid of these four), R = A or G, and Y = C or T.

The variable domain of the light chain was cloned into vector pRK.LPG3.HumanKappa containing the human kappa constant domain, while the heavy-chain variable domain was cloned into expression vector pRK.LPG4.HumanHC encoding the full-length human IgG1 constant domains. These vectors are derivatives of previously described vectors for expression of IgGs in 293 cells (Shields *et al.*, *J Biol Chem*, 276:6591-6604 (2000) and Gorman *et al.*, *DNA Prot Eng Tech*, 2:3-10 (1990)). For transient expression of antibody, these plasmids were co-transfected into 293 cells or CHO mammalian cell lines and the protein was purified as described in Example 1 above.

This chimeric antibody 2C8 was used in the animal experiments described below.

2. Humanization of 2C8

Humanization of the anti-LT α antibody 2C8 was carried out in a series of site-directed mutagenesis steps. Using the sequence of the variable domains of the chimera, the CDR residues of the light and heavy chains were identified by comparing the amino acid sequence of these domains with the sequences of known antibodies (Kabat *et al.*, *supra*). The CDRs were defined based on sequence hypervariability (Kabat *et al.*, *supra*) and are shown in Figure 2.

The CDR swap of 2C8 was made using oligonucleotide site-directed mutagenesis, wherein the CDR regions were put onto separate pRK5-based vectors for the light and heavy chains, respectively. These vectors contain the light-chain human kappa I constant domain, or for the heavy-chain vector, the human IgG1 constant domains CH1, hinge, CH2, and CH3 (Gorman, *Current Opinion in Biotechnology*, 1, (1), p36-47 (1990)).

Comparison to other humanized antibodies indicated that the light chain of 2C8 is very similar to the light chain of humanized anti-HER2 antibody 4D5 (US 5,821,337), while the heavy chain of 2C8 is similar in sequence to anti-TGF- β 2G7 (WO 2005/97832). Therefore, a vector containing the light chain of rhuMAb 4D5 was used as the template for the construction of the light chain of 2C8. Site-directed mutagenesis was performed on a deoxyuridine-containing template derived from this vector, using oligonucleotides with light-chain sequences shown in Table 3. Additionally, the oligonucleotide CA1730 was used to revert the framework 3 sequence to that of the human kappa I consensus.

TABLE 3

Oligonucleotides for 2C8 (light chain)

<u>Substitution</u>	<u>Oligonucleotide sequence</u>
5 CDR-L1	CC TGG TTT CTG TTG ATA CCA GGC TAC AGC GGA AGA CAC AGC CTG ACT GGC ACG GCA GG (SEQ ID NO:83)
CDR-L2	GCG AGA AGG GAC TCC AGT GTA ACG GTG GGA TGC AGA GTA AAT CTG TAG TTT CGG AGC (SEQ ID NO:84)
CDR-L3	GGT ACC CTG TCC GAA CGT CCA AGG AGT AGA ATA ATG TTG 10 CTG ACA GTA ATA AGT TGC (SEQ ID NO:85)
R66G (CA1730)	GGT CAG AGT GAA ATC CGT CCC AGA TCC GGA TCC AGA GAA GCG (SEQ ID NO:86)

(All the oligonucleotides were ordered as reverse complements to the coding strand, as the fl ori in pRK vectors is in the opposite orientation from that in pBR-based vectors.)

15 For the heavy chain, the deoxyuridine-containing template was derived from a vector containing the heavy chain of humanized antibody 2G7 (WO 2005/97832) using oligonucleotides shown in Table 4. The framework was converted to consensus using oligonucleotides KM55, KM56, and KM61.

TABLE 4

Oligonucleotides for 2C8 (heavy chain)

CDR-H1	GGC CTG ACG GAC CCA ATG GAT CAC ATA GCT GGT GAA TCT GTA GCC AGA AGC TGC (SEQ ID NO:87)
CDR-H2	CCC CTT GAA CTT CTC GTT ATA GTT GGT GCC GTC GTT ATA AGG ATT GTT ATA ACC AAC CCA CTC GAG GCC (SEQ ID NO:88) GGT TCC TTG ACC CCA GTA GGC GAA CCA TGG GAG CAT TGT
CDR-H3	GGG TCG AGA ACA ATA ATA GAC GGC AGT GTC CTC AGC (SEQ ID NO:89) CC GTC GTT ATA AGG ATT GTT ATA ACT AAC CCA CTC GAG
KM55	GCC (SEQ ID NO:90) CAT CTG CAG GTA TAG TGT GTT TTT CGA ATT GTC ACG ACT
KM56	GAT AGT GAA GCG CCC CTT GAA C (SEQ ID NO:91) CCA TGG GAG CAT TGT GGG TCG AGC ACA ATA ATA GAC GGC
KM61	AGT GTC C (SEQ ID NO:92)

The light and heavy chains comprising the CDR swap, v10, thus derived, were co-transfected into an adenovirus-transformed human embryonic kidney cell line, 293 (Graham
25 *et al., supra*). Antibodies were purified from culture supernatants using protein A-

SEPHAROSE CL-4BTM resin, then buffer-exchanged into 10 mM sodium succinate, 140 mM NaCl, pH 6.0, and concentrated using a CENTRICON-10TM concentrator (Amicon).

For measuring relative binding affinities of the CDR swap, and subsequent 2C8 variants, to human LT α , a plate-based ELISA was used as described above.

As shown in Table 5, the CDR swap, v10, shows no binding in the LT α ELISA. Version 2 and subsequent versions were constructed to regain this binding. Version 2 regains binding equivalent to the chimera, but contains seven non-consensus residues. Further variants were studied to obtain a framework as close to consensus as possible while still retaining or improving binding relative to the chimera. Version 12 has binding equivalent to (by ELISA), or slightly better than (by BIACORETM analysis), the chimera, and retains only five non-consensus residues. The light- and heavy-chain sequences of versions 7 and 12 are provided in Figure 2C.

TABLE 5

2C8 version	HC	LC	ELISA IC ₅₀ ratio	BIACORE TM (rhLT α)		mutations
				KD	ka/kd	
v0	v0	v0	1	0.6nM		
v2	v2	v1	1.7	0.2nM	6e5/ 1e-4	
v3	v3	v1	10.2			
v4	v4	v1	27.8			
v7	v2	v2	1.2	0.4nM	5e5/ 1.6e-4	LC.Q46L
v8	v8	v1	non- binding			HC.G49S.S93A
v10	v10	v1	non- binding			CDRswap
v12	v12	v2	1	0.07nM	2e6/ 0.9e-4	HC.A67F
v13	v13	v2	2.4			HC.S71R
v14	v14	v2	2			HC.K73N
v15	v15	v2	11.1			HC.A93S
v16	v12	v3	0.6			LC.Q46L.E89Q.S90H
vX	vX	v3	0.3			HC.D53A.A67F

3. Affinity maturation of 2C8

A humanized 2C8 monoclonal antibody with improved affinity to LT α was selected from a library of monovalent Fab displayed on phage. A template was generated that displays the humanized 2C8.v2 Fab and contains a stop codon in the CDR-L3.

Oligonucleotides (listed in Table 6) were designed to soft- randomize specific CDR-L3 and CDR-H3 residues.

TABLE 6

Soft randomization oligonucleotides for affinity maturation of 2C8 CDR-L3 and CDR-H3

Oligonucleotide name	Sequence ¹
KM73.2C8.phage.v3.LC3.soft	GGA AGA CTT CGC AAC TTA TTA CTG TCA G75 575 885 887 857 8CC T86 6AC GTT CGG ACA GGG TAC CAA GGT GG (SEQ ID NO:93)
KM74.2C8.phage.v3.HC3.soft	G GAC ACT GCC GTC TAT TAT TGT TCT CGA CCC 575 576 787 CCA 866 887 677 TAC TGG GGT CAA GGA ACC CTG G (SEQ ID NO:94)

¹ Soft randomization code (A=5, G= 6, C= 7 and T= 8): each degenerate base is 70% wild-type with 10% equimolar amounts of the remaining three nucleotides added.

5 Three rounds of sorting on LT α were used to selectively capture high-affinity Fab molecules with less than 2 nM binding to the antigen. For the first round, phage were captured for 2 hours against 2 μ g/ml plate-coated LT α at ambient temperature. The second and third rounds of selection were performed at ambient temperature by capturing phage for 30 minutes with 2 nM biotinylated LT α in solution phase, followed by capture on

10 neutravidin-coated plates. The highest affinity clone was identified using a competitive ELISA. Constant concentrations of phage clones (normalized by solid-phase LT α binding OD) were incubated with increasing concentrations of LT α (0-50 nM) in solution before capture on a plate coated with 2 μ g/ml LT α . One clone demonstrated a 10-fold improvement in competition with LT α over wild-type 2C8.v2. DNA sequencing of this phagemid clone

15 revealed two amino acid changes from the wild-type 2C8 sequence in CDR-L3: glutamine at position 89 was changed to a glutamic acid, and histidine at position 90 was changed to a serine. These two CDR-L3 changes were made by site-directed mutagenesis on the wild-type 2C8.v7 light chain. When v7 light chain was transfected into 293 cells with the 2C8.v12 heavy chain to give 2C8.v16, the resultant antibody demonstrated a two-fold improvement in

20 IgG binding to LT α by ELISA when compared to 2C8.v2 (Table 5).

To enhance stability, by removing a potential aspartic acid isomerization site, asp 53 in heavy-chain CDR2 was changed to alanine. The resultant 2C8 variant, vX, had a 3.5-fold and 3-fold improvement in binding to LT α by ELISA, compared to the 2C8.v2 and 2C8.chimera, respectively (Table 5).

25 Table 7 below shows how residue changes in CDR-L3 regions ranked as to ability to bind LT α by ELISA analysis.

TABLE 7

Affinity maturation of 2C8 by phage display: sequence changes in phagemid CDR-L3 regions ranked by ability to compete rhLT α

30

Phagemid clone	CDR-L3 Sequence ^{1,2}	rhLT α Competitive ELISA IC ₅₀ (nM)
Wild-type	QQHYSTPWT (SEQ ID NO:9)	3.14
A8	QESYSTPWT (SEQ ID NO:10)	0.32
G7	QENYSTPWT (SEQ ID NO:11)	0.57
H6	QEVYSTPWT (SEQ ID NO:12)	1.1
C12	QEHYSTPWT (SEQ ID NO:95)	3.41
H9	QQHYYPWT (SEQ ID NO:96)	3.80
G2	QQYYSTPWT (SEQ ID NO:97)	4.51
G12	QKTESTPWT (SEQ ID NO:98)	5.34
D8	QQFYSPWT (SEQ ID NO:99)	136.62
E2	QQKYSTPWT (SEQ ID NO:100)	not determined ³

¹ Boldface and italicized type shows residues randomized.

² All phagemids retained the wild-type CDR-H3 sequence

³ Clone E2 did not meet rhLT α direct ELISA binding threshold (of OD= 1) necessary for phagemid normalization in competitive ELISA.

5

It can be seen from this table that the best clones were A8, G7, and H6, which all bound rhLT α with higher affinity than the wild-type clone.

EXAMPLE 4: Anti-LT α antibody inhibits antibody-induced arthritis (AIA)

10 AIA differs from collagen-induced arthritis (CIA), described below, in that instead of injecting the antigen (bovine collagen type II), antibodies recognizing type II collagen are injected. In this way, adaptive B- and T-cell responses are circumvented to directly induce effector functions on macrophages and neutrophils through Fc receptor and complement-mediated activation.

15 AIA was induced in mice by i.v. injection of a combination of four different monoclonal antibodies generated by the ARTHROGEN-CIATM mouse B-hybridoma cell lines. Three of the monoclonal antibodies recognize autoantigenic epitopes clustered within an 84-amino-acid residue fragment, LyC2 (the smallest arthritogenic fragment of type II collagen) of CB11, and the fourth monoclonal antibody reacts with LyC1. All four

antibodies recognize the conserved epitopes shared by various species of type II collagen and crossreact with homologous and heterologous type II collagen.

All groups (n=5/group) received 2 mg of a cocktail of monoclonal antibodies i.v. in 100 µl PBS on day 0 followed by 25 µg LPS i.p. in 50 µl PBS on day 3. Animals were
5 treated on Day -1 (preventive) or Day 4 (therapeutic) with 10 mg/kg control isotype (anti-ragweed), hamster-murine chimeric LT α antibody S5H3 (as a surrogate for the anti-human LT α antibodies), anti-LT.Fc mutant (an anti-LT α antibody with DANA mutations in the Fc region as described below), or TNFRII.Fc (a TNFR.Ig immunoadhesin) intraperitoneally or subcutaneously. Swelling of the paws was monitored for 14 days. Each paw was given a
10 score of 0-4, for a total maximum of 16 per animal.

DANA mutations in the anti-LT.Fc mutant used in this Example and others below refer to two single-position changes in the Fc region of mouse or human IgG. These changes are D (Aspartic Acid) to A (Alanine) in position 265 and N (Asparagine) to A (Alanine) in position 297. The DANA mutant was constructed by introducing alanine residues into
15 positions 265 and 297 of mouse or human IgG by a PCR-based site-directed mutagenesis method (GENE TAILOR™, Invitrogen Corp.).

Figure 4A shows the average clinical score for the prophylactic treatment of each group described above as a function of days post-induction. It can be seen that the S5H3 antibody was the most effective in this model of all the treatment groups, including the
20 TNFRII.Fc and anti-LT.Fc mutant molecules. Thus, antibody S5H3 significantly inhibited the development of arthritis in this antibody-induced model. Anti-LT α with the Fc DANA mutation (anti-LT.Fc mutant) had no efficacy, indicating that Fc-mediated killing of LT α -expressing cells was required for efficacy (Figure 4A).

Figure 4B shows the average clinical score for therapeutic treatment of each group
25 described above as a function of days post-induction. The results show that the S5H3 antibody significantly inhibited the development of arthritis in this antibody-induced model when administered therapeutically, versus the other molecules tested.

In conclusion, animals treated with the hamster-mouse chimera S5H3 had significantly reduced clinical scores as compared to animals treated with S5H3.DANA or
30 control. S5H3 demonstrated both prophylactic and therapeutic efficacy in this animal model.

EXAMPLE 5: Anti-LT α inhibits collagen-induced arthritis (CIA)

In RA the synovial membrane of multiple joints can become inflamed, leading to destruction of joint tissues including bone and cartilage. The synovium of RA can be highly inflammatory in nature and is typically characterized by lymphocyte and mononuclear cell infiltration, T-cell and antigen-presenting cell (APC) activation, B-cell immunoglobulin (Ig) secretion, and pro-inflammatory cytokine production (Potocnik *et al.*, *Scand. J. Immunol.*, 31:213 (1990); Wernick *et al.*, *Arthritis Rheum.*, 28:742 (1985); Ridley *et al.*, *Br. J. Rheumatology*, 29:84 (1990); Thomas *et al.*, *J. Immunol.*, 152:2613 (1994); and Thomas *et al.*, *J. Immunol.*, 156:3074 (1996)). Chronically inflamed synovium is usually accompanied by a massive CD4 T- cell infiltration (Pitzalis *et al.*, *Eur. J. Immunol.*, 18:1397 (1988) and Morimoto *et al.*, *Am. J. Med.*, 84:817 (1988)).

Collagen-induced arthritis (CIA) is an animal model for human RA, which resembles human disease, and can be induced in susceptible strains of mice by immunization with heterologous type-II collagen (CII) (Courtenay *et al.*, *Nature*, 283:665 (1980) and Cathcart *et al.*, *Lab. Invest.*, 54:26 (1986)). Both CD4 T cells and antibodies to CII are required to develop CIA. Transfer of anti-CII to naïve animals only leads to partial histo-pathology that is quite different from CIA, and complete symptoms of CIA do not develop (Holmdahl *et al.*, *Agents Action*, 19:295 (1986)). In contrast, adoptive transfer of both CD4 T cells and anti-CII antibodies from CII-immunized mice to naïve recipients completely reconstitutes the symptoms of classical CIA (Seki *et al.*, *J. Immunol.*, 148:3093 (1992)). Involvement of both T cells and antibodies in CIA is also consistent with histo-pathological findings of inflamed joints in CIA. Thus, agents that block B- cell or T-cell functions, or inhibit pro-inflammatory cytokines induced by T cells, may be efficacious to prevent or treat arthritis. Indeed, depletion of CD4 T cells, blockade of CD40-CD40L interactions, neutralization of TNF- α , or blocking of IL-1 receptors can lead to prevention of CIA in mice (Maini *et al.*, *Immunol. Rev.*, 144:195 (1995); Joosten *et al.*, *Arthritis Rheum.*, 39:797 (1996); and Durie *et al.*, *Science*, 261:1328 (1993)).

In the CIA model used herein, DBA-1J mice were immunized with 100 μ g bovine collagen type II in 100 μ l of Complete Freund's Adjuvant (CFA) on Day 0 and Day 21 intradermally. At Day 24 post- immunization, mice were randomly divided into treatment groups. Animals were subcutaneously treated either with 6 mg/kg in 100 μ l anti-ragweed IgG2a monoclonal antibody (control antibody) or with hamster-mouse chimeric anti-LT α antibody (S5H3), anti-LT α .Fc mutant, or murine TNFRII-Ig at 4 mg/kg in 100 PBS.

Animals were treated three times weekly for the duration of the study. Limbs of animals were examined daily for signs of joint infiltration using a grading system of 1-4 for each joint, giving a maximum score of 16.

In a CIA-preventative model, the use of hamster-murine anti-LT α antibody S5H3 and TNFRILFc reduced the average clinical score over the period of days up through 70 days versus the anti-S5H3.DANA Fc mutant and isotype control (anti-ragweed IgG2a monoclonal antibody). See Figure 5A, showing comparable efficacy in the CIA preventative murine model of the antibody S5H3 with TNFR.Ig.

Figure 5B shows comparable efficacy of the hamster-murine chimeric anti-LT α antibodies (S5H3) with TNFRILFc over 90 days in this therapeutic CIA murine model, which efficacy is far better than the isotype control in reducing average clinical score. These results along with those in Figure 5A show that the antibodies herein are considered useful in preventing and being efficacious in autoimmune diseases such as RA. It is expected that the anti-LT α antibodies herein would be effective in preventing and treating RA in those who are non-responsive to TNF therapies in general, including TNFR.Ig and anti-TNF α antibodies, so that such non-responders could be treated with such antibodies prophylactically and efficaciously.

EXAMPLE 6: Anti-LT α delays onset and severity of encephalomyelitis (EAE) disease in MBP-TCR transgenic mice

Experimental autoimmune/allergic encephalomyelitis (EAE) is an inflammatory condition of the central nervous system with similarities to MS. In both diseases, circulating leukocytes penetrate the blood-brain barrier and damage myelin, resulting in impaired nerve conduction and paralysis. The EAE murine model (transgenic mouse preventative model) has been described as a model for human MS (Grewal *et al.*, *Science*, 273:1864-1867 (1996)).

MBP_{Ac1-11} T-cell receptor transgenic mice (10-to-15 week-old male and female adult MBP-TCR transgenic mice bred from an animal breeding pair obtained from Dr. Richard Flavell, Howard Hughes Medical Institute, Yale University) were immunized with 10 μ g of MBP_{Ac1-11} in 100 μ l of CFA subcutaneously. On Days 1 and 2 all mice were additionally injected intraperitoneally with pertussis toxin at 200 ng/100 μ l/mouse. On Day 0, mice (n=10/group) received either anti-gp120 IgG1 monoclonal antibody (control antibody) or hamster-mouse chimera anti-LT α antibody S5H3 at 6 mg/kg in 100 μ l i.p. and then 6 mg/kg in 100 μ l PBS s.c. three times weekly.

Animals were evaluated daily for clinical signs using the following grading system: 0 - Normal mouse; no overt signs of disease; 1- Limp-tail or hind-limb weakness, but not both; 2- Limp-tail and hind- limb weakness; 3- Partial hind-limb paralysis; 4- Complete hind-limb paralysis; 5- Moribund.

The results are shown in Figure 6. The disease score was lower for the anti-LT α -treated mice than for the control group, only reaching clinical scores of 3 (versus clinical scores of over 4 for the control) during the study. The results show that the antibody S5H3 delayed onset and severity of EAE disease in these transgenic mice, suggesting that the antibody treatment protected the mice from developing overt EAE.

EXAMPLE 7: Anti-LT α antibody treatment does not affect T-cell-dependent antibody isotype responses, indicating safety

The immune response to i.v.-injected trinitrophenol (TNP)-FICOLLTM is very high in most mouse strains, allowing one to assay the relative safety of an antibody administered to such mice by measuring various isotype levels in the mice.

BALB/c mice (n=12/group) were treated on Day 0 with either anti-ragweed isotype control, hamster-mouse anti-LT α antibody (S5H3), anti-LT.Fc mutant, or CTLA4-Fc (as a positive control) at 6 mg/kg in 100 μ l PBS intra-peritoneally. Treatment continued three times per week for 5 weeks. Mice were immunized on Day 1 with TNP-OVA (100 μ g) in 2 mg of alum per mouse intra-peritoneally, and on Day 29 with TNP-OVA (50 μ g) in PBS per mouse intra-peritoneally. Serum was collected on Days 0, 14, and 35 for determination of the anti-TNP Ig isotypes IgM, IgG1, and IgG2a by standard ELISA.

The results, shown in Figures 7A-7C, wherein the data are represented as Log10 titers of anti-TNP IgM, IgG1, and IgG2a, respectively, indicate that the isotype control, S5H3 antibody, and DANA Fc mutant antibody exhibited similar patterns for each of the isotype levels, whereas the CTLA4.Fc immunoadhesin acted differently. This indicates that there would be little, if any, immune response in a patient to the administration of the S5H3 antibody herein.

EXAMPLE 8: Anti-LT α antibody treatment does not affect T-cell-independent antibody isotype responses, indicating safety

BALB/c mice (n=10/group) were treated on Day 0 with either anti-ragweed (IgG2a isotype control), hamster-mouse anti-LT α antibody (S5H3), or TNFR1L.Fc at 6 mg/kg in 100 μ l PBS intra-peritoneally. Treatment continued three times per week for 10 days. Mice were

immunized on Day 1 with TNP-FICOLL™ (100 µg) i.p. in 100 µl PBS intra-peritoneally. Serum was collected on Days 0 and 10 for determination of the anti-TNP Ig isotypes IgM, IgG1, IgG2a, and IgG3 by standard ELISA.

Figure 8, wherein the data are represented as Log10 titers, shows that the isotype control, the S2C8 antibody, and the TNFRII.Fc immunoadhesin all behaved similarly for the IgM, IgG1, IgG2a, and IgG3 isotypes. This demonstrates further the safety of the antibodies herein for administration *in vivo*.

EXAMPLE 9: Anti-LT α antibody prevents human SCID graft-versus-host disease (GVHD)

Graft-versus-host disease (GVHD) occurs when immunocompetent cells are transplanted into immunosuppressed or tolerant patients. The donor T cells recognize host antigens and become activated, secrete cytokines, proliferate, and differentiate into effector cells. This response is known as graft-versus-host-reaction (GVHR). The GVHR response is a multi-organ syndrome, and the effects can vary from life-threatening severe inflammation to mild cases of diarrhea and weight loss. GVHD models in mice have been used to model the clinical disorders of acute and chronic GVHR that occur after bone-marrow transplantation and autoimmune diseases. A general procedure is described in *Current Protocols in Immunology, supra*, unit 4.3.

SCID mice were reconstituted with human PBMCs purified from a LEUKOPACK™ (available from blood banks such as Interstate Blood Bank, Memphis, TN) of a normal donor by FICOLL™ polysaccharide gradient. All mice (n=10/group) were sub-lethally irradiated with 350 rads using a Cesium 137 source. Two hours after irradiation, mice were injected with 50 million human PBMCs/mouse in 200 µl PBS intravenously. Immediately after cell injection, mice were treated i.p. either with 300 µg of trastuzumab (human IgG1 isotype control antibody), anti-LT α chimeric antibody 2C8, or CTLA4-Fc in 100 µl saline two times/week for three weeks. POLYMYXIN™ B (110 mg/liter) and NEOMYCIN™ (1.1 g/liter) antibiotics were added to the drinking water for five days post-irradiation. Mice were monitored for GVHD as indicated by survival.

The results are shown in Figure 9, and indicate that as compared to the isotype control, the 2C8 antibody significantly increased survival of the human SCID mice (preventing GVHD), as compared to anti-LT α 2C8 Fc mutant and the isotype control, with the CTLA4.Fc immunoadhesin being the best in this model of the molecules tested. Thus, the anti-LT α antibodies herein are expected to be useful in the prevention and/or treatment of

graft-versus-host disease. Further, the results show that depletion of LT α -positive cells is required for efficacy in this model, since the 2C8 Fc mutant (DANA) did not show efficacy.

EXAMPLE 10: Anti-LT α monoclonal antibodies bind to LT α 3

5 Microtiter wells were coated with 1 μ g/ml human LT α 3 in 50 mM carbonate buffer solution (100 μ L/well) overnight. The unabsorbed solution was decanted from the wells. Wells were blocked with 150 μ L PBS containing 5 mg/ml bovine serum albumin (PBS-BSA) for 1-2 hour. 100 μ L of appropriately diluted test sample (anti-LT α chimeric antibody 2C8 or 3F12) diluted in PBS-BSA was added to each well, incubated for one hour, and washed
10 with PBS containing 0.05% TWEENTM-20 surfactant. 100 μ L of biotin-labeled rat anti-mouse IgG in PBS-BSA buffer was then added to each well and incubated for one hour. The plate was then washed with PBS/0.05%TWEENTM-20 surfactant, and streptavidin-horseradish peroxidase (SA-HRP) was added for 30 minutes to each well. Each well was washed with PBS/0.05%TWEENTM-20 surfactant, and bound HRP was measured with a
15 solution of tetramethylbenzidine (TMB)/H₂O₂. After 15 minutes, the reaction was quenched by the addition of 100 μ L of 1M phosphoric acid. The absorbance at 450 nm was read with a reference wavelength of 650 nm.

FIG. 10A shows that chimeric anti-LT α antibodies 2C8 and 3F12 bound to human LT α 3.

20 The above procedure was repeated using murine LT α 3 instead of human LT α 3 and hamster-mouse chimeric anti-LT α antibody S5H3 and anti-LT.Fc mutant as the antibody tested for binding ability. FIG. 10B shows that both S5H3 and the anti-LT α .Fc mutant bound to murine LT α 3.

EXAMPLE 11: Anti-LT α antibodies bind to LT α 1 β 2

25 For the LT $\alpha\beta$ ELISA, microtiter wells were coated with 1 μ g/ml murine LT α 1 β 2 in 50 mM carbonate buffer solution (100 μ L/well) overnight. The unabsorbed solution was decanted from the wells. Wells were blocked with 150 μ L PBS containing 5 mg/ml bovine serum albumin (PBS-BSA) for 1-2 hours. 100 μ L of an appropriately diluted test sample (anti-LT α chimeric antibody S5H3 or anti-LT α .Fc mutant diluted in PBS-BSA) was added to each well, incubated for one hour, and washed with PBS containing 0.05% TWEENTM 20 surfactant. 100 μ L of biotin-labeled rat anti-mouse IgG in PBS-BSA buffer was then added to each well and incubated for one hour. The plate was then washed with PBS/0.05%
35 TWEENTM 20 surfactant, and streptavidin-horseradish peroxidase (SA-HRP) was added for

30 minutes to each well. Each well was washed with PBS/0.05% TWEENTM 20 surfactant, and bound HRP was measured with a solution of tetramethylbenzidine (TMB)/H₂O₂. After 15 minutes, the reaction was quenched by the addition of 100 µl of 1M phosphoric acid. The absorbance at 450 nm was read with a reference wavelength of 650 nm.

5 FIG. 11A shows that anti-LTα chimeric antibody S5H3 bound to the murine LTα1β2 complex, as did the anti-LT.Fc mutant.

 A FACS assay was used to determine binding of anti-LTα antibody to the LTα on the surface of cells complexed with LTβ. For human LTαβ, 293 cells were transfected with full-length human LTα and human LTαβ (human LTα sequence GenBank Ref NM_000595;
10 human LTβ sequence GenBank Ref NM_002341) to generate stable human LTαβ-expressing cell lines. Cells were incubated with 1-5 µg/ml of anti-LTα chimeric antibody 3F12 or 2C8, human LTβR.huIgG1, or huIgG1 isotype control (trastuzumab) for 20 minutes in PBS with 2% FBS. Cells were washed and incubated with anti-human Ig-PE secondary antibodies for detection.

15 For murine LTαβ, SVT2 cells were transfected with full-length murine LTα and murine LTαβ (murine LTα sequence GenBank Ref NM_010735; murine LTβ sequence GenBank Ref NM_008518) to generate stable murine LTαβ-expressing cell lines. Surface LT was detected with hamster-murine chimera S5H3, anti-LT.S5H3.Fc mutant, muLTβR.IgG2a, or isotype control (anti-IL122 muIgG2a) directly conjugated to ALEXA-
20 647TM fluorophore, for 20 minutes in PBS with 2% FBS. Cells were washed, aspirated, and resuspended in PBS with 2% FBS. Cells were analyzed on a FACSCALIBURTM (dual laser) using CELLQUESTTM software, and results analyzed on a FLOWJOTM analyzer (Treestar).

 FIG. 11B shows how well the chimeric anti-LTα antibodies 3F12 and 2C8 bound to the LTα on the surface of human cells complexed with LTβ. They are compared to LTβ-receptor.huIgG1 and isotype control huIgG1 (trastuzumab). All human antibodies bound
25 surface LTα.

 FIG. 11C shows how well hamster-murine anti-LTα antibody S5H3 bound to the LTα on the surface of murine cells complexed with LTβ. It is compared to LTβ-receptor murine IgG2a, anti-LT S5H3 Fc mutant, and isotype control (anti-IL122 muIgG2a). All murine
30 antibodies bound surface LTα.

EXAMPLE 12: Anti-LT α antibodies bind to human Th1, Th2, and Th17 cells

When CD4+ T cells mature from thymus and enter into the peripheral lymph system, they usually maintain their naive phenotype before encountering antigens specific for their T cell receptor (Sprent *et al.*, *Annu Rev Immunol.* 20:551-79 (2002)). The binding to specific antigens presented by APC, causes T cell activation. Depending on the environment and cytokine stimulation, CD4+ T cells differentiate into a Th1 or Th2 phenotype and become effector or memory cells (Sprent *et al.*, *supra* and Murphy *et al.*, *Nat Rev Immunol.* 2(12):933-44 (2000)). This process is known as primary activation. Having undergone primary activation, CD4+ T cells become effector or memory cells, they maintain their phenotype as Th1 or Th2. Once these cells encounter antigen again, they undergo secondary activation, but this time the response to antigen will be quicker than the primary activation and results in the production of effector cytokines as determined by the primary activation (Sprent *et al.*, *supra*, and Murphy *et al.*, *supra*).

For primary activation conditions, naïve T cells were activated by anti-CD3, anti-CD28, and specific cytokines depending on whether Th1 or Th2 was being examined. In particular, human Th1 and Th2 cell lines were generated by stimulating human PBMC with plate-bound anti-CD3 and anti-CD28 (10 μ g/ml and 5 μ g/ml, respectively; BD Pharmingen). On Day 1, to induce Th2 differentiation, interleukin (IL)-4 (5 ng/ml; R&D Systems Inc.), anti-IL-12 (5 μ g/ml; R&D Systems Inc., Minneapolis, MN), and anti-interferon (IFN)-gamma (5 μ g/ml; R&D Systems Inc.) were added. For Th1 differentiation, on Day 1 IL-12 (1 ng/ml; R&D Systems Inc.), IFN-gamma (10 ng/ml; R&D Systems Inc.), and anti-IL-4 (1 μ g/ml; R&D Systems Inc.) were added. Cells were restimulated twice at Days 7 and 14.

For FACS analysis cell-surface staining to determine binding of the antibodies to Th1 and Th2, two days after final stimulation, the activated human T-cells (Th1/Th2 cell lines) were incubated with anti-LT α antibody 2C8 (1 μ g/ml), 3F12 (1 μ g/ml), LT β R.Fc (1 μ g/ml), TNFR1L.Fc (1 μ g/ml) or hulgG1 isotype control (1 μ g/ml), all directly conjugated to ALEXA FLUOR® 647 fluorophore (Invitrogen Corp.), for 20 minutes in PBS with 2% FBS. Cells were washed, aspirated, and re-suspended in PBS with 2% FBS. Cells were analyzed on a FACSCALIBUR™ (dual laser) using CELLQUEST™ software, and results analyzed on a FLOWJO™ analyzer (Treestar).

FIG. 12A and 12B show that chimeric anti-LT α antibodies 2C8 and 3F12 bound to human Th1 and Th2 cells, respectively. The graphs also show the binding results for LT β R.Fc, TNFRII.Fc, and isotype control.

Since LT- α 3 and LT- α 1 β 2 were found to be expressed on human Th17 cells as well as human Th1 cells via FACS analysis, it would be expected that the anti-LT α antibodies herein, including chimeric 2C8, 2C8.v2, and 2C8.vX (described in Examples 3 and 18), would (similarly to Th1 and Th2) bind to human Th17 cells and hence be useful in treating many autoimmune diseases, including MS (they drive EAE in murine models) and lupus as well as RA and IBD, such as Crohn's disease and ulcerative colitis.

Hence, the same experiment described above was performed, except for using anti-LT α humanized antibody 2C8.vX as the LT α antibody and using a Th17 cell line as well as the Th1 cell line, but not a Th2 cell line. The human Th17 cell lines were generated by stimulating human PBMC with anti-CD3 and anti-CD28 as noted above and with IL-23 (10 ng/ml; R&D Systems Inc.), anti-IFN- γ (10 μ g/ml; R&D Systems Inc.), and anti-IL-12 (10 μ g/ml; R&D Systems Inc.). This experiment showed that, in fact, antibody 2C8.vX bound to human Th17 cells as well as human Th1 cells, but not to resting cells. See FIG. 12C-E.

EXAMPLE 13: Anti-LT α antibodies bind to human cells: T, B and NK A

A FACS assay was used to determine binding of anti-LT α antibodies to the LT α on the surface of primary human cells. Human PBMC were isolated and activated with anti-CD3 and anti-CD28 (10 μ g/ml and 5 μ g/ml, respectively; BD PharMingen) for two days for activated CD4 and CD8 cells; or anti-IgM (10 μ g/ml, R&D Systems Inc.) and IL-4 (20 ng/ml; R&D Systems Inc.) for two days for B cells. NK CD56+ cells were isolated by negative selection (Miltenyi Biotec) and incubated with IL-15 (20 ng/ml; R&D Systems Inc.) for 15 hours.

For FACS analysis, cells were incubated with chimeric anti-LT α antibody 3F12 (1 μ g/ml), human LT β R.huIgG1 (1 μ g/ml), or huIgG1 isotype control (trastuzumab, 1 μ g/ml), all directly conjugated to ALEXA FLUOR® 647 fluorophore (Invitrogen Corp.), for 20 minutes in PBS with 2% FBS. Cells were washed, aspirated, and re-suspended in PBS with 2% FBS. Cells were analyzed on a FACSCALIBUR™ (dual laser) using CELLQUEST™ software, and results analyzed on a FLOWJO™ analyzer (Treestar).

Figures 13A-13E show that the antibodies bound to the cells, versus the isotype control. Fig. 13A represents the unactivated cells, Fig. 13B shows the anti-CD3 and anti-CD28-activated CD4 T cells, Fig. 13C shows the CD8 T cells, Fig. 13D shows the IL15-activated CD56 NK cells, and Fig. 13E shows the IgM and CD40L-activated CD19 B cells.

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EXAMPLE 14: Anti-LT α monoclonal antibodies functionally block LT α 3

1. Blocking of human and murine LT α 3

Microtiter wells were coated with 0.7 μ g/ml human TNFRII.huIgG1 (ENBREL®) in 50 mM carbonate buffer solution (100 μ l/well) overnight. The unabsorbed solution was aspirated from the wells. Biotinylated LT α 3 (murine or human) was captured and detected with streptavidin-horse radish peroxidase (HRP). For neutralization of human LT α 3, increasing doses of chimeric anti-LT α antibodies 2C8 or 3F12 or control isotype (trastuzumab) were preincubated with the LT α 3 at indicated concentrations for one hour before adding to the coated microplate. The same procedure was carried out for neutralization of murine LT α 3 using the hamster-mouse chimeric anti-LT α antibody S5H3 or anti-LT.Fc mutant or control isotype (trastuzumab). Each well was washed with PBS/0.05%TWEENTM 20 surfactant, and bound HRP was measured with a solution of tetramethylbenzidine (TMB)/H₂O₂. After 15 minutes, the reaction was quenched by the addition of 100 μ l of 1M phosphoric acid to each well. The absorbance at 450 nm was read with a reference wavelength of 650 nm.

FIG. 14A shows that anti-LT α antibodies 2C8 and 3F12 functionally blocked human LT α 3 versus the isotype control. FIG. 14B shows that anti-LT α antibody S5H3 and the anti-LT.Fc mutant functionally blocked murine LT α 3 versus the isotype control.

2. Blocking of LT α 3-induced IL-8 in A549 cells

Epithelial cell lines (A549) were cultured in the presence of LT α 3 for 24 hours. Neutralization of the effect of LT α 3 with chimeric anti-LT α antibodies 2C8 and 3F12 was determined by serial dilutions of the anti-LT α antibodies (10-0 μ g/ml) added to the appropriate wells. Supernatant was used to detect IL-8 in a standard ELISA.

FIG. 14C shows that chimeric anti-LT α antibodies 2C8 and 3F12 blocked LT α 3-induced IL-8 in A549 cells versus the isotype control (trastuzumab IgG1).

3. Blocking of LT α 3-induced ICAM expression on HUVEC

HUVEC cells were cultured in the presence of LT α 3 for 24 hours. Neutralization of the effect of LT α 3 with chimeric anti-LT α antibodies 2C8 and 3F12 was determined by serial

dilutions of the anti-LT α antibodies (10-0 μ g/ml) added to the appropriate wells. Cell-surface expression of ICAM-1 was determined by FACS, shown as mean fluorescent intensity. TNFRILFc was used as a control.

FIG. 14D shows that anti-LT α antibodies 2C8 and 3F12, as well as the TNFRILFc mutant, blocked LT α 3-induced ICAM expression on HUVEC versus the isotype control (trastuzumab IgG1). The effect on unstimulated cells is also shown.

4. Blocking of LT α 3-induced NF κ B activation

To determine if anti-LT α antibodies block signaling of LT α 3 through the TNF receptors, 293 cell-lines were cultured in DMEM:F12 50:50 (+ 10% FBS, glutamine, P/S) to 75% confluency. Cells were transfected with a standard NF κ B-RE-luciferase reporter construct using standard transfection techniques (Fugene). After 24 hours, the cells were washed, and then stimulated with LT α 3 (100 ng/ml) or with serial dilutions of the chimeric anti-LT α antibody 2C8 (10-0 μ g/ml) that were preincubated with LT α 3 for 30 minutes. After six hours, the cells were washed with PBS, and luciferase activity was measured as follows: LYSIS BUFFERTM (Promega, 125 μ l/well) was added for 15 minutes. Lysates were collected and transferred at 20 μ l/well to a 96-well assay plate (high-binding, white polystyrene, COSTARTM) in triplicates and read in a LMAXIITM plate reader (Molecular Devices) after injection of LUCIFERASE ASSAY REAGENTTM and STOP AND GLO BUFFERSTM (Promega). The isotype control used was trastuzumab IgG1.

FIG. 14E shows that the anti-LT α antibody 2C8 blocked LT α 3-induced NF κ B activation, versus the isotype control. The effect on unstimulated cells is also shown.

EXAMPLE 15: Anti-LT α antibodies functionally block LT α 1 β 2

1. Blocking of LT α -induced NF κ B activation

To determine if anti-LT α antibody blocks signaling of LT α 1 β 2 through the LT β R, 293 cell lines were cultured in DMEM:F12 50:50 (+ 10% FBS, glutamine, P/S) to 75% confluency. Cells were transfected with standard NF κ B-RE-luciferase reporter construct using standard transfection techniques (Fugene). After 24 hours, the cells were washed, and then stimulated with LT α 1 β 2 (R&D Systems Inc.; 100 ng/ml) or with serial dilutions of the chimeric anti-LT α antibody 2C8 (10-0 μ g/ml) that were preincubated with LT α β for 30 minutes. After six hours, the cells were washed with PBS, and luciferase activity was measured as follows: LYSIS BUFFERTM (Promega, 125 μ l/well) was added for 15 minutes. Lysates were collected and transferred at 20 μ l/well to a 96-well assay plate (high-binding,

white polystyrene, COSTAR™) in triplicates and read in a LMAXII™ plate reader (Molecular Devices) after injection of LUCIFERASE ASSAY REAGENT™ and STOP AND GLO BUFFERS™ (Promega). The isotype control used was trastuzumab IgG1.

FIG. 15A shows that anti-LT α antibody 2C8 functionally blocked LT α 1 β 2-induced NF κ B activation, versus the isotype control. The response of unstimulated cells is also shown.

2. Blocking of LT α 3-, LT α 2 β 1-, and LT α 1 β 2-induced cytotoxicity

Test samples were assayed for ability to neutralize the cytolytic activity of LT α 3, LT α 2 β 1, and LT α 1 β 2 (R&D Systems Inc.) in a murine L929 cytotoxic assay. L929 cells were cultured in microtiter plates in the presence of one of the three LT α reagents (at the doses indicated in Figs. 15B-D) and the DNA inhibitor actinomycin-D. Neutralizing chimeric anti-LT α antibody 2C8 was added to the cultures at 10 μ g/ml. Cell lysis was determined by standard ALAMARBLUE™ stain of viable cells and represented as relative fluorescence units (RFU).

FIG. 15B, 15C, and 15D show that chimeric anti-LT α antibody 2C8 blocked, respectively, LT α 3-, LT α 2 β 1-, and LT α 1 β 2-induced cytotoxicity.

3. Blocking of LT α 1 β 2-induced ICAM expression on HUVEC

HUVEC cells were cultured in the presence of LT α 1 β 2 (R&D Systems Inc.) for 24 hours. Neutralization of the effect of LT α 1 β 2 with anti-LT α antibodies was determined by serial dilutions of chimeric anti-LT α antibodies 2C8 and 3F12 (10-0 μ g/ml) added to the appropriate wells. Cell-surface expression of ICAM-1 was determined by FACS.

FIG. 15E shows that chimeric anti-LT α antibodies 2C8 and 3F12 blocked LT α 1 β 2-induced ICAM expression on HUVEC, versus the isotype control (trastuzumab IgG1). The effect on unstimulated cells is also shown.

EXAMPLE 16: Anti-LT α monoclonal antibody can kill LT α β -expressing cells.

An ADCC assay was performed in a microtiter plate in duplicate as follows. NK cells were isolated from 100 ml of normal human donor whole blood using negative selection (ROSETTESEP™, #15065, StemCell Technologies). The assay diluent was RPMI™ 1640 and 0.25 mg/mL BSA. Chimeric antibody 2C8 and the Fc mutant thereof, in a serial dilution starting at 100 nM in 50 μ l, were incubated with 50 μ l of stable 293 cells expressing human LT α β (20,000) (see Example 11 for details) for 30 minutes at room temperature. 50 μ L of NK cells (120,000) were added and incubated for an additional four hours at 37°C. Plates

were centrifuged at 1500 rpm for 10 minutes, and 100 μ l of supernatant was transferred to a 96-flat-bottom microwell plate. The level of cell lysis was determined by measuring the amount of lactate dehydrogenase (LDH kit, #1-644-793, Roche) released from lysed cells. 100 μ l of LDH kit reaction mixture was added to 100 μ l of supernatant and incubated for up to 30 minutes. Plates were read at 490 nm. Controls included target:effector cells in the absence of antibody (for antibody-independent lysis), target cells alone with 1% TRITON X-100TM surfactant (for total lysis), and antibody ADCC negative and positive controls.

FIG. 16 shows that anti-LT α antibody 2C8 lysed a 293-human LT $\alpha\beta$ -expressing cell line in the ADCC assay, but the corresponding Fc mutant monoclonal antibody did not.

EXAMPLE 17: Anti-LT α monoclonal antibody blocks cytokine and chemokine secretion in 3T3 and HUVEC cells

HUVEC and 3T3 cells were cultured in the presence of human LT α 3 (100 ng/ml; R&D Systems Inc.) or human LT α 1 β 2 (200 ng/ml; R&D Systems Inc.) for 24 hours using human or murine reagents, respectively. Neutralization of the effect of LT α trimers with chimeric 2C8 or S5H3 anti-LT α antibodies (chimeric 2C8 with HUVEC, and hamster-mouse chimeric S5H3 with murine 3T3 cells) was determined using a static dose of 10-0 μ g/ml. Supernatants were assayed for human RANTES, IL-6, IP-10, and IL-8 for the HUVEC evaluation; or murine RANTES, IL-6, IP-10, and KC for the 3T3 evaluation, using standard LINCOPLEXTM kits and read on a LUMINEXTM plate reader.

FIG. 17A-H show that the anti-LT α antibody, versus an isotype control and as compared to unstimulated cells, blocked cytokine and chemokine secretion in HUVEC cells, with Figures 17A-17D, respectively, showing KC/IL-8, RANTES, IP10, and IL-6 with LT α 1 β 2, and Figures 17E-17H, respectively, showing KC/IL-8, RANTES, IP10, and IL-6 with LT α 3.

FIG. 17I-P show that the anti-LT α antibody, versus an isotype control and as compared to unstimulated cells, blocks cytokine and chemokine secretion in 3T3 cells, with Figures 17I-17L, respectively, showing KC, RANTES, IP10, and IL-6 with LT α 1 β 2, and Figures 17M-17P, respectively, showing KC, RANTES, IP10, and IL-6 with LT α 3.

In summary, the preferred antibodies herein against LT α :

- 1) Block LT α 3
- 2) Deplete LT α -positive cells by binding LT α as target, which is complexed with LT β on the cell surface. The data that depletion is important are the *in vivo* data

comparing wild-type with the Fc (DANA) mutant – for mouse: CIA, AIA (Figs 4 and 5); for human: human SCID mice with 2C8 (Fig. 9) and ADCC assay (Fig. 16)

3) Block LT α β function

4) Target any cell expressing LT α , including T-, B-, and possibly or likely any Th1- or Th17- or Th2-driven disease (and NK cells), without being limited to any one theory.

Diseases expected to benefit from treatment with the antibodies herein include RA, IBD, psoriasis, MS, Sjögren's syndrome, Hashimoto's thyroiditis, Graves' disease, myasthenia gravis, and lupus, especially, RA, MS, lupus (*e.g.*, SLE and LN), and IBD, including Crohn's disease and UC.

EXAMPLE 18: Affinity-matured anti-LT α antibody 2C8.vX

Affinity maturation of the antibody 2C8 noted in Example 3 led to the humanized antibody 2C8.vX. The light-chain and heavy-chain variable regions and CDRs of this antibody are shown in Figures 18A and 18B, respectively, along with the CDRs.

The following is the DNA sequence that encodes the full-length heavy chain of anti-LT α 2C8.vX:

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GAAGTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTC
CGTTTGTCTGTGCAGCTTCTGGCTACACATTCACCAGCTATGTGATCCATTGGGT
CCGTCAGGCCCCGGGTAAGGGCCTCGAGTGGGTGGTTATAACAATCCTTATAAC
GCCGGCACCAACTATAACGAGAAGTTCAAGGGGCGCTTCACTATCAGTTCTGAC
AAGTCGAAAAACACAGCATACCTGCAGATGAACAGCCTGCGTGCTGAGGACACT
GCCGTCTATTATTGTTCTCGACCCACAATGCTCCCATGGTTCGCCTACTGGGGTCA
AGGAACCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCC
CTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG
TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACTCAGGCGCCCTGAC
CAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC
AGCAGCGTGGTGACTGTGCCCTCTAGCAGCTTGGGCACCCAGACCTACATCTGCA
ACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT
CTTGTGACAAAACCTCACACATGCCCCACCGTGCCCAGCACCTGAACTCCTGGGGGG
ACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGG
ACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG
CGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCAC

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CGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA
 CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCC
 CCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAAGAGATGACCAAGAA
 CCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG
 5 GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTG
 CTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCA
 GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
 (SEQ ID NO:104)

10 The following is the DNA sequence that encodes the full-length light chain of anti-
 LT α 2C8.vX:

GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGG
 TCACCATCACCTGCCGTGCCAGTCAGGCTGTGTCTTCCGCTGTAGCCTGGTATCA
 ACAGAAACCAGGAAAAGCTCCGAAACTACTGATTTACTCTGCATCCCACCGTTAC
 15 ACTGGAGTCCCTTCTCGCTTCTCTGGATCCGGATCTGGGACGGATTTCACTCTGAC
 CATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTTATTACTGTCAGGAATCTTAT
 TCTACTCCTTGGACGTTTCGGACAGGGTACCAAGGTGGAGATCAAACGAACTGTG
 GCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
 CTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
 20 GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGA
 GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAA
 AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
 GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:105)

The following is the full-length heavy-chain amino acid sequence for 2C8.vX:

25 EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYVIHWVRQAPGKGLEWVGYNPNYN
 AGTNYNEKFKGRFTISSDKSKNTAYLQMNSLRAEDTAVYYCSRPTMLPWFAYWGQ
 GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
 HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
 CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
 30 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
 AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP
 PVLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ
 ID NO:106)

The following is the full-length light-chain amino acid sequence for 2C8.vX:

DIQMTQSPSSLSASVGDRVTITCRASQAVSSAVAWYQQKPGKAPKLLIYSASHRYTG
VPSRFGSGSGTDFTLTISLQPEDFATYYCQESYSTPWTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDY
5 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:107)

Epitope mapping indicates that this molecule has a partial overlap with the receptor binding site. Activated rhesus T and B cells express surface LT $\alpha\beta$. The antibody 2C8.vX bound (crossreacted with) rhesus surface LT $\alpha\beta$ (as determined by FACS) and rhesus soluble LT $\alpha\beta$ (as determined by FORTEBIO™ octet).

10 FIG. 19A and 19B show, respectively, ELISAs of LT $\alpha\beta$ and LT $\alpha\beta$ 2 binding of 2C8.vX antibodies in comparison to isotype control/TNFRILFc. These ELISAs were performed in the same way as described in Example 10 above using human LT $\alpha\beta$ and in Example 11 using LT $\alpha\beta$ 2. The EC₅₀ values of these same antibodies to LT $\alpha\beta$ and LT $\alpha\beta$ 2 are 1.03 nM for TNFRILFc and 0.05 nM for 2C8.vX to LT $\alpha\beta$, and no binding of TNFRILFc
15 and 0.34 nM for 2C8.vX to LT $\alpha\beta$ 2. The results show that 2C8.vX bound much better than TNFRILFc in both cases.

FIG. 20A and 20B show, respectively, blocking of human LT $\alpha\beta$ and LT $\alpha\beta$ 2 by 2C8.vX antibodies in comparison to isotype control/TNFRILFc using the assay for testing blocking of human LT-induced cytotoxicity described in Example 15 above. The IC₅₀ values
20 of these same antibodies to LT $\alpha\beta$ and LT $\alpha\beta$ 2 are 0.83 nM for TNFRILFc and 0.29 nM for 2C8.vX to LT $\alpha\beta$, with no isotype blocking, and 0.31 nM for 2C8.vX to LT $\alpha\beta$ 2, with no isotype blocking. The results show that 2C8.vX blocked LT $\alpha\beta$ better than TNFRILFc.

FIG. 21 relates to a functional assay showing inhibition of ICAM1 upregulation by LT $\alpha\beta$ on HUVEC cells, using the protocol described in Example 14 above. FIG. 21 shows a
25 comparison of how various molecules blocked LT $\alpha\beta$ in this functional assay. Specifically, isotype control, 2C8.vX, and TNFRILFc were compared. The results show that both antibodies were able to block LT $\alpha\beta$ in this assay.

FIG. 22 relates to a functional assay showing blocking of LT $\alpha\beta$ 2 using 293-LT β R cells, using the protocol described in Example 15 above. FIG. 22 shows a comparison of
30 how various molecules blocked LT $\alpha\beta$ 2 in this functional assay. Specifically, isotype control and 2C8.vX were compared along with unstimulated cells. The results show that 2C8.vX was able to block LT $\alpha\beta$ 2 in this assay, with an IC₅₀ of about 5 nM.

FIG. 23 shows ADCC activity of 2C8.vX using the protocol set forth in Example 16 involving 293-LT α 1 β 2 cells. 2C8.vX was compared with the 2C8.vX DANA mutant and isotype control. The results show that 2C8.vX had ADCC activity, whereas the DANA mutant did not.

FIG. 24 shows GVHD survival results with 2C8.vX and CTLA4-Fc, as compared to controls (Fc mutant and isotype control) in a human SCID model, using the same protocol as described above in Example 9, terminating in three to four weeks. The results indicate that CTLA4-Fc and 2C8.vX prolonged survival in this model, and thus were effective at depleting LT α cells, but not the controls 2C8.vX-DANA mutant and human IgG1 isotype control antibody.

FIG. 25A-D show that the 2C8.vX antibody depleted LT α 1 β 2-expressing T and B cells in the human SCID GVHD model. In this protocol, SCID mice were reconstituted with PBMCs purified from a LEUKOPACKTM (available from blood banks such as Interstate Blood Bank, Memphis, TN) of a normal donor by FICOLLTM polysaccharide gradient. All mice (n=10/group) were sub-lethally irradiated with 350 rads using a Cesium 137 source. Two hours after irradiation, mice were injected with 50 million human PBMCs/mouse in 200 μ l PBS intrasplenically. One day after cell injection, mice were treated i.p. either with 300 μ g of human IgG1 isotype control antibody, 2C8.vX, or 2C8.vX DANA mutant in 100 μ l saline for one day. On Day 2 after irradiation, the splenocytes of the mice were labeled with 5- (and 6-)carboxy fluorescein diacetate succinimidyl ester (CFSE), and the absolute cell number (total CFSE positive cells) was measured and the percentage of cells in CFSE peaks was measured after electronically gating on CD4⁺, CD8⁺, and CD19⁺ cells. For this three-color staining, the following monoclonal antibodies were used: PE-labeled anti-CD4, PE-labeled anti-CD8, and PE-labeled CD19. Stained cells were analyzed on a FACSCANTM or FACSCALIBURTM instrument.

The results in FIG. 25A show total CFSE-positive human cells at day 2 for isotype control, 2C8.vX, and 2C8.vX.DANA mutant, and indicate that depletion was best with the vX antibody. FIG. 25B-D show the results of gating using the CD4, CD8, and CD19 antibodies, respectively, indicating that the vX antibody was best at depleting LT α β -positive T and B cells as compared to isotype control and the DANA mutant.

In summary, the antibody 2C8.vX depletes LT-expressing cells in an *in vitro* ADCC assay and *in vivo* in two human SCID GVHD models. It also blocks LT α 3 and LT α 1 β 2 in functional cell assays using HUVEC cells and 293-LT β R cells, respectively. Further, it

cross-reacts with non-human primate (NHP) lymphotoxin as determined by activated rhesus primary cells and the LT α 3 rhesus protein.

EXAMPLE 19: Affinity matured anti-LT α antibody 3F12.v14

5 Affinity maturation of the antibody 3F12 noted above led to the humanized antibody 3F12.v14. The light-chain and heavy-chain variable regions and CDRs of this antibody are shown in Figures 26A and 26B, respectively, along with the CDRs.

When tested for binding and blocking LT α 3 and LT α β using the assays noted above, 3F12.v14 bound and blocked these molecules as well as did antibody 2c8.vX.

EXAMPLE 20: Afucosylated 2C8.vX

10 The antibodies herein may be altered so that they lack fucose by culturing them using a cell line or technology disclosed in US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; US 2006/0063254; US 2006/0064781; US 2006/0078990; US 2006/0078991; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); or Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614-622 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al., Arch. Biochem. Biophys.*, 249:533-545 (1986); US 2003/0157108 A1 (Presta) and WO 2004/056312 A1 (Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knock-out CHO cells (Yamane-Ohnuki *et al. supra*).

20 In fact, the antibody 2C8.vX was afucosylated to produce an antibody called anti-LT α 2C8.vX AF. This molecule was generated using a stable CHO cell line having a *FUT8* knock-out gene as described by the method in Yamane-Ohnuki *et al., supra*. It is 100% afucosylated.

30 The biophysical properties of 2C8.vX (including blocking and binding data) were not substantially changed upon afucosylation thereof, except for the ADCC. The ADCC properties of the wild-type and afucosylated molecules were determined by carrying out the experiments using the protocol set forth in Example 16 involving 293-LT α 1 β 2 cells. The results are shown in Fig. 27. The figure shows that the afucosylated antibody anti-LT α

2C8.vX AF has about a ten-fold increase over the wild-type (WT) antibody (produced in a normal CHO cell line as described above).

EXAMPLE 21: Underfucosylated 2C8.vX

5 As an alternative way to achieve high yields of non-fucosylated antibodies in mammalian cells, an RNAi approach may be employed to knock down the expression of the *FUT8* gene. A plasmid may be used to produce short hairpin siRNA consisting of 19nt (nucleotide) sense siRNA sequence specific to the gene of *FUT8*, linked to its reverse complementary antisense siRNA sequence by a short spacer (9nt hairpin loop), followed by
10 5-6 U's at the 3' end.

Four different RNAi probes are designed to target the different regions based on the available CHO *FUT8* DNA sequence.

For testing the efficacy of these RNAi probes, a FLAG-tagged *FUT8* fusion protein is constructed using the available CHO *FUT8* DNA sequence (Genbank accession no.

15 P_AAC63891). RT-PCR is performed with *FUT8* primers and the resulting PCR fragment fused with 5' FLAG tag sequence. The tagged *FUT8* fragment is cloned into an expression vector. The RNAi probe plasmid and flag-tagged *FUT8* plasmid are cotransfected into CHO cells. Cell lysate is extracted 24 hours after transfection and the *FUT8* fusion protein level analyzed using anti-flag M2 antibody by immuno-blotting. In the presence of RNAi probes,
20 the fusion protein expression is expected to be significantly inhibited in most of the cases.

The two probes showing the best inhibitory effect are transfected into a CHO cell line expressing an antibody as described herein, such as 2C8.vX. The expressed anti-LT-alpha antibody is purified by a protein A column and submitted for Matrix-Assisted Laser Desorption/Ionization Time-of-flight (MALDI-TOF) mass spectral analysis of asparagine-
25 linked oligosaccharides (including fucose content) and FcγR binding assay as described below.

Methods for analyzing the oligosaccharides by MALDI-TOF are conducted generally as follows: N-linked oligosaccharides are released from recombinant glycoproteins using the peptide-N-glycosidase -F (PNGase F) procedure of Papac *et al.*, *Glycobiology* 8, 445-454
30 (1998). Briefly, the wells of a 96-well polyvinylidene difluoride (PVDF)-lined microtitre plate (Millipore, Bedford, MA) are conditioned with 100 μl methanol that is drawn through the PVDF membranes by applying vacuum to the MILLIPORE™ multiscreen vacuum manifold. The conditioned PVDF membranes are washed with 3 X 250 μl water. Between

all wash steps the wells are drained completely by applying gentle vacuum to the manifold. The membranes are washed with reduction and carboxymethylation buffer (RCM) consisting of 6 M guanidine hydrochloride, 360 mM TRIS buffer, 2 mM EDTA, pH 8.6. Glycoprotein samples (50 µg) are applied to individual wells, again drawn through the PVDF membranes
5 by gentle vacuum and the wells washed with 2 X 50 µl of RCM buffer. The immobilized samples are reduced by adding 50 µl of a 0.1 M dithiothreitol (DTT) solution to each well and incubating the microtitre plate at 37°C for 1 hr. DTT is removed by vacuum and the wells are washed 4 x 250 µl water. Cysteine residues are carboxylmethylated by the addition of 50 µl of a 0.1 M iodoacetic acid (IAA) solution that is freshly prepared in 1 M NaOH and
10 diluted to 0.1 M with RCM buffer.

Carboxymethylation is accomplished by incubation for 30 min in the dark at ambient temperature. Vacuum is applied to the plate to remove the IAA solution and the wells are washed with 4 x 250 µl purified water. The PVDF membranes are blocked by the addition of 100 µl of 1% PVP360TM (polyvinylpyrrolidone 360,000 MW) (Sigma) solution and
15 incubation for 30 minutes at ambient temperature. The PVP-360TM solution is removed by gentle vacuum and the wells are washed 4 x 250 µl water. Peptide: N-Glycosidase F (PNGase FTM) amidase (New England Biolabs, Beverly, MA), at 25 µl of a 25 Unit/ml solution in 10 mM TRIS acetate, pH 8.3, is added to each well and the digest proceeds for 3 hr at 37°C. After digestion, the samples are transferred to 500 µl EPPENDORFTM tubes and
20 2.5 µl of a 1.5 M acetic acid solution is added to each sample. The acidified samples are incubated for two hrs at ambient temperature to convert the oligosaccharides from the glycosylamine to the hydroxyl form. Prior to MALDI-TOF mass spectral analysis, the released oligosaccharides are desalted using a 0.7-ml bed of cation-exchange resin (AG50W-X8 resin in the hydrogen form) (Bio-Rad, Hercules, CA) slurry packed into compact reaction
25 tubes (US Biochemical, Cleveland, OH).

For MALDI-TOF mass-spectral analysis of the samples in the positive mode, the desalted oligosaccharides (0.5 µl aliquots) are applied to the stainless target with 0.5 µl of the 2,5 dihydroxybenzoic acid matrix (sDHB) that is prepared by dissolving 2 mg 2,5 dihydroxybenzoic acid with 0.1 mg of 5-methoxysilylic acid in 1 ml of 1 mM NaCl in 25%
30 aqueous ethanol. The sample/matrix mixture is vacuum dried and then allowed to absorb atmospheric moisture prior to analysis. Released oligosaccharides are analyzed by MALDI-TOF on a PERSEPTIVE BIOSYSTEMS VOYAGER-ELITETM mass spectrometer. The mass spectrometer is operated in the positive mode at 20 kV with the linear configuration and

utilizing delayed extraction. Data are acquired using a laser power of approximately 1100 and in the data summation mode (240 scans) to improve the signal to noise ratio. The instrument is calibrated with a mixture of standard oligosaccharides and the data are smoothed using a 19-point Savitsky-Golay algorithm before the masses are assigned.

5 Integration of the mass-spectral data is achieved using the CAESAR 7.2TM data analysis software package (SciBridge Software).

Binding of control and test materials to the human Fcγ receptors can be assessed using modified versions of procedures originally described by Shields *et al.*, *J Biol Chem*, 276:6591-604 (2001).

10 For confirmation that the RNAi-transfected cells do have less FUT8 RNA expression, a Northern blot can be performed using RNA samples extracted from the transfected cells 24 hours after transfection. Total RNA from cells containing a control plasmid (random mouse DNA sequence, no homology to any known mouse proteins) and two RNAi plasmids can be purified and hybridized with a 300-bp probe. The knock down of endogenous α 1,6-

15 fucosyltransferase RNA can be further confirmed by quantitative PCR.

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
hybridoma murine Lymphotoxin alpha2 beta1 s5H3.2.2	PTA-7538	April 19, 2006

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S.

25 patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC § 122

30

and the Commissioner's rules pursuant thereto (including 37 CFR § 1.14 with particular reference to 886 OG 638).

5 The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

10 The foregoing written description is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the example presented herein. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated anti-lymphotoxin- α (LT α) antibody wherein the antibody comprises at least one complementarity-determining region (CDR) sequence selected from the group consisting of:

(a) a CDR-L1 sequence comprising amino acids A1-A11, wherein A1-A11 is KASQAVSSAVA (SEQ ID NO:1) or RASQAVSSAVA (SEQ ID NO:2), or comprising amino acids A1-A17, wherein A1-A17 is KSSQSLLYSTXQKXFLA (SEQ ID NO:3) or KSSQSLLYSAXQKXFLA (SEQ ID NO:4) or KSSQSLLYSTXQKXALA (SEQ ID NO:6), where X is any amino acid;

(b) a CDR-L2 sequence comprising amino acids B1-B7, wherein B1-B7 is SASHRYT (SEQ ID NO:7) or WASTRDS (SEQ ID NO:8);

(c) a CDR-L3 sequence comprising amino acids C1-C9, wherein C1-C9 is QQHYSTPWT (SEQ ID NO:9) or QEXYSTPWT (SEQ ID NO:11) or QQYYSYPRT (SEQ ID NO:13) or QQYASYPRT (SEQ ID NO:14) or QQYYAYPRT (SEQ ID NO:15), where X is any amino acid;

(d) a CDR-H1 sequence comprising amino acids D1-D10, wherein D1-D10 is GYTFTSYVIH (SEQ ID NO:16) or GYTFSSYWIE (SEQ ID NO:17);

(e) a CDR-H2 sequence comprising amino acids E1-E17, wherein E1-E17 is YXXPYXDGTXYXEKFKG (SEQ ID NO:18) or EISPGSGSTXYXEEFKG (SEQ ID NO:19) or YXXPYXAGTXYXEKFKG (SEQ ID NO:101) or EIXPGSGSTIYXEKFKG (SEQ ID NO:110), wherein X is any amino acid; and

(f) a CDR-H3 sequence comprising amino acids F1-F9, wherein F1-F9 is PTMLPWFAY (SEQ ID NO:20), or comprising amino acids F1-F5, wherein F1-F5 is GYHGY (SEQ ID NO:21) or GYHGA (SEQ ID NO:22);

wherein the antibody:

(i) binds to lymphotoxin- α_3 trimer (LT α_3) and blocks the interaction of LT α_3 with tumor necrosis factor receptor-I (TNFRI) and tumor necrosis factor receptor-II (TNFRII); and

(ii) blocks the interaction of LT $\alpha\beta$ with lymphotoxin-beta receptor (LT β -R).

2. The antibody of claim 1, for use in treating an autoimmune disorder selected from the group consisting of rheumatoid arthritis, lupus such as systemic lupus erythematosus or lupus nephritis, Wegener's disease, inflammatory bowel disease (IBD), such as Crohn's disease or ulcerative colitis, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), autoimmune thrombocytopenia, multiple sclerosis (MS), psoriasis, IgA nephropathy, IgM polyneuropathies, myasthenia gravis, vasculitis, diabetes mellitus,

Reynaud's syndrome, Sjögren's syndrome, glomerulonephritis, Hashimoto's thyroiditis, Graves' disease, helicobacter-pylori gastritis, and chronic hepatitis C.

3. The antibody of claim 1 or claim 2, for use in treating an autoimmune disorder wherein the autoimmune disorder is rheumatoid arthritis.

5 4. The antibody of any one of claims 1 to 3 wherein:

(i) SEQ ID NO:3 is KSSQSLLYSTAQKXFLA (SEQ ID NO:5); and/or

(ii) SEQ ID NO:11 is QESYSTPWT (SEQ ID NO:10) or QEVYSTPWT (SEQ ID NO:12).

5. The antibody of any one of claims 1-4 wherein the CDR-L1 sequence is SEQ ID NO:2 or 3 or 4 or 6.

10 6. The antibody of any one of claims 1-4 comprising:

(i) either all of SEQ ID NOS:1 or 2 and 7 together with 9, or all of SEQ ID NOS:1 or 2 together with 7 or 8 and together with 11, or all of SEQ ID NOS:16 and 18 and 20, or all of SEQ ID NOS:16 and 101 and 20; or

15 (ii) either all of SEQ ID NOS:3 and 8 and 13, or all of SEQ ID NOS:4, 5 or 6 together with 8 and 13, or all of SEQ ID NOS:3 and 8 together with 14 or 15, or all of SEQ ID NOS:4, 5, or 6 together with 8 and together with 14 or 15, or all of SEQ ID NOS:17 and 19 together with 21 or 22, or all of SEQ ID NOS:17 and 110 and 21.

20 7. The antibody of any one of claims 1-6 comprising (i) all of the CDR-L1 to CDR-L3 amino acid sequences of SEQ ID NOS:1 or 2 together with 7 and 9, or of SEQ ID NOS:1 or 2 together with 7 or 8 and together with 11, or of SEQ ID NOS:3, 8, and 13; or of SEQ ID NOS:4, 5 or 6 together with 8 and 13, or of SEQ ID NOS:3 and 8 together with 14 or 15, or of SEQ ID NOS:4, 5 or 6 together with 8 and together with 14 or 15, and (ii) all of the CDR-H1 to CDR-H3 amino acid sequences of SEQ ID NOS:16, 18, and 20, or of SEQ ID NOS:16, 101, and 20, or of SEQ ID NOS:17 and 19, together with 21 or 22, or of SEQ ID NOS:17, 110, and 21.

25 8. The antibody of any one of claims 1-7 comprising:

(i) a human κ subgroup 1 consensus framework sequence (SEQ ID NO: 40); or

(ii) a heavy-chain human subgroup III consensus framework sequence (SEQ ID NO: 41).

9. The antibody of claim 8 part(ii) wherein the framework sequence comprises a substitution at position 71, 73, or 78, such as R71A, N73T, or N78A or a combination thereof.

30 10. The antibody of any one of claims 1-9 that:

(i) has an Fc region, and decreases levels of inflammatory cytokines associated with rheumatoid arthritis in an *in vitro* arthritis assay; and/or

(ii) binds to a lymphotoxin- $\alpha\beta$ heterodimer (LT $\alpha\beta$) complex; and/or

(iii) depletes LT α -positive cells.

35 11. The antibody of any one of claims 1-10 that is chimeric or humanized.

12. The antibody of claim 11 wherein the framework sequence is a human consensus framework sequence.

13. The antibody of any one of claims 1-12 that is of the IgG isotype, such as IgG1 or IgG2a.

14. The antibody of claim 10-13 wherein the Fc region is a wild-type Fc region.

15. The antibody of claim 10-13 further comprising one or more amino acid substitutions in its Fc region that result in it having increased antibody-dependent cell-mediated cytotoxicity (ADCC) function as compared to the same antibody having a wild-type-sequence Fc region such as one to three amino acid substitutions in its Fc region.

16. The antibody of claim 15, wherein the Fc region has the amino acid substitutions at any one or any combination of positions that are 268D, or 298A, or 326D, or 333A, or 334A, or 298A together with 333A, or 298A together with 334A, or 239D together with 332E, or 239D together with 298A and 332E, or 239D together with 268D and 298A and 332E, or 239D together with 268D and 298A and 326A and 332A, or 239D together with 268D and 298A and 326A and 332E, or 239D together with 268D and 283L and 298A and 332E, or 239D together with 268D and 283L and 298A and 326A and 332E, or 239D together with 330L and 332E and 272Y and 254T and 256E, or 250Q together with 428L, or 265A, or 297A, wherein the 265A substitution is in the absence of 297A and the 297A substitution is in the absence of 265A.

17. The antibody of any of claims 10-16 having a monovalent affinity to human LT α that is about the same as or greater than the monovalent affinity to human LT α of a murine antibody produced by a hybridoma cell line deposited under American Type Culture Collection Accession Number PTA-7538 (hybridoma murine Lymphotoxin alpha2 beta1 s5H3.2.2).

18. The antibody of claim 17, wherein the monovalent affinity is expressed as a Kd value, optionally as measured by optical biosensor or radioimmunoassay.

19. The anti-lymphotoxin- α (LT α) antibody of claim 1 having:

(i) a light-chain variable domain comprising SEQ ID NO:23 or 24, or a heavy-chain variable domain comprising SEQ ID NO:25 or 26, or having light-chain and heavy-chain variable domains comprising SEQ ID NOS:23 and 25, or SEQ ID NOS:24 and 26; or

(ii) a light-chain variable domain comprising SEQ ID NO:27 or 28, or a heavy-chain variable domain comprising SEQ ID NO:29 or 30 or 31, or having light-chain and heavy-chain variable domains comprising (a) SEQ ID NOS:27 and 29, or (b) SEQ ID NOS:27 and 30, or (c) SEQ ID NOS:27 and 31, or (d) SEQ ID NOS:28 and 30, or (e) SEQ ID NOS: 28 and 31; or

(iii) a light-chain variable domain comprising SEQ ID NO:102, or a heavy-chain variable domain comprising SEQ ID NO:103, or having light-chain and heavy-chain variable domains comprising SEQ ID NOS:102 and 103; or

(iv) a light-chain variable domain comprising SEQ ID NO:108, or a heavy-chain variable domain comprising SEQ ID NO:109, or having light-chain and heavy-chain variable domains comprising SEQ ID NOS:108 and 109.

20. An antibody of any one of claims 1, 14 or 15 having reduced fucose relative to the amount of fucose on the same antibody produced in a wild-type Chinese hamster ovary cell.

21. The antibody of claim 20 having no fucose.

22. An antibody composition comprising the antibodies of any one of claims 1-21 having an Fc region, wherein about 20-100% of the antibodies in the composition comprise a mature core carbohydrate structure in the Fc region that lacks a fucose.

23. The composition of claim 22 wherein:

(i) the antibody comprises an Fc region that has been altered to change one or more of the antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), or pharmacokinetic properties of the antibody compared to a wild-type IgG Fc sequence by substituting an amino acid selected from the group consisting of A, D, E, L, Q, T, and Y at any one or any combination of positions of the Fc region selected from the group consisting of: 238, 239, 246, 248, 249, 250, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 297, 298, 301, 303, 305, 307, 309, 312, 314, 315, 320, 322, 324, 326, 327, 329, 330, 331, 332, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 428, 430, 434, 435, 437, 438 and 439; or

(ii) the antibody has antibody-dependent cell-mediated cytotoxicity (ADCC) activity in the presence of human effector cells or has increased ADCC activity in the presence of human effector cells compared to the otherwise same antibody comprising a human wild-type IgG1 Fc; or

(iii) the antibody further comprises an Fc substitution that is 268D or 326D or 333A together with 334A, or 298A together with 333A, or 298A together with 334A, or 239D together with 332E, or 239D together with 298A and 332E, or 239D together with 268D and 298A and 332E, or 239D together with 268D and 298A and 326A and 332A, or 239D together with 268D and 298A and 326A and 332E, or 239D together with 268D and 283L and 298A and 332E, or 239D together with 268D and 283L and 298A and 326A and 332E, or 239D together with 330L and 332E.

24. The composition of claim 22 or claim 23 wherein:

(i) the antibody binds an FcγRIII; or

(ii) the antibody binds the FcγRIII with better affinity, or mediates antibody-dependent cell-mediated cytotoxicity (ADCC) more effectively, than a glycoprotein with a mature core carbohydrate structure including fucose attached to the Fc region of the glycoprotein.

25. The composition of any one of claims 22-24 wherein:

(i) the antibody has been produced by a Chinese hamster ovary (CHO) cell, such as a Lec13 cell; or

(ii) the antibody has been produced by a mammalian cell lacking a fucosyltransferase gene, such as the *FUT8* gene.

26. The composition of claim 22 wherein:

(i) the antibody is free of bisecting N-acetylglucosamine (GlcNAc) attached to its mature core carbohydrate structure; or

(ii) the antibody has bisecting N-acetylglucosamine (GlcNAc) attached to its mature core carbohydrate structure.

27. The composition of claim 22 wherein:

(i) the antibody has one or more galactose residues attached to its mature core carbohydrate structure; or

(ii) the antibody is free of one or more galactose residues attached to its mature core carbohydrate structure; or

(iii) the antibody has one or more sialic acid residues attached to its mature core carbohydrate structure; or

(iv) the antibody is free of one or more sialic acid residues attached to its mature core carbohydrate structure.

28. The composition of claim 22 that comprises:

(i) at least about 2% afucosylated antibodies; or

(ii) at least about 4% afucosylated antibodies; or

(iii) at least about 10% afucosylated antibodies; or (iv) at least about 19% afucosylated antibodies; or (v) about 100% afucosylated antibodies.

29. A composition comprising the antibody of any one of claims 1-21 and a carrier.

30. The composition of claim 29 further comprising a second medicament, wherein the anti-LTα antibody is a first medicament, such as an immunosuppressive agent, an antagonist that binds a B-cell surface marker, a BAFF antagonist, a disease-modifying anti-rheumatic drug (DMARD), an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, or a combination thereof.

31. A hybridoma deposited at the American Type Culture Collection (ATCC) on April 19, 2006 under the Deposit No. PTA-7538.

32. An antibody that is secreted by a hybridoma of claim 31.

33. An *ex vivo* method of inhibiting lymphotoxin- α -activated cell proliferation, said method comprising contacting a cell or tissue with an effective amount of the antibody of any one of claims 1-21.

34. An isolated anti-lymphotoxin- α (LT α) antibody of any one of claims 1 to 21 for use in a method of treating an autoimmune disorder in a subject.

35. Use of an isolated anti-lymphotoxin- α (LT α) antibody of any one of claims 1 to 21 in the manufacture of a medicament for treating an autoimmune disorder in a subject.

36. The antibody of claim 34 or the use of claim 35 wherein the autoimmune disorder is selected from the group consisting of rheumatoid arthritis, lupus such as systemic lupus erythematosus or lupus nephritis, Wegener's disease, inflammatory bowel disease (IBD), such as Crohn's disease or ulcerative colitis, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), autoimmune thrombocytopenia, multiple sclerosis (MS), psoriasis, IgA nephropathy, IgM polyneuropathies, myasthenia gravis, vasculitis, diabetes mellitus, Reynaud's syndrome, Sjögren's syndrome, glomerulonephritis, Hashimoto's thyroiditis, Graves' disease, helicobacter-pylori gastritis, and chronic hepatitis C.

37. The antibody of claim 34 or 36 or the use of claim 35 or claim 36, wherein the autoimmune disorder is rheumatoid arthritis.

38. The antibody of claim 34, 36 or 37, or the use of any one of claims 35-37, wherein at least one further medicament is to be administered in an effective amount, wherein the anti-LT α antibody is a first medicament, wherein the second medicament is an immunosuppressive agent, an antagonist that binds a B-cell surface marker, a BAFF antagonist, a disease-modifying anti-rheumatic drug (DMARD), an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, or a combination thereof.

39. The antibody or the use of claim 38 wherein the second medicament is a DMARD or methotrexate.

40. The antibody or the use of any one of claims 34-39 wherein:

- (i) the subject has never been previously treated with a medicament for the disorder; or
- (ii) the subject has never been previously treated with a TNF antagonist; or
- (iii) the subject has been previously treated with a medicament for the disorder; or
- (iv) the subject has been previously treated with a TNF antagonist or a DMARD.

41. The antibody or the use of any one of claims 34-39, wherein the antibody is a naked antibody or the antibody is conjugated with another molecule such as a cytotoxic agent.

42. The antibody or the use of any one of claims 34-41 wherein the antibody is to be administered intravenously or subcutaneously.

43. The antibody or the use of any one of claims 34-42 wherein the subject has rheumatoid arthritis and the antibody induces a major clinical response in the subject.

44. The antibody or the use of any one of claims 34-43 wherein the subject has an abnormal level of one or more regulatory cytokines, anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), infiltrating CD20 cells, anti-double stranded DNA (dsDNA) antibodies, anti-

- phospholipid antibodies, anti-Ro/SS-A antibodies, anti-Ro antibodies, anti-La antibodies, antibodies directed against Sjögren's-associated antigen A or B (SS-A or SS-B), antibodies directed against centromere protein B (CENP B) or centromere protein C (CENP C), autoantibodies to ICA69, anti-Smith antigen (Sm) antibodies, anti-nuclear ribonucleoprotein antibodies, anti-
5 ribosomal P antibodies, autoantibodies staining the nuclear or perinuclear zone of neutrophils (pANCA), anti-Saccharomyces cerevisiae antibodies, cross-reactive antibodies to GM1 ganglioside or GQ1b ganglioside, anti-acetylcholine receptor (AChR), anti-AChR subtype, or anti-muscle specific tyrosine kinase (MuSK) antibodies, serum anti-endothelial cell antibodies, IgG or anti-desmoglein (Dsg) antibodies, anti-centromere, anti-topoisomerase-1 (Scl-70), anti-RNA
10 polymerase or anti-U3-ribonucleoprotein (U3-RNP) antibodies, anti-glomerular basement membrane (GBM) antibodies, anti-mitochondrial (AMA) or anti-mitochondrial M2 antibodies, anti-thyroid peroxidase (TPO), anti-thyroglobin (TG) or anti-thyroid stimulating hormone receptor (TSHR) antibodies, anti-nucleic (AN), anti-actin (AA) or anti-smooth muscle antigen (ASM) antibodies, IgA anti-endomysial, IgA anti-tissue transglutaminase, IgA anti-gliadin or IgG anti-
15 gliadin antibodies, anti-CYP21A2, anti-CYP11A1 or anti-CYP17 antibodies, anti-ribonucleoprotein (RNP), or myositis-specific antibodies, anti-myelin associated glycoprotein (MAG) antibodies, anti-hepatitis C virus (HCV) antibodies, anti-GM1 ganglioside, anti-sulfate-3-glycuronyl paragloboside (SGPG), or IgM anti-glycoconjugate antibodies, IgM anti-ganglioside antibody, anti-thyroid peroxidase (TPO), anti-thyroglobin (TG) or anti-thyroid stimulating
20 hormone receptor (TSHR) antibodies, anti-myelin basic protein or anti-myelin oligodendrocytic glycoprotein antibodies, IgM rheumatoid factor antibodies directed against the Fc portion of IgG, anti-Factor VIII antibodies, or a combination thereof.
45. The antibody or the use of any one of claims 34-44 wherein:
- (a) the antibody is to be administered no more than about once every other week; or
25 (b) the antibody is to be administered about once a month.

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 <223> Sequence is synthesized

<400> 17

Gly Tyr Thr Phe Ser Ser Tyr Trp Ile Glu
5 10

<210> 18

<211> 17

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<220>

<221> Xaa

<222> 2-3

<223> any amino acid

<220>

<221> Unsure

<222> 2-3,6,10,12

<223> Unknown amino acid

<220>

<221> Xaa

<222> 6

<223> any amino acid

<220>

<221> Xaa

<222> 10

<223> any amino acid

<220>

<221> Xaa

<222> 12

<223> any amino acid

<400> 18

Tyr Xaa Xaa Pro Tyr Xaa Asp Gly Thr Xaa Tyr Xaa Glu Lys Phe
1 5 10 15

Lys Gly

<210> 19

<211> 17

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<220>

<221> Xaa

<222> 10

<223> any amino acid

<220>

<221> Xaa

<222> 12

<223> any amino acid

<400> 19

Glu Ile Ser Pro Gly Ser Gly Ser Thr Xaa Tyr Xaa Glu Glu Phe
1 5 10 15

Lys Gly

<210> 20
 <211> 9
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 20
 Pro Thr Met Leu Pro Trp Phe Ala Tyr
 5

<210> 21
 <211> 5
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 21
 Gly Tyr His Gly Tyr
 5

<210> 22
 <211> 5
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 22
 Gly Tyr His Gly Ala
 5

<210> 23
 <211> 108
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 23
 Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
 1 5 10 15
 Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Ala Val Ser
 20 25 30
 Ser Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys
 35 40 45
 Leu Gln Ile Tyr Ser Ala Ser His Arg Tyr Thr Gly Val Pro Asp
 50 55 60
 Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile
 65 70 75
 Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
 80 85 90
 His Tyr Ser Thr Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu
 95 100 105

<210> 24
<211> 108
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 24
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15
Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ala Val Ser
20 25 30
Ser Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45
Leu Gln Ile Tyr Ser Ala Ser His Arg Tyr Thr Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75
Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90
His Tyr Ser Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105
Ile Lys Arg

<210> 25
<211> 118
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 25
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15
Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
20 25 30
Ser Tyr Val Ile His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu
35 40 45
Glu Trp Ile Gly Tyr Asn Asn Pro Tyr Asn Asp Gly Thr Asn Tyr
50 55 60
Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser
65 70 75
Ser Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Ser Glu Asp
80 85 90
Ser Ala Val Tyr Tyr Cys Ser Arg Pro Thr Met Leu Pro Trp Phe
95 100 105
Ala Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ala
110 115

<210> 26
 <211> 118
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 26
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1 5 10 15
 Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr
 20 25 30
 Ser Tyr Val Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 35 40 45
 Glu Trp Val Gly Tyr Asn Asn Pro Tyr Asn Asp Gly Thr Asn Tyr
 50 55 60
 Asn Glu Lys Phe Lys Gly Arg Ala Thr Ile Ser Ser Asp Lys Ser
 65 70 75
 Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
 80 85 90
 Thr Ala Val Tyr Tyr Cys Ser Arg Pro Thr Met Leu Pro Trp Phe
 95 100 105
 Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 110 115

<210> 27
 <211> 114
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 27
 Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val
 1 5 10 15
 Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu
 20 25 30
 Tyr Ser Thr Asn Gln Lys Asn Phe Leu Ala Trp Tyr Gln Gln Lys
 35 40 45
 Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
 50 55 60
 Asp Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
 65 70 75
 Asp Phe Thr Leu Thr Ile Ser Ser Val Lys Ala Glu Asp Leu Ala
 80 85 90
 Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe Gly
 95 100 105
 Gly Gly Thr Lys Leu Glu Ile Lys Arg
 110

<210> 28

<211> 114
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 28
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 1 5 10 15
 Gly Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu
 20 25 30
 Tyr Ser Thr Asn Gln Lys Asn Phe Leu Ala Trp Tyr Gln Gln Lys
 35 40 45
 Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
 50 55 60
 Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
 65 70 75
 Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala
 80 85 90
 Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe Gly
 95 100 105
 Gln Gly Thr Lys Val Glu Ile Lys Arg
 110

<210> 29
 <211> 116
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 29
 Gln Gly Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly
 1 5 10 15
 Ala Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser
 20 25 30
 Ser Tyr Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu
 35 40 45
 Glu Trp Ile Gly Glu Ile Ser Pro Gly Ser Gly Ser Thr Asn Tyr
 50 55 60
 Asn Glu Glu Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Lys Ser
 65 70 75
 Ser Asn Thr Ala Tyr Ile Gln Leu Ser Ser Leu Ser Thr Ser Glu
 80 85 90
 Asp Ser Ala Val Tyr Tyr Cys Ala Asp Gly Tyr His Gly Tyr Trp
 95 100 105
 Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala
 110 115

<210> 30
 <211> 115
 <212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 30

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

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<210> 31

<211> 115

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 31

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

```

<210> 32

<211> 214

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 32

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Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val
 1           5           10           15
Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Ala Val Ser
          20           25           30
Ser Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys
          35           40           45
Leu Gln Ile Tyr Ser Ala Ser His Arg Tyr Thr Gly Val Pro Asp
          50           55           60
Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile
          65           70           75
Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
          80           85           90
His Tyr Ser Thr Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu
          95          100          105
Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
          110          115          120
Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
          125          130          135
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
          140          145          150
Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
          155          160          165
Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
          170          175          180
Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
          185          190          195
Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
          200          205          210
Arg Gly Glu Cys
    
```

<210> 33

<211> 448

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 33

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

Asn	Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ser	Asp	Lys	Ser	50	55	60
				65					70					75			
Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp			
				80					85					90			
Ser	Ala	Val	Tyr	Tyr	Cys	Ser	Arg	Pro	Thr	Met	Leu	Pro	Trp	Phe			
				95					100					105			
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ala	Ala	Ser			
				110					115					120			
Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser			
				125					130					135			
Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr			
				140					145					150			
Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr			
				155					160					165			
Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu			
				170					175					180			
Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly			
				185					190					195			
Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr			
				200					205					210			
Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His			
				215					220					225			
Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser			
				230					235					240			
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser			
				245					250					255			
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu			
				260					265					270			
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val			
				275					280					285			
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr			
				290					295					300			
Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu			
				305					310					315			
Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro			
				320					325					330			
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg			
				335					340					345			
Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr			
				350					355					360			
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro			
				365					370					375			
Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn			
				380					385					390			

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Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
395 400 405

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
410 415 420

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
425 430 435

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
440 445

<210> 34

<211> 220

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 34

Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val
1 5 10 15

Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu
20 25 30

Tyr Ser Thr Asn Gln Lys Asn Phe Leu Ala Trp Tyr Gln Gln Lys
35 40 45

Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
50 55 60

Asp Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
65 70 75

Asp Phe Thr Leu Thr Ile Ser Ser Val Lys Ala Glu Asp Leu Ala
80 85 90

Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe Gly
95 100 105

Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser
110 115 120

Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
125 130 135

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
140 145 150

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
155 160 165

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
170 175 180

Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
185 190 195

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
200 205 210

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
215 220

<210> 35

<211> 445

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 35

Gln Gly Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser
20 25 30

Ser Tyr Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu
35 40 45

Glu Trp Ile Gly Glu Ile Ser Pro Gly Ser Gly Ser Thr Asn Tyr
50 55 60

Asn Glu Glu Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Lys Ser
65 70 75

Ser Asn Thr Ala Tyr Ile Gln Leu Ser Ser Leu Ser Thr Ser Glu
80 85 90

Asp Ser Ala Val Tyr Tyr Cys Ala Asp Gly Tyr His Gly Tyr Trp
95 100 105

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Gly
110 115 120

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
125 130 135

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
140 145 150

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
155 160 165

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
170 175 180

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
185 190 195

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
200 205 210

Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro
215 220 225

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275 280 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
Page 14

305 310 315

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
320 325 330

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
335 340 345

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
350 355 360

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
365 370 375

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
380 385 390

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
395 400 405

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
410 415 420

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
425 430 435

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
440 445

<210> 36

<211> 113

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 36

Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val
1 5 10 15

Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu
20 25 30

Tyr Ser Thr Asn Gln Lys Asn Phe Leu Ala Trp Tyr Gln Gln Lys
35 40 45

Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
50 55 60

Asp Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
65 70 75

Asp Phe Thr Leu Thr Ile Ser Ser Val Lys Ala Glu Asp Leu Ala
80 85 90

Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe Gly
95 100 105

Gly Gly Thr Lys Leu Glu Ile Lys
110

<210> 37

<211> 107

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 37

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

<210> 38

<211> 114

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 38

```

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

<210> 39

<211> 335

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 39

Ala	Lys	Thr	Thr	Pro	Pro	Ser	Val	Tyr	Pro	Leu	Ala	Pro	Gly	Cys	
1				5					10					15	
Gly	Asp	Thr	Thr	Gly	Ser	Ser	Val	Thr	Leu	Gly	Cys	Leu	Val	Lys	
				20					25					30	
Gly	Tyr	Phe	Pro	Glu	Ser	Val	Thr	Val	Thr	Trp	Asn	Ser	Gly	Ser	
				35					40					45	
Leu	Ser	Ser	Ser	Val	His	Thr	Phe	Pro	Ala	Leu	Leu	Gln	Ser	Gly	
				50					55					60	
Leu	Tyr	Thr	Met	Ser	Ser	Ser	Val	Thr	Val	Pro	Ser	Ser	Thr	Trp	
				65					70					75	
Pro	Ser	Gln	Thr	Val	Thr	Cys	Ser	Val	Ala	His	Pro	Ala	Ser	Ser	
				80					85					90	
Thr	Thr	Val	Asp	Lys	Lys	Leu	Glu	Pro	Ser	Gly	Pro	Ile	Ser	Thr	
				95					100					105	
Ile	Asn	Pro	Cys	Pro	Pro	Cys	Lys	Glu	Cys	His	Lys	Cys	Pro	Ala	
				110					115					120	
Pro	Asn	Leu	Glu	Gly	Gly	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Asn	
				125					130					135	
Ile	Lys	Asp	Val	Leu	Met	Ile	Ser	Leu	Thr	Pro	Lys	Val	Thr	Cys	
				140					145					150	
Val	Val	Val	Asp	Val	Ser	Glu	Asp	Asp	Pro	Asp	Val	Gln	Ile	Ser	
				155					160					165	
Trp	Phe	Val	Asn	Asn	Val	Glu	Val	His	Thr	Ala	Gln	Thr	Gln	Thr	
				170					175					180	
His	Arg	Glu	Asp	Tyr	Asn	Ser	Thr	Ile	Arg	Val	Val	Ser	Thr	Leu	
				185					190					195	
Pro	Ile	Gln	His	Gln	Asp	Trp	Met	Ser	Gly	Lys	Glu	Phe	Lys	Cys	
				200					205					210	
Lys	Val	Asn	Asn	Lys	Asp	Leu	Pro	Ser	Pro	Ile	Glu	Arg	Thr	Ile	
				215					220					225	
Ser	Lys	Ile	Lys	Gly	Leu	Val	Arg	Ala	Pro	Gln	Val	Tyr	Ile	Leu	
				230					235					240	
Pro	Pro	Pro	Ala	Glu	Gln	Leu	Ser	Arg	Lys	Asp	Val	Ser	Leu	Thr	
				245					250					255	
Cys	Leu	Val	Val	Gly	Phe	Asn	Pro	Gly	Asp	Ile	Ser	Val	Glu	Trp	
				260					265					270	
Thr	Ser	Asn	Gly	His	Thr	Glu	Glu	Asn	Tyr	Lys	Asp	Thr	Ala	Pro	
				275					280					285	
Val	Leu	Asp	Ser	Asp	Gly	Ser	Tyr	Phe	Ile	Tyr	Ser	Lys	Leu	Asn	
				290					295					300	
Met	Lys	Thr	Ser	Lys	Trp	Glu	Lys	Thr	Asp	Ser	Phe	Ser	Cys	Asn	
				305					310					315	
Val	Arg	His	Glu	Gly	Leu	Lys	Asn	Tyr	Tyr	Leu	Lys	Lys	Thr	Ile	
				320					325					330	
Ser	Arg	Ser	Pro	Gly											

<210> 40
<211> 108
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 40
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15
Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
20 25 30
Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45
Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75
Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90
Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105
Ile Lys Arg

<210> 41
<211> 118
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 41
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30
Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45
Glu Trp Val Ser Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
50 55 60
Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
65 70 75
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90
Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Gly Gly Gly Ser Asp
95 100 105
Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
110 115

<210> 42
 <211> 214
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 42
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 1 5 10 15
 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ala Val Ser
 20 25 30
 Ser Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
 35 40 45
 Leu Leu Ile Tyr Ser Ala Ser His Arg Tyr Thr Gly Val Pro Ser
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
 65 70 75
 Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
 80 85 90
 His Tyr Ser Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
 95 100 105
 Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
 110 115 120
 Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 125 130 135
 Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
 140 145 150
 Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
 155 160 165
 Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
 170 175 180
 Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
 185 190 195
 Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
 200 205 210

Arg Gly Glu Cys

<210> 43
 <211> 448
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 43
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1 5 10 15
 Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr
 20 25 30

Ser	Tyr	Val	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	35	40	45
Glu	Trp	Val	Gly	Tyr	Asn	Asn	Pro	Tyr	Asn	Asp	Gly	Thr	Asn	Tyr	50	55	60
Asn	Glu	Lys	Phe	Lys	Gly	Arg	Ala	Thr	Ile	Ser	Ser	Asp	Lys	Ser	65	70	75
Lys	Asn	Thr	Ala	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	80	85	90
Thr	Ala	Val	Tyr	Tyr	Cys	Ser	Arg	Pro	Thr	Met	Leu	Pro	Trp	Phe	95	100	105
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	110	115	120
Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	125	130	135
Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	140	145	150
Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	155	160	165
Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	170	175	180
Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	185	190	195
Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	200	205	210
Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	215	220	225
Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	230	235	240
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	245	250	255
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	260	265	270
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	275	280	285
His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	290	295	300
Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	305	310	315
Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	320	325	330
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	335	340	345
Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	350	355	360
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro			

	365		370		375
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn					
	380		385		390
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe					
	395		400		405
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln					
	410		415		420
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn					
	425		430		435
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys					
	440		445		

<210> 44

<211> 214

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 44

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val					
1	5		10		15
Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ala Val Ser					
	20		25		30
Ser Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys					
	35		40		45
Leu Leu Ile Tyr Ser Ala Ser His Arg Tyr Thr Gly Val Pro Ser					
	50		55		60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile					
	65		70		75
Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln					
	80		85		90
His Tyr Ser Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu					
	95		100		105
Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro					
	110		115		120
Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu					
	125		130		135
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val					
	140		145		150
Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu					
	155		160		165
Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr					
	170		175		180
Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu					
	185		190		195
Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn					
	200		205		210

<210> 45
 <211> 448
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 45
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1 5 10 15
 Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr
 20 25 30
 Ser Tyr Val Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 35 40 45
 Glu Trp Val Gly Tyr Asn Asn Pro Tyr Asn Asp Gly Thr Asn Tyr
 50 55 60
 Asn Glu Lys Phe Lys Gly Arg Phe Thr Ile Ser Ser Asp Lys Ser
 65 70 75
 Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
 80 85 90
 Thr Ala Val Tyr Tyr Cys Ser Arg Pro Thr Met Leu Pro Trp Phe
 95 100 105
 Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser
 110 115 120
 Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser
 125 130 135
 Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 140 145 150
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
 155 160 165
 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu
 170 175 180
 Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
 185 190 195
 Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
 200 205 210
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 215 220 225
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
 230 235 240
 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 245 250 255
 Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
 260 265 270
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 275 280 285

```

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
      290      295      300
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
      305      310      315
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
      320      325      330
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
      335      340      345
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
      350      355      360
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
      365      370      375
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
      380      385      390
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
      395      400      405
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
      410      415      420
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
      425      430      435
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
      440      445

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<210> 46
 <211> 25
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

```

<400> 46
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
  1          5          10          15
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser
          20          25

```

<210> 47
 <211> 14
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

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<400> 47
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly
          5          10

```

<210> 48
 <211> 32
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

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<220>
<221> xaa
<222> 8
<223> any amino acid

<220>
<221> xaa
<222> 11
<223> any amino acid

<220>
<221> xaa
<222> 18
<223> any amino acid

<400> 48
Arg Phe Thr Ile Ser Arg Asp Xaa Ser Lys Xaa Thr Leu Tyr Leu
 1           5           10           15
Gln Met Xaa Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
          20           25           30
Ala Arg

<210> 49
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 49
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
          5           10

<210> 50
<211> 32
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<220>
<221> xaa
<222> 8
<223> any amino acid

<220>
<221> xaa
<222> 11
<223> any amino acid

<220>
<221> xaa
<222> 18
<223> any amino acid

<400> 50
Arg Ala Thr Phe Ser Ala Asp Xaa Ser Lys Xaa Thr Ala Tyr Leu
 1           5           10           15
Gln Met Xaa Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
          20           25           30

```

Ala Asp

<210> 51
 <211> 23
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 51
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys
 20

<210> 52
 <211> 15
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 52
 Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Gln Ile Tyr
 1 5 10 15

<210> 53
 <211> 32
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 53
 Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
 1 5 10 15

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
 20 25 30

Tyr Cys

<210> 54
 <211> 11
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 54
 Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
 5 10

<210> 55
 <211> 15
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 55
 Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
 1 5 10 15

<210> 56
 <211> 35
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<220>
 <221> Unknown
 <222> 15, 30
 <223> R = A or G

<220>
 <221> Unknown
 <222> 18, 24, 27, 33
 <223> N= A, G, C, and T

<220>
 <221> Unknown
 <222> 22
 <223> Y = C or T

<400> 56
 ggatcatcga tacarctngt vytncancar tcncc 35

<210> 57
 <211> 33
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<220>
 <221> Unknown
 <222> 3
 <223> R = A or G

<220>
 <221> Unknown
 <222> 18
 <223> Y = C or T

<400> 57
 aarggtggta gaagactygc cgcgcggcaa tgg 33

<210> 58
 <211> 33
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 58
 ggatccgata tccagctggg attgacccaa tct 33

<210> 59
 <211> 33
 <212> DNA
 <213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 59

cccagcacac aaaaaccgtc gccatggact agg 33

<210> 60

<211> 30

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<220>

<221> Unknown

<222> 14, 24, 30

<223> R = A or G

<220>

<221> Unknown

<222> 17, 27

<223> N = A, G, C, and T

<400> 60

tgataatcga tgargtncca tttrgtngar 30

<210> 61

<211> 36

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<220>

<221> Unknown

<222> 6

<223> R = A or G

<220>

<221> Unknown

<222> 9, 12

<223> N = A, G, C, and T

<400> 61

accttragnc cnagggactg gtccgcgcgg aatgat 36

<210> 62

<211> 32

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 62

tgatcgcgta cgctgagggtt caattggttg ag 32

<210> 63

<211> 35

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 63

ggcagaggag tcggtgttgt ttcccgggtg ctagt 35

<210> 64
 <211> 220
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 64
 Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val
 1 5 10 15
 Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu
 20 25 30
 Tyr Ser Thr Asn Gln Lys Asn Phe Leu Ala Trp Tyr Gln Gln Lys
 35 40 45
 Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
 50 55 60
 Asp Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
 65 70 75
 Asp Phe Thr Leu Thr Ile Ser Ser Val Lys Ala Glu Asp Leu Ala
 80 85 90
 Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Arg Thr Phe Gly
 95 100 105
 Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr
 110 115 120
 Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly
 125 130 135
 Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
 140 145 150
 Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val
 155 160 165
 Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser
 170 175 180
 Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His
 185 190 195
 Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
 200 205 210
 Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 215 220

<210> 65
 <211> 220
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<220>
 <221> Xaa
 <222> 59
 <223> any amino acid

<220>
 <221> Xaa
 <222> 61
 <223> any amino acid

<220>
 <221> Xaa
 <222> 77
 <223> any amino acid

<220>
 <221> Xaa
 <222> 157
 <223> any amino acid

<400> 65
 Gln Gly Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly
 1 5 10 15
 Ala Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser
 20 25 30
 Ser Tyr Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu
 35 40 45
 Glu Trp Ile Gly Glu Ile Ser Pro Gly Ser Gly Ser Thr Xaa Tyr
 50 55 60
 Xaa Glu Glu Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Lys Ser
 65 70 75
 Ser Xaa Thr Ala Tyr Ile Gln Leu Ser Ser Leu Ser Thr Ser Glu
 80 85 90
 Asp Ser Ala Val Tyr Tyr Cys Ala Asp Gly Tyr His Gly Tyr Trp
 95 100 105
 Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro
 110 115 120
 Pro Ser Val Tyr Pro Leu Ala Pro Gly Cys Gly Asp Thr Thr Gly
 125 130 135
 Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu
 140 145 150
 Ser Val Thr Val Thr Trp Xaa Ser Gly Ser Leu Ser Ser Ser Val
 155 160 165
 His Thr Phe Pro Ala Leu Leu Gln Ser Gly Leu Tyr Thr Met Ser
 170 175 180
 Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr Val
 185 190 195
 Thr Cys Ser Val Ala His Pro Ala Ser Ser Thr Thr Val Asp Lys
 200 205 210
 Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr
 215 220

<210> 66
 <211> 78
 <212> DNA
 <213> Artificial sequence

<220>
 <223> sequence is synthesized

<400> 66
ggtcatagt accttttctc caacagacac agccagagat gatggcgact 50
gtgacatcac gatatctgaa tgtactcc 78

<210> 67
<211> 66
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 67
gctgtaagtc cagtcaaagt cttttataca gtaccaatca gaagaacttc 50
ttggcctggt accagc 66

<210> 68
<211> 35
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 68
gcgatcaggg acaccagatt ccctagtgga tgccc 35

<210> 69
<211> 31
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 69
gccacgtctt cagcttttac actgctgatg g 31

<210> 70
<211> 63
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 70
ggccccctt ccgaacgtgc gcgggtagga gtagtattgc tgacagtaat 50
aaactgccag gtc 63

<210> 71
<211> 28
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 71
gctgcagctg accttctgaa tgtactcc 28

<210> 72
<211> 50
<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 72

gccttgcagg agatcttcac tgaagcccca ggcttcatca gctcagctcc 50

<210> 73

<211> 48

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 73

cccactctat ccagtaacta gagaagggtgt atccagtagc cttgcagg 48

<210> 74

<211> 49

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 74

ccacttccag gactaatctc tccaatccac tcaaggccat gtccaggcc 49

<210> 75

<211> 42

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 75

gcccttgaac tcctcattgt aattagtact accacttcca gg 42

<210> 76

<211> 85

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 76

ccgcagagtc ctcagatgtg atcaggctgc tgagctggat gtaggcagtg 50

ttggaggatt tgtctgcagt gaatgttgcc ttgcc 85

<210> 77

<211> 72

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 77

ggctgaggag acggtgactg tggcgccttg gccccagtag ccatggtacc 50

cgtctgcaca gtaatagacc tc 72

<210> 78

<211> 41
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Sequence is synthesized

 <400> 78
 ccagactgct gcagctgacc ttgtgaatgt actccagttg c 41

 <210> 79
 <211> 28
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Sequence is synthesized

 <220>
 <221> Unknown
 <222> 15
 <223> R = A or G

 <220>
 <221> Unknown
 <222> 21
 <223> N = T, G, A or C

 <400> 79
 gccatagata tcgtratgca ncagtctc 28

 <210> 80
 <211> 21
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Sequence is synthesized

 <220>
 <221> Unknown
 <222> 15
 <223> Y = C or T

 <220>
 <221> Unknown
 <222> 18
 <223> K = G or T

 <400> 80
 ccatggttcg acctytaktt t 21

 <210> 81
 <211> 43
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Sequence is synthesized

 <220>
 <221> Unknown
 <222> 20
 <223> Y = C or T

 <220>
 <221> Unknown
 <222> 26

<223> S = C or G

<400> 81

gatcgacgta cgctgaggty cagctscagc tscagcagtc tgg 43

<210> 82

<211> 44

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<220>

<221> Unknown

<222> 3, 5, 18

<223> R = A or G

<220>

<221> Unknown

<222> 4, 12

<223> D = A or G or T

<220>

<221> Unknown

<222> 6

<223> H = A or C or T

<220>

<221> Unknown

<222> 7

<223> S = C or G

<220>

<221> Unknown

<222> 19

<223> M = A or C

<400> 82

tgrdrhsagt gdcagagrmg tcggagggtg ttcccgggta gaca 44

<210> 83

<211> 58

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 83

cctgggtttct gttgatacca ggctacagcg gaagacacag cctgactggc 50

acggcagg 58

<210> 84

<211> 57

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 84

gcgagaaggg actccagtgt aacggtggga tgcagagtaa atctgtagtt 50

tcggagc 57

<210> 85

<211> 57
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 85
 ggtaccctgt ccgaacgtcc aaggagtaga ataatgttgc tgacagtaat 50
 aagttgc 57

<210> 86
 <211> 42
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 86
 ggtcagagtg aaatccgtcc cagatccgga tccagagaag cg 42

<210> 87
 <211> 54
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 87
 ggcctgacgg acccaatgga tcacatagct ggtgaatctg tagccagaag 50
 ctgc 54

<210> 88
 <211> 69
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 88
 ccccttgaac ttctcggtat agttggtgcc gtcgttataa ggattgttat 50
 aaccaaccca ctcgaggcc 69

<210> 89
 <211> 75
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 89
 ggttccttga cccagtagg cgaaccatgg gagcattgtg ggtcgagaac 50
 aataatagac ggcagtgtcc tcagc 75

<210> 90
 <211> 41
 <212> DNA
 <213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 90

ccgtcgttat aaggattgtt ataactaacc cactcgaggc c 41

<210> 91

<211> 61

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 91

catctgcagg tatagtgtgt ttttgaatt gtcacgactg atagtgaagc 50

gcccccttgaa c 61

<210> 92

<211> 46

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 92

ccatgggagc attgtgggtc gagcacaata atagacggca gtgtcc 46

<210> 93

<211> 74

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 93

ggaagacttc gcaacttatt actgtcagca acattattct actccttgga 50

cgttcggaca gggtagcaag gtgg 74

<210> 94

<211> 74

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 94

ggacactgcc gtctattatt gttctcgacc cacaacgctc ccatgggttcg 50

cctactggggg tcaaggaacc ctgg 74

<210> 95

<211> 9

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 95

Gln Glu His Tyr Ser Thr Pro Trp Thr

5

<210> 96

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<211> 9
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<220>
<223> Sequence is synthesized

<400> 96
  Gln Gln His Tyr Tyr Thr Pro Trp Thr
      5

<210> 97
<211> 9
<212> PRT
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<223> Sequence is synthesized

<400> 97
  Gln Gln Tyr Tyr Ser Thr Pro Trp Thr
      5

<210> 98
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 98
  Gln Lys Thr Phe Ser Thr Pro Trp Thr
      5

<210> 99
<211> 9
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<400> 99
  Gln Gln Phe Tyr Ser Val Pro Trp Thr
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<210> 100
<211> 9
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<400> 100
  Gln Gln Lys Tyr Ser Thr Pro Trp Thr
      5

<210> 101
<211> 17
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<221> Xaa
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<223> any amino acid

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<222> 2-3,6,10,12
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<222> 6
<223> any amino acid

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<221> Xaa
<222> 10
<223> any amino acid

<220>
<221> Xaa
<222> 12
<223> any amino acid

<400> 101
Tyr Xaa Xaa Pro Tyr Xaa Ala Gly Thr Xaa Tyr Xaa Glu Lys Phe
1 5 10 15
Lys Gly

<210> 102
<211> 108
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 102
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
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Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ala Val Ser
20 25 30
Ser Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45
Leu Leu Ile Tyr Ser Ala Ser His Arg Tyr Thr Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75
Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Glu
80 85 90
Ser Tyr Ser Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105
Ile Lys Arg

<210> 103
<211> 118
<212> PRT
<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 103

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

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<210> 104

<211> 1704

<212> DNA

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 104

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tccattgggt ccgtcaggcc ccgggtaagg gcctcgagtg ggttggttat 150
aacaatcctt ataacgccgg caccaactat aacgagaagt tcaaggggcg 200
cttcactatc agttctgaca agtcgaaaaa cacagcatac ctgcagatga 250
acagcctgcg tgctgaggac actgccgtct attattgttc tcgaccaca 300
atgctcccat ggctgccta ctgggggtcaa ggaaccctgg tcaccgtctc 350
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caggggggctc actccgtttg tcctgtgcag cttctggcta cacattcacc 450
agctatgtga tccattgggt ccgtcaggcc ccgggtaagg gcctcgagtg 500
ggttggttat aacaatcctt ataacgccgg caccaactat aacgagaagt 550
tcaaggggcg cttcactatc agttctgaca agtcgaaaaa cacagcatac 600
ctgcagatga acagcctgcg tgctgaggac actgccgtct attattgttc 650
tcgaccaca atgctcccat ggctgccta ctgggggtcaa ggaaccctgg 700
tcaccgtctc ctcagcctcc accaagggcc catcggtctt cccctggca 750

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ccctcctcca agagcacctc tgggggcaca gcggccctgg gctgcctggt 800
 caaggactac ttccccgaac cggtgacggt gtcgtggaac tcaggcgccc 850
 tgaccagcgg cgtgcacacc ttcccggctg tcctacagtc ctcaggactc 900
 tactccctca gcagcgtggt gactgtgccc tctagcagct tgggcaccca 950
 gacctacatc tgcaacgtga atcacaagcc cagcaacacc aaggtggaca 1000
 agaaagttga gccc aaatct tgtgacaaaa ctcacacatg cccaccgtgc 1050
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 tggtgagcgt gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg 1200
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 acaggtgtac accctgcccc catcccgga agagatgacc aagaaccagg 1450
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 acaagagcag gtggcagcag gggaaagtct tctcatgctc cgtgatgcat 1650
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 taaa 1704

<210> 105
 <211> 642
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 105
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 tagggtcacc atcacctgcc gtgccagtca ggctgtgtct tccgctgtag 100
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 gcatcccacc gttacactgg agtcccttct cgcttctctg gatccggatc 200
 tgggacggat ttactctga ccatcagcag tctgcagccg gaagacttcg 250
 caacttatta ctgtcaggaa tcttattcta ctcttggac gttcggacag 300
 ggtaccaagg tggagatcaa acgaactgtg gctgcacat ctgtcttcat 350
 cttcccgcca tctgatgagc agttgaaatc tggaactgcc tctgttgtgt 400
 gcctgctgaa taacttctat cccagagagg ccaaagtaca gtggaagggtg 450
 gataacgccc tccaatcggg taactcccag gagagtgtca cagagcagga 500

cagcaaggac agcacctaca gcctcagcag caccctgacg ctgagcaaag 550

cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600

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<210> 106

<211> 448

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 106

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr
20 25 30

Ser Tyr Val Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Gly Tyr Asn Asn Pro Tyr Asn Ala Gly Thr Asn Tyr
50 55 60

Asn Glu Lys Phe Lys Gly Arg Phe Thr Ile Ser Ser Asp Lys Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ser Arg Pro Thr Met Leu Pro Trp Phe
95 100 105

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser
110 115 120

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser
125 130 135

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
140 145 150

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
155 160 165

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu
170 175 180

Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
185 190 195

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
200 205 210

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
215 220 225

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
230 235 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
Page 40

260	265	270
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val		
275	280	285
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
290	295	300
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu		
305	310	315
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro		
320	325	330
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg		
335	340	345
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr		
350	355	360
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro		
365	370	375
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn		
380	385	390
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe		
395	400	405
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln		
410	415	420
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn		
425	430	435
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		
440	445	

<210> 107
 <211> 214
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 107
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
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20 25 30
Ser Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45
Leu Leu Ile Tyr Ser Ala Ser His Arg Tyr Thr Gly Val Pro Ser
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75
Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Glu
80 85 90
Ser Tyr Ser Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	
				110					115					120	
Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	
				125					130					135	
Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	
				140					145					150	
Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	
				155					160					165	
Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	
				170					175					180	
Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	
				185					190					195	
Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	
				200					205					210	

Arg Gly Glu Cys

<210> 108
 <211> 114
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

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

<210> 109
 <211> 114
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	
1				5					10					15	

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

<210> 110

<211> 17

<212> PRT

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<220>

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<221> Xaa

<222> 3

<223> any amino acid

<220>

<221> Xaa

<222> 12

<223> any amino acid

<400> 110

Glu Ile Xaa Pro Gly Ser Gly Ser Thr Ile Tyr Xaa Glu Lys Phe
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Lys Gly