METHODS FOR THE TREATMENT OF AUTOIMMUNE DISORDERS USING IMMUNOSUPPRESSIVE MONOCLONAL ANTIBODIES WITH REDUCED TOXICITY

[0001] This application claims priority to U.S. Provisional Application Serial No. 60/698,517, filed on July 11, 2005, which is incorporated by reference herein in its entirety.

1. INTRODUCTION

[0002] The present invention provides methods of treating, preventing or ameliorating the symptoms of T cell-mediated immunological diseases, particularly autoimmune diseases, through the use of anti-CD3 antibodies. In particular, the methods of the invention provide for administration of antibodies that specifically bind the epsilon subunit within the human CD3 complex. Such antibodies modulate the T cell receptor/alloantigen interaction and, thus, regulate the T cell mediated cytotoxicity associated with autoimmune disorders. Additionally, the invention provides for modification of the anti-CD3 antibodies such that they exhibit reduced or eliminated effector function and T cell activation as compared to non-modified anti-CD3 antibodies.

2. BACKGROUND OF THE INVENTION

2.1 Autoimmune Diseases

[0003] Autoimmune diseases are caused when the body's immune system, which normally defends the body against bacteria, viruses and other infective agents, attacks "self" tissue, cells and organs. The mobilization of the immune system against such "self" targets is termed autoimmunity. Although some autoimmunity is present in every individual, rigid control systems suppress the self-recognizing cells of the immune system to an extent that the autoimmunity is normally asymptomatic. Disease states arise when there is some interruption in the control system, allowing the autoimmune cells to escape suppression, or when there is some change in a target tissue such that it is no longer recognized as self. The mechanisms underlying these changes are not well understood, but have been theorized to be the result of aberrant immune stimulation in genetically predisposed individuals.

[0004] Autoimmune diseases can be organ specific or systemic and are provoked by differing pathogenic mechanisms. Organ specific autoimmunization is characterized by tolerance and suppression within the T cell compartment, aberrant expression of major-histocompatibility complex (MHC) antigens, antigenic mimicry and allelic variations in MHC genes. Systemic autoimmune diseases involve polyclonal B cell activation and abnormalities of immunoregulatory T cells, T cell receptors and MHC genes. Examples of organ specific

autoimmune diseases are diabetes, cutaneous psoriasis, ulcerative colitis, hyperthyroidism, autoimmune adrenal insufficiency, hemolytic anemia, multiple sclerosis and rheumatic carditis. Representative systemic autoimmune diseases are systemic lupus, erythematosus, rheumatoid arthritis, psoriatic arthritis, Sjogren's syndrome polymyositis, dermatomyositis and scleroderma.

[0005] Also, while not an autoimmune disorder, organ transplant recipients often experience similar symptoms and require therapies similar to autoimmune patients. Immune system attacks on the transplanted organs can lead to organ failure or more serious systemic complications, *i.e.* bone-marrow transplant recipients and graft-vs.-host disease (GVHD).

[0006] There is a clear need for improved strategies to treat autoimmune disorders and/or modulate immune response. Currently, immune system disorders are treated with immunosuppressive agents such as cortisone, aspirin derivatives, hydroxychloroquine, methotrexate, azathioprine,cyclophsophamide and various biologics such as anti TNF antibodies, or combinations thereof. The treatments are varyingly successful, depending on the individual patient and disorder. However, a dilemma in the use of such general immunosuppressive therapies arises in that the greater the immune-suppression, and thus the potential for successful treatment of the autoimmune disorder, the more at-risk the patient becomes for developing opportunistic infections. Further, due to the compromised nature of the patient's immune system, even a minor infection can rapidly become of serious concern.

2.2 Diabetes

[0007] One of the most common autoimmune diseases is type 1 diabetes, also known as juvenile diabetes or insulin-dependent diabetes mellitus. It affects 15 million people in the United States with an estimated additional 12 million people who are currently asymptomatic, and thus are unaware that they have this disease. Diabetes is caused by an autoimmune response in which the insulin producing β-cells of the pancreas (also known as islet cells) are gradually destroyed. The early stage of the disease, termed insulitis, is characterized by infiltration of leukocytes into the pancreas and is associated with both pancreatic inflammation and the release of anti- β-cell cytotoxic antibodies. As the disease progresses, the injured tissue may also attract lymphocytes, causing yet further damage to the β-cells. Also, subsequent general activation of lymphocytes, for example in response to viral infection, food allergy, chemical, or stress, may result in yet more islet cells being destroyed. Early stages of the disease are often overlooked or misdiagnosed as clinical symptoms of diabetes typically manifest only after about 80 % of the β-cells have been destroyed. Once symptoms occur, the type-1 diabetic is normally insulin dependent for life. The dysregulation of blood-glucose levels associated with diabetes can lead to blindness, kidney failure, nerve damage and is a major contributing factor in the etiology of stroke, coronary heart disease and other blood vessel disorders.

2.3 T cell Functionality in Diabetes and other Autoimmune Disorders

[0008] Destruction of β-cells in diabetes, or of the target cells of other autoimmune disorders, is believed largely mediated by cytotoxic T-lymphocytes (CTLs - also known as CD8+ T cells) that specifically recognize antigenic, target cell derived peptides. CTLs, as well as other types of T cells, recognize these antigenic peptides through their specific T cell receptor (TcR). Unlike antibodies which recognize soluble whole foreign proteins as antigen, the TcR instead interacts with small peptidic antigens presented only in complex with major histocompatibility complex (MHC) proteins.

[0009] Most cells of the body express MHC molecules of various classes on their surface and, depending on the class of MHC expressed, will present either soluble antigens, those dispersed within the lymph and/or circulatory systems, or fragments of their cytoplasmic proteins. MHC molecules (called human leukocyte antigens or HLA in humans) and TcRs are extremely polymorphic, each clonal variation recognizing and binding to a single peptidic sequence, or set of similar peptidic analogs. Apart from cells specific to the immune system, *i.e.* B cells and T cells, cells of the body express multiple variants of the MHC molecule, each variant binding to a different peptide sequence. In contrast, during maturation, B and T cells lose the ability to express multiple variants of MHC and TcR, respectively. Mature T cells, therefore, will express only one of the possible variants of the TcR and will thus recognize/bind a single MHC/antigen complex.

[0010] Binding of a TcR to a MHC/antigen complex releases an intracellular signal cascade within the T cell, termed activation, which results in clonal proliferation of the T cell and class-specific T cell responses. For example, in CTLs the response to activation also includes the release of cytotoxic enzymes that result in apoptosis/destruction of the target cell.

2.4 Modulation of T cell Activation by Monoclonal Antibodies

[0011] The finding that autoimmune diseases are at least partially caused by aberrant T cell action has lead to the investigation of therapies that either eliminate problematic T cell clones (those expressing TcRs against self antigens) or selectively reduce undesired T cell activity/activation. T cell activation due to TcR binding is, however, an unexpectedly complex phenomenon due to the participation of a variety of cell surface molecules expressed on the responding T cell population (Billadeau et al., 2002, J. Clin. Invest. 109:161-168; Weiss, 1990, J. Clin. Invest. 86:1015-1022; Leo et al., 1987, PNAS 84:1374-1378; Weiss et al., 1984, PNAS 81:4169-4173; Hoffman et al., 1985, J. Immunol. 135:5-8).

[0012] Targeted therapies directed against general T cell activation were problematic in that the TcR is composed of a disulfide-linked heterodimer, containing two clonally distributed, integral membrane glycoprotein chains, α and β, or γ and δ. Most of the research in modulation

of T cell activation was done in connection with improving immune suppression in organ transplant recipients. One of the first clinically successful methods of selectively reducing T cell activation was the use of monoclonal antibodies. U.S. Pat. No. 4,658,019, describes a novel hybridoma (designated OKT3, ATCC Accession No. CRL-8001) which is capable of producing a murine monoclonal antibody against an antigen found on essentially all normal human peripheral T cells. Binding of OKT3 to T cells in vivo produces pronounced, reversible immunosuppression. OKT3 was found to recognize an epitope on the ε-subunit within the human CD3 complex (Salmeron et al., 1991, J. Immunol. 147:3047-3052; Transy et al., 1989, Eur. J. Immunol. 19:947-950; see also, U.S. Pat. No. 4,658,019). The CD3 complex (also known as T3) is comprised of low molecular weight invariant proteins, which non-covalently associate with the TcR (Samelson et al., 1985, Cell 43:223-231). The CD3 structures are thought to represent accessory molecules that may be the transducing elements of activation signals initiated upon binding of the TcR α-β to its ligand.

[0013] OKT3 possesses potent T cell activating and suppressive properties (Landgren, 1982; Van Seventer, 1987; Weiss, 1986). Fc receptor-mediated cross-linking of TcR-bound anti-CD3 mAb results in T cell activation marker expression, and proliferation (Weiss et al., 1986, Ann. Rev. Immunol. 4:593). Similarly, in vivo administration of OKT3 results in both T cell activation and suppression of immune responses (Ellenhorn et al., 1992, Transplantation; Chatenoud, 1990, Transplantation 49:697). Repeated daily administration of OKT3 results in profound immunosuppression, and provides effective treatment of rejection following renal transplantation (Thistlethwaite, 1984, Transplantation 38:695).

[0014] The use of therapeutic mAbs, including for example OKT3, is limited by problems of "first dose" side effects, ranging from mild flu-like symptoms to severe toxicity. The first dose side effects are believed to be caused by cytokine production stimulated by T cell activation. It has been shown that the activating properties of mAbs result from TcR cross-linking mediated by the mAb bound to T cells (via its $F(ab')^2$ portion) and to FcγR-bearing cells via its Fc portion) (Palacios et al., 1985, Eur. J. Immunol. 15:645-651; Ceuppens et al., 1985, J. Immunol. 134:1498-1502; Kan et al., 1986, Cell Immunol. 98:181-185). For example, the use of OKT3 was found to trigger activation of mAb-bound T cells and FcγR-bearing cells prior to achieving immune suppression, resulting in a massive systemic release of cytokines (Abramowicz, 1989, Transplantation 47:P606; Chatenoud, 1989). Reported side effects of OKT3 therapy include flu-like symptoms, respiratory distress, neurological symptoms, and acute tubular necrosis that may follow the first and sometimes the second injection of the mAb (Abramowicz, 1989; Chatenoud, 1989; Toussaint, 1989, Transplantion 48:524; Thistlethwaite, 1988, Am. J. Kid. Dis. 11:112; Goldman, 1990, Transplantation 50:148).

[0015] Data obtained using experimental models in chimpanzees and mice have suggested that preventing or neutralizing the cellular activation induced by anti-CD3 mAbs reduces the toxicity of these agents (Parleviet, 1990, Transplantion 50:889; Rao, 1991, Transplantion 52:691; Alegre, Eur. J. Immunol., 1990, 20:707; Alegre, 1990, Transplant Proc. 22:1920.; Alegre, Transplantation. 52:674, 1991; Alegre, J. Immun.; 1991; Ferran, 1990, Transplantation, 50:642). In addition, previous results reported in mice using $F(ab')_2$ fragments of 145-2C11, a hamster anti-mouse CD3, have suggested that, in the absence of FcγR binding and cellular activation, anti-CD3 mAbs retain at least some immunosuppressive properties in vivo (Hirsch, Transplant Proc., 1991, 23:270; Hirsch, J. Immunol., 1991, 147:2088).

2.5 Immunosuppressive Monoclonal Antibodies Exhibiting Reduced T cell Activation

[0016] U.S. Pat. No. 6,491,916, U.S. Pat. application Pub. No. 2005/0064514 and U.S. Pat. application Pub. No. 2005/0037000 describe the modification of the Fc regions of immunoglobulins such that the variant molecules exhibit enhanced or reduced binding to various Fc receptors when compared to immunoglobulins with wild type Fc domains. In particular the patents/applications describe modifications to the Fc regions of IgG antibodies such that the affinity for the FcγR is selectively enhanced or reduced. By tailoring the affinity for activating or suppressive Fc receptors, the specific immune response elicited by the therapeutic mAb may be more selectively controlled. For example, mutations in the CH_2 portion of a humanized OKT3 IgG4 have been identified (P234A and L235A) that significantly reduced binding of the mAb to human and murine FcγRI and II and lead to a markedly reduced activating phenotype in vitro (Alegre et al., 1992, 8th International Congress of Immunology 23-28; Alegre et al., 1994, Transplantation 57: 1537-1543; Xu et al., 2000, Cell Immunol. 200:16-26). Importantly, this variant mAb retained the capacity to induce TcR modulation and immunosupression (Xu et al., 2000).

3. SUMMARY OF THE INVENTION

[0017] The present invention provides methods of treating, preventing and ameliorating the symptoms of T cell-mediated immunological diseases, particularly autoimmune diseases, by administering to a subject a therapeutically or prophylactically acceptable amount of an anti-CD3 antibody. In particular, the methods of the invention provide for administration of antibodies that specifically bind the epsilon subunit within the human CD3 complex. For example, such antibodies may be or may be derived from (*e.g.*, humanized or chimerized versions of) one of the antibodies Leu-4, 500A2, CLB-T3/3, M291, YTH 12.5 or BMA030, or compete with one of Leu-4, 500A2, CLB-T3/3, M291, YTH 12.5 or BMA030 for binding, and

may be based on an IgG1, IgG2, IgG3 or IgG4 backbone. In a preferred embodiment, the antibody has the binding specificity of the murine monoclonal antibody OKT3 (see, e.g., U.S. Patent Nos. 4,658,019 and 6,113,901, which are incorporated by reference herein in their entireties), e.g., binds to the same epitope as OKT3 and/or competes for binding with OKT3, such as a humanized version of the antibody OKT3, such as OKT3-7 (see the antibodies disclosed in U.S. Patent No. 6,491,916, which is incorporated herein by reference in its entirety). In certain embodiments, the anti-CD3 antibody has diminished (such as, but not limited to, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5% or less than 1% as compared to binding by an antibody having a wild-type, glycosylated Fc domain) or, more preferably, no detectable binding to at least one FcγR as determined by assays routine in the art. In other embodiments, the anti-CD3 antibody has diminished (such as, but not limited to, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5% or less than 1% as compared to binding by an antibody having a wild-type, glycosylated Fc domain) or, more preferably, no detectable binding to one or more FcγR as determined by assays routine in the art. In addition or alternatively, the anti-CD3 antibody has diminished (such as, but not limited to, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5% or less than 1% as compared to binding by an antibody having a wild-type, glycosylated Fc domain) or, more preferably, no detectable binding to any complement receptors, such as, C1q, as determined in routinely used assays. In particular embodiments, the antibody is aglycosylated. In other embodiments, the antibody lacks an Fc domain (*e.g.*, is a Fab fragment, $F(ab')_2$ or single chain antibody). In other embodiments, the antibody comprises an Fc domain having one or more amino acid modifications that reduce or abolish binding of the Fc domain to at least one FcγR and/or to one or more FcγRs, which Fc domain may be derived from IgG1, IgG2, IgG3, or IgG4 and/or contain one or more domains (*e.g.*, CH2, CH3) from one or more of IgG1, IgG2, IgG3, or IgG4. In certain embodiments, the antibody of the invention comprises an Fc domain having a modification (*e.g.*, substitution, insertion, deletion) at position 234 of the Fc region (CH2) and at position 235 (CH2) of the Fc region. In a specific example in accordance with this embodiment, the antibody of the invention comprises an Fc domain having a substitution at position 234 (CH2) of the Fc region with alanine and a substitution at position 235 (CH2) of the Fc region with alanine. In another example in accordance with this embodiment, the antibody of the invention comprises an Fc domain having a substitution at position 234 of the Fc region (CH2) with alanine and a substitution at position 235 of the Fc region (CH2) with glutamic acid. In other embodiments, the antibody of the invention comprises an Fc domain comprising an alanine at position 234 of the Fc region (CH2) and an alanine at position 235 of the Fc region (CH2), also known as an "ala-ala" antibody. In yet other

embodiments, the antibody of the invention comprises an Fc domain having an alanine at position 234 of the Fc region (CH2) and a glutamic acid at position 235 of the Fc region (CH2). In particular embodiments, the antibody of the invention is OKT3-7 comprising the mutations and/or Fc regions described herein (*e.g.*, OKT3γ1(ala-ala), see U.S. Patent No. 6,491,916, which is hereby incorporated by reference in its entirety). In other embodiments, the antibody of the invention comprises and Fc domain having a glutamate at position 235.

[0018] The invention particularly provides methods of treating, preventing or ameliorating the symptoms of autoimmune diseases such as Type I Diabetes, psoriasis, rheumatoid arthritis, lupus (particularly, cutaneous), inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, multiple sclerosis, effects from organ transplantation, graft vs. host disease (GVHD), etc. Particularly, the methods of the invention are advantageous in subjects with early stage disease to slow or reduce the damage from the autoimmunity and maintain a high level of function and/or reduce the need for other therapy. In addition, the methods of the invention advantageously reduce the incidence of or result in no incidence of cytokine release syndrome previously associated with administration of therapeutic antibodies, and, in particular, anti-CD3 antibodies. Cytokine release syndrome is manifested by, for example, headache, nausea, vomiting, fever, myalgias, arthralgias and shaking and may be caused by increased serum levels of, for example, IL-2, IL-6, IL-10, TNFα, and IFNγ. In certain embodiments, the methods of the invention reduce the incidence and/or reduce the severity of the symptoms of cytokine release syndrome associated with administration of an antibody of the invention relative a similar administration of murine OKT3. The methods also reduce the incidence and severity of other adverse effects, such as, but not limited to, EBV activation, immunogenicity (production of anti-idiotype antibodies, particularly IgE anti-idiotype antibodies), lymphopenia, thrombocytopenia or neutropenia.

[0019] In certain embodiments, following administration of one or more doses of anti-CD3 antibody to a subject according to methods of the invention, cytokines (*e.g.*, IL-2, IL-6, IL-10, TNFα, IFNγ) are undetectable in the serum of said subject by ELISA or any other method known in the art for detection of serum proteins. In other embodiments, one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, TNFα, IFNγ) may be detected in the serum of a subject following administration of one or more doses of an anti-CD3 antibody to said subject according to methods of the invention at concentrations of less than about 250 pg/ml, less than about 225 pg/ml, less than about 200 pg/ml, less than about 175 pg/ml, less than about 150 pg/ml, less than about 125 pg/ml, less than about 100 pg/ml, less than about 90 pg/ml, less than about 80 pg/ml, less than about 70 pg/ml, less than about 60 pg/ml, less than about 50 pg/ml, less than about 45 pg/ml, less than about 40 pg/ml, less than about 35 pg/ml, less than about 30 pg/ml, less than

about 25 pg/ml, less than about 20 pg/ml, less than about 15 pg/ml, less than about 14 pg/ml, less than about 13 pg/ml, less than about 12 pg/ml, less than about 11 pg/ml, less than about 10 pg/ml, less than about 9 pg/ml, less than about 8 pg/ml, less than about 7 pg/ml, less than about 6 pg/ml, less than about 5 pg/ml, less than about 4 pg/ml, less than about 3 pg/ml, less than about 2 pg/ml, or less than about 1 pg/ml. In still other embodiments, one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, TNFα, IFNγ) may be detected in the serum of a subject (*e.g.*, by ELISA or other method known in the art) following administration of one or more doses of an anti-CD3 antibody to said subject according to methods of the invention at a concentration range from undetectable to less than about 10 pg/ml, from undetectable to less than about 15 pg/ml, from undetectable to less than about 20 pg/ml, from undetectable to less than about 25 pg/ml, from undetectable to less than about 30 pg/ml, from undetectable to less than about 40 pg/ml, from undetectable to less than about 50 pg/ml, from undetectable to less than about 75 pg/ml, from undetectable to less than about 100 pg/ml, from undetectable to less than about 150 pg/ml, from undetectable to less than about 200 pg/ml, from undetectable to less than about 250 pg/ml, from undetectable to less than about 300 pg/ml, from undetectable to less than about 350 pg/ml, or from undetectable to less than about 400 pg/ml. In still other embodiments, one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, TNFα, IFNγ) may be detected in the serum of a subject (*e.g.*, by ELISA or other method known in the art) following administration of one or more doses of an anti-CD3 antibody to said subject according to methods of the invention at a concentration range from about 5 pg/ml to less than about 10 pg/ml, from about 5 pg/ml to less than about 15 pg/ml, from about 5 pg/ml to less than about 20 pg/ml, from about 5 pg/ml to less than about 25 pg/ml, from about 5 pg/ml to less than about 30 pg/ml, from about 5 pg/ml to less than about 35 pg/ml, from about 5 pg/ml to less than about 40 pg/ml, from about 5 pg/ml to less than about 50 pg/ml, from about 5 pg/ml to less than about 75 pg/ml, from about 5 pg/ml to less than about 100 pg/ml, from about 5 pg/ml to less than about 150 pg/ml, from about 5 pg/ml to less than about 200 pg/ml, from about 5 pg/ml to less than about 250 pg/ml, from about 5 pg/ml to less than about 300 pg/ml, or from about 5 pg/ml to less than about 350 pg/ml.

[0020] In still other embodiments, the methods of the invention reduce the detectable, *e.g.* by ELISA, concentration of one or more cytokines in serum of a subject following the administration of one or more doses of an anti-CD3 antibody to said subject by at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 325%, at least about 350%, at least about 375%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000% relative to the

concentration of said one or more cytokines in serum of the subject as a result of a similar administration of murine OKT3. In another embodiment, the method of the invention reduces the detectable, *e.g.* by ELISA, concentration of one or more cytokines in the serum of a subject following the administration of one or more doses of an anti-CD3 antibody to said subject by at least about 1 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 12 fold, at least about 14 fold, at least about 16 fold, at least about 18 fold, at least about 20 fold, at least about 25 fold, at least about 30 fold, at least about 35 fold, at least about 40 fold, at least about 45 fold, at least about 50 fold, at least about 75 fold or at least about 100 fold relative to the concentration of said one or more cytokines in the serum of a subject as a result of a similar administration of murine OKT3.

[0021] With respect to treatment of Type 1 diabetes, the anti-CD3 antibody is administered to achieve, or maintain a level of glycosylated hemoglobin (HA1 or HA1c) less than 8%, less than 7.5%, less than 7%, less than 6.5% , less than 6%, less than 5.5% or 5% or less. At the initiation of treatment, patients have a HA1 or HA1c level of less than 8%, less than 7.5%, less than 7%, less than 6.5%, less than 6%, or, more preferably, from 4%-6% (preferably measured in the absence of other treatment for diabetes, such as administration of exogenous insulin). Such patients preferably have retained at least 95%, 90%, 80%, 70%, 60%, 50%, 40% 30% or 20% of beta-cell function prior to initiation of treatment. The administration of the anti-CD3 antibodies prevents damage, thereby slowing onset or progression of the disease and reducing the need for insulin administration. In addition, the invention provides methods of treatment such that a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody (preferably, without any intervening treatment with anti-CD3 antibodies), results in a level of HA1 or HA1c is 7% or less, 6.5% or less, 6% or less, 5.5% or less, or 5% or less 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment. Specifically, in such methods of the invention a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody (preferably, without any intervening treatment with anti-CD3 antibodies), decreases the average level of HA1 or HA1c in the patient by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65% or about 70% as compared to pre-treatment levels at 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment. In addition, after treatment with a CD3 antibody according to the invention in a single dose or repeated every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24

months (preferably, without any intervening treatment with anti-CD3 antibodies), the average level of HA1 or HA1c in the patient only increases by about 0.5%, about 1%, about 2.5%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50% as compared to pre-treatment levels at 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment. In other embodiments, after a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody according to the methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), the average level of HA1 or HA1c in the patient is greater than about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70% or greater than about 100% less than the levels in a patient that initiated conventional treatment with similar clinical parameters and was administered conventional treatment after the same amount of time, which levels were determined at 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment.

[0022] In another embodiment, the anti-CD3 antibody is administered to achieve, or maintain the C-peptide response in a subject as determined by a mixed-meal tolerance test (MMTT), oral glucose tolerance test (OGTT), intravenous tolerance test (IGTT) or two phase glucose clamp procedure. Preferably, at the initiation of treatment, patients have residual β cell function as determined by the C-peptide response to MMTT, OGTT, IGTT, or two phase glucose clamp procedure as is known to one skilled in the art. In preferred embodiments, the patients have a C-peptide response to MMTT, OGTT, IGTT or two phase glucose clamp procedure (preferably MMTT) resulting in an area under curve (AUC) of at least 80 pmol/ml/240 min., preferably, at least 90 pmol/ml/240 min., more preferably at least 100 pmol/ml/240 min., or even at least 110 pmol/ml/240 min. In addition, the invention provides methods of treatment such that after a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody (preferably, without any intervening treatment with anti-CD3 antibodies), the level of C-peptide response in the patient is at least 99%, at least 98%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65% or at least 60% of the pre-treatment response as determined at 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment. Specifically, in such methods of the invention, after a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody according to methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), the average level of C-peptide response to a MMTT, OGTT, IGTT, or two-phase glucose clamp

procedure in the patient decreases by less than 1%, less than 5%, less than 10%, less than 20%, less than 30%, less than 40%, less than 50% of the pre-treatment levels as determined at 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment. In addition, after a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody according to methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), the average level of C-peptide response to a MMTT, OGTT, IGTT or two-phase glucose clamp procedure in the patient is at least 10%, 20%, 30%, 40%, 50%, 70% or 100% greater than the levels in a patient who initiated conventional diabetes therapy with similar clinical parameters and was administered conventional diabetes therapy over the 6 month, 9 month, 12 month, 15 month or 18 month period, said peptide response being determined at 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment

[0023] In specific embodiments, after a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody according to the methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), the patients have a C-peptide response to MMTT, OGTT, IGTT or two-phase glucose clamp procedure (preferably, MMTT) resulting in an AUC of at least 40 pmol/ml/240 min., 50 pmol/ml/240 min, 60 pmol/ml/240 min, 70 pmol/ml/240 min., 80 pmol/ml/240 min., preferably, at least 90 pmol/ml/240 min., more preferably at least 100 pmol/ml/240 min., or even at least 110 pmol/ml/240 min, said response determined 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment.

[0024] The determination of C-peptide response is a measure of β-cell function as is known to one skilled in the art. In other embodiments, β-cell function or residual β-cell function is determined by First-Phase Insulin Release (FPIR). In preferred embodiments, the patients greater than 8 years of age and prior to administration of an anti-CD3 antibody of the invention have a FPIR of at least 300 pmol/l, at least 350 pmol/l ,at least 400 pmol/l, at least 450 pmol/l, at least 500 pmol/l, preferably, at least 550 pmol/l, more preferably at least 600 pmol/l, or even at least 700 pmol/l. In preferred embodiments for patients less than 8 years of age and prior to administration of an anti-CD3 antibody, the FPIR is at least 50 pmol/l, at least 100 pmol/l, at least 200 pmol/l, at least 300 pmol/l, at least 350 pmol/l, preferably at least 400 pmol/l, at least 500 pmol/l, preferably, at least 550 pmol/l, more preferably at least 600 pmol/l, or even at least 700 pmol/l. In addition, the invention provides methods of treatment such that after a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months,

every 18 months, or every 24 months with an anti-CD3 antibody according to the methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), the FPIR is at least 99%, at least 98%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65% or at least 60% of the pre-treatment response, said FPIR determined 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment. Specifically, in such methods of the invention, after a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody according to the methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), the average FPIR in the patient decreases by less than 1%, less than 5%, less than 10%, less than 20%, less than 30%, less than 40%, less than 50% of the pre-treatment levels, said FPIR determined 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment. In addition, after a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody according to the methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), the average FPIR in the patient is at least 10%, 20%, 30%, 40%, 50%, 70% or 100% greater than the levels in a patient who initiated conventional diabetes therapy with similar clinical parameters and was administered conventional diabetes therapy over the 6 month, 9 month, 12 month, 15 month or 18 month period, said FPIR determined 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment. In specific embodiments, after a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody according to the methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), the patients have a FPIR of at least 300 pmol/l, at least 400 pmol/l, preferably, at least 500 pmol/l, more preferably at least 600 pmol/l, or even at least 700 pmol/l (for patients greater than 8 years of age) or at least 50 pmol/l, at least 100 pmol/l, at least 200 pmol/l, more preferably at least 350 pmol/l, or even at least 400 pmol/l (for patients under 8 years of age), said FPIR determined at 6 months, 9 months, 12 months, 15 months, 18 months, or 24 months after the previous treatment.

[0025] In other specific embodiments of the invention with respect to the treatment of type 1 diabetes, at the initiation of treatment the subject does not require administration of insulin or requires less than 30 U/kg/day, less than 20 U/kg/day, less than 10 U/kg/day, less than 5 U/kg/day, less than 1 U/kg/day, preferably less than 0.5 u/kg/day, even more preferably less than 0.25 U/kg/day. In other embodiments, a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with

an anti-CD3 antibody according to the methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), results in either a decrease (for example, of 10%, 20%, 30%, 40%, or 50%) in the amount of insulin required on average per day, or no change in the average amount of insulin required per day, or an increase of less than 1%, less than 5%, less than 10%, less than 20% or less than 30% of insulin administered, on average, per day as compared to the pre-treatment average dose of insulin per day. In certain embodiments, a single treatment or treatment every 6 months, every 9 months, every 12 months, every 15 months, every 18 months, or every 24 months with an anti-CD3 antibody according to the methods of the invention (preferably, without any intervening treatment with anti-CD3 antibodies), results in an average daily dose of insulin that is 10%, 20%, 50%, 75%, 90%, 99% less than the average daily dose of insulin required for a patient similarly situated (i.e., similar chemical parameters at the beginning of the month or year period) that had not received the anti-CD3 antibody treatment.

[0026] In other embodiments, the methods of the invention result in a reduction in hypoglycemic episodes by 1, 2, 3, 4, 5, 6 or more episodes in a one-day, two-day, 5-day, 10-day or 15-day period as compared to similarly situated patients not having been administered the anti-CD3 antibody according to the invention.

[0027] In preferred embodiments, the patient is under 21 years of age, 18 years of age, under 15 years of age, under 12 years of age, under 9 years of age, or under 5 years of age or from infancy to 3 years of age, from 2 to 5 years of age, from 5 to 9 years of age, from 9 to 12 years of age, from 12 to 20 years of age. In other embodiments, the patient is an adult.

[0028] In specific embodiments, the subject has received transplanted islet cells and is administered a prophylactically or therapeutically effective amount of the anti-CD3 antibody according to the methods of the invention. In a specific embodiment, the subject having received transplanted islet cells is an adult. In other specific embodiments, the subject having received the transplanted islet cells is younger than 21 years of age or is younger than 18 years of age.

[0029] In some embodiments of the invention, subjects suffering from an autoimmune disease, preferably in early stages of the disease, are administered anti-CD3 antibodies to reduce or slow progression of the disease and to reduce or prevent an increase in the level of therapy, for example, administration of an immunosuppressant or anti-inflammatory, required to manage the disease.

[0030] The invention also provides combination therapy methods. The methods of the invention can be carried out in combination with any standard treatment for the particular indication, such as standard immunosuppressant and/or anti-inflammatory treatments

administered for the treatment or amelioration of autoimmune diseases. For example, with respect to Type 1 diabetes, the anti-CD3 antibody therapy of the invention may be administered along with other therapies for diabetes, such as, but not limited to, administration of insulin, GLP-1 analogs, GLP-1 agonists (*e.g.*, exenatide), pramlintide or a combination thereof. The CD3 antibodies of the invention may further be administered with other therapies such as anti-inflammatory agents, steroidal therapies (for example, but not limited to, glucocorticoids, dexamethasone, cortisone, hydrocortisone, prednisone, prednisolone, triamcinolone, azulfidine, etc.), non-steroidal anti-inflammatories (NSAIDS) (for example, but not limited to, COX-2 inhibitors, aspirin, ibuprofen, diclofenac, etodolac, fenoprofen, indomethacin, ketolorac, oxaprozin, nabumetone, sulindac, tolmentin, naproxen, ketoprofen, etc.), beta-agonists, anti-cholinergic agents, immunomodulatory agents (for example, but not limited to, T cell receptor modulators, cytokine receptor modulators, T cell depleting agents, cytokine antagonists, monokine agonists, lymphokine inhibitors, etc.), immunosuppressants (such as, but not limited to, methotrexate or cyclosporin), anti-angiogenic agents (*e.g.*, angiostatin and TNF-α antagonists and/or inhibitors (for example, but not limited to, etanercept and infliximab)), dapsone and psoralens. In certain embodiments of the invention, subjects which have become refractory to conventional treatments are treated using methods of the invention.

[0031] According to the invention, the anti-CD3 antibody is administered so as to reduce adverse effects, such as the cytokine release associated with antibody administration, EBV activation (as evidenced by EBV-induced lymphoproliferative diseases, *e.g.*, mononucleosis) or lymphopenia (defined as<1000 lymphocytes/μL serum), with administration of anti-CD3 antibodies, and also reduce the number and duration of the administration. In particular embodiments, the invention provides a treatment regimen of administration of a dose of the anti-CD3 antibody for 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days or 14 days. In preferred embodiments, the administration is carried out on consecutive days, *e.g.*, administration occurs on at least 4 consecutive days, at least 5 consecutive days, at least 6 consecutive days, at least 7 consecutive days, at least 8 consecutive days, at least 9 consecutive days, at least 10 consecutive days, at least 11 consecutive days, or at least 12 consecutive days. In other embodiments, the administration of a dose of the anti-CD3 antibody of the invention occurs on no more than 2 consecutive days, no more than 3 consecutive days, no more than 4 consecutive days, no more than 5 consecutive days, no more than 6 consecutive days, no more than 7 consecutive days, no more than 8 consecutive days, no more than 9 consecutive days, no more than 10 consecutive days, no more than 11 consecutive days, or on no more than 12 consecutive days. In yet other embodiments, the dosage regimen can be 2 days or less, 3 days or less, 4 days or less, 5 days or less, 6 days or less, 7 days or less,

8 days or less, 9 days or less, 10 days or less, 11 days or less, 12 days or less, 13 days or less, or 14 days or less. In certain, embodiments, the dose administered is the same each day of the regimen. However, in preferred embodiments the dose administered escalates over the first few days, *e.g.*, over at least the first three days, of the regimen to reduce or eliminate the incidence of cytokine release syndrome.

[0032] In specific embodiments, the dose administered is 5-50 μg/kg/day, preferably, 20-30 μg/kg/day, more preferably, approximately 22-28 μg/kg/day, or approximately 25-26 μg/kg/day. In other specific embodiments, the dose on day 1 of the regimen is 1-3 μg/kg/day, preferably 1.6 μg/kg/day and escalates to the daily dose as recited immediately above by day 3, 4, 5, 6 or 7. For example, on day 1, the subject is administered a dose of approximately 1.6 μg/kg/day, on day 2 approximately 3.2 μg/kg/day, on day 3 approximately 6.5 μg/kg/day, on day 4 approximately 13 μg/kg/day and on subsequent days of the regimen 26 μg/kg/day. In another embodiment, on day 1, the subject is administered a dose of approximately 1.42 μg/kg/day, on day 2 approximately 5.7 μg/kg/day, on day 3 approximately 11 μg/kg/day, on day 4 approximately 22.6 μg/kg/day and on subsequent days of the regimen 45.4 μg/kg/day.

[0033] In other specific embodiments, the dose administered is based on surface area. For example the dose administered is 177-1412 $\mu g/m^2$/day, preferably, 706-1059 $\mu g/m^2$/day, more preferably, approximately 781-990 $\mu g/m^2$/day, or approximately 884-916 $\mu g/m^2$/day. In other embodiments, the dose on day 1 of the regimen is 35-106 $\mu g/m^2$/day, preferably 57 $\mu g/m^2$/day and escalates to the daily dose as recited immediately above by day 3, 4, 5, 6 or 7. For example, on day 1, the subject is administered a dose of approximately 57 $\mu g/m^2$/day, on day 2 approximately 115 $\mu g/m^2$/day, on day 3 approximately 230 $\mu g/m^2$/day, on day 4 approximately 460 $\mu g/m^2$/day and on subsequent days of the regimen 919 $\mu g/m^2$/day. In another embodiment, on day 1, the subject is administered a dose of approximately 455 $\mu g/m^2$/day, on day 2 approximately 919 $\mu g/m^2$/day, on day 3 and subsequent days, approximately 1818 $\mu g/m^2$/day. In another embodiment, on day 1, the subject is administered a dose of approximately 227 $\mu g/m^2$/day, on day 2 approximately 459 $\mu g/m^2$/day, on day 3 and subsequent days, approximately 919 $\mu g/m^2$/day. In another embodiment, on day 1, the subject is administered a dose of approximately 284 $\mu g/m^2$/day, on day 2 approximately 574 $\mu g/m^2$/day, on day 3 and subsequent days, approximately 1148 $\mu g/m^2$/day.

[0034] In specific embodiments, to reduce the possibility of cytokine release and other adverse effects, the initial the first 1, 2, 3, or 4 doses are administered more slowly by IV. For example, a dose 13 mg/kg/day may be administered over about 5 minutes, about 15 minutes, about 30 minutes, about 45 minutes, about 1 hour, about 2 hours, about 4 hours, or about 6 hours.

[0035] In a more specific embodiment, the initial dose is ¼ to ½ to equal to the daily dose at the end of the regimen but is administered in portions at intervals of 6, 8, 10 or 12 hours. For example, a 13 ug/kg/day dose is administered in four doses of 3-4 μg/kg at intervals of 6 hours to reduce the level of cytokine release caused by administration of the antibody. In certain embodiments, the treatment dose is delivered in equal portions at 6, 8, 10 or 12 hour intervals. In other embodiments, the portions of the treatment dose increase at each subsequent interval such that a dose escalation occurs at 6, 8 10, or 12 hour intervals.

[0036] In preferred embodiments, the anti-CD3 antibodies are administered parenterally, for example, intravenously, intramuscularly or subcutaneously, or, alternatively, are administered orally. The anti-CD3 antibodies may also be administered as a sustained release formulation.

[0037] Additionally, in certain embodiments, the invention provides methods and regimens of administering anti-CD3 antibodies that reduce the severity and/or incidence of adverse effects such as, but not limited to, cytokine release, apoptosis, activation of EBV, immune reaction against the anti-CD3 antibody, lymphopenia, anemia, neutropenia, thrombocytopenia or secondary infection. In other embodiments, the invention provides methods and regimens of administering anti-CD3 antibodies that reduce the severity and/or incidence of adverse effects such as, but not limited to, cytokine release, apoptosis, activation of EBV, immune reaction against the anti-CD3 antibody, lymphopenia, anemia, neutropenia, thrombocytopenia or secondary infection relative to the side effects associated with a similar regimen of administering to a subject with similar clinical parameters murine OKT3 antibody..

[0038] The invention, in other embodiments, provides methods of producing anti-CD3 antibodies, particularly OKT3 derived antibodies, such as, but not limited to, humanized OKTγ1 (ala-ala), in CHO cells. In particular embodiments, the invention provides methods of producing anti-CD3 antibodies comprising (a) culturing CHO cells that have been transfected with the expression vector pMGX1303, or progeny thereof, in media under conditions suitable for expression of said anti-CD3 antibody; and (b) recovering said anti-CD3 antibody from said media.

3.1 TERMINOLOGY

[0039] As used herein, the term "about" or "approximately," when used in conjunction with a number, refers to any number within 1, 5 or 10% of the referenced number.

[0040] As used herein, the term "analog" in the context of polypeptides refers to a polypeptide that possesses a similar or identical function as a second polypeptide but does not necessarily comprise a similar or identical amino acid sequence of the second polypeptide, or possess a similar or identical structure of the second polypeptide. A polypeptide that has a

similar amino acid sequence refers to a second polypeptide that satisfies at least one of the following: (a) a polypeptide having an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a second polypeptide; (b) a polypeptide encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding a second polypeptide of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, or at least 150 contiguous amino acid residues; and (c) a polypeptide encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence encoding a second polypeptide. A polypeptide with similar structure to a second polypeptide refers to a polypeptide that has a similar secondary, tertiary or quaternary structure to the second polypeptide. The structure of a polypeptide can be determined by methods known to those skilled in the art, including but not limited to, peptide sequencing, X-ray crystallography, nuclear magnetic resonance, circular dichroism, and crystallographic electron microscopy.

[0041] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

[0042] The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin

and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873-5877, each of which is hereby incorporated by reference herein in its entirety. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score-50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, the NCBI website). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17, which is hereby incorporated by reference herein in its entirety. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[0043] The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

[0044] As used herein, the term "analog" in the context of a non-proteinaceous analog refers to a second organic or inorganic molecule which possess a similar or identical function as a first organic or inorganic molecule and is structurally similar to the first organic or inorganic molecule.

[0045] As used herein, the terms "antagonist" and "antagonists" refer to any protein, polypeptide, peptide, antibody, antibody fragment, large molecule, or small molecule (less than 10 kD) that blocks, inhibits, reduces or neutralizes the function, activity and/or expression of another molecule. In various embodiments, an antagonist reduces the function, activity and/or expression of another molecule by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% relative to a control such as phosphate buffered saline (PBS).

[0046] As used herein, the terms "antibody" and "antibodies" refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass.

[0047] As used herein, the term "C-peptide" refers to a 31-amino acid peptide cleaved from proinsulin as it is converted to insulin. Proinsulin consists of an A chain, a connecting peptide (C-peptide), and a B chain. After proinsulin is cleaved, C-peptide remains in the secretory granule of beta cells in the pancreas with insulin and is cosecreted with insulin in response to glucose stimulation. C-peptide is thus released from the pancreas in equi-molar amounts with insulin and may be used as a marker of endogenous insulin production.

[0048] As used herein, the term "derivative" in the context of polypeptides refers to a polypeptide that comprises an amino acid sequence which has been altered by the introduction of amino acid residue substitutions, deletions or additions. The term "derivative" as used herein also refers to a polypeptide which has been modified, *i.e*, by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, an antibody may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative polypeptide may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative polypeptide may contain one or more non-classical amino acids. A polypeptide derivative possesses a similar or identical function as the polypeptide from which it was derived.

[0049] As used herein, the terms "disorder" and "disease" are used interchangeably to refer to a condition in a subject. In particular, the term "autoimmune disease" is used interchangeably with the term "autoimmune disorder" to refer to a condition in a subject characterized by cellular, tissue and/or organ injury caused by an immunologic reaction of the subject to its own cells, tissues and/or organs.

[0050] As used herein, the term "epitopes" refers to fragments of a polypeptide or protein having antigenic or immunogenic activity in an animal, preferably in a mammal, and

most preferably in a human. An epitope having immunogenic activity is a fragment of a polypeptide or protein that elicits an antibody response in an animal. An epitope having antigenic activity is a fragment of a polypeptide or protein to which an antibody immunospecifically binds as determined by any method well-known to one of skill in the art, for example by immunoassays. Antigenic epitopes need not necessarily be immunogenic.

[0051] As used herein, the term "Fc region" is used to define a C-terminal region of an IgG heavy chain. Although the boundaries may vary slightly, the human IgG heavy chain Fc region is defined to stretch from Cys226 to the carboxy terminus. The Fc region of an IgG comprises two constant domains, CH2 and CH3. The CH2 domain of a human IgG Fc region (also referred to as "Cγ2" domain) usually extends from amino acids 231 to amino acid 341. The CH3 domain of a human IgG Fc region usually extends from amino acids 342 to 447. 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.

[0001] Throughout the present specification, the numbering of the residues in an IgG heavy chain is that of the EU index as in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, NH1, MD (1991), expressly incorporated herein by references. The "EU index as in Kabat" refers to the numbering of the human IgG1 EU antibody.

[0052] The "hinge region" is generally defined as stretching from Glu216 to Pro230 of human IgG1. Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S binds in the same positions.

[0053] As used herein the term "early stage" with respect to an autoimmune disorder or inflammatory disorder refers to clinical states meeting at least the minimum criteria for positive diagnosis of said disorder as defined herein or known in the art. Additionally, early stages autoimmune disorders or inflammatory disorders typically exhibit minimal or mild tissue damage resulting from the autoimmune reaction and require only minimal medical intervention, e.g., low doses of standard therapy, to manage the disease.

[0054] As used herein, the term "fragment" refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least

contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of another polypeptide. In a specific embodiment, a fragment of a polypeptide retains at least one function of the polypeptide.

[0055] As used herein, the term "functional fragment" refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of second, different polypeptide, wherein said peptide or polypeptide retains at least one function of the second, different polypeptide.

[0056] As used herein, the term "fusion protein" refers to a polypeptide that comprises an amino acid sequence of a first protein or functional fragment, analog or derivative thereof, and an amino acid sequence of a heterologous protein (*i.e.*, a second protein or functional fragment, analog or derivative thereof different than the first protein or functional fragment, analog or derivative thereof). In particular embodiments, a fusion protein comprises a CD3 binding molecule and a heterologous protein, polypeptide, or peptide.

[0057] As used herein, the term "host cell" refers to the particular subject cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0058] As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing under which nucleotide sequences at least 60% (65%, 70%, preferably 75%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, which is hereby incorporated by reference herein in its entirety. In one, non-limiting example stringent

hybridization conditions are hybridization at 6X sodium chloride/sodium citrate (SSC) at about 45o C, followed by one or more washes in 0.1 X SSC, 0.2% SDS at about 68o C. In a preferred, non-limiting example stringent hybridization conditions are hybridization in 6 X SSC at about 45o C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65o C (*i.e.*, one or more washes at 50o C, 55o C, 60o C or 65o C). It is understood that the nucleic acids of the invention do not include nucleic acid molecules that hybridize under these conditions solely to a nucleotide sequence consisting of only A or T nucleotides.

[0059] As used herein, the term "hypoglycemic episode" refers to a blood glucose level in a subject of less than 60 mg/dL that results in typical symptoms of hypoglycemia such as sweatiness, nausea, blurred vision (e.g., seeing spots), shakiness, numb lips and/or tongue, irritability, fainting, clammy skin, confusion, nervousness, weakness, and/or rapid heart beat.

[0060] As used herein, the term "immunomodulatory agent" and variations thereof refer to an agent that modulates a host's immune system. In certain embodiments, an immunomodulatory agent is an immunosuppressant agent. In certain other embodiments, an immunomodulatory agent is an immunostimulatory agent. Immunomodulatory agents include, but are not limited to, small molecules, peptides, polypeptides, fusion proteins, antibodies, inorganic molecules, mimetic agents, and organic molecules.

[0061] As used herein, the term "immunospecifically binds to an antigen" and analogous terms refer to peptides, polypeptides, fusion proteins and antibodies or fragments thereof that specifically bind to an antigen or a fragment and do not specifically bind to other antigens. A peptide or polypeptide that immunospecifically binds to an antigen may bind to other peptides or polypeptides with lower affinity as determined by, *e.g.*, immunoassays, BIAcore, or other assays known in the art. Antibodies or fragments that immunospecifically bind to an antigen may cross-reactive with related antigens. Preferably, antibodies or fragments that immunospecifically bind to an antigen do not cross-react with other antigens.

[0062] As used herein, the term "immunospecifically binds to a CD3 polypeptide" and analogous terms refer to peptides, polypeptides, fusion proteins and antibodies or fragments thereof that specifically bind to a CD3 polypeptide or a fragment thereof and do not specifically bind to other polypeptides. A peptide or polypeptide that immunospecifically binds to a CD3 polypeptide may bind to other peptides or polypeptides with lower affinity as determined by, *e.g.*, immunoassays, BIAcore, or other assays known in the art. Antibodies or fragments that immunospecifically bind to a CD3 polypeptide may be cross-reactive with related antigens. Preferably, antibodies or fragments that immunospecifically bind to a CD3 polypeptide or fragment thereof do not cross-react with other antigens. Antibodies or fragments that immunospecifically bind to a CD3 polypeptide can be identified, for example, by

immunoassays, BIAcore, or other techniques known to those of skill in the art. An antibody or fragment thereof binds specifically to a CD3 polypeptide when it binds to a CD3 polypeptide with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISAs). See, e.g., Paul, ed., 1989, Fundamental Immunology Second Edition, Raven Press, New York at pages 332-336 (which is hereby incorporated by reference herein in its entirety) for a discussion regarding antibody specificity.

[0063] As used herein, the term "in combination" refers to the use of more than one prophylactic and/or therapeutic agents. The use of the term "in combination" does not restrict the order in which prophylactic and/or therapeutic agents are administered to a subject with a disorder. A first prophylactic or therapeutic agent can be administered prior to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second prophylactic or therapeutic agent to a subject with a disorder.

[0064] As used herein, the term "isolated" in the context of a peptide, polypeptide, fusion protein or antibody refers to a peptide, polypeptide, fusion protein or antibody which is substantially free of cellular material or contaminating proteins from the cell or tissue source from which it is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a peptide, polypeptide, fusion protein or antibody in which the peptide, polypeptide, fusion protein or antibody is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a peptide, polypeptide, fusion protein or antibody that is substantially free of cellular material includes preparations of a peptide, polypeptide, fusion protein or antibody having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the peptide, polypeptide, fusion protein or antibody is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the peptide, polypeptide, fusion protein or antibody is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the peptide, polypeptide, fusion protein or antibody. Accordingly such preparations of a peptide, polypeptide, fusion protein or antibody have less

than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the peptide, polypeptide, fusion protein or antibody of interest. In a preferred embodiment, a CD3 binding molecule is isolated. In another preferred embodiment, an anti-CD3 antibody is isolated.

[0065] As used herein, the term "isolated" in the context of nucleic acid molecules refers to a nucleic acid molecule which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a preferred embodiment, a nucleic acid molecule encoding a CD3 binding molecule is isolated. In another preferred embodiment, a nucleic acid molecule encoding an anti-CD3 antibody is isolated.

[0066] As used herein, the term "minimal toxicity" with respect to a method of the invention (*e.g.* anti-CD3 antibody therapy) refers to a state in which the patient may suffer from one or more side effects associated with said method (*e.g.*, first dose effects, cytokine release syndrome), however the patient derives benefit from and/or will continue (or is advised to continue) therapy because the benefits of the therapy outweigh the adverse effects and/or the harm from said one or more side effects.

[0067] As used herein, the terms "non-responsive" and refractory" describe patients treated with a currently available prophylactic or therapeutic agent for an autoimmune disorder which is not clinically adequate to relieve one or more symptoms associated with the autoimmune disorder. Typically, such patients suffer from severe, persistently active disease and require additional therapy to ameliorate the symptoms associated with their autoimmune disorder.

[0068] As used herein, the terms "nucleic acids" and "nucleotide sequences" include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), combinations of DNA and RNA molecules or hybrid DNA/RNA molecules, and analogs of DNA or RNA molecules. Such analogs can be generated using, for example, nucleotide analogs, which include, but are not limited to, inosine or tritylated bases. Such analogs can also comprise DNA or RNA molecules comprising modified backbones that lend beneficial attributes to the molecules such as, for example, nuclease resistance or an increased ability to cross cellular membranes. The nucleic acids or nucleotide sequences can be single-stranded, double-stranded, may contain both single-stranded and double-stranded portions, and may contain triple-stranded portions, but preferably is double-stranded DNA.

[0069] As used herein, the terms "prophylactic agent" and "prophylactic agents" refer to CD3 binding molecules which can be used in the prevention, treatment, management or amelioration of one or more symptoms of an autoimmune disease. In certain embodiments, the term "prophylactic agent" refers to anti-CD3 antibodies (*e.g.*, OKT3 and variants and derivatives thereof).

[0070] As used herein, the term "prophylactically effective amount" refers to that amount of a CD3 binding molecule sufficient to prevent the development, recurrence or onset of one or more symptoms of a disorder. In certain embodiments, the term "prophylactically effective amount" refers to the amount of an anti-CD3 antibody sufficient to prevent the development, recurrence or onset of one or more symptoms of a disorder.

[0071] As used herein, the terms "prevent", " preventing" and prevention refer to the prevention of the recurrence or onset of one or more symptoms of an autoimmune or inflammatory disorder in a subject resulting from the administration of a prophylactic or therapeutic agent.

[0072] A used herein, a "protocol" includes dosing schedules and dosing regimens. The protocols herein are methods of use and include prophylactic and therapeutic protocols.

[0073] As used herein, the phrase "side effects" encompasses unwanted and adverse effects of a therapy (*e.g.*, a prophylactic or therapeutic agent). Adverse effects are always unwanted, but unwanted effects are not necessarily adverse. An adverse effect from a therapy (*e.g.*, a prophylactic or therapeutic agent) might be harmful or uncomfortable or risky. Examples of side effects associated with the administration of anti-CD3 antibodies include first dose side effects and/ or cytokine release syndrome, both of which may manifest as, *e.g.*, flu-like symptoms, respiratory distress, neurological symptoms, acute tubular necrosis, apoptosis, activation of EBV, lymphopenia, anemia, neutropenia, thrombocytopenia or secondary infection, high fever, chills/rigors, headache, tremor, nausea/vomiting, diarrhea, abdominal pain, malaise, muscle/joint aches and pains, and generalized weakness. headache, nausea, vomiting, fever, myalgias, arthralgias, shaking and increased serum levels of, *e.g.*, IL-2, IL-6, IL-10, TNFα, IFNγ, and lymphokines, as is known in the art. Additional undesired effects experienced by patients are numerous and known in the art.

[0074] As used herein, the terms "subject" and "patient" are used interchangeably. As used herein, the terms "subject" and "subjects" refer to an animal, preferably a mammal including a non-primate (*e.g.*, a cow, pig, horse, cat, dog, rat, and mouse) and a primate (*e.g.*, a monkey or a human), and more preferably a human.

[0075] As used herein, the term "synergistic" refers to a combination of prophylactic or therapeutic agents which is more effective than the additive effects of any two or more single

agents. A synergistic effect of a combination of prophylactic or therapeutic agents permits the use of lower dosages of one or more of the agents and/or less frequent administration of said agents to a subject with an autoimmune disorder. The ability to utilize lower dosages of prophylactic or therapeutic agents and/or to administer said agents less frequently reduces the toxicity associated with the administration of said agents to a subjected without reducing the efficacy of said agents in the prevention or treatment of autoimmune disorders. In addition, a synergistic effect can result in improved efficacy of agents in the prevention or treatment of autoimmune disorders. Finally, synergistic effect of a combination of prophylactic or therapeutic agents may avoid or reduce adverse or unwanted side effects associated with the use of combination therapy.

[0076] As used herein, the terms "therapeutic agent" and "therapeutic agents" refer to CD3 binding molecules which can be used in the prevention, treatment, management or amelioration of one or more symptoms of an autoimmune or inflammatory disease. In certain embodiments, the term "therapeutic agent" refers to anti-CD3 antibodies (*e.g.*, OKT3 and variants or derivatives thereof).

[0077] As used herein, the term "therapeutically effective amount" refers to that amount of a therapeutic agent sufficient to result in amelioration of one or more symptoms of a disorder. With respect to diabetes, a therapeutically effective amount preferably refers to the amount of therapeutic agent that reduces a subject's average daily insulin requirements by at least 20%, by at least 25%, by at least 30%, by at least 35%, by at least 40%, by at least 45%, by at least 50%, by at least 55%, by at least 60%, by at least 65%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%. With respect to the treatment of psoriasis, a therapeutically effective amount preferably refers to the amount of a therapeutic agent that reduces a human's Psoriasis Area and Severity Index (PASI) score by at least 20%, at least 35%, at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 85%. Alternatively, with respect to the treatment of psoriasis, a therapeutically effective amount preferably refers to the amount of a therapeutic agent that improves a human's global assessment score by at least 25%, at least 35%, at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%.

[0078] As used herein, the terms "treat", "treatment" and "treating" refer to the amelioration of one or more symptoms associated with an autoimmune or inflammatory disorder that results from the administration of one or more CD3 binding molecules. In particular, such terms refer to the amelioration of one or more symptoms associated with an autoimmune disorders that results from the administration of one or more anti-CD3 antibodies. In certain

embodiments, such terms refer to a reduction in the swelling of one or more joints, or a reduction in the pain associated with an autoimmune or inflammatory disorder resulting from the administration of one or more CD3 binding molecules, preferably one or more anti-CD3 antibodies, to a subject with such a disorder. In other embodiments, such terms refer to a reduction in a human's PASI score. In other embodiments, such terms refer to an improvement in a human's global assessment score. In other embodiments, such terms refer to a reduction in a human's average number of hypoglycemic episodes. In other embodiments, such terms refer to the maintenance of a reference level of C-peptide in the peripheral blood.

4. DESCRIPTION OF THE FIGURES

[0079] FIGS. 1A - 1B. Sequences of humanized OKT3 variable regions. Figure 1A and Figure 1B show the alignment of the amino acid sequences of OKT3 light chain variable domain (FIG. 1A) (SEQ ID NO:1) and heavy chain variable domain (FIG. 1B) (SEQ ID NO:5); (row 1 in each of FIG. 1A and FIG. 1B). Also shown in row 2 of FIG. 1A and FIG. 1B are the amino acid sequences of the variable domains from the human antibodies chosen as acceptor framework, REI (SEQ ID NO:2) for the light chain variable domain (FIG. 1A) and KOL (SEQ ID NO:6) of the heavy chain variable domain. Rows 3-4 of FIG. 1A (SEQ ID NO:3 and SEQ ID NO:4) and rows 3-5 of FIG. 1B (SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9) present the amino acid sequences of alternate humanized OKT3 light chain and heavy chain variable domains, respectively. The CDR choices are singly underlined. Rows 3-4 FIG. 1A and rows 3-5 FIG. 1B show only differences from the human acceptor sequence, with the non-CDR differences shown double underlined. Dashes indicate gaps introduced in the sequences to maximize the alignment. Numbering is as Kabat et al., (1987).

[0080] FIGS. 2A – 2D. Figure 2A and Figure 2B, nucleotide and amino acid sequence, respectively of the light chain of a humanized OKT3γ1 (ala-ala). Figure 2C and Figure 2D, nucleotide and amino acid sequence, respectively, of the heavy chain of a humanized OKT3.

[0081] FIG. 3. Schematic representation of mammalian expression vector pMGX1303, containing coding regions for humanized OKT3γ1 and capable of promoting expression of the humanized antibody in CHO cells.

5. DETAILED DESCRIPTION OF THE INVENTION

[0082] The present invention provides for monoclonal antibodies directed against the CD3 complex associated with the human TcR and methods of administration and/or use thereof for the treatment of autoimmune disorders. In particular embodiments, the antibody binds to the epsilon subunit of the CD3 complex. The methods of the invention may be used with any anti-

CD3 antibody presented herein or known in the art, *e.g.* OKT3, ChAglyCD3 (TRX4™), HUM291 (visilizumab; Nuvion™), UCHT1, Leu4, 500A2, CLB-T3/3, BMA030, BH11, T2/30, AG3, BC3, SP34, RIV-9, UCHT1, 64.1, M291 and YTH 12.5, and variations or derivatives thereof. In one embodiment of the invention the antibody is OKT3, preferably humanized versions of OKT3 or an antibody that competes for binding with OKT3. In another embodiment, the antibody is humanized OKT3, which has been modified at one or more amino acid residues as described herein to exhibit reduced T cell activation and/or FcR binding when compared to a non-modified humanized OKT3 antibody.

[0083] Anti-CD3 mAbs are potent immunosuppressive agents directed against an invariant protein complex associated with the human TCR (Van Wauwe, 1980, J. Immunol. 124:2708, which is hereby incorporated by reference herein in its entirety). The CD3 complexes are believed to be accessory structures that transduce the activation signals initiated upon binding of the TcR to its ligand. Binding of the anti-CD3 antibody OKT3 to the TcR mediates TcR blockade and inhibits alloantigen recognition and cell-mediated cytotoxicity (Landegren et al., 1982, J. Exp. Med. 155:1579; van Seventer et al., 1987, J. Immunol. 139:2545; Weiss et al., 1986, Ann. Rev. Immunol. 4:593, each of which is hereby incorporated by reference herein in its entirety). However, the administration of some immune-cell directed mAbs, including OKT3, may induce T cell activation, including the systematic release of several cytokines, including IL-2, IL-6, TNF-α and IFN-γ (Abramowicz, 1989; Chatenoud, 1989). This production of cytokines has been correlated with the adverse side-effects frequently observed after the first injection of mAbs (Van Wauwe, 1980; Chatenoud, 1989; Thistlethwaite, 1988), and may augment the production of anti-isotopic and anti-idiotypic antibodies occurring in some patents after one or two weeks of treatment. This immune response can neutralize the specific mAb, as well as other antibodies of the same class (isotype), and preclude subsequent treatments (Thistlethwaite, 1988).

[0084] Several pieces of evidence strongly suggest that these side-effects are a consequence of the cross-linking between T lymphocytes and Fc receptor (FcR)-bearing cells through the Fc portion of mAbs, including for example, OKT3, resulting in activation of both cell types (Debets, 1990, J. Immunol. 144:1304; Krutman, 1990, J. Immunol. 145:1337, each of which is hereby incorporated by reference herein in its entirety): 1) anti-CD3 mAbs did not stimulate T cell proliferation in vitro, unless the Ab was immobilized to plastic or bound to FcR+ antigen presenting cells included in the culture (van Lier, 1989, Immunol. 68:45, which is hereby incorporated by reference herein in its entirety); 2) the cross-linking of OKT3 through FcRs I and II enhanced proliferation in response to IL-2, in vitro (van Lier, 1987, J. Immunol. 139:2873, which is hereby incorporated by reference herein in its entirety); 3) proliferation of

murine T cells induced by 145-2C11, a hamster mAb directed against the murine CD3 complex, could be blocked by the anti-FcR Ab, 2.4G2; 4) the injection into mice of $F(ab')_2$ fragments of 145-2C11 induced significant immunosuppression without triggering full T cell activation (Hirsch, 1990) and was less toxic in mice than the whole mAb (Alegre, 1990); and 5) the administration of an OKT3 IgA switch variant that displayed a reduced FcR-mediated T cell activation as compared with OKT3 IgG2a, resulted in fewer side effects in chimpanzees in vivo (Parleviet, 1990).

[0085] Administration of anti-CD3 antibodies has also been associated with transient retrovirus activation, specifically activation of dormant Epstein-Barr Virus (EBV) infection. Anti-CD3 antibody treatment has also been found to be lytic to activated T cells and apoptotic to some T cell populations. The reasons for these effects are unclear, but they may be dose related and are probably the result of the modulation of the TcR complex resulting in suboptimal signaling.

[0086] Thus improvement of anti-CD3 mAb therapy can be obtained by molecularly modifying the antibody to reduce its affinity for FcRs. The mutated Ab obtained could lead to lower cellular activation and reduced toxicity in vivo, but retain the original immunosuppressive properties of the antibody.

5.1 Antibodies that Immunospecifically Bind to CD3 Polypeptides

[0087] It should be recognized that antibodies that immunospecifically bind to a CD3 polypeptide are known in the art. Examples of known antibodies that immunospecifically bind to a CD3 polypeptide include, but are not limited to OKT3, HuM291, M291, ChAglyCD3, UCHT1, Leu4, 500A2, CLB-T3/3, BMA030, YTH 12.5, SP34, RIV-9, UCHT1 (*See* Herold et al., 2005, Diabetes 54:1763-1769; Carpenter et al., 2005, Biol. Blood Marrow Transplant 11:465-471; Cole et al., 1999, Transplantation 68:563-571; Keymeulen et al., 2005, N. Engl. J. Med. 352:26422644; Schwinzer et al., 1992, J. Immunol. 148:1322-1328; Tsoukas et al., 1985, J. Immunol. 135:1719-1723; Brams et al., 1989, Immunol., 66:348-353; van Lier et al., 1989, Immunol. 68:45-50; Walker et al., 1987, Eur. J. Immunol. 17:1611-1618; Routledge et al., 1991, Eur. J. Immunol. 21:2717-2725, Yang et al., 1986, J. Immunol. 137:1097-1100; Vaessen et al., 1989, Transplant. Proc. 21:1026-1037; Callard et al., 1981, Clin. Exp. Immunol. 43:497-505; Tsoukas et al., 1984, Cell. Immunol. 89:66-74, respectively, each of which is hereby incorporated by reference herein in its entirety) and BH11, T2/30, AG3, and BC3 (*See* Takas et al., 1987, J. Immunol. 138:687-698, which is hereby incorporated by reference herein in its entirety).

[0088] The present invention provides antibodies that immunospecifically bind to a CD3 polypeptide expressed by an immune cell such as a T cell, wherein said antibodies modulate an

activity or function of said T cell. In a specific embodiment, antibodies that immunospecifically bind to a CD3 polypeptide directly or indirectly modulate the activity of lymphocytes, preferably peripheral blood T cells. In particular, the present invention provides antibodies that immunospecifically bind to a CD3 polypeptide expressed by a T cell, and said antibodies modulate the activity of peripheral blood T cell.

[0089] In a specific embodiment, antibodies that immunospecifically bind to a CD3 polypeptide inhibit T cell activation by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% and inhibit T cell proliferation by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% in an *in vivo* or *in vitro* assay described herein or well-known to one of skill in the art. In another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide inhibit alloantigen recognition by T cells by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% in an *in vivo* or *in vitro* assay described herein or well-known to one of skill in the art. In another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide inhibit T cell mediated cytotoxicity by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% in an *in vivo* or *in vitro* assay described herein or well-known to one of skill in the art.

[0090] In another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide do not induce or reduce cytokine expression and/or release in an *in vivo* or *in vitro* assay described herein or well-known to one of skill in the art. In still other embodiments, antibodies of the invention that immunospecifically bind to a CD3 polypeptide reduce cytokine expression and/or release relative to the cytokine expression and/or release associated with murine OKT3 in an *in vivo* or *in vitro* assay described herein or well-known to one of skill in the art. In a specific embodiment, antibodies that immunospecifically bind to a CD3 polypeptide do not induce an increase in the concentration cytokines such as, *e.g.*, IFN-γ, IL-2, IL-4, IL-6, IL-9, IL-12, and IL-15 in the serum of a subject administered such an antibody. In an alternative embodiment, antibodies that immunospecifically bind to a CD3 polypeptide induce cytokine expression and/or release in an *in vitro* or *in vivo* assay described herein or well-known to one of skill in the art. Serum concentrations of a cytokine can be measured by any technique well-known to one of skill in the art such as, *e.g.*, ELISA.

[0091] In certain embodiments, following administration of one or more doses of anti-CD3 antibody to a subject according to methods of the invention, cytokines (*e.g.*, IL-2, IL-6, IL-10, TNFα, IFNγ) are undetectable in the serum of said subject by ELISA or any other method known in the art for detection of serum proteins. In other embodiments, one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, TNFα, IFNγ) may be detected in the serum of a subject following administration of one or more doses of an anti-CD3 antibody to said subject according to methods of the invention at concentrations of less than about 250 pg/ml, less than about 225 pg/ml, less than about 200 pg/ml, less than about 175 pg/ml, less than about 150 pg/ml, less than about 125 pg/ml, less than about 100 pg/ml, less than about 90 pg/ml, less than about 80 pg/ml, less than about 70 pg/ml, less than about 60 pg/ml, less than about 50 pg/ml, less than about 45 pg/ml, less than about 40 pg/ml, less than about 35 pg/ml, less than about 30 pg/ml, less than about 25 pg/ml, less than about 20 pg/ml, less than about 15 pg/ml, less than about 14 pg/ml, less than about 13 pg/ml, less than about 12 pg/ml, less than about 11 pg/ml, less than about 10 pg/ml, less than about 9 pg/ml, less than about 8 pg/ml, less than about 7 pg/ml, less than about 6 pg/ml, less than about 5 pg/ml, less than about 4 pg/ml, less than about 3 pg/ml, less than about 2 pg/ml, or less than about 1 pg/ml. In still other embodiments, one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, TNFα, IFNγ) may be detected in the serum of a subject (*e.g.*, by ELISA or other method known in the art) following administration of one or more doses of an anti-CD3 antibody to said subject according to methods of the invention at a concentration range from undetectable to less than about 10 pg/ml, from undetectable to less than about 15 pg/ml, from undetectable to less than about 20 pg/ml, from undetectable to less than about 25 pg/ml, from undetectable to less than about 30 pg/ml, from undetectable to less than about 40 pg/ml, from undetectable to less than about 50 pg/ml, from undetectable to less than about 75 pg/ml, from undetectable to less than about 100 pg/ml, from undetectable to less than about 150 pg/ml, from undetectable to less than about 200 pg/ml, from undetectable to less than about 250 pg/ml, from undetectable to less than about 300 pg/ml, from undetectable to less than about 350 pg/ml, or from undetectable to less than about 400 pg/ml. In still other embodiments, one or more cytokines (*e.g.*, IL-2, IL-6, IL-10, TNFα, IFNγ) may be detected in the serum of a subject (*e.g.*, by ELISA or other method known in the art) following administration of one or more doses of an anti-CD3 antibody to said subject according to methods of the invention at a concentration range from about 5 pg/ml to less than about 10 pg/ml, from about 5 pg/ml to less than about 15 pg/ml, from about 5 pg/ml to less than about 20 pg/ml, from about 5 pg/ml to less than about 25 pg/ml, from about 5 pg/ml to less than about 30 pg/ml, from about 5 pg/ml to less than about 35 pg/ml, from about 5 pg/ml to less than about 40 pg/ml, from about 5 pg/ml to less than about 50 pg/ml, from about 5 pg/ml to less than about 75 pg/ml, from about 5 pg/ml to less

than about 100 pg/ml, from about 5 pg/ml to less than about 150 pg/ml, from about 5 pg/ml to less than about 200 pg/ml, from about 5 pg/ml to less than about 250 pg/ml, from about 5 pg/ml to less than about 300 pg/ml, or from about 5 pg/ml to less than about 350 pg/ml.

[0092] In still other embodiments, the methods of the invention reduce the detectable, *e.g.* by ELISA, concentration of one or more cytokines in serum of a subject following the administration of one or more doses of an anti-CD3 antibody to said subject by at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 325%, at least about 350%, at least about 375%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000% relative to the concentration of said one or more cytokines in serum of the subject as a result of a similar administration of murine OKT3. In another embodiment, the method of the invention reduces the detectable, *e.g.* by ELISA, concentration of one or more cytokines in the serum of a subject following the administration of one or more doses of an anti-CD3 antibody to said subject by at least about 1 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 12 fold, at least about 14 fold, at least about 16 fold, at least about 18 fold, at least about 20 fold, at least about 25 fold, at least about 30 fold, at least about 35 fold, at least about 40 fold, at least about 45 fold, at least about 50 fold, at least about 75 fold or at least about 100 fold relative to the concentration of said one or more cytokines in the serum of a subject as a result of a similar administration of murine OKT3.

[0093] In another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide induce T cell anergy in an *in vivo* or *in vitro* assay described herein or well-known to one of skill in the art. In an alternative embodiment, antibodies that immunospecifically bind to a CD3 polypeptide do not induce T cell anergy in an *in vivo* or *in vitro* assay described herein or well-known to one of skill in the art. In another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide elicit a state of antigen-specific unresponsiveness for at least 30 minutes, at least 1 hour, at least 2 hours, at least 6 hours, at least 12 hours, at least 24 hours, at least 2 days, at least 5 days, at least 7 days, at least 10 days or more in an *in vitro* assay described herein or known to one of skill in the art.

[0094] In another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide inhibit T cell activation by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% and inhibit T cell proliferation by at least

25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% in an *in vivo* or *in vitro* assay described herein or well-known to one of skill in the art.

[0095] In another embodiment, the Fc domain of an antibody that immunospecifically binds to a CD3 polypeptide does not detectably bind to at least one Fc receptor ("FcR") expressed by an immune cell such as a T cell, monocyte, and macrophage. Additionally or alternatively, the Fc domain of an antibody that immunospecifically binds to a CD3 polypeptide does not detectably bind to one or more FcRs expressed by an immune cell such as a T cell, monocyte, and macrophage.

[0096] Antibodies that immunospecifically bind to a CD3 polypeptide include, but are not limited to, monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. In particular, antibodies that immunospecifically bind to a CD3 polypeptide include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds to a CD3 polypeptide. The immunoglobulin molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass of immunoglobulin molecule. In a specific embodiment, the antibodies that immunospecifically bind to a CD3 polypeptide and mediate the activity of T cells comprise an Fc domain or a fragment thereof (*e.g.*, the CH2, CH3, and/or hinge regions of an Fc domain), or may comprise one or more fragments and/or domains from one or more type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass of immunoglobulin molecule. In a preferred embodiment, the antibodies that immunospecifically bind to a CD3 polypeptide and mediate the activity of T cells comprise an Fc domain or fragment thereof that does not bind to an FcR expressed by an immune cell.

[0097] The antibodies that immunospecifically bind to a CD3 polypeptide may be from any animal origin including birds and mammals (*e.g.*, human, murine, donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken). Preferably, the antibodies of the invention are human or humanized monoclonal antibodies. Human antibodies that immunospecifically bind to a CD3 polypeptide include antibodies having the amino acid sequence of a human immunoglobulin and antibodies isolated from human immunoglobulin libraries or from mice that express antibodies from human genes.

[0098] The antibodies that immunospecifically bind to a CD3 polypeptide may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a CD3 polypeptide or may be specific for both a CD3 polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, *e.g.*, PCT publications WO 93/17715, WO 92/08802, WO 91/00360, and WO 92/05793; Tutt, et al., J. Immunol. 147:60-69(1991); U.S. Patent Nos. 4,474,893, 4,714,681, 4,925,648, 5,573,920, and 5,601,819; and Kostelny et al., J. Immunol. 148:1547-1553 (1992), each of which is hereby incorporated by reference herein in its entirety.

[0099] The present invention provides for antibodies that have a high binding affinity for a CD3 polypeptide. In a specific embodiment, an antibody that immunospecifically binds to a CD3 polypeptide has an association rate constant or k_{on} rate (antibody (Ab) + antigen (Ag)$^{k_{on}} \rightarrow$ Ab-Ag) of at least $10^5\ M^{-1}s^{-1}$, at least $5 \times 10^5\ M^{-1}s^{-1}$, at least $10^6\ M^{-1}s^{-1}$, at least $5 \times 10^6\ M^{-1}s^{-1}$, at least $10^7\ M^{-1}s^{-1}$, at least $5 \times 10^7\ M^{-1}s^{-1}$, or at least $10^8\ M^{-1}s^{-1}$. In a preferred embodiment, an antibody that immunospecifically binds to a CD3 polypeptide has a k_{on} of at least $2 \times 10^5\ M^{-1}s^{-1}$, at least $5 \times 10^5\ M^{-1}s^{-1}$, at least $10^6\ M^{-1}s^{-1}$, at least $5 \times 10^6\ M^{-1}s^{-1}$, at least $10^7\ M^{-1}s^{-1}$, at least $5 \times 10^7\ M^{-1}s^{-1}$, or at least $10^8\ M^{-1}s^{-1}$.

[00100] In another embodiment, an antibody that immunospecifically binds to a CD3 polypeptide has a k_{off} rate (antibody (Ab) + antigen (Ag)$^{K_{off}}$ Ab-Ag) of less than $10^{-1}\ s^{-1}$, less than $5 \times 10^{-1}\ s^{-1}$, less than $10^{-2}\ s^{-1}$, less than $5 \times 10^{-2}\ s^{-1}$, less than $10^{-3}\ s^{-1}$, less than $5 \times 10^{-3}\ s^{-1}$, less than $10^{-4}\ s^{-1}$, less than $5 \times 10^{-4}\ s^{-1}$, less than $10^{-5}\ s^{-1}$, less than $5 \times 10^{-5}\ s^{-1}$, less than $10^{-6}\ s^{-1}$, less than $5 \times 10^{-6}\ s^{-1}$, less than $10^{-7}\ s^{-1}$, less than $5 \times 10^{-7}\ s^{-1}$, less than $10^{-8}\ s^{-1}$, less than $5 \times 10^{-8}\ s^{-1}$, less than $10^{-9}\ s^{-1}$, less than $5 \times 10^{-9}\ s^{-1}$, or less than $10^{-10}\ s^{-1}$. In a preferred embodiment, an antibody that immunospecifically binds to a CD3 polypeptide has a k_{on} of less than $5 \times 10^{-4}\ s^{-1}$, less than $10^{-5}\ s^{-1}$, less than $5 \times 10^{-5}\ s^{-1}$, less than $10^{-6}\ s^{-1}$, less than $5 \times 10^{-6}\ s^{-1}$, less than $10^{-7}\ s^{-1}$, less than $5 \times 10^{-7}\ s^{-1}$, less than $10^{-8}\ s^{-1}$, less than $5 \times 10^{-8}\ s^{-1}$, less than $10^{-9}\ s^{-1}$, less than $5 \times 10^{-9}\ s^{-1}$, or less than $10^{-10}\ s^{-1}$.

[00101] In another embodiment, an antibody that immunospecifically binds to a CD3 polypeptide has an affinity constant or K_a (k_{on}/k_{off}) of at least $10^2\ M^{-1}$, at least $5 \times 10^2\ M^{-1}$, at least $10^3\ M^{-1}$, at least $5 \times 10^3\ M^{-1}$, at least $10^4\ M^{-1}$, at least $5 \times 10^4\ M^{-1}$, at least $10^5\ M^{-1}$, at least $5 \times 10^5\ M^{-1}$, at least $10^6\ M^{-1}$, at least $5 \times 10^6\ M^{-1}$, at least $10^7\ M^{-1}$, at least $5 \times 10^7\ M^{-1}$, at least $10^8\ M^{-1}$, at least $5 \times 10^8\ M^{-1}$, at least $10^9\ M^{-1}$, at least $5 \times 10^9\ M^{-1}$, at least $10^{10}\ M^{-1}$, at least $5 \times 10^{10}\ M^{-1}$, at least $10^{11}\ M^{-1}$, at least $5 \times 10^{11}\ M^{-1}$, at least $10^{12}\ M^{-1}$, at least $5 \times 10^{12}\ M^{-1}$, at least $10^{13}\ M^{-1}$, at least $5 \times 10^{13}\ M^{-1}$, at least $10^{14}\ M^{-1}$, at least $5 \times 10^{14}\ M^{-1}$, at least $10^{15}\ M^{-1}$, or at least $5 \times 10^{15}\ M^{-1}$. In yet another embodiment, an antibody that immunospecifically binds to a CD3 polypeptide has a dissociation constant or K_d (k_{off}/k_{on}) of less than 10^{-2} M, less than 5×10^{-2} M,

less than 10^{-3} M, less than 5 X 10^{-3} M, less than 10^{-4} M, less than 5 X 10^{-4} M, less than 10^{-5} M, less than 5 X 10^{-5} M, less than 10^{-6} M, less than 5 X 10^{-6} M, less than 10^{-7} M, less than 5 X 10^{-7} M, less than 10^{-8} M, less than 5 X 10^{-8} M, less than 10^{-9} M, less than 5 X 10^{-9} M, less than 10^{-10} M, less than 5 X 10^{-10} M, less than 10^{-11} M, less than 5 X 10^{-11} M, less than 10^{-12} M, less than 5 X 10^{-12} M, less than 10^{-13} M, less than 5 X 10^{-13} M, less than 10^{-14} M, less than 5 X 10^{-14} M, less than 10^{-15} M, or less than 5 X 10^{-15} M.

[00102] In a specific embodiment, an antibody that immunospecifically binds to a CD3 polypeptide is humanized OKT3 or an antigen-binding fragment thereof *e.g.*, (one or more complementarity determining regions (CDRs) of humanized OKT3). OKT3 has the amino acid sequence disclosed, *e.g.*, in U.S. Patent Nos. 4,658,019, 6,113,901 and 6,491,916 (each of which is incorporated herein by reference in its entirety), or the amino acid sequence of the monoclonal antibody produced by the cell line deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, Virginia 20110-2209 on July 28, 1993 as Accession Number CRL-8001. Several humanized versions of OKT3 are also reported in U.S. Patent No. 6,491,916. In an alternative embodiment, an antibody that immunospecifically binds to a CD3 polypeptide is not OKT3, a derivative of OKT3, *e.g.* humanized OKT3, an antigen-binding fragment of OKT3, or, more preferably, a humanized or chimeric version thereof.

[00103] In a specific embodiment, the present invention also provides antibodies that immunospecifically bind a CD3 polypeptide, said antibodies comprising a variable heavy ("VH") domain having an amino acid sequence of a VH domain of a humanized OKT3 (*e.g.*, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9; FIG. 1B). In a preferred embodiment, the humanized OKT3 antibody comprises a heavy chain comprising the amino acid sequence of the hOKT3γ1 (ala-ala) heavy chain set forth in SEQ ID NO:13 (FIG. 2D) and/or a heavy chain encoded by a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:12 (FIG. 2C).

[00104] In a specific embodiment, the present invention also provides antibodies that immunospecifically bind to a CD3 polypeptide, said antibodies comprising a variable light ("VL") domain having an amino acid sequence of the VL domain of a humanized OKT3 (*e.g.*, SEQ ID NO:3 or SEQ ID NO:4; FIG 1A). In a preferred embodiment, the humanized OKT3 antibody comprises a light chain comprising the amino acid sequence of the hOKT3γ1 light chain set forth in SEQ ID NO:11 (FIG. 2B) and/or a light chain encoded by a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:10 (FIG. 2A).

[00105] The present invention also provides antibodies that immunospecifically bind to a CD3 polypeptide, said antibodies comprising a VH domain disclosed herein, or a VH domain of an antibody disclosed herein, combined with a VL domain disclosed herein, or other VL

domain. The present invention further provides antibodies that immunospecifically bind to a CD3 polypeptide, said antibodies comprising a VL domain disclosed herein, or a VL domain of an antibody disclosed herein, combined with a VH domain disclosed herein, or other VH domain.

[00106] In certain embodiments, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to a CD3 polypeptide, said antibody comprising a VH domain having the amino acid sequence of a VH domain of humanized OKT3 (SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9; FIG. 1B).

[00107] In a preferred embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to a CD3 polypeptide, said antibody comprising a heavy chain having the amino acid sequence of the heavy chain of hOKT3γ-1 (ala-ala) disclosed in FIG. 2D (SEQ ID NO:13).

[00108] In one embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to a CD3 polypeptide, said antibody comprising a VL domain having the amino acid sequence of the VL domain of a humanized OKT3, for example, SEQ ID NO:3 or SEQ ID NO:4 (FIG. 1A).

[00109] In a preferred embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to a CD3 polypeptide, said antibody comprising a light chain having the amino acid sequence of the light chain of hOKT3γ-1 disclosed in FIG. 2B (SEQ ID NO:11).

[00110] In another embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to a CD3 polypeptide, said antibody comprising a VH domain having the amino acid sequence of the VH domain of a humanized OKT3, for example, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9 (FIG. 1B) and a VL domain having the amino acid sequence of the VL domain of a humanized OKT3, for example, SEQ ID NO:3 or SEQ ID NO:4 (FIG. 1A). In another embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to a CD3 polypeptide, said antibody comprising a heavy chain comprising the amino acid sequence of the heavy chain of a humanized OKT3, for example the amino acid sequence of hOKT3γ-1 (ala-ala) disclosed in FIG. 2D (SEQ ID NO:13), and/or a light chain comprising the amino acid sequence of the light chain of a humanized OKT3, for example the amino acid sequence of hOKT3γ-1 disclosed in FIG. 2B (SEQ ID NO:11). In other embodiments, the invention provides an antibody that immunospecifically binds to a CD3 polypeptide, said antibody comprising a heavy chain encoded by a nucleic acid molecule comprising the nuleotide sequence of SEQ ID NO:12 (FIG. 2C) and/or a light chain encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:10 (FIG. 2A).

[00111] In one embodiment, antibodies that immunospecifically bind to a CD3 polypeptide comprise a one or more VH CDRs disclosed in FIG. 1B. In another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide comprise more than one of the VH CDRs disclosed in FIG. 1B.

[00112] In one embodiment, antibodies that immunospecifically bind to a CD3 polypeptide comprise a one or more of the VL CDRs disclosed in FIG. 1A. In another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide comprise more than one of the VL CDRs disclosed in FIG. 1A.

[00113] In another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide comprise one or more VH CDRs disclosed in FIG. 1B and one or more VL CDRs disclosed in FIG. 1A. In yet another embodiment, antibodies that immunospecifically bind to a CD3 polypeptide comprise more than one of the VH CDRs disclosed in FIG. 1B and more than one of the VL CDRs disclosed in FIG. 1A.

[00114] In another embodiment, antibodies of the invention that immunospecifically bind to a CD3 polypeptide comprise an Fc domain comprising an alanine at position 234 and an alanine at position 235 according to the numbering of Kabat et al. (1987).

[00115] The present invention also provides antibodies that immunospecifically bind to a CD3 polypeptide, said antibodies comprising derivatives of the VH domains, VH CDRs, VL domains, or VL CDRs described herein, or available to one of ordinary skill in the art, that immunospecifically bind to a CD3 polypeptide. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding an antibody of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which results in amino acid substitutions. Preferably, the derivatives include less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the original molecule. In a preferred embodiment, the derivatives have conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues (*i.e.*, amino acid residues which are not critical for the antibody to immunospecifically bind to a CD3 polypeptide). A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine,

leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded antibody can be expressed and the activity of the antibody can be determined.

[00116] In a specific embodiment, the present invention provides for antibodies that immunospecifically bind to a CD3 polypeptide, said antibodies comprising the amino acid sequence of a humanized OKT3 with one or more amino acid residue substitutions in the variable light (VL) domain and/or variable heavy (VH) domain. The present invention also provides for antibodies that immunospecifically bind to a CD3 polypeptide, said antibodies comprising the amino acid sequence of murine OKT3 with one or more amino acid residue substitutions in one or more VL CDRs and/or one or more VH CDRs. The antibody generated by introducing substitutions in the VH domain, VH CDRs, VL domain and/or VL CDRs of humanized OKT3 can be tested *in vitro* and *in vivo,* for example, for its ability to bind to a CD3 polypeptide, or for its ability to inhibit T cell activation, or for its ability to inhibit T cell proliferation, or for its ability to induce T cell lysis, or for its ability to prevent, treat or ameliorate one or more symptoms associated with an autoimmune disorder.

[00117] In a specific embodiment, an antibody that immunospecifically binds to a CD3 polypeptide comprises a nucleotide sequence that hybridizes to the nucleotide sequence encoding the monoclonal antibody produced by the cell line deposited with the ATCC® as Accession Number CRL-8001 under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 ° C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45°C followed by one or more washes in 0.1 X SSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3, which is hereby incorporated by reference herein in its entirety).

[00118] In a specific embodiment, an antibody that immunospecifically binds to a CD3 polypeptide comprises a nucleotide sequence that hybridizes to the nucleotide sequence encoding a humanized OKT3 under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 ° C, under highly stringent conditions, *e.g.*, hybridization to

filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1 X SSC/0.2% SDS at about 68°C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3, which is hereby incorporated by reference herein in its entirety).

[00119] In a specific embodiment, an antibody that immunospecifically binds to a CD3 polypeptide comprises an amino acid sequence of a VH domain or an amino acid sequence a VL domain encoded by a nucleotide sequence that hybridizes to the nucleotide sequence encoding the VH or VL domains of a humanized OKT3 under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 ° C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1 X SSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3, which is hereby incorporated by reference herein in its entirety).

[00120] In another embodiment, an antibody that immunospecifically binds to a CD3 polypeptide comprises an amino acid sequence of a VH CDR or an amino acid sequence of a VL CDR encoded by a nucleotide sequence that hybridizes to the nucleotide sequence encoding any one of VH CDRs or VL CDRs of the monoclonal antibody produced by the cell line deposited with the ATCC® as Accession Number CRL-8001 under stringent conditions *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 ° C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1 X SSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art.

[00121] In another embodiment, an antibody that immunospecifically binds to a CD3 polypeptide comprises an amino acid sequence of a VH CDR and an amino acid sequence of a VL CDR encoded by nucleotide sequences that hybridizes to the nucleotide sequences encoding the monoclonal antibody produced by the cell line deposited with the ATCC® as Accession Number CRL-8001 under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 ° C, under highly stringent conditions, *e.g.*, hybridization to

filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1 X SSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art.

[00122] In a specific embodiment, an antibody that immunospecifically binds to a CD3 polypeptide comprises an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of the monoclonal antibody produced by the cell line deposited with the ATCC® as Accession Number CRL-8001. In another embodiment, an antibody that immunospecifically binds to a CD3 polypeptide comprises an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of a humanized OKT3 (*e.g.*, hOKT3γ-1).

[00123] In another embodiment, an antibody that immunospecifically binds to a CD3 polypeptide comprises an amino acid sequence of a VH domain that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the VH domain of a humanized OKT3 (*e.g.*, hOKT3γ-1).

[00124] In another embodiment, an antibody that immunospecifically binds to a CD3 polypeptide comprises an amino acid sequence of a VL domain that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the VL domain of a humanized OKT3 (*e.g.*, hOKT3γ-1).

[00125] The present invention encompasses antibodies that compete with an antibody described herein for binding to a CD3 polypeptide. In a specific embodiment, the present invention encompasses antibodies that compete with anti-CD3 antibodies known in the art, derivatives thereof or antigen binding fragments thereof. For example, antibodies provided by the invention compete with OKT3 or a derivative thereof, *e.g.* humanized OKT3, or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with ChAglyCD3 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with HuM291 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with UCHT1 or a derivative thereof or an antigen-binding fragment thereof for binding

to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with Leu4 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with YTH 12.5 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with 500A2 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with CLB-T3/3 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with BMA030 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with BH11 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with T2/30 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with AG3 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with BC3 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with SP34 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with RIV-9 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with 64.1 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide. In another specific embodiment, the present invention encompasses antibodies that compete with M291 or a derivative thereof or an antigen-binding fragment thereof for binding to the CD3 polypeptide.

[00126] The present invention also encompasses VH domains that compete with the VH domain of the antibodies disclosed herein, or with the VH domains of other anti-CD3 antibodies known in the art, or derivatives or variants thereof for binding to a CD3 polypeptide. In a specific embodiment, the present invention encompasses VH domains that compete with the VH domain of OKT3 or a derivative thereof, *e.g.* a humanized OKT3, for binding to a CD3 polypeptide. The present invention also encompasses VL domains that compete with the VL

domain of the antibodies disclosed herein, or with the VL domains of other anti-CD3 antibodies known in the art, or derivatives or variants thereof for binding to a CD3 polypeptide. In a specific embodiment, the present invention encompasses VL domains that compete with a VL domain of OKT3 or a derivative thereof, *e.g.* a humanized OKT3, for binding to a CD3 polypeptide.

[00127] The antibodies that immunospecifically bind to a CD3 polypeptide include derivatives that are modified, *i.e*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[00128] The present invention also provides antibodies that immunospecifically bind to a CD3 polypeptide, said antibodies comprising a framework region known to those of skill in the art. Preferably, the fragment region of an antibody of the invention is human.

[00129] The present invention also encompasses antibodies which immunospecifically bind to a CD3 polypeptide, said antibodies comprising the amino acid sequence of OKT3 or a derivative thereof, *e.g.* a humanized OKT3 (*e.g.*, hOKT3γ-1), with mutations (*e.g.*, one or more amino acid substitutions) in the framework regions. In certain embodiments, antibodies which immunospecifically bind to a CD3 polypeptide comprise the amino acid sequence of OKT3 or a derivative thereof, *e.g.* a humanized OKT3 (*e.g.*, hOKT3γ-1), with one or more amino acid residue substitutions in the framework regions of the VH and/or VL domains.

[00130] The present invention also encompasses antibodies which immunospecifically bind to a CD3 polypeptide, said antibodies comprising the amino acid sequence of OKT3 or a derivative thereof, *e.g.* a humanized OKT3 (*e.g.*, hOKT3γ-1), with mutations (*e.g.*, one or more amino acid residue substitutions) in the variable and framework regions.

[00131] The present invention also provides for fusion proteins comprising an antibody that immunospecifically binds to a CD3 polypeptide and a heterologous polypeptide. Preferably, the heterologous polypeptide that the antibody is fused to is useful for targeting the antibody to T cells.

[00132] The antibodies of the invention include derivatives that are otherwise modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding antigen and/or generating an anti-

idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

5.1.1 Polypeptides and Antibodies with variant Fc Regions

[00133] The use of therapeutic monoclonal antibodies is limited by problems of "first dose" side effects. First dose side effects, range from mild flu-like symptoms to severe toxicity, can be mild to severe, and include symptoms, such as, high fever, chills/rigors, headache, tremor, nausea/vomiting, diarrhea, abdominal pain, malaise, muscle/joint aches and pains, and generalized weakness. The first dose side effects are believed to be caused by lymphokine production and cytokine release stimulated by the Fc region of a MAb binding to and activating an FcγR on an FcγR-containing cell.

[00134] The FcR recognizes immunoglobulins of one or more isotypes through a recognition domain on the α chain of the Fc receptor. Fc receptors are defined by their specificity for immunoglobulin subtypes. For example, Fc receptors for IgG are referred to as FcγR. Different accessory cells bear Fc receptors for antibodies of different isotype, and the isotype of the antibody determines which accessory cells will be engaged in a given response (reviewed by Ravetch J.V. *et al.* 1991, *Annu. Rev. Immunol.* 9: 457-92; Gerber J.S. *et al.* 2001 *Microbes and Infection*, 3: 131-139; Billadeau D.D. *et al.* 2002, *The Journal of Clinical Investigation*, 2(109): 161-168l; Ravetch J.V. *et al.* 2000, *Science*, 290: 84-89; Ravetch J.V. *et al.*, 2001 *Annu. Rev. Immunol.* 19:275-90; Ravetch J.V. 1994, *Cell*, 78(4): 553-60, each of which is hereby incorporated by reference herein in its entirety). The different Fc receptors, the cells that express them, and their isotype specificity is summarized in Table 1 (adapted from Immunobiology: The Immune System in Health and Disease, 4th ed. 1999, Elsevier Science Ltd/Garland Publishing, New York, which is hereby incorporated by reference herein in its entirety).

[00135] The invention thus encompasses CD3 binding molecules that reduce or eliminate at least one symptom associated with first dose side effects and/or minimize toxicity associated with administration of an antibody of the invention by reducing or eliminating binding of the Fc to one or more FcγR. Such CD3 binding proteins comprise a variant Fc region having one or more amino acid modifications, relative to a wild type Fc region, wherein said Fc region may be derived from any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3,

IgG$_4$, IgA$_1$ and IgA$_2$) or subclass of immunoglobulin molecule. The modification decreases or eliminates binding of the Fc to one or more FcγRs, relative to a comparable wild type Fc region. The modification is typically an amino acid substitution. However, the modification can be an amino acid insertion and/or deletion. Typically, the modification occurs in the CH2 and/or hinge region. Alternatively, binding of Fc to one or more FcγRs can be reduced or eliminated by altering or eliminating one or more glycosyl groups on one in more Fc regions. Fc glycosylation can be altered or eliminated by methods well know in the art. For example, Fc glycosylation can be altered by producing the Fc in a cell that is deficient in fucosylation (*e.g.*, *fuc6* null cells), or eliminated by deglycosylation enzymes or an amino acid modification that alters or eliminates a glycosylation site (*e.g.*, the N-X-S/T glycosylation site at positions 297-299 in the CH2 domain). FcγR binding can be measured using standard methods known in the art and exemplified herein. The antibodies of the invention are thus particularly useful because they have reduced or no in vivo toxicity caused by lymphokine production or cytokine release. The affinities and binding properties of the molecules of the invention for an FcR are initially determined using *in vitro* assays (biochemical or immunological based assays) known in the art for determining Fc-FcR interactions, *i.e.*, specific binding of an Fc region to an FcR including but not limited to ELISA assay, surface plasmon resonance assay, immunoprecipitation assays (*See* Section 5.4). Preferably, the binding properties of the molecules of the invention are also characterized by in vitro functional assays for determining one or more FcγR mediator effector cell functions (*See* Section 5.4). In most preferred embodiments, the molecules of the invention have similar binding properties in in vivo models (such as those described and disclosed herein) as those in in vitro based assays However, the present invention does not exclude molecules of the invention that do not exhibit the desired phenotype in in vitro based assays but do exhibit the desired phenotype in vivo.

[00136] *Fcγ Receptors*

[00137] Each member of this family is an integral membrane glycoprotein, possessing extracellular domains related to a C2-set of immunoglobulin-related domains, a single membrane spanning domain and an intracytoplasmic domain of variable length. There are three known FcγRs, designated FcγRI(CD64), FcγRII(CD32), and FcγRIII(CD16), which exhibit extensive homology but are encoded by distinct genes. Both activating and inhibitory signals are transduced through the FcγRs following ligation. These diametrically opposing functions result from structural differences among the different receptor isoforms. In general, the binding of a complimentary Fc domain to FcγRI, FcγRIIA and FcγRIIIA results in activation of downstream substrates (*e.g.*, PI_3K) and leading to the release of proinflammatory mediators. In contrast, the binding of a complimentary Fc domain to FcγRIIB results in phosphorylation of

FcγRIIB and association with the SH2 domain of the inosital polyphosphate 5'-phosphatase (SHIP). SHIP hydrolyzes phosphoinositol messengers released as a consequence of FcγRI mediated tyrosine kinase activation, consequently preventing the influx of intracellular Ca^{++}. Thus crosslinking of FcγRIIB dampens the activating response to FcγR ligation and inhibits cellular responsiveness.

[00138] Methods of measuring lymphokine production and cytokine release are known and routine in the art and encompassed herein. For example, cytokine release may be measured by measuring secretion of cytokines including but not limited to TNF-α, GM-CSF, IFN-γ. See, e.g., U.S. Patent No. 6,491,916; Isaacs et al., 2001, Rheumatology, 40: 724-738; each of which is incorporated herein by reference in its entirety. Lymphokine production may be measured by measuring secretion of lymphokines including but not limited to Interleukin -2 (IL-2), . Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-12 (IL-12), Interleukin-16 (IL-16), PDGF, TGF-α, TGF-β, TNF- α, TNF- β, GCSF, GM-CSF, MCSF, IFN- α, IFN- β, TFN-γ, IGF-I, IGF-II. For example, see, Isaacs et al., 2001, Rheumatology, 40: 724-738; Soubrane et al., 1993, Blood, 81(1): 15-19; each of which is incorporated herein by reference in its entirety.

[00139] As used herein, the term "Fc region" is used to define a C-terminal region of an IgG heavy chain. Although the boundaries may vary slightly, the human IgG heavy chain Fc region is defined to stretch from Cys226 to the carboxy terminus. The Fc region of an IgG comprises two constant domains, CH2 and CH3. The CH2 domain of a human IgG Fc region (also referred to as "Cγ2" domain) usually extends from amino acids 231 to amino acid 341. The CH3 domain of a human IgG Fc region usually extends from amino acids 342 to 447. 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.

[00140] In preferred embodiments, the invention encompasses molecules comprising a variant Fc region, wherein said variant Fc region may be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass of immunoglobulin molecule and wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region, which variant Fc region does not bind any FcγR, as determined by standard assays known in the art and disclosed herein, relative to a comparable molecule comprising the wild type Fc region. In a specific embodiment, the one or more amino acid modifications which abolish binding to all FcγRs comprise a substitution at position 233 with phenylalanine; or a substitution at position 238 with arginine; or a substitution at position 265 with alanine; or a substitution at position 265 with glutamic acid; or a substitution at position 270 with alanine; or a substitution at position 270 with asparagine; or a substitution at position

297 with alanine; or a substitution at position 297 with glutamine; or a substitution at position 298 with phenylalanine; or a substitution at position 298 with asparagine; or a substitution at position 299 with any amino acid other than serine or threonine; or a substitution at position 265 with alanine and at position 297 with alanine; or a substitution at position 265 with alanine and at position 297 with glutamine; or a substitution at position 265 with glutamic acid and at position 297 with alanine; or a substitution at position 265 with glutamic acid and at position 297 with glutamine; or a substitution at position 234 with alanine and at position 235 with alanine; or a substitution at position 234 with alanine and at position 235 with glutamic acid. In another embodiment, the one or more amino acid modifications which abolish binding to all FcγRs comprise combinations of the modifications listed herein or combinations of the modifications listed herein with any that may confer null binding to CD32A, CD32B and CD16A as determined by the methods disclosed herein or known to one skilled in the art.

[00141] The invention encompasses methods for reducing or eliminating at least one symptom associated with first dose side effect in a patient and/or minimizing toxicity associated with administration of an antibody of the invention comprising administering an effective amount of one or more antibodies of the invention. The methods of the invention reduce at least one symptom associated with cytokine release syndrome including but not limited to high fever, chills/rigors, headache, tremor, nausea/vomiting, diarrhea, abdominal pain, malaise, muscle/joint aches and pains, and generalized weakness. In certain embodiments, the invention encompasses methods for reducing at least one symptom associated with first dose side effect and/or minimizing toxicity associated with administration of an antibody of the invention (*e.g.*, minimizing at least one symptom associated with cytokine release syndrome) comprising administering an effective amount of one or more antibodies of the invention, wherein said reduction is determined relative to that associated with the administration of a similar amount of murine OKT3 by an *in vitro* or *in vivo* assay described herein or known in the art.

[00142] The present invention provides for antibodies that immunospecifically bind to a CD3 polypeptide which have a extended half-life *in vivo*. In particular, the present invention provides antibodies that immunospecifically bind to a CD3 polypeptide which have a half-life in an animal, preferably a mammal and most preferably a human, of greater than 3 days, greater than 7 days, greater than 10 days, preferably greater than 15 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months.

[00143] To prolong the serum circulation of antibodies (*e.g.*, monoclonal antibodies, single chain antibodies and Fab fragments) *in vivo*, for example, inert polymer molecules such as high molecular weight polyethyleneglycol (PEG) can be attached to the antibodies with or

without a multifunctional linker either through site-specific conjugation of the PEG to the – or C-terminus of the antibodies or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation can be closely monitored by SDS-PAGE and mass spectrometry to ensure proper conjugation of PEG molecules to the antibodies. Unreacted PEG can be separated from antibody-PEG conjugates by size-exclusion or by ion-exchange chromatography. PEG-derivatized antibodies can be tested for binding activity as well as for *in vivo* efficacy using methods well-known to those of skill in the art, for example, by immunoassays described herein.

[00144] Antibodies having an increased half-life *in vivo* can also be generated introducing one or more amino acid modifications (*i.e.*, substitutions, insertions or deletions) into an IgG constant domain, or FcRn binding fragment thereof (preferably a Fc or hinge-Fc domain fragment). See, *e.g.*, International Publication No. WO 98/23289; International Publication No. WO 97/34631; and U.S. Patent No. 6,277,375, each of which is incorporated herein by reference in its entirety.

5.1.2 Antibody Conjugates

[00145] The present invention encompasses antibodies or antigen-binding fragments thereof that immunospecifically bind to a CD3 polypeptide recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a heterologous polypeptide (or a fragment thereof, preferably at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 contiguous amino acids of the polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. For example, antibodies may be used to target heterologous polypeptides to particular cell types (*e.g.*, T cells), either *in vitro* or *in vivo*, by fusing or conjugating the antibodies to antibodies specific for particular cell surface receptors such as, *e.g.*, CD4 and CD8.

[00146] The present invention also encompasses antibodies or antigen-binding fragments thereof that immunospecifically bind to a CD3 polypeptide fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., 1989, Proc. Natl. Acad. Sci. USA 86:821-824 (which is hereby incorporated by reference herein in its entirety), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin" HA" tag, which corresponds to an epitope derived

from the influenza hemagglutinin protein (Wilson et al., 1984, Cell 37:767, which is hereby incorporated by reference herein in its entirety) and the "flag" tag.

[00147] The present invention further encompasses antibodies or antigen-binding fragments thereof that immunospecifically bind to a CD3 polypeptide conjugated to an agent which has a potential therapeutic benefit. An antibody or an antigen-binding fragment thereof that immunospecifically binds to a CD3 polypeptide may be conjugated to a therapeutic moiety such as a cytotoxin, *e.g.*, a cytostatic or cytocidal agent, an agent which has a potential therapeutic benefit, or a radioactive metal ion, *e.g.*, alpha-emitters. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples of a cytotoxin or cytotoxic agent include, but are not limited to, paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Agents which have a potential therapeutic benefit include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[00148] Further, an antibody or an antigen-binding fragment thereof that immunospecifically binds to a CD3 polypeptide may be conjugated to a therapeutic agent or drug moiety that modifies a given biological response. Agents which have a potential therapeutic benefit or drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, interferon-α ("IFN-α"), interferon-β ("IFN-β"), nerve growth factor ("NGF"), platelet derived growth factor ("PDGF"), tissue plasminogen activator ("TPA"), an apoptotic agent, *e.g.*, TNF-α, TNF-β, a thrombotic agent or an anti-angiogenic agent, *e.g.*, angiostatin or endostatin; or, a biological response modifier such as, for example, a lymphokine (*e.g.*, interleukin-1 ("IL- 1"), IL-2, IL-6, IL-10, granulocyte macrophage colony stimulating factor ("GM-CSF"), and granulocyte colony stimulating factor ("G-CSF")), or a growth factor (*e.g.*, growth hormone

("GIF"). Such proteins may also include, for example, AIM I, AIM II, Fas Ligand, and/or VEGF (*See*, International Publication No. WO 97/33899; International Publication No. WO 97/34911; Takahashi et al., 1994, J. Iminunol., 6:1567-1574; and International Publication No. WO 99/23105, respectively, each of which is hereby incorporated by reference herein in its entirety).

[00149] Techniques for conjugating such therapeutic moieties to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 *(1985)*; "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985); and Thorpe et al., 1982, Immunol. Rev. 62:119-58, each of which is hereby incorporated by reference herein in its entirety.

[00150] An antibody or an antigen-binding fragment thereof that immunospecifically binds to a CD3 polypeptide can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[00151] Antibodies or antigen-binding fragments thereof that immunospecifically bind to a CD3 polypeptide may be attached to solid supports, which are particularly useful for the purification of $CD3^+$ immune cells such as T cells. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

5.2 Prophylactic and Therapeutic Methods

[00152] The present invention is directed to therapies which involve administering CD3 binding molecules, particularly anti-CD3 antibodies, to a subject, preferably a human subject, for preventing, treating, or ameliorating an autoimmune disorder or one or more symptoms thereof. In particular, the present invention is directed to therapies which involve administering CD3 binding molecules, particularly anti-CD3 antibodies, more particularly humanized forms of OKT3 that have Fc domains that do not bind to Fc receptors, to a subject, preferably a human subject, for preventing, treating, or ameliorating one or more symptoms of diabetes.

[00153] Examples of autoimmune disorders that may be treated by administering the molecules of the present invention include, but are not limited to, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barre, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), irritable bowel disease (IBD), IgA neuropathy, juvenile arthritis, lichen planus, lupus erthematosus, Ménière's disease, mixed connective tissue disease, multiple sclerosis, type 1 or immune-mediated diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychrondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynauld's phenomenon, Reiter's syndrome, Rheumatoid arthritis, sarcoidosis, scleroderma, Sjögren's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, takayasu arteritis, temporal arteristis/ giant cell arteritis, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis. Some autoimmune disorders are also associated with an inflammatory condition. Thus, there is overlap between what is considered an autoimmune disorder and an inflammatory disorder. Therefore, some autoimmune disorders may also be characterized as inflammatory disorders. Examples of inflammatory disorders which can be prevented, treated or managed in accordance with the methods of the invention include, but are not limited to, asthma, encephilitis, inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), allergic disorders, pulmonary fibrosis, undifferentitated spondyloarthropathy, undifferentiated arthropathy, arthritis, inflammatory osteolysis, and chronic inflammation resulting from chronic viral or bacteria infections. Examples of the types of psoriasis which can be treated in accordance with the compositions and methods of the invention include, but are not limited to, plaque psoriasis, pustular psoriasis, erythrodermic psoriasis, guttate psoriasis and inverse psoriasis.

5.2.1 Diabetes

[00154] Type 1 diabetes or immune-mediated diabetes mellitus is caused by an autoimmune response in which the insulin producing β-cells of the pancreas are gradually destroyed. Destruction of the β-cells is believed largely mediated by CTLs (CD8+ T cells). The

early stage of the disease, termed insulitis, is characterized by infiltration of leukocytes into the pancreas and is associated with both pancreatic inflammation and the release of anti- β-cell cytotoxic antibodies. Early stages of the disease are often overlooked or misdiagnosed as clinical symptoms of diabetes typically manifest only after about 80 % of the β-cells have been destroyed. Even with immunosuppressive therapy, β-cell populations do not recover to a significant extent; therefore, once clinical symptoms occur, the type-1 diabetic is normally insulin dependent for life. Insulin is currently the only standard therapy for treating symptoms of type 1 diabetes. Although immunosuppressive drugs such as methotrexate and cyclosporin showed early clinical promise in the treatment of type I diabetes, *e.g.* maintenance of β cell function, as with all general immunosuppressants, their prolonged use was associated with a number of severe side effects.

[00155] Use of the invention in the context of diabetes therefore encompasses methods to sustain/protect the levels and functionality of β cells which exist at the time of treatment. In a specific embodiment, anti-CD3 antibody therapy is not used for the treatment of acute diabetes but rather to prevent progression of the disease in recently diagnosed individuals, including children diagnosed with juvenille diabetes (*See* Herold et al., 2005, Diabetes 54:1763-1769). In a specific embodiment anti-CD3 therapy is used only in patients that have residual β cell function as compared to an individual in the same population (*i.e* age, sex, race, and general health) and determined by methods described herein or known to one of ordinary skill in the art. In another embodiment, anti-CD3 antibody therapy is used to maintain transplanted β cell function in pancreatic transplant recipients.

[00156] β cell function prior to, during, and after therapy may be assessed by methods described herein or by any method known to one of ordinary skill in the art. For example, the Diabetes Control and Complications Trial (DCCT) research group has established the monitoring of percentage glycoslyated hemoglobin (HA1 and HA1c) as the standard for evaluation of blood glucose control (DCCT, 1993, N. Engl. J. Med. 329:977-986). Alternatively, characterization of daily insulin needs, C-peptide levels/response, hypoglycemic episodes, and/or FPIR may be used as markers of β cell function or to establish a therapeutic index (*See* Keymeulen et al., 2005, N. Engl. J. Med. 352:2598-2608; Herold et al., 2005, Diabetes 54:1763-1769; U.S. Pat. Appl. Pub. No. 2004/0038867 A1; and Greenbaum et al., 2001, Diabetes 50:470-476, respectively). For example, FPIR is calculated as the sum of insulin values at 1 and 3 minutes post IGTT, which are performed according to Islet Cell Antibody Register User's Study protocols (*see, e.g.*, Bingley et al., 1996, Diabetes 45:1720-1728 and McCulloch et al., 1993, Diabetes Care 16:911-915) (each of said references being hereby incorporated by reference herein in its entirety).

5.2.2 Psoriasis

[00157] Psoriasis is a chronic, inflammatory, hyperproliferative skin disease that affects approximately 1-2% of the general population with men and women affected in equal numbers. (Nevitt, G.J. et al., 1996, British J. of Dermatology 135:533-537, which is hereby incorporated by reference herein in its entirety). Approximately 150,000 new cases of psoriasis and approximately 400 deaths from psoriasis are reported each year (Stern, R.S., 1995, Dermatol. Clin. 13:717-722, which is hereby incorporated by reference herein in its entirety). The most common type of psoriasis is chronic plaque syndrome. The condition is chronic for many sufferers and consists of periods of remission and relapse during the course of the disease (Ashcroft, D.M., et al., 2000, J. of Clin. Pharm. And Therap. 25:1-10).

[00158] Psoriasis is characterized by indurated, erythematous scaling plaques most commonly located on the scalp or the extensor aspects of the elbows and knees, but may occur at any skin site. Present treatment options currently available for psoriasis include topical agents, phototherapy and systemic agents. Topical treatments are first-line therapy for patients with mild to moderate plaque psoriasis. Systemic treatment is generally prescribed for severe cases of psoriasis where topical therapy is either impractical or ineffective. Phototherapy can be administered either alone or in combination with either topical or systemic agents. Unfortunately, each of these treatment options is associated with severe side effects. Most of the topical agents available for the treatment of psoriasis are associated with skin irritation, toxicity and possible carcinogenicity (Ashcroft, D.M., et al., 2000, J. of Clin. Pharm. and Therap. 25:1-10). Phototherapy, either broadband (UVB) or long wave (UVA), is associated with short term risks such as vesiculation, nausea, erythema, headache and skin pain as well as long-term risks of actinic keratoses, premature ageing of the skin, irregular pigmentation and squamous cell carcinoma which is reported in a quarter of patients (Stern, R.S., 1994, Cancer 73:2759-2764, which is hereby incorporated by reference herein in its entirety). Systemic agents are also associated with adverse side effects, and most are unavailable to pregnant patients. In particular, methotrexate, which is considered to be the 'gold standard' for treatment of severe psoriasis, carries a risk of hepatotoxicity with long-term use. In addition, it is recommended that patients have a liner biopsy performed at or near the start of each treatment and after each cumulative dose of 1.0-1.5 mg MTX (Roenigk, H.H. et al., 1988, J. of the Am. Acad. Of Dermatology, which is hereby incorporated by reference herein in its entirety).

[00159] When patients are provided with information regarding the possible adverse effects of the currently available therapies for psoriasis, many often choose to live with the condition rather than undergo treatment (Greaves M.W., 1995, New England J. of Medicine

332:381-388, which is hereby incorporated by reference herein in its entirety). Thus, there remains a need for better methods of treating psoriasis than currently available therapies.

[00160] Use of the invention in the context of psoriasis therefore encompasses methods to treat the acute phases of the disease as well as to prevent recurrence of symptoms of psoriasis. The response to anti-CD3 therapy in the context of psoriasis may be assessed by methods described herein or by any method known to one of ordinary skill in the art. Common methods used to monitor the sysmtoms of psoraisis include, but are not limited to, the Psoriasis Area and Severity Index (PASI), Physician Global Assessment (PGA) and NPF Psoriasis Score (NPF-PS) (*See* Ashcroft et al., 1999, Br. J. Dermatol. 141:185-191; van der Kerkhof et al., 1997, Br. J. Dermatol. 137:661-662 and Krueger et al., 1999, National Psoriasis Foundation Psoriasis Forum 5:1-5., respectively, each of which is hereby incorporated by reference herein in its entirety)

5.2.3 Rheumatoid Arthritis

[00161] Rheumatoid arthritis (RA) is an autoimmune disorder where the body's immune system improperly identifies the synovial membranes that secrete the lubricating fluid in the joints as foreign. Inflammation results, and the cartilage and tissues in and around the joints are damaged or destroyed. In severe cases, this inflammation extends to other joint tissues and surrounding cartilage, where it may erode or destroy bone and cartilage and lead to joint deformities. The body replaces damaged tissue with scar tissue, causing the normal spaces within the joints to become narrow and the bones to fuse together. Rheumatoid arthritis creates stiffness, swelling, fatigue, anemia, weight loss, fever, and often, crippling pain. Some common symptoms of rheumatoid arthritis include joint stiffness upon awakening that lasts an hour or longer; swelling in a specific finger or wrist joints; swelling in the soft tissue around the joints; and swelling on both sides of the joint. Swelling can occur with or without pain, and can worsen progressively or remain the same for years before progressing. Besides rheumatoid arthritis, other types of arthritis associated with autoimmune inflammation include the following: psoriatic arthritis, Reiter's syndrome and ankylosing spondylitis arthritis. Rheumatoid arthritis occurs in joints on both sides of the body (such as both hands, wrists or knees). This symmetry helps distinguish rheumatoid arthritis from other types of arthritis. In addition to affecting the joints, rheumatoid arthritis may occasionally affect the skin, eyes, lungs, heart, blood or nerves.

[00162] Rheumatoid arthritis affects about 1% of the world's population and is potentially disabling. There are approximately 2.9 million incidences of rheumatoid arthritis in the United States. Two to three times more women are affected than men. The typical age that rheumatoid arthritis occurs is between 25 and 50. Juvenile rheumatoid arthritis affects 71,000 young Americans (aged eighteen and under), affecting six times as many girls as boys.

[00163] Currently available therapy for arthritis focuses on reducing inflammation of the joints with anti-inflammatory or immunosuppressive medications. The first line of treatment of any arthritis is usually anti-inflammatories, such as aspirin, ibuprofen and Cox-2 inhibitors such as celecoxib and rofecoxib. "Second line drugs" include gold, methotrexate and steroids. Although these are well-established treatments for arthritis, very few patients remit on these lines of treatment alone. Recent advances in the understanding of the pathogenesis of rheumatoid arthritis have led to the use of methotrexate in combination with antibodies to cytokines or recombinant soluble receptors. However, only about 50% of the patients treated with a combination of methotrexate and anti-TNF-α agents such as recombinant soluble receptors for TNF-α show clinically significant improvement. Many patients remain refractory despite treatment. Difficult treatment issues still remain for patients with rheumatoid arthritis. Many current treatments have a high incidence of side effects or cannot completely prevent disease progression.

[00164] Use of the invention in the context of RA therefore excompasses methods to treat the acute phases of the disease as well as to prevent recurrence of symptoms of RA. The response to the therapeutic methods of the invention in the context of RA may be assessed by methods described herein or by any method known to one of ordinary skill in the art. For example, many, but not all, people with rheumatoid arthritis have rheumatoid-factor antibody in their blood; however, the presence of rheumatoid factor is not in itself definitive for a positive diagnosis of RA as other conditions are know which cause the rheumatoid factor to be produced. Therefore, the diagnosis and assessment of rheumatoid arthritis is most commonly based on a combination of factors, including, but not limited to: the specific location and symmetry of painful joints, the presence of joint stiffness in the morning, the presence of bumps and nodules under the skin (rheumatoid nodules), results of X-ray tests that suggest rheumatoid arthritis.

5.2.4 Tissue Transplantation

[00165] Tissue transplantation between genetically nonidentical individuals results in immunological rejection of the tissue through T cell-dependent mechanisms. To prevent allograft rejection, immunosuppression is achieved with agents that generally interfere with T cell function by modulating TcR signal transduction (see, e.g., Borel, J. F., 1989, Pharmacol. Rev. 42:260-372; Morris, P. J., 1991, Curr. Opin. Immunol. 3:748-751; Sigal et al., 1992, Ann. Rev. Immunol. 10:519-560; and L'Azou et al., 1999, Arch. Toxicol. 73:337-345, each of which is hereby incorporated by reference herein in its entirety). Further, since the effect of the immunosuppressive agents is short-lasting, transplant recipients normally require life-long treatment of immunosuppressive agents to prevent transplant rejection. Transplant recipients receiving long-term immunosuppressive treatment have a high risk of developing infections and

tumors. For example, patients receiving immunotherapy are at higher risk of developing lymphomas, skin tumors and brain tumors (see, e.g., Fellstrom et al., 1993, Immunol. Rev. 134:83-98, which is hereby incorporated by reference herein in its entirety). As an alternative to the general immunosuppressive agents currently used for the prevention of allograft rejection, monoclonal antibodies, including OKT3, have been successfully used to specifically block receptors involved in T cell stimulation/activation.

[00166] Use of the invention in the context of tissue transplantation therefore excompasses methods to treat the acute phases of the rejection as well as to prevent recurrence of symptoms of rejection. The response to the therapeutic methods of the invention in the context of tissue may be assessed by methods described herein or by any method known to one of ordinary skill in the art; however, no general methods, other than detecting an increasing frequency of CTL that recognize donor antigens (described *infra*), presently exist to monitor whether a transplant is being rejected by a recipient. Although the function of some transplants may be directly monitored (*i.e.* kidney or liver), often the first overt sign of rejection is a complete physiologic failure of the tissue, at which point the tissue is normally beyond rescue.

5.2.5 Diagnosis, Prediction and Assessment of Autoimmune Disorders

[00167] Patients with autoimmune disorders generally have an increasing frequency of CTL that recognize autoantigens. In the context of tissue transplantation, the patients will exhibit an increasing frequency of CTL that recognize donor-specific antigens. Such autoreactive or donor-reactive CTL may be detected in peripheral blood or target tissues. For example, in the diabetic patient, autoreactive CTL may be detected in pancreatic islet cell tissues; in the psoriatic patient, autoreactive CTL may be detected in epidermal or dermal tissues; in the arthritic patient, autoreactive CTL may be detected in synovial cell tissues and in the organ transplant recipient, donor-reactive CTL may be detected in the transplant graft. Since the generation of autoreactive or donor-reactive CTL is thought to precede the development of auto/donor antibodies and other indicia of the clinical symptoms of immune disorders, detection of specific CTL may in some cases enable more sensitive and specific diagnosis of the disorder.

[00168] The assays can also be used to quantify both the absolute number and the proportion of autoreactive CTL present in a sample, such as a peripheral blood sample, in both pre-clinical subjects and patients that have received therapy. In some embodiments, both the severity and course of the autoimmune or allograft disorder may be predicted and followed using such assays. For example, the human MHC class I molecule HLA-A 0201 can be used in combination with the a diabetic autoantigen, for example IA-2, to detect autoreactive CTL

present in a peripheral blood sample of a pre-diabetic subject or diabetic patient currently undergoing therapy using the methods of the invention.

[00169] The compounds of the invention can also be used in vivo in combination with, for example, imaging techniques or other in vivo detection methods for detecting CTLs labeled by binding with compounds or formulations of the invention.

[00170] Antigen-specific CTLs can be detected using a wide variety of assays, including immunospot (e.g., ELISPOT) assays, MHC class I tetramer assays, or other assays, as described herein or as known to a person skilled in the art.

[00171] The time period over which any biomarker of disorder progression or therapeutic effectiveness may be evaluated may be the time period of a single dose or an extended treatment time period, *e.g.* hours, days, weeks or months.

5.2.6 Therapeutic and Prophylactic Methods

[00172] The compositions and methods of the invention are particularly useful for the prevention, treatment or amelioration of T cell mediated diseases such as autoimmune disorders characterized by increased T cell infiltration of lymphocytes into affected tissues, or autoimmune disorders characterized by increased T cell activation and/or abnormal antigen presentation and/or recognition. The compositions and methods are also useful for the prevention, treatment or amelioration of inflammatory disorders characterized by increased T cell activation and/or abnormal antigen presentation. In specific embodiments, the invention provides methods of treating, preventing, managing or ameliorating the symptoms of an autoimmune disease, particularly, Type I Diabetes, ulcerative colitis, psoriasis, rheumatoid arthritis, lupus (particularly, cutaneous), psoriatic arthritis, inflammatory bowel disease (IBD), ulcerative colitis, multiple sclerosis, effects from organ transplantation, and graft vs. host disease (GVHD). Preferably, the treatment regimens described herein are administered to patients in early stages of the autoimmune disease, exhibiting mild tissue damage resulting from the autoimmune reaction and requiring minimal medical intervention, e.g., low doses of standard therapy, to manage the disease. The treatment regimens describe herein maintain high level functioning and prevent, slow or reduce additional tissue damage. Thus, the methods of the invention may reduce the need for additional therapy to treat, manage or ameliorate the disease or disorder.

[00173] In a preferred embodiment, pharmaceutical compositions comprising one or more CD3 binding molecules (*e.g.*, one or more anti-CD3 antibodies) are administered one or more times to prevent or reduce a decrease, or slow or reduce a decrease in β cell function associated with Type 1 diabetes in a subject. In yet another embodiment, one or more pharmaceutical compositions comprising one or more CD3 binding molecules (*e.g.*, one or more anti-CD3

antibodies) are administered one or ore times to prevent decrease in β cell function associated with diabetes in a subject that has had an allograft comprising pancreatic islet cell tissue. In accordance with these embodiments, changes in a subject's β cell function may be assessed by characterization of daily insulin requirements, HA1c levels, C-peptide function/levels or frequency of hypoglycemic episodes as discussed in Section 5.2.1.

[00174] In a specific embodiment anti-CD3 therapy is used in patients that have at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 75%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20% or at least 10% residual β cell function as compared to an individual in the same population (*i.e* age, sex, race, and general health) and determined by methods described herein or known to one of ordinary skill in the art. In another embodiment, after one or more courses of treatment with an anti-CD3 antibody according to the invention the level of β cell function of the patient decreases by less than 1%, less than 5%, less than 10%, less than 20%, less than 30%, less than 40% or less than 50% of the pretreatment levels. In yet another embodiment of the invention, after one or more courses of treatment with an anti-CD3 antibody according to the invention the level of β cell function of the patient is maintained at at least 99%, at least 95%, at least 90%, at least 80%, at least 70%, at least 60%, or at least 50% of pretreatment levels for at least 4 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months, or at least 30 months after the end of treatment. In another embodiment of the invention, after one or more courses of treatment with an anti-CD3 antibody according to the invention the level of β cell function of the patient is maintained at at least 99%, at least 95%, at least 90%, at least 80%, at least 70%, at least 60%, or at least 50% of pretreatment levels for at least 4 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months, or at least 30 months after the end of treatment and does not reduce the mean lymphocyte count of the patient to less than 800 cells/ml, less than 750 cells/ml, less than 700 cells/ml, less than 650 cells/ml, less than 600 cells/ml, less than 550 cells/ml, less than 500 cells/ml, less than 400 cells/ml, less than 300 cells/ml or 200 cells/ml or less. n another embodiment of the invention, after one or more courses of treatment with an anti-CD3 antibody according to the invention the level of β cell function of the patient is maintained at at least 99%, at least 95%, at least 90%, at least 80%, at least 70%, at least 60%, or at least 50% of pretreatment levels for at least 4 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months, or at least 30 months after the end of treatment and does not reduce the mean platelet count of the patient to less than 100,000,000 platelets/ml, less than 75,000,000 platelets/ml, less than 50,000,000 platelets/ml, less than 25,000,000 platelets/ml, less than 1,000,000 platelets/ml,

less than 750,000 platelets/ml, less than 500,000 platelets/ml, less than 250,000 platelets/ml, less than 150,000 platelets/ml or 100,000 platelets/ml or less.

[00175] In a specific embodiment anti-CD3 therapy is used in patients that have average insulin requirements of at least 0.2 U/kg/day, least 0.25 U/kg/day, least 0.5 U/kg/day, least 1 U/kg/day, least 4 U/kg/day, least 10 U/kg/day, least 50 U/kg/day, least 100 U/kg/day or 200 U/kg/day or more. In another embodiment, a human is administered doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies to achieve a reduction in said human's daily insulin requirements by at least 10%, at least 15%, at least 20%, at least 35%, at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% of pretreatment levels. In yet another embodiment of the invention, after one or more courses of treatment with an anti-CD3 antibody according to the invention the reduction of a patient's daily insulin requirements by at least 10%, at least 15%, at least 20%, at least 35%, at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 85% of pretreatment levels is maintained for at least 4 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months, or at least 30 months after the end of treatment. In yet another embodiment of the invention, after one or more courses of treatment with an anti-CD3 antibody according to the invention the reduction of a patient's daily insulin requirements by at least 10%, at least 15%, at least 20%, at least 35%, at least 30%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 85% of pretreatment levels is maintained for at least 4 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months, or at least 30 months after the end of treatment and does not reduce the mean lymphocyte count of the patient to less than 800 cells/ml, less than 750 cells/ml, less than 700 cells/ml, less than 650 cells/ml, less than 600 cells/ml, less than 550 cells/ml, less than 500 cells/ml, less than 400 cells/ml, less than 300 cells/ml or 200 cells/ml or less.

[00176] In yet another embodiment, a human subject having Type 1 diabetes is administered one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies to preserve said human's C-peptide or FPIR response to MMTT, OGTT or IGTT over about 2 weeks, about 1 month, about 2 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, about 21 months or about 24 months. In another embodiment of the invention, after one or more courses of treatment with an anti-CD3 antibody according to the invention the C-peptide response or FPIR of the patient to MMTT, OGTT or IGTT decreases by less than 1%, less than 5%, less than

10%, less than 20%, less than 30%, less than 40% or less than 50% of the pretreatment levels. In yet another embodiment of the invention, after one or more courses of treatment with an anti-CD3 antibody according to the invention the C-peptide response or FPIR of the patient to MMTT, OGTT or IGTT is maintained at at least 99%, at least 95%, at least 90%, at least 80%, at least 70%, at least 60%, or at least 50% of pretreatment levels for at least 4 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months, or at least 30 months after the end of treatment. In another embodiment of the invention, after one or more courses of treatment with an anti-CD3 antibody according to the invention the C-peptide response or FPIR of the patient to MMTT, OGTT or IGTT is maintained at at least 99%, at least 95%, at least 90%, at least 80%, at least 70%, at least 60%, or at least 50% of pretreatment levels for at least 4 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months, or at least 30 months after the end of treatment and does not reduce the mean lymphocyte count of the patient to less than 800 cells/ml, less than 750 cells/ml, less than 700 cells/ml, less than 650 cells/ml, less than 600 cells/ml, less than 550 cells/ml, less than 500 cells/ml, less than 400 cells/ml, less than 300 cells/ml or 200 cells/ml or less.

[00177] In another embodiment, a subject is administered one or more unit doses of approximately 0.5-50 μg/kg, approximately 0.5-40 μg/kg, approximately 0.5-30 μg/kg, approximately 0.5-20 μg/kg, approximately 0.5-15 μg/kg, approximately 0.5-10 μg/kg, approximately 0.5-5 μg/kg, approximately 1-5 μg/kg, approximately 1-10 μg/kg, approximately 20-40 μg/kg, approximately 20-30 μg/kg, approximately 22-28 μg/kg or approximately 25-26 μg/kg of one or more anti-CD3 antibody to prevent, treat or ameliorate one or more symptoms of an autoimmune disorder, particularly diabetes. In another embodiment, a subject is administered one or more unit doses of 200 μg/kg, 175 μg/kg, 150 μg/kg, 125 μg/kg, 100 μg/kg, 95 μg/kg, 90 μg/kg, 85 μg/kg, 80 μg/kg, 75 μg/kg, 70 μg/kg, 65 μg/kg, 60 μg/kg, 55 μg/kg, 50 μg/kg, 45 μg/kg, 40 μg/kg, 35 μg/kg, 30 μg/kg, 26 μg/kg, 25 μg/kg, 20 μg/kg, 15 μg/kg, 13 μg/kg, 10 μg/kg, 6.5 μg/kg, 5 μg/kg, 3.2 μg/kg, 3 μg/kg, 2.5 μg/kg, 2 μg/kg, 1.6 μg/kg, 1.5 μg/kg, 1 μg/kg, 0.5 μg/kg, 0.25 μg/kg, 0.1 μg/kg, or 0.05 μg/kg of one or more anti-CD3 antibodies to prevent, treat or ameliorate one or more symptoms of an autoimmune disorder, particularly diabetes.

[00178] In a one embodiment, a subject is administered one or more doses of 200 μg/kg or less, 175 μg/kg or less, 150 μg/kg or less, 125 μg/kg or less, 100 μg/kg or less, 95 μg/kg or less, 90 μg/kg or less, 85 μg/kg or less, 80 μg/kg or less, 75 μg/kg or less, 70 μg/kg or less, 65 μg/kg or less, 60 μg/kg or less, 55 μg/kg or less, 50 μg/kg or less, 45 μg/kg or less, 40 μg/kg or less, 35 μg/kg or less, 30 μg/kg or less, 25 μg/kg or less, 20 μg/kg or less, 15 μg/kg or less, 10 μg/kg or less, 5 μg/kg or less, 2.5 μg/kg or less, 2 μg/kg or less, 1.5 μg/kg or less, 1 μg/kg or

less, 0.5 μg/kg or less, 0.25 μg/kg or less, 0.1 μg/kg or less, or 0.05 μg/kg or less of one or more anti-CD3 antibody of the invention to prevent, treat or ameliorate one or more symptoms of an autoimmune disorder, such as but not limited to diabetes.

[00179] In certain embodiments, the subject is administered a treatment regimen comprising one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies, wherein the course of treatment is administered over 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days or 14 days. In another embodiment, the subject is administered a treatment regimen comprising one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies, wherein the course of treatment is administered over 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 consecutive days. In still another embodiment, the subject is administered a treatment regimen comprising one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies, wherein the course of treatment is administered over no more than 2, no more than 3, no more than 4, no more than 5, no more than 6, no more than 7, no more than 8, no more than 9, no more than 10, no more than 11, no more than 12, no more than 13 or no more than 14 consecutive days. In still other embodiments, the subject is administered a treatment regimen comprising one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies, wherein the course of treatment is administered over 2 days or less, 3 days or less, 4 days or less, 5 days or less, 6 days or less, 7 days or less, 8 days or less, 9 days or less, 10 days or less, 11 days or less, 12 days or less, 13 days or less or 14 days or less. In one embodiment, the treatment regimen comprises administering doses of the prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies every day, every 2nd day, every 3rd day or every 4th day. In certain embodiments the dose administered is the same each day of the regimen. In certain embodiments, a subject is administered a treatment regimen comprising one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies, wherein the prophylactically or therapeutically effective amount is 200 μg/kg/day, 175 μg/kg/day, 150 μg/kg/day, 125 μg/kg/day, 100 μg/kg/day, 95 μg/kg/day, 90 μg/kg/day, 85 μg/kg/day, 80 μg/kg/day, 75 μg/kg/day, 70 μg/kg/day, 65 μg/kg/day, 60 μg/kg/day, 55 μg/kg/day, 50 μg/kg/day, 45 μg/kg/day, 40 μg/kg/day, 35 μg/kg/day, 30 μg/kg/day, 26 μg/kg/day, 25 μg/kg/day, 20 μg/kg/day, 15 μg/kg/day, 13 μg/kg/day, 10 μg/kg/day, 6.5 μg/kg/day, 5 μg/kg/day, 3.2 μg/kg/day, 3 μg/kg/day, 2.5 μg/kg/day, 2 μg/kg/day, 1.6 μg/kg/day, 1.5 μg/kg/day, 1 μg/kg/day, 0.5 μg/kg/day, 0.25 μg/kg/day, 0.1 μg/kg/day, or 0.05 μg/kg/day. In certain embodiments, the dose escalates over the first fourth, first half or first 2/3 of the doses of the treatment regimen until the daily prophylactically or therapeutically effective amount of one

or more anti-CD3 antibodies is achieved. In preferred embodiments, the dose escalates over the first three days of the treatment regimen until the daily prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies is achieved. In certain embodiments, a subject is administered a treatment regimen comprising one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies, wherein the prophylactically or therapeutically effective amount is increased by, *e.g.*, 0.01 μg/kg, 0.02 μg/kg, 0.04 μg/kg, 0.05 μg/kg, 0.06 μg/kg, 0.08 μg/kg, 0.1 μg/kg, 0.2 μg/kg, 0.25 μg/kg, 0.5 μg/kg, 0.75 μg/kg, 1 μg/kg, 1.5 μg/kg, 2 μg/kg, 4 μg/kg, 5 μg/kg, 10 μg/kg, 15 μg/kg, 20 μg/kg, 25 μg/kg, 30 μg/kg, 35 μg/kg, 40 μg/kg, 45 μg/kg, 50 μg/kg, 55 μg/kg, 60 μg/kg, 65 μg/kg, 70 μg/kg, 75 μg/kg, 80 μg/kg, 85 μg/kg, 90 μg/kg, 95 μg/kg, 100 μg/kg, or 125 μg/kg, as treatment progresses. In certain embodiments, a subject is administered a treatment regimen comprising one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies, wherein the prophylactically or therapeutically effective amount is increased by a factor of 1.25, a factor of 1.5, a factor of 2, a factor of 2.25, a factor of 2.5, or a factor of 5 until the daily prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies is achieved.

[00180] In a specific embodiment, a subject is intramuscularly administered one or more doses of a 200 μg/kg or less, preferably 175 μg/kg or less, 150 μg/kg or less, 125 μg/kg or less, 100 μg/kg or less, 95 μg/kg or less, 90 μg/kg or less, 85 μg/kg or less, 80 μg/kg or less, 75 μg/kg or less, 70 μg/kg or less, 65 μg/kg or less, 60 μg/kg or less, 55 μg/kg or less, 50 μg/kg or less, 45 μg/kg or less, 40 μg/kg or less, 35 μg/kg or less, 30 μg/kg or less, 25 μg/kg or less, 20 μg/kg or less, 15 μg/kg or less, 10 μg/kg or less, 5 μg/kg or less, 2.5 μg/kg or less, 2 μg/kg or less, 1.5 μg/kg or less, 1 μg/kg or less, 0.5 μg/kg or less, or 0.5 μg/kg or less of one or more anti-CD3 antibodies to prevent, treat or ameliorate one or more symptoms of an autoimmune disorder.

[00181] In another embodiment, a subject is subcutaneously administered one or more doses of a 200 μg/kg or less, preferably 175 μg/kg or less, 150 μg/kg or less, 125 μg/kg or less, 100 μg/kg or less, 95 μg/kg or less, 90 μg/kg or less, 85 μg/kg or less, 80 μg/kg or less, 75 μg/kg or less, 70 μg/kg or less, 65 μg/kg or less, 60 μg/kg or less, 55 μg/kg or less, 50 μg/kg or less, 45 μg/kg or less, 40 μg/kg or less, 35 μg/kg or less, 30 μg/kg or less, 25 μg/kg or less, 20 μg/kg or less, 15 μg/kg or less, 10 μg/kg or less, 5 μg/kg or less, 2.5 μg/kg or less, 2 μg/kg or less, 1.5 μg/kg or less, 1 μg/kg or less, 0.5 μg/kg or less, or 0.5 μg/kg or less of one or more anti-CD3 antibodies to prevent, treat or ameliorate one or more symptoms of an autoimmune disorder.

[00182] In another embodiment, a subject is intravenously administered one or more doses of a 100 μg/kg or less, preferably 95 μg/kg or less, 90 μg/kg or less, 85 μg/kg or less, 80 μg/kg or less, 75 μg/kg or less, 70 μg/kg or less, 65 μg/kg or less, 60 μg/kg or less, 55 μg/kg or

less, 50 μg/kg or less, 45 μg/kg or less, 40 μg/kg or less, 35 μg/kg or less, 30 μg/kg or less, 25 μg/kg or less, 20 μg/kg or less, 15 μg/kg or less, 10 μg/kg or less, 5 μg/kg or less, 2.5 μg/kg or less, 2 μg/kg or less, 1.5 μg/kg or less, 1 μg/kg or less, 0.5 μg/kg or less, or 0.5 μg/kg or less of one or more anti CD3 antibodies to prevent, treat or ameliorate one or more symptoms of an autoimmune disorder. In another embodiment, the intravenous dose of 100 μg/kg or less, 95 μg/kg or less, 90 μg/kg or less, 85 μg/kg or less, 80 μg/kg or less, 75 μg/kg or less, 70 μg/kg or less, 65 μg/kg or less, 60 μg/kg or less, 55 μg/kg or less, 50 μg/kg or less, 45 μg/kg or less, 40 μg/kg or less, 35 μg/kg or less, 30 μg/kg or less, 25 μg/kg or less, 20 μg/kg or less, 15 μg/kg or less, 10 μg/kg or less, 5 μg/kg or less, 2.5 μg/kg or less, 2 μg/kg or less, 1.5 μg/kg or less, 1 μg/kg or less, 0.5 μg/kg or less, or 0.5 μg/kg or less of one or more anti CD3 antibodies is administered over about 6 hours, about 4 hours, about 2 hours, about 1.5 hours, about 1 hour, about 50 minutes, about 40 minutes, about 30 minutes, about 20 minutes, about 10 minutes, about 5 minutes, about 2 minutes, about 1 minute, about 30 seconds or about 10 seconds to prevent, treat or ameliorate one or more symptoms of an autoimmune disorder.

[00183] In a specific embodiment, the mean absolute lymphocyte count in a subject with an autoimmune disorder is assessed before and/or after the administration of one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies to determine whether one or more subsequent doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies should be administered to said subject. In another embodiment, the mean absolute lymphocyte count in a subject with an autoimmune disorder is assessed before and/or after the administration of one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies to determine whether one or more subsequent doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies should be administered to said subject. Preferably, a subsequent dose of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies is not administered to said subject if the lymphocyte count is less than 800 cells/mm^3, less than 750 cells/mm^3, less than 700 cells/mm^3, less than 650 cells/mm^3, less than 600 cells/mm^3, less than 500 cells/mm^3, less than 400 cells/mm^3 or 300 cells/mm^3 or less.

[00184] In another embodiment, the mean absolute lymphocyte count in a subject with an autoimmune disorder is determined prior to the administration of a first dose of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies and the mean absolute lymphocyte count is monitored prior to the administration of one or more subsequent doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies. Preferably, the mean absolute lymphocyte count in the subject is at least 900 cells/mm^3, preferably at least 950 cells/mm^3, at least 1000 cells/mm^3, at least1050 cells/mm^3, at

least 1100 cells/mm^3, at least 1200 cells/mm^3, or at least 1250 cells/ mm^3 prior to the administration of a first dose of one or more anti-CD3 antibodies.

[00185] In another embodiment, a mean absolute lymphocyte count of approximately 700 cells/ml to approximately 1200 cells/ml, approximately 700 cells/ml to approximately 1100 cells/ml, approximately 700 cells/ml to approximately 1000 cells/ml, approximately 700 to approximately 900 cells/ml, approximately 750 cells/ml to approximately 1200 cells/ml, approximately 750 cells/ml to approximately 1100 cells/ml, approximately 750 cells/ml to approximately 1000 cells/ml, approximately 750 cells/ml to approximately 900 cells/ml, approximately 800 cells/ml to approximately 1200 cells/ml, approximately 800 cells/ml to approximately 1100 cells/ml, approximately 800 cells/ml to approximately 1000 cells/ml, approximately 900 cells/ml to approximately 1200 cells/ml, approximately 900 cells/ml to approximately 1100 cells/ml, approximately 900 cells/ml to approximately 1000 cells/ml, or approximately 1000 cells to approximately 1200 cells/ml is maintained in a subject with an autoimmune disorder by administering one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies. In another embodiment, a mean absolute lymphocyte count of approximately 700 cells/ml to below 1000 cells/ml is maintained in a subject with an autoimmune disorder by administering one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies.

[00186] In a specific embodiment, the administration of one or more doses or a dosage regimen of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies does not induce or reduces relative to other immunosuppressive agents one or more of the following unwanted or adverse effects: vital sign abnormalities (fever, tachycardia, bardycardia, hypertension, hypotension), hematological events (anemia, lymphopenia, leukopenia, thrombocytopenia), headache, chills, dizziness, nausea, asthenia, back pain, chest pain (chest pressure), diarrhea, myalgia, pain, pruritus, psoriasis, rhinitis, sweating, injection site reaction, vasodilatation, an increased risk of opportunistic infection, activation of Epstein Barr Virus, apoptosis of T cells and an increased risk of developing certain types of cancer. In another specific embodiment, the administration of one or more doses of a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies does not induce or reduces relative to other immunosuppressive agents one or more of the following unwanted or adverse effects: vital sign abnormalities (fever, tachycardia, bardycardia, hypertension, hypotension), hematological events (anemia, lymphopenia, leukopenia, thrombocytopenia), headache, chills, dizziness, nausea, asthenia, back pain, chest pain (chest pressure), diarrhea, myalgia, pain, pruritus, psoriasis, rhinitis, sweating, injection site reaction, vasodilatation, an increased risk of

opportunistic infection, Epstein Barr Virus activation, apoptosis of T cells, and an increased risk of developing certain types of cancer.

[00187] In accordance with the invention, the dose or dosage regimen comprising a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies for the treatment of an autoimmune disorder may be repeated at 1 month, 2 months, 4 months, 6 months, 8 months, 12 months, 15 months, 18 months or 24 months or longer after the initial or previous dose or dosage regimen comprising a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies. The repeat dose or dosage regimen may be administered as a matter of course, when symptoms associated with said autoimmune disorder recur after an improvement following the initial or previous dose or dosage regimen, or when symptoms associated with said autoimmune disorder do not improve after the initial dose or dosage regimen of anti-CD3 antibodies according to methods of the invention. With respect to diabetes, a repeat dose or dosage regimen comprising a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies may be administered to a subject when, for example, the subject's average daily insulin use at 1 month, 2 months, 4 months, 6 months, 8 months, 12 months, 15 months, 18 months or 24 months or longer after initial or previous treatment with anti-CD3 antibodies does not decrease by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% compared to pre-treatment levels. Alternatively, with respect to diabetes, a repeat dose or dosage regimen comprising a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies may be administered to a subject when, for example, the subject's HA1 or HA1C levels at 1 month, 2 months, 4 months, 6 months, 8 months, 12 months, 15 months, 18 months or 24 months or longer after initial or previous treatment with anti-CD3 antibodies do not decrease by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% compared to pre-treatment levels. In another embodiment, with respect to diabetes, a repeat dose or dosage regimen comprising a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies may be administered to a subject when, for example, the subject's C-peptide response at 1 month, 2 months, 4 months, 6 months, 8 months, 12 months, 15 months, 18 months or 24 months or longer after initial or previous treatment with anti-CD3 antibodies decreases by more than 5%, more than 10%, more than 20%, more than 30%, more than 40%, more than 50%, more than 60%, more than 70%, more than 80% or more than 90% compared to pre-treatment levels.

5.2.7 Combinatorial Therapy

[00188] The present invention provides compositions comprising one or more anti-CD3 antibody and one or more prophylactic or therapeutic agents other than anti-CD3 antibodies, and methods for preventing, treating or ameliorating one or more symptoms associated with an inflammatory or autoimmune disorder in a subject comprising administering to said subject one or more of said compositions. Therapeutic or prophylactic agents include, but are not limited to, peptides, polypeptides, fusion proteins, nucleic acid molecules, small molecules, mimetic agents, synthetic drugs, inorganic molecules, and organic molecules. Any agent which is known to be useful, or which has been used or is currently being used for the prevention, treatment or amelioration of one or more symptoms associated with an inflammatory or autoimmune disorder can be used in combination with an anti-CD3 antibody in accordance with the invention described herein. Examples of such agents include, but are not limited to antibody fragments, GLP-1 analogs or derivatives, GLP-1 agaonists (*e.g.* exendin-4; exentatide), amylin analogs or dericatives, insulin, dermatological agents for rashes and swellings (*e.g.*, phototherapy (*i.e.*, ultraviolet B radiation), photochemotherapy (*e.g.*, PUVA) and topical agents such as emollients, salicylic acid, coal tar, topical steroids, topical corticosteroids, topical vitamin D3 analogs (*e.g.*, calcipotriene), tazarotene, and topical retinoids), anti-inflammatory agents (*e.g.*, corticosteroids (*e.g.*, prednisone and hydrocortisone), glucocorticoids, steroids, non-steriodal anti-inflammatory drugs (*e.g.*, aspirin, ibuprofen, diclofenac, and COX-2 inhibitors), beta-agonists, anticholinergic agents and methyl xanthines), immunomodulatory agents (*e.g.*, small organic molecules, a T cell receptor modulators, cytokine receptor modulators, T cell depleting agents, cytokine antagonists, monokine antagonists, lymphocyte inhibitors, or anti-cancer agents), gold injections, sulphasalazine, penicillamine, anti-angiogenic agents (*e.g.*, angiostatin, TNF-α antagonists (*e.g.*, anti-TNFα antibodies), and endostatin), dapsone, psoralens (*e.g.*, methoxalen and trioxsalen), anti-malarial agents (*e.g.*, hydroxychloroquine), anti-viral agents, and antibiotics (*e.g.*, erythomycin and penicillin). Any immunomodulatory agent well-known to one of skill in the art may also be used in the methods and compositions of the invention. Immunomodulatory agents can affect one or more or all aspects of the immune response in a subject. Aspects of the immune response include, but are not limited to, the inflammatory response, the complement cascade, leukocyte and lymphocyte differentiation, proliferation, and/or effector function, monocyte and/or basophil counts, and the cellular communication among cells of the immune system. In certain embodiments of the invention, an immunomodulatory agent modulates one aspect of the immune response. In other embodiments, an immunomodulatory agent modulates more than one aspect of the immune response. In a preferred embodiment of the invention, the administration of an immunomodulatory agent to a subject inhibits or reduces one or more aspects of the subject's immune response capabilities. In a specific embodiment of the

invention, the immunomodulatory agent inhibits or suppresses the immune response in a subject. In accordance with the invention, an immunomodulatory agent is not an anti-CD3 antibody. In certain embodiments, an immunomodulatory agent is not an anti-inflammatory agent. In other embodiments, an immunomodulatory agent is not a CD3 binding molecule. In yet other embodiments, an immunomodulatory agent is not OKT3 or a derivative thereof.

[00189] An immunomodulatory agent may be selected to interfere with the interactions between the T helper subsets (TH1 or TH2) and B cells to inhibit neutralizing antibody formation. An immunomodulatory agent may be selected to inhibit the interaction between TH1 cells and CTLs to reduce the occurrence of CTL-mediated killing. An immunomodulatory agent may be selected to alter (*e.g.*, inhibit or suppress) the proliferation, differentiation, activity and/or function of the $CD4^+$ and/or $CD8^+$ T cells. For example, antibodies specific for T cells can be used as immunomodulatory agents to deplete, or alter the proliferation, differentiation, activity and/or function of $CD4^+$ and/or $CD8^+$ T cells.

[00190] In a preferred embodiment, proteins, polypeptides or peptides (including antibodies) that are utilized as prophylactic, therapeutic or immunomodulatory agents are derived from the same species as the recipient of the proteins, polypeptides or peptides so as to reduce the likelihood of an immune response to those proteins, polypeptides or peptides. In another preferred embodiment, when the subject is a human, the proteins, polypeptides, or peptides that are utilized as immunomodulatory agents are human or humanized.

[00191] In accordance with the invention, one or more prophylactic, therapeutic or immunomodulatory agents are administered to a subject with an inflammatory or autoimmune disease prior to, subsequent to, or concomitantly with the therapeutic and/or prophylactic agents of the invention. Preferably, one or more prophylactic, therapeutic or immunomodulatory agents are administered to a subject with an inflammatory or autoimmune disease to reduce or inhibit one or more symptoms of the disease or aspects of the immune response as necessary. Any technique well-known to one skilled in the art can be used to measure one or more aspects of the immune response in a particular subject, and thereby determine when it is necessary to administer an immunomodulatory agent to said subject. In a preferred embodiment, an absolute lymphocyte count of approximately 500 cells/mm^3, preferably 600 cells/mm^3, more 700 cells/mm^3, and most preferably 800 cells/mm^3 is maintained in a subject. In another preferred embodiment, a subject with an autoimmune or inflammatory disorder is not administered an immunomodulatory agent if their absolute lymphocyte count is 500 cells/mm^3 or less, 550 cells/mm^3 or less, 600 cells/mm^3 or less, 650 cells/mm^3 or less, 700 cells/mm^3 or less, 750 cells/mm^3 or less, or 800 cells/mm^3 or less.

[00192] In a preferred embodiment, one or more prophylactic, therapeutic or immunomodulatory agents are administered to a subject with an inflammatory or autoimmune disease so as to transiently reduce or inhibit one or more aspects of the disease or of the immune response. Such a transient inhibition or reduction of one or more aspects of the disease or of the immune system can last for hours, days, weeks, or months. Preferably, the transient inhibition or reduction in one or more aspects of the disease or of the immune response last for a few hours (*e.g.*, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 14 hours, 16 hours, 18 hours, 24 hours, 36 hours, or 48 hours), a few days (*e.g.*, 3 days, 4 days, 5 days, 6 days, 7 days, or 14 days), or a few weeks (*e.g.*, 3 weeks, 4 weeks, 5 weeks or 6 weeks). The transient reduction or inhibition of one or more aspects of the disease or of the immune response enhances the prophylactic and/or therapeutic capabilities of an anti-CD3 antibody.

[00193] Nucleic acid molecules encoding proteins, polypeptides, or peptides with prophylactic, therapeutic or immunomodulatory activity or proteins, polypeptides, or peptides with prophylactic, therapeutic or immunomodulatory activity can be administered to a subject with an inflammatory or autoimmune disease in accordance with the methods of the invention. Further, nucleic acid molecules encoding derivatives, analogs, fragments or variants of proteins, polypeptides, or peptides with prophylactic, therapeutic or immunomodulatory activity, or derivatives, analogs, fragments or variants of proteins, polypeptides, or peptides with prophylactic, therapeutic or immunomodulatory activity can be administered to a subject with an inflammatory or autoimmune disease in accordance with the methods of the invention. Preferably, such derivatives, analogs, variants and fragments retain the prophylactic, therapeutic or immunomodulatory activity of the full-length wild-type protein, polypeptide, or peptide.

[00194] Proteins, polypeptides, or peptides that can be used as prophylactic, therapeutic or immunomodulatory agents can be produced by any technique well-known in the art or described herein. See, *e.g.*, Chapter 16 Ausubel et al. (eds.), 1999, Short Protocols in Molecular Biology, Fourth Edition, John Wiley & Sons, NY (hereby incorporated by reference herein in its entirety), which describes methods of producing proteins, polypeptides, or peptides, and which is incorporated herein by reference in its entirety. Antibodies which can be used as prophylactic, therapeutic or immunomodulatory agents can be produced by, *e.g.*, methods described in U.S. Patent No. 6,245,527 and in Harlow and Lane Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988, which are incorporated herein by reference in their entirety. Preferably, agents that are commercially available and known to function as prophylactic, therapeutic or immunomodulatory agents are used in the compositions and methods of the invention. The prophylactic, therapeutic or immunomodulatory activity of an agent can be determined *in vitro* and/or *in vivo* by any technique well-known to one skilled in

the art, including, e.g., by CTL assays, proliferation assays, and immunoassays (*e.g.* ELISAs) for the expression of particular proteins such as co-stimulatory molecules and cytokines.

[00195] The combination of one or more anti-CD3 antibodies and one or more prophylactic or therapeutic agents other than anti-CD3 antibodies produces a better prophylactic or therapeutic effect in a subject than either treatment alone. In certain embodiments, the combination of an anti-CD3 antibody and a prophylactic or therapeutic agent other than an anti-CD3 antibody achieves a 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98% better prophylactic or therapeutic effect in a subject with an autoimmune or inflammatory disorder than either treatment alone. In particular embodiments, the combination of one or more anti-CD3 antibodies and a prophylactic or therapeutic agent other than an anti-CD3 antibody achieves a 20%, preferably a 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98% greater reduction in the inflammation of a particular organ, tissue or joint in a subject with an inflammatory disorder or an autoimmune disorder which is associated with inflammation than either treatment alone. In other embodiments, the combination of one or more anti-CD3 antibodies and one or more prophylactic or therapeutic agents other than anti-CD3 antibodies has an a more than additive effect or synergistic effect in a subject with an autoimmune or inflammatory disorder.

[00196] The combination therapies of the invention enable lower dosages of anti-CD3 antibodies and/or less frequent administration of anti-CD3 antibodies, preferably humanized OKT3, to a subject with an autoimmune or inflammatory disorder to achieve a prophylactic or therapeutic effect. The combination therapies of the invention enable lower dosages of the prophylactic or therapeutic agents utilized in conjunction with anti-CD3 antibodies for the prevention or treatment of an autoimmune or inflammatory disorder and/or less frequent administration of such prophylactic or therapeutic agents to a subject with an autoimmune or inflammatory disorder to achieve a prophylactic or therapeutic effect. The combination therapies of the invention reduce or avoid unwanted or adverse side effects associated with the administration of current single agent therapies and/or existing combination therapies for autoimmune or inflammatory disorders, which in turn improves patient compliance with the treatment protocol.

[00197] The prophylactic or therapeutic agents of the combination therapies of the present invention can be administered concomitantly, concurrently or sequentially. The prophylactic or therapeutic agents of the combination therapies of the present invention can also be cyclically administered. Cycling therapy involves the administration of a first prophylactic or therapeutic agent for a period of time, followed by the administration of a second prophylactic or therapeutic agent for a period of time and repeating this sequential administration, *i.e.*, the cycle, in order to

reduce the development of resistance to one of the agents, to avoid or reduce the side effects of one of the agents, and/or to improve the efficacy of the treatment.

5.2.7.1 Methods of Use of Combination Therapy

[00198] In a specific embodiment, the present invention provides a method for preventing, treating, managing, or ameliorating one or more symptoms associated with an autoimmune or inflammatory disorder in a subject, said method comprising administering to said subject one or more anti-CD3 antibodies and one or more prophylactic or therapeutic agents other than anti-CD3 antibodies. In a preferred embodiment, the present invention provides a method for preventing, treating, managing, or ameliorating one or more symptoms associated with an autoimmune or inflammatory disorder in a subject, said method comprising administering to said subject one or more anti-CD3 antibodies and one or more prophylactic or therapeutic agents other than anti-CD3 antibodies, wherein at least one of the anti-CD3 antibodies is a humanized OKT3.

[00199] The present invention provides methods of preventing, treating, managing or ameliorating one or more symptoms associated with an autoimmune or inflammatory disorder in a subject, said methods comprising administering to said subject one or more anti-CD3 antibodies and one or more prophylactic, therapeutic or immunomodulatory agents. Preferably, the immunomodulatory agents are not administered to a subject with an autoimmune or inflammatory disorder whose absolute lymphocyte count is less than 500 cells/mm^3, less than 550 cells/mm^3, less than 600 cells/mm^3, less than 650 cells/mm^3, less than 700 cells/mm^3, less than 750 cells/mm^3, less than 800 cells/mm^3, less than 850 cells/mm^3 or less than 900 cells/mm^3. Thus, in a preferred embodiment, prior to or subsequent to the administration of one or more dosages of one or more immunomodulatory agents to a subject with an autoimmune or inflammatory disorder, the absolute lymphocyte count of said subject is determined by techniques well-known to one of skill in the art, including, *e.g.*, flow cytometry or trypan blue counts.

[00200] In one embodiment, the present invention provides a method for preventing, treating, managing or ameliorating one or more symptoms associated with diabetes, said method comprising administering to said subject a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies and a prophylactically or therapeutically effective amount of insulin. In one embodiment, the present invention provides a method for preventing, treating, managing or ameliorating one or more symptoms associated with diabetes, said method comprising administering to said subject a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies and a prophylactically or therapeutically effective amount of GLP1 or GLP1 analog. In one embodiment, the present invention provides a method for

preventing, treating, managing or ameliorating one or more symptoms associated with diabetes, said method comprising administering to said subject a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies and a prophylactically or therapeutically effective amount of exendin-4 or analog thereof. In one embodiment, the present invention provides a method for preventing, treating, managing or ameliorating one or more symptoms associated with diabetes, said method comprising administering to said subject a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies and a prophylactically or therapeutically effective amount of amylin or an analog thereof. In another embodiment, the present invention provides a method for preventing, treating, managing or ameliorating one or more symptoms associated with diabetes, said method comprising administering to said subject a prophylactically or therapeutically effective amount of the humanized anti-CD3 antibody OKT3 and a prophylactically or therapeutically effective amount of insulin. In another embodiment, the present invention provides a method for preventing, treating, managing or ameliorating one or more symptoms associated with psoriasis, said method comprising administering to said subject a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies and a prophylactically or therapeutically effective amount of methotrexate. In another embodiment, the present invention provides a method for preventing, treating, managing or ameliorating one or more symptoms associated with psoriasis in a subject, said method comprising administering to said subject a prophylactically or therapeutically effective amount of the humanized anti-CD3 antibody OKT3 and a prophylactically or therapeutically effective amount of methotrexate.

5.3 Pharmaceutical Compositions

[00201] The present invention provides compositions for the treatment, prophylaxis, and amelioration of one or more symptoms associated with an autoimmune or inflammatory disorder. In a specific embodiment, a composition comprises one or more anti-CD3 antibodies. In another embodiment, a composition comprises one or more nucleic acid molecules encoding one or more anti-CD3 antibodies.

[00202] In a specific embodiment, a composition comprises an anti-CD3 antibody, wherein said anti-CD3 antibody is a human or humanized monoclonal antibody. In yet another preferred embodiment, a composition comprises humanized OKT3, a analog, derivative, fragment thereof that immunospecifically binds to CD3 polypeptides.

[00203] In a preferred embodiment, a composition of the invention is a pharmaceutical composition. Such compositions comprise a prophylactically or therapeutically effective amount of one or more anti-CD3 antibodies, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory

agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete)), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like (*See*, for example, Handbook of Pharmaceutical Excipients, Arthur H. Kibbe (ed., 2000, which is incorporated by reference herein in its entirety), Am. Pharmaceutical Association, Washington, DC. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained release formulations and the like. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a prophylactically or therapeutically effective amount of a prophylactic or therapeutic agent preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. In a preferred embodiment, the pharmaceutical compositions are sterile and in suitable form for administration to a subject, preferably an animal subject, more preferably a mammalian subject, and most preferably a human subject.

[00204] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering an anti-CD3 antibody, care must be taken to use materials to which the anti-CD3 antibody does not absorb.

[00205] In another embodiment, the composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat *et al.*, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New

York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 3 17-327; see generally ibid., each of which is hereby incorporated by reference herein in its entirety).

[00206] In yet another embodiment, the composition can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:20; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574, each of which is hereby incorporated by reference herein in its entirety). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the antibodies of the invention or fragments thereof (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 7 1:105); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253, each of which is hereby incorporated by reference herein in its entirety. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the therapeutic target, *i.e.*, the lungs, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984), which is hereby incorporated by reference herein in its entirety).

[00207] Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533, which is hereby incorporated by reference herein in its entirety). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more antibodies of the invention or fragments thereof. See, *e.g.*,.U.S. Patent No. 4,526,938, .PCT publication WO 91/05548, PCT publication WO 96/20698,.Ning *et al.*, 1996, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," Radiotherapy & Oncology 39:179-189,.Song *et al.*, 1995, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," PDA Journal of Pharmaceutical

Science & Technology 50:372-397, Cleek *et al.*, 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam *et al.*, 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in their entirety.

[00208] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), intranasal, transdermal (topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal or topical administration to human beings. In a preferred embodiment, a pharmaceutical composition is formulated in accordance with routine procedures for subcutaneous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocamne to ease pain at the site of the injection.

[00209] If the compositions of the invention are to be administered topically, the compositions can be formulated in the form of, *e.g.*, an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, *e.g.*, Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 4th ed., Lea & Febiger, Philadelphia, PA (1985), which is hereby incorporated by reference herein in its entirety. For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity preferably greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (*e.g.*, a gaseous propellant, such as freon), or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

[00210] If the compositions of the invention are to be administered intranasally, the compositions can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00211] If the compositions of the invention are to be administered orally, the compositions can be formulated orally in the form of, *e.g.*, tablets, capsules, cachets, gelcaps, solutions, suspensions and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release or sustained release of a prophylactic or therapeutic agent(s).

[00212] The compositions of the invention may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[00213] The compositions of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[00214] In addition to the formulations described previously, the compositions of the invention may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[00215] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[00216] Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00217] In particular, the invention provides that one or more anti-CD3 antibodies, or pharmaceutical compositions of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the anti-CD3 antibodies, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, one or more of the anti-CD3 antibodies, or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be stored at between 2 and 8°C in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be administered within 1 week, preferably within 5 days, within 72 hours, within 48 hours,

within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the anti-CD3 antibodies, or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. Preferably, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, more preferably at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml or at least 100 mg/ml. The liquid form should be stored at between 2°C and 8°C in its original container.

[00218] In a preferred embodiment, the invention provides that the composition of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of anti-CD3 antibody.

[00219] The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack.

[00220] Generally, the ingredients of the compositions of the invention are derived from a subject that is the same species origin or species reactivity as recipient of such compositions. Thus, in a preferred embodiment, human or humanized antibodies are administered to a human patient for therapy or prophylaxis.

[00221] The amount of the composition of the invention which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with an inflammatory disease or autoimmune disorder can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[00222] For antibodies, proteins, polypeptides, peptides and fusion proteins encompassed by the invention, the dosage administered to a patient is typically 0.0001 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.0001 mg/kg and 20 mg/kg, 0.0001 mg/kg and 10 mg/kg, 0.0001 mg/kg and 5 mg/kg, 0.0001 and 2 mg/kg, 0.0001 and 1 mg/kg, 0.0001 mg/kg and 0.75 mg/kg, 0.0001 mg/kg and 0.5 mg/kg, 0.0001 mg/kg to 0.25 mg/kg, 0.0001 to 0.15 mg/kg, 0.0001 to 0.10 mg/kg, 0.001 to 0.5 mg/kg, 0.01 to 0.25 mg/kg or 0.01 to 0.10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies

and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention or fragments thereof may be reduced by enhancing uptake and tissue penetration of the antibodies by modifications such as, for example, lipidation.

5.4 Characterization of Anti-CD3 Therapeutic or Prophylactic Utility

[00223] CD3 binding molecules may be characterized in a variety of ways. In particular, CD3 binding molecules may be assayed for the ability to immunospecifically bind to a CD3 polypeptide. Such an assay may be performed in solution (*e.g.*, Houghten, 1992, Bio/Techniques 13:412-421), on beads (Lam, 1991, Nature 354:82-84), on chips (Fodor, 1993, Nature 364:555-556), on bacteria (U.S. Patent No. 5,223,409), on spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (Cull et al., 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869) or on phage (Scott and Smith, 1990, Science 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici, 1991, J. Mol. Biol. 222:301-310) (each of these references is incorporated herein in its entirety by reference). CD3 binding molecules that have been identified to immunospecifically bind to a CD3 polypeptide can then be assayed for their specificity and affinity for a CD3 polypeptide.

[00224] CD3 binding molecules may be assayed for immunospecific binding to a CD3 polypeptide and cross-reactivity with other polypeptides by any method known in the art. Immunoassays which can be used to analyze immunospecific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[00225] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the CD3 binding molecule of interest to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the CD3 binding molecule of

interest to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the CD3 binding molecule to a CD3 polypeptide and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1, which is hereby incorporated by reference herein in its entirety.

[00226] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), blocking the membrane with CD3 binding molecule of interest (*e.g.*, an antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with an antibody (which recognizes the CD3 binding molecule) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*, ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the CD3 polypeptide. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1, which is hereby incorporated by reference herein in its entirety.

[00227] ELISAs comprise preparing CD3 polypeptide, coating the well of a 96 well microtiter plate with the CD3 polypeptide, adding the CD3 binding molecule of interest conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the CD3 polypeptide. In ELISAs the CD3 binding molecule of interest does not have to be conjugated to a detectable compound; instead, an antibody (which recognizes the CD3 binding molecule of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the CD3 polypeptide, the CD3 binding molecule may be coated to the well. In this case, an antibody conjugated to a detectable compound may be added following the addition of the CD3 polypeptide to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion

regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1, which is hereby incorporated by reference herein in its entirety.

[00228] The binding affinity of a CD3 binding molecule to a CD3 polypeptide and the off-rate of an CD3 binding molecule-CD3 polypeptide interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled CD3 polypeptide (*e.g.*, ^{3}H or ^{125}I) with the CD3 binding molecule of interest in the presence of increasing amounts of unlabeled CD3 polypeptide, and the detection of the CD3 binding molecule bound to the labeled CD3 polypeptide. The affinity of a CD3 binding molecule for a CD3 polypeptide and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second CD3 binding molecule can also be determined using radioimmunoassays. In this case, a CD3 polypeptide is incubated with a CD3 binding molecule conjugated to a labeled compound (*e.g.*, ^{3}H or ^{125}I) in the presence of increasing amounts of a second unlabeled CD3 binding molecule.

[00229] In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of CD3 binding molecules to a CD3 polypeptide. BIAcore kinetic analysis comprises analyzing the binding and dissociation of a CD3 polypeptide from chips with immobilized CD3 binding molecules on their surface.

[00230] The CD3 binding molecules, in particular anti-CD3 antibodies, and compositions of the invention can also be assayed for their ability to modulate T cell activation. T cell activation can be determined by measuring, *e.g.*, changes in the level of expression of cytokines and/or T cell activation markers. Techniques known to those of skill in the art, including, but not limited to, immunoprecipitation followed by western blot analysis, ELISAs, flow cytometry, Northern blot analysis, and RT-PCR can be used to measure the expression cytokines and T cell activation markers. In a preferred embodiment, a CD3 binding molecule or composition of the invention is tested for its ability to induce the expression of IFN-γ and/or IL-2.

[00231] The anti-CD3 antibodies, and compositions of the invention can also be assayed for their ability to induce T cell signaling. The ability of an anti-CD3 antibody or a composition of the invention induce T cell signaling can be assayed, *e.g.*, by kinase assays and electrophoretic shift assays (EMSAs).

[00232] The anti-CD3 antibodies, and compositions of the invention can be tested *in vitro* or *in vivo* for their ability to modulate T cell proliferation. For example, the ability of an anti-CD3 antibody or a composition of the invention to modulate T cell proliferation can be assessed by, *e.g.*, ^{3}H-thymidine incorporation, trypan blue cell counts, and fluorescence activated cell sorting (FACS).

[00233] The anti-CD3 antibodies, and compositions of the invention can be tested *in vitro* or *in vivo* for their ability to induce cytolysis. For example, the ability of a an anti-CD3 antibody or a composition of the invention to induce cytolysis can be assessed by, *e.g.*, ^{51}Cr-release assays.

[00234] The anti-CD3 antibodies, and compositions of the invention can be tested *in vitro* or *in vivo* for their ability to mediate the depletion of peripheral blood T cells. For example, the ability of an anti-CD3 antibody or a composition of the invention to mediate the depletion of peripheral blood T cells can be assessed by, *e.g.*, measuring T cell counts using flow cytometry analysis.

[00235] The anti-CD3 antibodies, and compositions of the invention can be tested *in vivo* for their ability to mediate peripheral blood lymphocyte counts. For example, the ability of an anti-CD3 antibody or a composition of the invention to mediate peripheral blood lymphocyte counts can be assessed by, *e.g.*, obtaining a sample of peripheral blood from a subject, separating the lymphocytes from other components of peripheral blood such as plasma using, *e.g.*, a Ficoll gradient, and counting the lymphocytes using trypan blue.

5.4.1 Characterization of Immunoglobulin Molecules with Variant Fc Regions

[00236] In preferred embodiments, characterization of molecules comprising variant Fc regions with altered FcγR affinities (*e.g.*, null FcγR binding) are done with one or more biochemical based assays, preferably in a high throughput manner. The one or more biochemical assays can be any assay known in the art for identifying Fc-FcγR interaction, *i.e.*, specific binding of an Fc region to an FcγR, including, but not limited to, an ELISA assay, surface plasmon resonance assays, immunoprecipitation assay, affinity chromatography, and equilibrium dialysis. The functional based assays can be any assay known in the art for characterizing one or more FcγR mediated effector cell functions. Comparison of antibodies with altered Fc regions of the invention to control antibodies provides a measure of the extent of decrease or elimination of Fc-FcγR interaction. Non-limiting examples of effector cell functions that can be used in accordance with the methods of the invention, include but are not limited to, antibody-dependent cell mediated cytotoxicity (ADCC), antibody-dependent phagocytosis, phagocytosis, opsonization, opsonophagocytosis, cell binding, rosetting, C1q binding, and complement dependent cell mediated cytotoxicity. In preferred embodiments, characterization of molecules comprising variant Fc regions with altered FcγR affinities (*e.g.*, null FcR binding) are done with one or more biochemical based assays in combination or in parallel with one or more functional based assays, preferably in a high throughput manner.

[00237] In some embodiments, characterization of molecules comprising variant Fc regions with altered FcγR affinities (*e.g.*, null FcγR binding) comprise: characterizing the binding of the molecule comprising the variant Fc region to a FcγR (one or more), using a biochemical assay for determining Fc-FcγR interaction, preferably, an ELISA based assay followed by comparision of the results to the results of the same assay obtained with a control, *i.e.* non-modified, antibody. Once the molecule comprising a variant Fc region has been characterized for its interaction with one or more FcγRs and determined to have null binding to one or more FcγRs, by at least one biochemical based assay, *e.g.*, an ELISA assay, the molecule maybe engineered into a complete immunoglobulin, using standard recombinant DNA technology methods known in the art, and the immunoglobulin comprising the variant Fc region expressed in mammalian cells for further biochemical characterization. The immunoglobulin into which a variant Fc region of the invention is introduced (*e.g.*, replacing the Fc region of the immunoglobulin) can be any immunoglobulin including, but not limited to, polyclonal antibodies, monoclonal antibodies, bispecific antibodies, multi-specific antibodies, humanized antibodies, and chimeric antibodies. In preferred embodiments, a variant Fc region is introduced into an immunoglobulin specific for the CD3 complex associated with the human TCR.

[00238] The variant Fc regions, preferably in the context of an immunoglobulin, can be further characterized using one or more biochemical assays and/or one or more functional assays, preferably in a high throughput manner. In some alternate embodiments, the variant Fc regions are not introduced into an immunoglobulin and are further characterized using one or more biochemical based assays and/or one or more functional assays, preferably in a high throughput manner. The one or more biochemical assays can be any assay known in the art for identifying Fc-FcγR interactions, including, but not limited to, an ELISA assay, and surface plasmon resonance-based assay for determining the kinetic parameters of Fc-FcγR interaction, *e.g.*, BIAcore assay. The one or more functional assays can be any assay known in the art for characterizing one or more FcγR mediated effector cell function as known to one skilled in the art or described herein. In specific embodiments, the immunoglobulins comprising the variant Fc regions are assayed in an ELISA assay for binding to one or more FcγRs, *e.g.*, FcγRIIIA, FcγRIIA, FcγRIIA; followed by one or more ADCC assays. In some embodiments, the immunoglobulins comprising the variant Fc regions are assayed further using a surface plasmon resonance-based assay, *e.g.*, BIAcore. For further a detailed discussion of characterization of immunoglubulins comprising variant Fc regions see U.S. Pat. Appl. Pub. No. 2005/0064514 A1 and U.S. Pat. Appl. Pub. No. 20050037000 A1.

[00239] The immunoglobulin comprising the variant Fc regions may be analyzed at any point using a surface plasmon based resonance based assay, *e.g.*, BIAcore, for defining the kinetic parameters of the Fc-FcγR interaction, using methods known to those of skill in the art.

[00240] In most preferred embodiments, the immunoglobulin comprising the variant Fc regions is further characterized in an animal model for interaction with an FcγR. Preferred animal models for use in the methods of the invention are, for example, transgenic mice expressing human FcγRs, *e.g.*, any mouse model described in U.S. Patent No. 5,877,397, which is incorporated herein by reference in its entirety. Transgenic mice for use in the methods of the invention include, but are not limited to, nude knockout FcγRIIIA mice carrying human FcγRIIIA; nude knockout FcγRIIIA mice carrying human FcγRIIA; nude knockout FcγRIIIAmice carrying human FcγRIIB and human FcγRIIIA; nude knockout FcγRIIIA mice carrying human FcγRIIB and human FcγRIIA.

5.4.2 In Vitro and In Vivo Characterization

[00241] Several aspects of the pharmaceutical compositions or the anti-CD3 antibodies of the invention are preferably tested *in vitro*, in a cell culture system, and in an animal model organism, such as a rodent animal model system, for the desired therapeutic activity prior to use in humans. For example, assays which can be used to determine whether administration of a specific pharmaceutical composition is indicated, include cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise contacted with a pharmaceutical composition, and the effect of such composition upon the tissue sample is observed. The tissue sample can be obtained by biopsy from the patient. This test allows the identification of the therapeutically most effective tumor-targeted bacteria and the therapeutically most effective therapeutic molecule(s) for each individual patient. In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in an autoimmune or inflammatory disorder (*e.g.*, T cells), to determine if a pharmaceutical composition of the invention has a desired effect upon such cell types.

[00242] In accordance with the invention, clinical trials with human subjects need not be performed in order to demonstrate the prophylactic and/or therapeutic efficacy of anti-CD3 antibodies. *In vitro* and animal model studies using anti-CD3 antibodies can be extrapolated to humans and are sufficient for demonstrating the prophylactic and/or therapeutic utility of said anti-CD3 antibodies.

[00243] Anti-CD3 antibodies can be tested in suitable animal model systems prior to use in humans. Such animal model systems include, but are not limited to, rats, mice, chicken, cows, monkeys, pigs, dogs, rabbits, etc. Any animal system well-known in the art may be used. In a specific embodiment of the invention, CD3 binding molecules are tested in a mouse model

system. Such model systems are widely used and well-known to the skilled artisan. CD3 binding molecules can be administered repeatedly. Several aspects of the procedure may vary. Said aspects include the temporal regime of administering CD3 binding molecules, and whether such agents are administered separately or as an admixture.

[00244] The anti-inflammatory activity of anti-CD3 antibodies or pharmaceutical compositions of invention can be determined by using various experimental animal models of inflammatory arthritis known in the art and described in Crofford L.J. and Wilder R.L., "Arthritis and Autoimmunity in Animals", in Arthritis and Allied Conditions: A Textbook of Rheumatology, McCarty *et al.*(eds.), Chapter 30 (Lee and Febiger, 1993), which is hereby incorporated by reference herein in its entirety. Experimental and spontaneous animal models of inflammatory arthritis and autoimmune rheumatic diseases can also be used to assess the anti-inflammatory activity of anti-CD3 antibodies or pharmaceutical compositions of invention. The following are some assays provided as examples and not by limitation.

[00245] The principle animal models for arthritis or inflammatory disease known in the art and widely used include: adjuvant-induced arthritis rat models, collagen-induced arthritis rat and mouse models and antigen-induced arthritis rat, rabbit and hamster models, all described in Crofford L.J. and Wilder R.L., "Arthritis and Autoimmunity in Animals", in Arthritis and Allied Conditions: A Textbook of Rheumatology, McCarty *et al.*(eds.), Chapter 30 (Lee and Febiger, 1993), incorporated herein by reference in its entirety. A collagen-induced arthritis (CIA) is an animal model for the human autoimmune disease rheumatoid arthritis (RA) (Trenthorn et al., 1977, J. Exp. Med.146:857, which is hereby incorporated by reference herein in its entirety). This disease can be induced in many species by the administration of heterologous type II collagen (Courtenay et al., 1980, Nature 283:665; and Cathcart et at, 1986, Lab. Invest.54:26); with respect to animal models of arthritis see, in addition, *e.g.*, Holmdahl, R., 1999, Curr. Biol. 15:R528-530 (each of these references is hereby incorporated by reference herein in its entirety).

[00246] Additionally, animal models for inflammatory bowel disease can also be used to assess the efficacy of the anti-CD3 antibodies or pharmaceutical compositions of invention (Kim eta l., 1992, Scand. J. Gastroentrol. 27:529-537; Strober, 1985, Dig. Dis. Sci. 30(12 Suppl):3S-10S, each of which is hereby incorporated by reference herein in its entirety). Ulcerative cholitis and Crohn's disease are human inflammatory bowel diseases that can be induced in animals. Sulfated polysaccharides including, but not limited to amylopectin, carrageen, amylopectin sulfate, and dextran sulfate or chemical irritants including but not limited to trinitrobenzenesulphonic acid (TNBS) and acetic acid can be administered to animals orally to induce inflammatory bowel diseases.

[00247] Animal models for asthma can also be used to assess the efficacy of anti-CD3 antibodies or pharmaceutical compositions of invention. An example of one such model is the murine adoptive transfer model in which aeroallergen provocation of TH1 or TH2 recipient mice results in TH effector cell migration to the airways and is associated with an intense neutrophilic (TH1) and eosinophilic (TH2) lung mucosal inflammatory response (Cohn et al., 1997, J. Exp. Med. 1861737-1747, which is hereby incorporated by reference herein in its entirety).

[00248] Animal models for autoimmune disorders can also be used to assess the efficacy of anti-CD3 antibodies or pharmaceutical compositions of invention. Animal models for autoimmune disorders such as type 1 diabetes, thyroid autoimmunity, sytemic lupus eruthematosus, and glomerulonephritis have been developed (Bluestone et al., 2004, PNAS 101:14622-14626; Flanders et al., 1999, Autoimmunity 29:235-246; Krogh et al., 1999, Biochimie 81:511-515; Foster, 1999, Semin. Nephrol. 19:12-24, each of which is hereby incorporated by reference herein in its entirety).

[00249] The efficacy of anti-CD3 antibodies or pharmaceutical compositions of invention can also be tested in such autoimmune disorder models as an experimental allergic encephalomyelitis (EAE) model. EAE is an experimental autoimmune disease of the central nervous system (CNS) (Zamvil et al, 1990, Ann. Rev, Immunol. 8:579, which is hereby incorporated by reference herein in its entirety) and is a disease model for the human autoimmune condition, multiple sclerosis (MS). EAE is an example of a cell-mediated autoimmune disorder that is mediated via T cells. EAE is readily induced in mammalian species by immunizations of myelin basic protein (MBP) purified from the CNS or an encephalitogenic proteolipid (PLP). SJL/J mice are a susceptible strain of mice (H-2u) and, upon induction of EAE, these mice develop an acute paralytic disease and an acute cellular infiltrate is identifiable within the CNS. EAE spontaneously develops in MBP1-17 peptide-specific T cell receptor (TCR) transgenic mice (TgMBP+) of a RAG-1-deficient background (Lafaille et al., 1994, Cell 78:399, which is hereby incorporated by reference herein in its entirety).

[00250] Further, any assays known to those skilled in the art can be used to evaluate anti-CD3 antibodies or the pharmaceutical compositions disclosed herein for autoimmune and/or inflammatory diseases.

[00251] The toxicity and/or efficacy of anti-CD3 antibodies or pharmaceutical compositions of invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Anti-CD3 antibodies that exhibit large therapeutic indices are preferred.

While anti-CD3 antibodies that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00252] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage of anti-CD3 antibodies for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00253] Efficacy in preventing or treating an autoimmune disorder may be demonstrated, *e.g.*, by detecting the ability of a anti-CD3 antibodies or composition of the invention to reduce one or more symptoms of the autoimmune disorder, to reduce mean absolute lymphocyte counts, to decrease T cell activation, to decrease T cell proliferation, to reduce cytokine production, or to modulate one or more particular cytokine profiles. Efficacy in treating diabetes may be demonstrated, *e.g.* by detecting the ability of a anti-CD3 antibodies or composition of the invention to reduce one or more symptoms of dibetes, to preserve the C-peptide response to MMTT, to reduce the level HA1 or HA1c, to reduce the daily requirement for insulin, or to decrease T cell activation in pancreatic islet tissue. Efficacy in preventing or treating an inflammatory disorder may be demonstrated, *e.g.*, by detecting the ability of a an anti-CD3 antibody to reduce one or more symptoms of the inflammatory disorder, to decrease T cell activation, to decrease T cell proliferation, to modulate one or more cytokine profiles, to reduce cytokine production, to reduce inflammation of a joint, organ or tissue or to improve quality of life.

[00254] Changes in inflammatory disease activity may be assessed through tender and swollen joint counts, patient and physician global scores for pain and disease activity, and the ESR/CRP. Progression of structural joint damage may be assessed by quantitative scoring of X-rays of hands, wrists, and feet (Sharp method). Changes in functional status in humans with inflammatory disorders may be evaluated using the Health Assessment Questionnaire (HAQ), and quality of life changes are assessed with the SF-36.

5.5 Methods of Monitoring Lymphocyte Counts and Percent Binding

[00255] The effect of one or more doses of one or more the ability of a anti-CD3 antibodies or composition of the invention to reduce one or more symptoms of an autoimmune or donor-specific immune disorder on peripheral blood lymphocyte counts can be monitored/assessed using standard techniques known to one of skill in the art. Peripheral blood lymphocytes counts in a mammal can be determined by, *e.g.*, obtaining a sample of peripheral blood from said mammal, separating the lymphocytes from other components of peripheral blood such as plasma using, *e.g.*, Ficoll-Hypaque (Pharmacia) gradient centrifugation, and counting the lymphocytes using trypan blue. Peripheral blood T cell counts in mammal can be determined by, *e.g.*, separating the lymphocytes from other components of peripheral blood such as plasma using, *e.g.*, a use of Ficoll-Hypaque (Pharmacia) gradient centrifugation, labeling the T cells with an antibody directed to a T cell antigen such as CD2, CD3, CD4, and CD8 which is conjugated to FITC or phycoerythrin, and measuring the number of T cells by FACS. Further, the effect on a particular subset of T cells (*e.g.*, $CD2^+$, $CD4^+$, $CD8^+$, $CD4^+RO^+$, $CD8^+RO^+$, $CD4^+RA^+$, or $CD8^+RA^+$) cells can be determined using standard techniques known to one of skill in the art such as FACS.

[00256] The percentage of CD3 polypeptides expressed by peripheral blood lymphocytes bound by anti-CD3 antibodies prior or after, or both prior to and after the administration of one or more doses of anti-CD3 antibodies can be assessed using standard techniques known to one of skill in the art. The percentage of CD3 polypeptides expressed by peripheral blood T cells bound by anti-CD3 antibodies can be determined by, *e.g.*, obtaining a sample of peripheral blood from a mammal, separating the lymphocytes from other components of peripheral blood such as plasma using, *e.g.*, Ficoll-Hypaque (Pharmacia) gradient centrifugation, and labeling the T cells with an anti-CD3 binding molecule antibody other than that of the invention conjugated to FITC and an antibody directed to a T cell antigen such as CD3, CD4 or CD8 which is conjugated to phycoerythrin, and determining the number of T cells labeled with anti-CD3 binding molecule antibody relative to the number of T cells labeled with an antibody directed to a T cell antigen using FACS.

5.6 Methods of Producing Antibodies

[00257] Antibodies that immunospecifically bind to an CD3 polypeptide can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[00258] Polyclonal antibodies that immunospecifically bind to an antigen can be produced by various procedures well-known in the art. For example, a human antigen can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to

induce the production of sera containing polyclonal antibodies specific for the human antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[00259] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T cell Hybridomas 563 681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[00260] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. Briefly, mice can be immunized with a CD3 antigen and once an immune response is detected, *e.g.*, antibodies specific for a CD3 antigen (preferably, CD3 ε antigen) are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[00261] Accordingly, the present invention provides methods of generating antibodies by culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with a CD3 antigen with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind to a CD3 antigen (preferably, CD3 ε antigen).

[00262] Antibody fragments which recognize specific CD3 antigens (preferably, CD3 ε antigen) may be generated by any technique known to those of skill in the art. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain. Further, the antibodies of the present invention can also be generated using various phage display methods known in the art.

[00263] In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of affected tissues). The DNA encoding the VH and VL domains are recombined together with an scFv linker by PCR and cloned into a phagemid vector. The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to a particular antigen can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., 1995, J. Immunol. Methods 182:41-50; Ames et al., 1995, J. Immunol. Methods 184:177-186; Kettleborough et al., 1994, Eur. J. Immunol. 24:952-958; Persic et al., 1997, Gene 187:9-18; Burton et al., 1994, Advances in Immunology 57:191-280; PCT Application No. PCT/GB91/O1 134; International Publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and WO97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[00264] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described below. Techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication No. WO 92/22324; Mullinax et al., 1992, BioTechniques 12(6):864-869; Sawai et al., 1995, AJRI 34:26-34; and

Better et al., 1988, Science 240:1041-1043 (said references incorporated by reference in their entireties).

[00265] To generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a VH constant region, *e.g.*, the human gamma 4 constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, *e.g.*, human kappa or lamba constant regions. Preferably, the vectors for expressing the VH or VL domains comprise an EF-1α promoter, a secretion signal, a cloning site for the variable domain, constant domains, and a selection marker such as neomycin. The VH and VL domains may also cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

[00266] For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use human or chimeric antibodies. Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also U.S. Patent Nos. 4,444,887 and 4,716,111; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[00267] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the J_H region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then be bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a polypeptide

of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93, which is hereby incorporated by reference herein in its entirety). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.*, PCT publication Nos. WO 98/24893, WO 96/34096, and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318, and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[00268] A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison, 1985, Science 229:1202; Oi et al., 1986, BioTechniques 4:214; Gillies et al., 1989, J. Immunol. Methods 125:191-202; and U.S. Patent Nos. 5,807,715, 4,816,567, 4,816,397, and 6,331,415, which are incorporated herein by reference in their entirety.

[00269] A humanized antibody is an antibody or its variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a framework region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immuoglobulin. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non human immunoglobulin (*i.e.*, donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and lgG4. Usually the constant domain is a complement

fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG2 class. Examples of VL and VH constant domains that can be used in certain embodiments of the invention include, but are not limited to, C-kappa and C-gamma-1 (nG1m) described in Johnson et al. (1997) *J. Infect. Dis.* 176, 1215-1224 and those described in U.S. Patent No. 5,824,307, each of which is hereby incorporated by reference herein in its entirety. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art. The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, *e.g.*, the donor CDR or the consensus framework may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or framework residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often 90%, and most preferably greater than 95%. Humanized antibody can be produced using variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239,400; International publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering 7(6):805-814; and Roguska et al., 1994, PNAS 91:969-973), chain shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, *e.g.*, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, WO 9317105, Tan et al., J. Immunol. 169:1119 25 (2002), Caldas et al., Protein Eng. 13(5):353-60 (2000), Morea et al., Methods 20(3):267 79 (2000), Baca et al., J. Biol. Chem. 272(16):10678-84 (1997), Roguska et al., Protein Eng. 9(10):895 904 (1996), Couto et al., Cancer Res. 55 (23 Supp):5973s- 5977s (1995), Couto et al., Cancer Res. 55(8):1717-22 (1995), Sandhu JS, Gene 150(2):409-10 (1994), Pedersen et al., J. Mol. Biol. 235(3):959-73 (1994). and U.S. Patent Pub. No. US 2005/0042664 A1 (Feb. 24, 2005), each of which is incorporated by reference herein in its entirety. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen et al., U.S. Patent No. 5,585,089; and Riechmann et al., 1988, Nature 332:323, which are incorporated herein by reference in their entireties.)

[00270] Single domain antibodies, for example, antibodies lacking the light chains, can be produced by methods well-known in the art. See Riechmann et al., 1999, J. Immuno. 231:25-38; Nuttall et al., 2000, Curr. Pharm. Biotechnol. 1(3):253-263; Muylderman, 2001, J. Biotechnol. 74(4):277302; U.S. Patent No. 6,005,079; and International Publication Nos. WO 94/04678, WO 94/25591, and WO 01/44301, each of which is incorporated herein by reference in its entirety.

5.7 Polynucleotides Encoding Antibodies

[00271] The invention provides polynucleotides comprising a nucleotide sequence encoding an antibody that immunospecifically binds to a CD3 polypeptide. The invention also encompasses polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions, *e.g.*, as defined *supra*, to polynucleotides that encode an antibody of the invention.

[00272] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. The nucleotide sequence of antibodies immunospecific for a CD3 polypeptide can be obtained, *e.g.*, from the literature or a database such as GenBank. Since the amino acid sequences of, *e.g.*, humanized OKT3 is known, nucleotide sequences encoding these antibodies can be determined using methods well known in the art, *i.e.*, nucleotide codons known to encode particular amino acids are assembled in such a way to generate a nucleic acid that encodes the antibody. Such a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, BioTechniques 17:242, which is hereby incorporated by reference herein in its entirety), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[00273] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and *5'* ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone

from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[00274] Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[00275] In a specific embodiment, one or more of the CDRs is inserted within framework regions using routine recombinant DNA techniques. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, *e.g.*, Chothia et al., 1998, J. Mol. Biol. 278: 457-479 (which is hereby incorporated by reference herein in its entirety) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds to a CD3 polypeptide. Preferably, as discussed *supra*, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

5.8 RECOMBINANT EXPRESSION OF MOLECULES OF THE INVENTION

[00276] Once a nucleic acid sequence encoding molecules of the invention (*i.e.*, antibodies) has been obtained, the vector for the production of the molecules may be produced by recombinant DNA technology using techniques well known in the art. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequences for the molecules of the invention and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. (*See*, for example, the techniques described in Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.* eds., 1998,

Current Protocols in Molecular Biology, John Wiley & Sons, NY, each of which is hereby incorporated by reference herein in its entirety).

[00277] An expression vector comprising the nucleotide sequence of a molecule identified by the methods of the invention (*i.e.*, an antibody) can be transferred to a host cell by conventional techniques (*e.g.*, electroporation, liposomal transfection, and calcium phosphate precipitation) and the transfected cells are then cultured by conventional techniques to produce the molecules of the invention. In specific embodiments, the expression of the molecules of the invention is regulated by a constitutive, an inducible or a tissue, specific promoter. In specific embodiments the expression vector pMGX1303 (FIG. 4).

[00278] The host cells used to express the molecules identified by the methods of the invention may be either bacterial cells such as *Escherichia coli,* or, preferably, eukaryotic cells, especially for the expression of whole recombinant immunoglobulin molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for immunoglobulins (Foecking *et al.*, 1998, *Gene* 45:101; Cockett *et al.*, 1990, *Bio/Technology* 8:2, each of which is hereby incorporated by reference herein in its entirety).

[00279] A variety of host-expression vector systems may be utilized to express the molecules identified by the methods of the invention. Such host-expression systems represent vehicles by which the coding sequences of the molecules of the invention may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the molecules of the invention *in situ.* These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing coding sequences for the molecules identified by the methods of the invention; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing sequences encoding the molecules identified by the methods of the invention; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the sequences encoding the molecules identified by the methods of the invention; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing sequences encoding the molecules identified by the methods of the invention; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 293T, 3T3 cells, lymphotic cells (*see* U.S. 5,807,715), Per C.6 cells (human retinal cells developed by Crucell) harboring recombinant expression constructs containing

promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[00280] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, *EMBO J.* 2:1791, which is hereby incorporated by reference herein in its entirety), in which the antibody coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509, each of which is hereby incorporated by reference herein in its entirety); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free gluta-thione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00281] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (*e.g.*, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (*e.g.*, the polyhedrin promoter).

[00282] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the immunoglobulin molecule in infected hosts (*e.g.*, *see* Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359, which is hereby incorporated by reference herein in its entirety). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous

translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, *etc.* (*see* Bittner *et al.*, 1987, *Methods in Enzymol.* 153:51-544, which is hereby incorporated by reference herein in its entirety).

[00283] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 293T, 3T3, WI38, BT483, Hs578T, HTB2, BT20 and T47D, CRL7030 and Hs578Bst.

[00284] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express an antibody of the invention may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibodies of the invention. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibodies of the invention.

[00285] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler *et al.*, 1977, *Cell* 11: 223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1992, *Proc. Natl. Acad. Sci. USA* 48: 202), and adenine phosphoribosyltransferase (Lowy *et al.*, 1980, *Cell* 22: 817) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler *et al.*, 1980, *Proc. Natl. Acad. Sci. USA* 77:357; O'Hare *et al.*, 1981, *Proc. Natl. Acad. Sci. USA*

78: 1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci.* USA 78: 2072); neo, which confers resistance to the aminoglycoside G-418 *Clinical Pharmacy* 12: 488-505; Wu and Wu, 1991, 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIB TECH* 11(5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli *et al.* (eds), 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY.; Colberre-Garapin *et al.*, 1981, *J. Mol. Biol.* 150:1; and hygro, which confers resistance to hygromycin (Santerre *et al.*, 1984, *Gene* 30:147) (each of said references is hereby incorporated by reference herein in its entirety).

[00286] The expression levels of an antibody of the invention can be increased by vector amplification (for a review, *see* Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3 (Academic Press, New York, 1987) (which is hereby incorporated by reference herein in its entirety). When a marker in the vector system expressing an antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse *et al.*, 1983, *Mol. Cell. Biol.* 3:257).

[00287] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197, each of which is hereby incorporated by reference herein in its entirety). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[00288] Once a molecule of the invention (*i.e.*, antibodies) has been recombinantly expressed, it may be purified by any method known in the art for purification of polypeptides or antibodies, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation,

differential solubility, or by any other standard technique for the purification of polypeptides or antibodies.

6. EQUIVALENTS

[00289] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00290] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

WHAT IS CLAIMED:

1. A method of treating an autoimmune disorder or ameliorating the symptoms thereof in a patient in need thereof, said method comprising administering to said patient a therapeutically effective amount of an anti-CD3 antibody that has at least 50% reduced binding to at least one FcγR than an antibody with a wild type Fc domain, wherein said administration results in minimal toxicity.

2. A method of treating an autoimmune disorder or ameliorating the symptoms thereof in a patient in need thereof, said method comprising administering to said patient a therapeutically effective amount of an anti-CD3 antibody that has at least 50% reduced binding to at least one FcγR than an antibody with a wild type Fc domain, wherein said administration reduces the symptoms of cytokine release syndrome relative to an equivalent administration of murine OKT3.

3. The method of claim 1 or 2 wherein said antibody does not detectably bind to any FcγR.

4. The method of claim 1 or 2, wherein said antibody has at least 50% reduced binding to C1q.

5. The method of claim 1 or 2, wherein said antibody does not detectably bind to any complement related receptors.

6. The method of claim 1 or 2, wherein said antibody does not detectably bind to C1q.

7. The method of claim 1 or 2, wherein six months after said administration, said patient requires no increase in adjunctive therapy to manage said autoimmune disorder.

8. The method of claim 1 or 2, wherein said patient is in the early stages of said autoimmune disease.

9. The method of claim 1 or 2, wherein said anti-CD3 antibody is chimeric or humanized.

10. The method of claim 9, wherein said antibody is a humanized or chimerized version of OKT3, Leu-4, 500A2, CLB-T3/3, M291, YTH 12.5 or BMA030.

11. The method of claim 9, wherein said antibody is aglycosylated.

12. The method of claim 10, wherein said antibody is aglycosylated.

13. The method of claim 9, wherein said antibody comprises an Fc domain having an amino acid modification, wherein said modified Fc domain does not bind any FcγR.

14. The method of claim 10, wherein said antibody comprises an Fc domain having an amino acid modification, wherein said modified Fc domain does not bind any FcγR.

15. The method of claim 14, wherein said antibody is humanized OKT3γ1 ala-ala.

16. The method of claim 1 or 2, wherein said autoimmune disorder is Type 1 diabetes, psoriasis, rheumatoid arthritis, lupus, inflammatory bowel disease (IBD), ulcerative colitis, Crohn's disease, multiple sclerosis, effects form organ transplantation, or graft vs. host disease (GVHD).

17. The method of claim 16, wherein said antibody is aglycosylated.

18. The method of claim 16, wherein said antibody comprises an Fc domain comprising at least one amino acid modification, and wherein said modified Fc domain does not bind any FcγR.

19. The method of claim 16 wherein said autoimmune disorder is Type 1 diabetes.

20. The method of claim 19, wherein prior to said treatment, the HA1c of said patient is less than 7.5% or the C-peptide response of said patient to a mixed-meal tolerance test (MMTT) results in a mean area under the curve for the initial 4 hours of at least 100 pmol/ml.

21. The method of claim 19, wherein six months after said treatment the average daily dose of insulin of said patient increases by no more than 30 U/kg/day relative to the pre-treatment average daily dose.

22. The method of claim 19, wherein six months after said treatment the average daily dose of insulin of said patient increases by no more than 10 U/kg/day relative to the pre-treatment average daily dose.

23. The method of claim 19, wherein six months after said treatment the average daily dose of insulin of said patient has not increased relative to the pre-treatment average daily dose.

24. The method of claim 19, wherein six months after said treatment said patient requires an average daily dose of insulin of no more than 0.5 U/kg/day.

25. The method of claim 19, wherein six months after said treatment said patient requires an average daily dose of insulin of no more than 0.2 U/kg/day.

26. The method of claim 19, wherein six months after said treatment the HA1c of said patient is less than 7.5%.

27. The method of claim 19, wherein twelve months after said treatment the HA1c of said patient is less than 7.5%.

28. The method of claim 19, wherein twelve months after said treatment the C-peptide response of said patient to a MMTT decreases by no more than 10% relative to the pre-treatment response.

29. The method of claim 19, wherein twelve months after said treatment the C-peptide response of said patient to a MMTT decreases by no more than 5% relative to the pre-treatment response.

30. The method of claim 20, wherein six months after said treatment the HA1c of said patient is less than 7.5%.

31. The method of claim 20, wherein twelve months after said treatment the HA1c of said patient is less than 7.5%.

32. The method of claim 20, wherein twelve months after said treatment the C-peptide response of said patient to a MMTT decreases by no more than 10% relative to the pre-treatment response.

33. The method of claim 20, wherein twelve months after said treatment the C-peptide response of said patient to a MMTT decreases by no more than 5% relative to the pre-treatment response.

34. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on at least 4 consecutive days.

35. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on at least 5 consecutive days.

36. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on at least 6 consecutive days.

37. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on at least 7 consecutive days.

38. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on at least 8 consecutive days.

39. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on at least 9 consecutive days.

40. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on at least 10 consecutive days.

41. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on at least 11 consecutive days.

42. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on no more than 11 consecutive days.

43. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on no more than 10 consecutive days.

44. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on no more than 9 consecutive days.

45. The method of claim 16, wherein said treatment comprises administration of doses of said antibody on no more than 8 consecutive days.

46. The method of claim 16, wherein said treatment comprises administration of doses of said antibody on no more than 7 consecutive days.

47. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on no more than 6 consecutive days.

48. The method of claim 16, wherein said treatment comprises administration of doses of said antibody on no more than 5 consecutive days.

49. The method of claim 1 or 2, wherein said treatment comprises administration of doses of said antibody on no more than 4 consecutive days.

50. The method of claim 16, wherein said treatment comprises a dosage regimen comprising doses of increasing amounts of said antibody on at least the initial 3 days of said dosage regimen.

51. The method of claim 50, wherein said dosage regimen is 4 days or less.

52. The method of claim 50, wherein said dosage regimen is 5 days or less.

53. The method of claim 50, wherein said dosage regimen is 6 days or less.

54. The method of claim 50, wherein said dosage regimen is 7 days or less.

55. The method of claim 50, wherein said dosage regimen is 8 days or less.

56. The method of claim 50, wherein said dosage regimen is 9 days or less.

57. The method of claim 50, wherein said dosage regimen is 10 days or less.

58. The method of claim 50, wherein said dosage regimen is 11 days or less.

59. The method of claim 50, wherein said dosage regimen is 12 days or less.

60. The method of claim 50, wherein said dosage regimen is 13 days or less.

61. The method of claim 50, wherein said dosage regimen is 14 days or less.

62. The method of claim 50, wherein the dose on day 1 is approximately 1.6 μg/kg, the dose on day 2 is approximately 3.2 μg/kg, the dose on day 3 is approximately 6.5 μg/kg, the dose on day 4 is approximately 13 μg/kg, and the dose on subsequent days is approximately 26 μg/kg.

63. The method of claim 62, wherein said dosage regimen is 5 days or less.

64. The method of claim 62, wherein said dosage regimen is 6 days or less.

65. The method of claim 62, wherein said dosage regimen is 7 days or less.

66. The method of claim 62, wherein said dosage regimen is 8 days or less.

67. The method of claim 62, wherein said dosage regimen is 9 days or less.

68. The method of claim 62, wherein said dosage regimen is 10 days or less.

69. The method of claim 62, wherein said dosage regimen is 11 days or less.

70. The method of claim 62, wherein said dosage regimen is 12 days or less.

71. The method of claim 62, wherein said dosage regimen is 13 days or less.

72. The method of claim 62, wherein said dosage regimen is 14 days or less.

73. The method of claim 1 or 2, wherein said treatment comprises a multi-day dosage regimen in which, on at least the first day of said multi-day dosage regimen, said antibody is administered in roughly equal portions at intervals of at least 6 hours, at intervals of at least 8 hours, or at intervals of at least 12 hours.

74. The method of claim 1 or 2, wherein said treatment comprises a multi-day dosage regimen in which, on at least the first day of said multi-day dosage regimen, said antibody is administered in increasing portions at intervals of at least 6 hours, at intervals of at least 8 hours, or at intervals of at least 12 hours.

75. The method of claim 1 or 2, wherein said administering is selected from the group consisting of intravenous, intramuscular, subcutaneous or oral.

76. The method of claim 75, wherein said administering is intravenous and provided over a period of at least 30 minutes.

77. The method of claim 19, in which said administration is in combination with administration of insulin.

78. The method of claim 1 or 2, in which said administration is in combination with administration of an immunosuppressant.

79. The method of claim 19, in which said administration is in combination with administration of exenatide.

80. The method of claim 19, in which said administration is in combination with administration of pramlintide.

81. The method of claim 1 or 2, in which said administering does not result in EBV-induced lymphoproliferative diseases or lymphocyte counts less than 1000 lymphocytes/μl serum.

82. A method of producing a humanized anti-CD3 antibody, said method comprising:

(a) culturing CHO cells that have been transfected with the expression vector pMGX1303, or progeny thereof, in media under conditions suitable for expression of said anti-CD3 antibody; and

(b) recovering said anti-CD3 antibody from said media.

83. The method of claim 82, wherein said anti-CD3 antibody is OKT3γ1 (ala-ala).