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(54) Title: MOLECULES PREFERENTIALLY ASSOCIATED WITH EFFECTOR T CELLS AND METHODS OF THEIR USE

(57) Abstract: The present invention is based, at least in part, on the discovery of certain genes which are absent from T regulatory cells and present on effector T cells (Th1 and Th2), *e.g.*, Protein Kinase C Theta (PKC theta). Furthermore, a pathway essential for the production of inflammatory cytokines and cellular proliferation of inflammatory, effector T cells is not utilized by regulatory T cells. Accordingly, in one aspect the invention provides methods for promoting regulatory T cell function in immune cells relative to effector T cell function, comprising contacting immune cells with an agent that inhibits a protein kinase C theta pathway in the immune cells. In another aspect, the invention provides methods for treating a subject having a condition that would benefit from promoting regulatory T cell function relative to effector T cell function in the subject, comprising administering an agent that inhibits a protein kinase C theta pathway in immune cells of the subject. In still another aspect, the invention provides assays for screening compounds that specifically modulate an effector T cell function without modulating regulatory T cell function comprising contacting a protein kinase C theta pathway molecule with a test compound and determining the ability of the test compound to modulate the protein kinase C theta pathway molecule activity, wherein modulation of a protein kinase C theta pathway molecule activity indicates that the test compound is a specific modulator of an effector T cell function.

MOLECULES PREFERENTIALLY ASSOCIATED WITH EFFECTOR T CELLS AND METHODS OF THEIR USE

Related Applications

This application claims the benefit of U.S. Provisional Application, 60/467,477, filed May 2, 2003, titled "Methods for Promoting Regulatory T Cell Function in Immune Cells Relative to Effector T Cell Function". This application also claims the benefit of U.S. Provisional Application, 60/424,777, filed November 8, 2002, titled "Intracellular Proteins of Th1 and/or TH2 Cells and Regulation of Immune Responses." The entire contents of each of these applications are incorporated herein by reference

Background of the Invention

Protein kinase C (PKC) is a family of enzymes that are physiologically activated by 1,2-diacylglycerol (DAG) and other lipids. When activated, the isozymes bind to membrane phospholipids or to membrane receptors and anchor the enzymes in a subcellular compartment (reviewed in Liu and Heckman, *Cell. Signal.*, 1998, 10, 529-542).

Protein kinase C isozymes differ in number and expression level in different cell lines and tissues. To date, 11 different isozymes (alpha, betaI, betaII, gamma, delta, epsilon, nu, lambda, mu, theta and zeta) have been identified and they have been divided into three groups based on their differential expression patterns and cofactor requirements. Interest in protein kinase C as a therapeutic target was generated by the finding that it is the major cellular receptor through which a class of tumor-promoting agents, called phorbol esters, exert their pleiotropic effects on cells (Liu and Heckman, *Cell. Signal.*, 1998, 10, 529-542).

Protein kinase C theta (also known as PKC-theta, PKCT, PRKCT, nPKC-theta and PRKCQ), one of the novel serine/threonine protein kinase C isoforms (nPKC), is expressed ubiquitously in tissues with the highest levels found in hematopoietic cell lines, including T-cells and thymocytes (Baier *et al.*, *J. Biol. Chem.*, 1993, 268, 4997-5004; Keenan *et al.*, *Immunology*, 1997, 90, 557-563; Meller *et al.*, *Cell. Immunol.*, 1999, 193, 185-193; Wang *et al.*, *Biochem. Biophys. Res. Commun.*, 1993, 191, 240-246). This isozyme has been shown to be specifically responsible for antigen driven

activation events in peripheral T cells. Protein kinase C theta is not required for the development of T cells in the thymus, as Protein kinase C theta knock-out mice develop normal numbers of peripheral T cells. However, when these mice are challenged with an antigen, they fail to make a T cell response.

Summary of the Invention

The present invention is based, at least in part, on the finding that certain molecules are preferentially associated with effector T cells (Th1 andTh2) or regulatory T cells. For example, it has been found that protein kinase C theta (PKC theta) is preferentially expressed by cells of the Th1 and Th2 lineages. Accordingly, immune responses by one or the other subset of cells can be preferentially modulated. The invention pertains, *e.g.*, to methods of modulating (*e.g.*, up- or down-modulating), the balance between the activation of regulatory T cells and effector T cells leading to modulation of immune responses and to compositions useful in modulating those responses. The invention also pertains to methods useful in diagnosing, treating, or preventing conditions that would benefit from modulating effector T cell function relative to regulatory T cell function or from modulating regulatory T cell function relative to effector T cell function in a subject. The subject methods and compositions are especially useful in the diagnosis, treatment or prevention of conditions characterized by a too-vigorous effector T cell response to antigens associated with the condition, in the diagnosis, treatment or prevention of conditions characterized by a weak effector T cell response, in the diagnosis, treatment or prevention of conditions characterized by a too-vigorous regulatory T cell response, or in the diagnosis, treatment, or prevention of conditions characterized by a weak regulatory T cell response.

Accordingly, in one aspect, the invention pertains to a method for treating a condition in a subject in need of such treatment, comprising administering an agent that modulates the expression or activity of a protein kinase C theta pathway component, wherein the effect of such treatment is to modulate the balance of effector T cell function relative to regulatory T cell function in the subject. In one embodiment, the component is a nucleic acid selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, and 11.In another embodiment, the component is a polypeptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, and 12. In yet another embodiment, the

agent is a protein, peptide, small molecule or nucleic acid. In a further embodiment, the condition is a transplant, an allergic disorder, an autoimmune disorder, a viral infection, a microbial infection, a parasitic infection or cancer.

In another aspect, the invention pertains to a method for modulating the expression or activity of a protein kinase C theta pathway component, comprising: contacting a population of cells, the population of cells comprising one or more of the following: T cells; naïve T cells; regulatory T cells; effector T cells; or peripheral blood leukocytes, with an agent that modulates the expression or activity of a PKC theta pathway component, wherein the effect of such contacting is to modulate the balance of effector T cell function relative to regulatory T cell function in the population of cells. In one embodimemt the method further comprises administering the population of cells that have been contacted with an agent to a subject suffering from a condition, the effect of which is treat the condition. In another embodiment, the agent is protein, peptide, small molecule or nucleic acid. In a further embodiment, the condition is a transplant, an allergic disorder, an autoimmune disorder, a viral infection, a microbial infection, a parasitic infection or cancer.

In another aspect, the invention pertains to an assay for identifying agents modulating the expression or activity of a protein kinase C theta pathway component, comprising: contacting an indicator composition comprising a protein kinase C theta pathway component with a plurality of test agents; and, determining the ability of a test agent to modulate the expression or activity of a protein kinase C theta pathway component,

wherein the agent identified is able to modulate the balance of effector T cell function relative to regulatory T cell function. In one embodiment, the agent is a protein, peptide, small molecule or nucleic acid. In another embodiment, the indicator composition is a cell expressing a protein kinase C theta pathway component.

Brief Description of the Drawings

Figure 1 is a diagram of T cell activation pathways.

Figures 2A-C depict graphs which illustrate signals observed on the Affymetrix™ Gene Chip indicating expression of genes associated with the PKC theta signaling pathway in three cell types, Th1, Th2, and regulatory T cells. Figure 1A shows expression of PKC theta in Th1 and Th2 cells, but not regulatory T cells. Figure

1B shows expression of Bcl 10 in Th1 and Th2 cells, but not regulatory T cells. Figure 1C shows expression of CARMA1 in Th1 cells, but not regulatory T cells. "Absent" calls are indicated as no signal.

Figure 3 depicts results of staining of human lymphocytes with anti-TCR and anti-PKC theta antibodies in peripheral blood lymphocytes (PBL), Th1, Th2, and regulatory T cells. PBL or differentiated Th1, Th2 and regulatory T cells were stained with FITC- anti-TCR or HRP-anti-PKC theta followed by TRITC anti-HRP.

Figure 4 depicts inhibition of proliferation of Th1 and Th2 cells, but not regulatory T cells, by Rottlerin, a commercially available inhibitor of PKC enzymes. Differentiated cells were stimulated with CD3 and CD28 in the presence of absence of the PKC inhibitor Rottlerin. Incorporation of ^{3}H-thymidine was used to monitor cell proliferation. Proliferation of each cell type is normalized to the proliferation observed in the absence of inhibitor.

Figure 5 graphically depicts representative data showing that the antennapedia-PKCθ peptide selectively inhibits the proliferation of Th1 and Th2 but not TGFβ-derived Treg cells.

Detailed Description of the Invention

In classical immune responses, effector T cell (Teff) responses dominate over responses of T regulatory cells (Treg) resulting in antigen removal. Tolerance initiates with the same steps as the classical activation pathway (*i.e.*, antigen presentation and T cell activation), but factors including, but not limited to, the abundance of antigen, the means by which it is presented to the T cell, and the relative availability of CD4+ cell help lead to the proliferation of a distinct class of lymphocytes called regulatory T cells. Just as effector T cells mediate classical immune responses, regulatory T cells mediate tolerogenic responses. However, unwanted or misdirected immune responses, such as those associated with allergy, autoimmune diseases, organ rejection, chronic administration of therapeutic proteins and the like, can lead to conditions in the body which are undesirable and which, in some instances, can prove fatal. The dominance or shifting of balance of regulatory T cells over effector T cells results in antigen preservation and immunological tolerance.

The present invention is based, at least in part, on the identification of genes which are expressed differentially between effector T cells (Th1 and Th2) and regulatory T cells. Among the genes preferentially expressed by effector T cells are the genes for PKC theta and other protein members known to be required for signal transduction from PKC theta through NFκB in T cells (Figure 1). Protein members of the PKC theta pathway, including PKC theta, can be utilized to identify compounds, including but not limited to compounds which would be capable of blocking an unwanted immune response. A desired property of the identified compounds could include, but is not limited to the ability to affect the balance between effector T cells and regulatory T cells such that a regulatory T cell-mediated response is dominant. Development of such a dominant regulatory response would be capable of controlling and/or preventing future unwanted immune responses.

Because regulatory T cells are capable of activating and dividing in response to T cell receptor stimulation, but do not appear to utilize the PKC theta signaling system, compounds which selectively target and modulate, *e.g.*, downmodulate, PKC theta and members of this pathway are useful as preferential modulators of effector T cell responses. These compounds are useful in the treatment or prevention of conditions that would benefit from preferential modulation of, *e.g.*, promoting effector T cell function. In one embodiment, such compounds do not modulate a regulatory T cell response (or modulating such responses in a favorable direction, e.g., through the use of an additional agent or protocol) function in a subject. Likewise, these compounds are useful in the treatment of prevention of conditions characterized by too-vigorous effector T cell responses and which would be helped by the simultaneous development of a robust regulatory T cell response to antigens associated with the condition.

In one embodiment of the invention, any of the members of the PKC theta pathway (*e.g.*, see Figure 1) may be expressed and used in screening assays, *e.g.*, high throughput screening assays, to identify compounds which would bind to and inhibit the function of these proteins. Blockade of this pathway preferentially inhibits inflammatory responses. Therefore, compounds directed to this pathway would be capable of reducing, preventing or halting unwanted inflammatory responses, *e.g.*, the destruction of organ transplants, while minimally affecting the T regulatory cell population, or resulting in a net positive effect on the T regulatory population. In one

embodiment, such compounds allow the desirable expansion of the regulatory T cell population, which would ultimately control all future attacks on the transplanted organ without additional compounds.

These compounds would also be useful in halting autoimmune attack in a number of diseases such as Multiple Sclerosis, Systemic Lupus, or inflammatory bowel syndromes, for example. As in the case of transplant rejection, for example, these drugs would halt tissue destruction by the effector T cells, while permitting the regulatory arm of the immune system to re-exert dominance and eventually control the disease in the absence of additional drug treatment.

Regulatory T cells have also been shown to function to control antibody responses. Some autoimmune diseases are mediated in large part by autoantibodies. Because this therapy would inhibit the T cell help provided to B cells by effector T cells, it would also be useful in treating autoantibody mediated autoimmune diseases such as Myasthenia Gravis.

In one embodiment of the invention, unlike currently used immunosuppressives, the compounds described herein only need to be administered over a short term course of therapy, rather than an intermediate course of therapy or an extended or prolonged course of therapy, to control unwanted immune responses, because they foster development of a homeostatic immunoregulatory mechanism. In one embodiment of the
invention the compounds described herein may be administered in multiple rounds of a short course of therapy. The compounds described herein may be administered in two rounds of therapy or three rounds of therapy or more than three rounds of therapy. In another embodiment of the invention a test may be administered to a patient receiving the therapy
to determine the efficacy of said therapy to determine if an additional course of therapy is needed. The tests administered may include by are not limited to a biopsy, a blood test, an assay to determine the proper functioning of, e.g., a renal transplant, an X-ray, an MRI or a physical examination. Because the resulting immunoregulation would be mediated by natural T cell mechanisms, the need for additional drugs is needed to maintain immunoregulation can be reduced or eliminated once the dominant regulatory T cell response is established. In one embodiment, elimination of prolonged or life-long treatment with immunosuppressants is achieved and will eliminate many, if not all, side

effects currently associated with treatment of, for example, autoimmunity and organ grafts.

As can been seen in Figure 1, activation of the T cell requires signaling through both the T cell receptor for antigen (TCR) and CD28. The CD4 molecule provides additional kinase signals resulting in a complete, strong cellular response. Among the molecules phosphorylated by these early T cell activating events is the adaptor protein vav. Phosphorylated vav has been shown to interact with adhesion molecules to alter cell shape and also serves to activate PKC theta. Activated PKC theta migrates to the cell membrane where it attaches to a scaffolding protein, CARMA1. Also interacting with CARMA1 is the protein Bcl 10. Bcl 10 is phosphorylated by PKC theta and is then able to release IκB, an inhibitory molecule, from NFκB, thereby activating NFκB. Activated NFκB then enters the nucleus where it binds to specific sites on the DNA, resulting in transcription of mRNA for genes coding for many of the molecules characteristic of and mediating the inflammatory immune response.

I. *Definitions*

As used herein, the term "protein kinase C theta" refers to the serine/threonine protein kinase also known as PKCT, PRKCT, nPKC-theta and PRKCQ. The nucleotide sequence of protein kinase C theta is shown in SEQ ID NO:1 and the amino acid sequence of protein kinase C theta is shown in SEQ ID NO:2. PKC theta is expressed ubiquitously in tissues with the highest levels found in hematopoietic cell lines, including T-cells and thymocytes (Baier *et al.*, *J. Biol. Chem.*, 1993, 268, 4997-5004; Keenan *et al.*, *Immunology*, 1997, 90, 557-563; Meller *et al.*, *Cell. Immunol.*, 1999, 193, 185-193; Wang, *et al., Biochem. Biophys. Res. Commun.*, 1993, 191, 240-246). This isozyme has been shown to function in a calcium-independent fashion, and transient overexpression of the protein in murine thymoma cells resulted in transcriptional activation of an interleukin-2 promoter-driven construct (Baier *et al., Eur. J. Biochem.*, 1994, 225, 195-203).

The term "protein kinase C theta pathway" includes the means by which a cell converts an extracellular influence or signal (*e.g.*, a signal transduced by a receptor on the surface of a cell, such as a cytokine receptor or an antigen receptor) into a cellular response (*e.g.*, modulation of gene transcription), wherein PKC theta is one of the

molecules involved in transduction of the signal. As used herein, a "PKC theta pathway component" or "pathway component" includes a molecule in a signal transduction pathway involving PKC theta, e.g., PKC theta or molecules upstream or downstream of PKC theta that are involved in transducing the extracellular influence or signal into a cellular response. Preferably, modulation of a PKC theta pathway component results in the modulation of a biological activity of PKC theta. Exemplary components of a PKC theta pathway are known to the skilled artisan and generally outlined in Figure 1 and include: PKC theta, vav, CARMA1, Bcl10, IκB and NFκB. The nucleotide sequence of vav is shown in SEQ ID NO:3 and the amino acid sequence of vav is shown in SEQ ID NO:4; the nucleotide sequence of CARMA1 is shown in SEQ ID NO:5 and the amino acid sequence of CARMA1 is shown in SEQ ID NO:6; the nucleotide sequence of Bcl 10 is shown in SEQ ID NO:7 and the amino acid sequence of Bcl 10 is shown in SEQ ID NO:8; the nucleotide sequence of IκB is shown in SEQ ID NO:9 and the amino acid sequence of IκB is shown in SEQ ID NO:10; the nucleotide sequence of NFκB is shown in SEQ ID NO:11 and the amino acid sequence of NFκB is shown in SEQ ID NO:12.

As used herein, the term "CARMA1" refers to the lipid raft-associated regulator of TCR-induced NFκB activation and CD28 costimulation-dependent Jnk activation, also known as CARD 11. CARMA is a scaffolding protein. CARMA1 belongs to the membrane-associated guanylate kinase (MAGUK) family, a class of proteins that functions as molecular scaffolds for the assembly of multiprotein complexes at specialized regions of the plasma membrane. This protein is also a member of the CARD protein family, which is defined by carrying a characteristic caspase-associated recruitment domain (CARD). This protein has a domain structure similar to that of CARD14 protein. The CARD domains of both proteins have been shown to specifically interact with BCL10, a protein known to function as a positive regulator of cell apoptosis and NF-kappaB activation. When expressed in cells, this protein activates NFkB and induced the phosphorylation of BCL10. Gaide, O.*et al. Nat. Immunol.* 3 (9), 836-843 (2002) Wang, D., *et al. Nat. Immunol.* 3 (9), 830-835 (2002); Gaide, O., *et al. FEBS Lett.* 496 (2-3), 121-127 (2001); Bertin, J., *et al. J. Biol. Chem.* 276 (15), 11877-11882 (2001)

As used herein the term "Bcl 10" refers to the protein containing a caspase recruitment domain (CARD) and has been shown to induce apoptosis and to activate NF-kappaB. This protein is reported to interact with other CARD domain

containing proteins including CARD9, 10, 11 and 14, which are thought to function as upstream regulators in NF-kappaB signaling. The Bcl10 gene was identified by its translocation in a case of mucosa-associated lymphoid tissue (MALT) lymphoma. This protein is found to form a complex with MALT1, a protein encoded by another gene known to be translocated in MALT lymphoma. MALT1 and this protein are thought to synergize in the activation of NF-kappaB, and the deregulation of either of them may contribute to the same pathogenetic process that leads to the malignancy. (see, *e.g.*, GenBank accession No. NM_003921; Maes, B. *et al. Blood* 99 (4), 1398-1404 (2002); Kawano, T. *et al. Anticancer Res.* 22 (1A), 305-309 (2002); Wang, L., *et al. J. Biol. Chem.* 276 (24), 21405-21409 (2001); Lucas, P.C., *et al. J. Biol. Chem.* 276 (22), 19012-19019 (2001); Bertin, J., *et al. J. Biol. Chem.* 276 (15), 11877-11882 (2001); Ruland, J., *et al. Cell* 104 (1), 33-42 (2001); Bertin, J., *et al. J. Biol. Chem.* 275 (52), 41082-41086 (2000)).

As used herein, the term "effector T cell" includes T cells which function to eliminate antigen (*e.g.*, by producing cytokines which modulate the activation of other cells or by cytotoxic activity). The term "effector T cell" includes T helper cells (*e.g.*, Th1 and Th2 cells) and cytotoxic T cells. Th1 cells mediate delayed type hypersensitivity responses and macrophage activation while Th2 cells provide help to B cells and are critical in the allergic response (Mosmann and Coffman, 1989, *Annu. Rev. Immunol.* 7, 145-173; Paul and Seder, 1994, *Cell* 76, 241-251; Arthur and Mason, 1986, *J. Exp. Med.* 163, 774-786; Paliard *et al.*, 1988, *J. Immunol.* 141, 849-855; Finkelman *et al.*, 1988, *J. Immunol.* 141, 2335-2341). As used herein, the term " T helper type 1 response" (Th1 response) refers to a response that is characterized by the production of one or more cytokines selected from IFN-γ, IL-2, TNF, and lymphotoxin (LT) and other cytokines produced preferentially or exclusively by Th1 cells rather than by Th2 cells. As used herein, a "T helper type 2 response" (Th2 response) refers to a response by $CD4^+$ T cells that is characterized by the production of one or more cytokines selected from IL-4, IL-5, IL-6 and IL-10, and that is associated with efficient B cell "help" provided by the Th2 cells (*e.g.*, enhanced IgG1 and/or IgE production).

As used herein, the term "regulatory T cell" includes T cells which produce low levels of IL-2, IL-4, IL-5, and IL-12. Regulatory T cells produce TNFα, TGFβ, IFN-γ, and IL-10, albeit at lower levels than effector T cells. Although TGFβ is the predominant cytokine produced by regulatory T cells, the cytokine is produced at

levels less than or equal to that produced by Th1 or Th2 cells, *e.g.*, an order of magnitude less than in Th1 or Th2 cells. Regulatory T cells can be found in the CD4+CD25+ population of cells (see, *e.g.*, Waldmann and Cobbold. 2001. *Immunity.* 14:399). Regulatory T cells actively suppress the proliferation and cytokine production of Th1, Th2, or naïve T cells which have been stimulated in culture with an activating signal (*e.g.*, antigen and antigen presenting cells or with a signal that mimics antigen in the context of MHC, *e.g.*, anti-CD3 antibody, plus anti-CD28 antibody).

As used herein the phrase, "modulating the balance of regulatory T cell function relative to effector T cell function" or "modulating regulatory T cell function relative to effector T cell function" includes preferentially altering at least one regulatory T cell function (in a population of cells including both T effector cells and T regulatory cells) such that there is a shift in the balance of T effector/T regulatory cell activity as compared to the balance prior to treatment.

As used herein the phrase, "modulating the balance of effector T cell function relative to regulatory T cell function" or "modulating effector T cell function relative to regulatory T cell function" includes preferentially altering at least one effector T cell function (in a population of cells including both T effector cells and T regulatory cells) is altered such that there is a shift in the balance of T effector/T regulatory cell activity as compared to the balance prior to treatment.

As used herein, the term "agent" includes compounds that modulate, *e.g.*, up-modulate or stimulate and down-modulate or inhibit, the expression and/or activity of a molecule of the invention. As used herein the term "inhibitor" or "inhibitory agent" includes agents which inhibit the expression and/or activity of a molecule of the invention. Exemplary inhibitors include antibodies, RNAi, compounds that mediate RNAi (*e.g.*, siRNA), antisense RNA, dominant/negative mutants of molecules of the invention, peptides, and/or peptidomimetics.

The term "stimulator" or "stimulatory agent" includes agents, *e.g.*, agonists, which increase the expression and/or activity of molecules of the invention. Exemplary stimulating agents include active protein and nucleic acid molecules, peptides and peptidomimetics of molecules of the invention. The agents of the invention can directly modulate, *i.e.*, increase or decrease, the expression and/or activity of a molecule of the invention. Exemplary agents are described herein or can be identified using screening assays that select for such compounds, as described in detail below.

For screening assays of the invention, preferably, the "test compound or agent" screened includes molecules that are not known in the art to modulate the balance of T cell activation, *e.g.*, the relative activity of T effector cells as compared to the relative activity of T regulatory cells or vice versa. Preferably, a plurality of agents is tested using the instant methods.

In one embodiment, a screening assay of the invention can be performed in the presence of an activating agent. As used herein, the term "activating agent" includes one or more agents that stimulate T cell activation (*e.g.*, effector functions such as cytokine production, proliferation, and/or lysis of target cells). Exemplary activating agents are known in the art and include, but are not limited to, *e.g.*, mitogens (e.g., phytohemagglutinin or concanavalin A), antibodies that react with the T cell receptor or CD3 (in some cases combined with antigen presenting cells or antibodies that react with CD28), or antigen plus antigen presenting cells.

Preferably, the modulating agents of the invention are used for a short term or course of therapy rather than an extended or prolonged course of therapy. As used herein the language "short term or course of therapy" includes a therapeutic regimen that is of relatively short duration relative to the course of the illness being treated. For example a short course of therapy may last between about one week to about eight weeks. In contrast, "an intermediate course of therapy" includes a therapeutic regimen that is of longer duration than a short course of therapy. For example, an intermediate course of therapy can last from more than two months to about four months (*e.g.*, between about eight to about 16 weeks). An "extended or prolonged course of therapy" includes those therapeutic regimens that last longer than about four months, *e.g.*, from about five months on. For example, an extended course of therapy may last from about six months to as long as the illness persists. The appropriateness of one or more of the courses of therapy described above for any one individual can readily be determined by one of ordinary skill in the art. In addition, the treatment appropriate for a subject may be changed over time as required.

As used herein, the term "tolerance" includes refractivity to activating receptor-mediated stimulation. Such refractivity is generally antigen-specific and persists after exposure to the tolerizing antigen has ceased. For example, tolerance is characterized by lack of cytokine production, *e.g.*, IL-2. Tolerance can occur to self antigens or to foreign antigens.

As used herein, the term "T cell" (*i.e.*, T lymphocyte) is intended to include all cells within the T cell lineage, including thymocytes, immature T cells, mature T cells and the like, from a mammal (*e.g.*, human). Preferably, T cells are mature T cells that express either CD4 or CD8, but not both, and a T cell receptor. The various T cell populations described herein can be defined based on their cytokine profiles and their function.

As used herein, the term "naïve T cells" includes T cells that have not been exposed to cognate antigen and so are not activated or memory cells. Naïve T cells are not cycling and human naïve T cells are CD45RA+. If naïve T cells recognize antigen and receive additional signals depending upon but not limited to the amount of antigen, route of administration and timing of administration, they may proliferate and differentiate into various subsets of T cells, e.g. effector T cells.

As used herein, the term "memory T cell" includes lymphocytes which, after exposure to antigen, become functionally quiescent and which are capable of surviving for long periods in the absence of antigen. Human memory T cells are CD45RA-.

The "molecules of the invention" (*e.g.*, nucleic acid or polypeptide molecules) are preferentially expressed (and/or preferentially active in modulating the balance between T effector cells and T regulatory cells) in a particular cell type, *e.g.*, effector T cells, or in regulatory T cells. Such molecules may be necessary in the process that leads to differentiation of the cell type and may be expressed prior to or at an early stage of differentiation to the cell type. Such molecules may be secreted by the cell, extracellular (expressed on the cell surface) or expressed intracellularly, and may be involved in a signal transduction pathway that leads to differentiation. Modulator molecules of the invention include molecules of the invention as well as molecules (*e.g.*, drugs) which modulate the expression of a molecule of the invention.

As used herein the term "T effector (Teff) molecule" includes molecules that are preferentially expressed and/or preferentially active in effector T cells.

As used herein the term "T regulatory (Treg) molecule" includes molecules that are preferentially expressed and/or preferentially active in regulatory T cells.

In one embodiment, small molecules can be used as test compounds. The term "small molecule" is a term of the art and includes molecules that are less than about 1000 molecular weight or less than about 500 molecular weight. In one embodiment, small molecules do not exclusively comprise peptide bonds. In another embodiment, small molecules are not oligomeric. Exemplary small molecule compounds which can be screened for activity include, but are not limited to, peptides, peptidomimetics, nucleic acids, carbohydrates, small organic molecules (*e.g.*, polyketides) (Cane *et al.* 1998. *Science* 282:63), and natural product extract libraries. In another embodiment, the compounds are small, organic non-peptidic compounds. In a further embodiment, a small molecule is not biosynthetic.

As used herein, the term "oligonucleotide" includes two or more nucleotides covalently coupled to each other by linkages (*e.g.*, phosphodiester linkages) or substitute linkages.

As used herein, the term "peptide" includes relatively short chains of amino acids linked by peptide bonds. The term "peptidomimetic" includes compounds containing non-peptidic structural elements that are capable of mimicking or antagonizing peptides.

As used herein, the term "reporter gene" includes genes that express a detectable gene product, which may be RNA or protein. Preferred reporter genes are those that are readily detectable. The reporter gene may also be included in a construct in the form of a fusion gene with a gene that includes desired transcriptional regulatory sequences or exhibits other desirable properties. Examples of reporter genes include, but are not limited to CAT (chloramphenicol acetyl transferase) (Alton and Vapnek (1979), *Nature* 282: 864-869) luciferase, and other enzyme detection systems, such as beta-galactosidase; firefly luciferase (deWet *et al.* (1987), *Mol. Cell. Biol.* 7:725-737); bacterial luciferase (Engebrecht and Silverman (1984), *Proc. Natl. Acad. Sci., USA* 1: 4154-4158; Baldwin *et al.* (1984), *Biochemistry* 23: 3663-3667); alkaline phosphatase (Toh *et al.* (1989) *Eur. J. Biochem.* 182: 231-238, Hall *et al.* (1983) *J. Mol. Appl. Gen.* 2: 101), human placental secreted alkaline phosphatase (Cullen and Malim (1992) *Methods in Enzymol.* 216:362-368) and green fluorescent protein (U.S. patent 5,491,084; WO 96/23898).

"Treatment", as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to

an isolated tissue or cell line from a patient, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving or affecting the disease or disorder, at least one symptom of disease or disorder or modulating the balance of effector T cell function relative to regulatory T cell function.

II. *Modulatory Agents*

A. Stimulatory Agents

According to a modulatory method of the invention, expression and/or activity of a protein kinase C theta pathway and/or expression and/or activity of a protein kinase C theta pathway component is stimulated in a cell by contacting the cell with a stimulatory agent. Examples of such stimulatory agents include active protein and nucleic acid molecules that are introduced into the cell to increase expression and/or activity of a protein kinase C theta pathway component in the cell.

A preferred stimulatory agent is a nucleic acid molecule encoding a protein product of a protein kinase C theta pathway component, wherein the nucleic acid molecule is introduced into the cell in a form suitable for expression of the active protein of a protein kinase C theta pathway in the cell. To express a protein in a cell, typically a nucleic acid molecule encoding a polypeptide of a pathway component is first introduced into a recombinant expression vector using standard molecular biology techniques, *e.g.*, as described herein. A nucleic acid molecule encoding a polypeptide of a pathway component can be obtained, for example, by amplification using the polymerase chain reaction (PCR), using primers based on the nucleotide sequence of a pathway component. Following isolation or amplification of the nucleic acid molecule encoding a polypeptide of a pathway component, the DNA fragment is introduced into an expression vector and transfected into target cells by standard methods, as described herein.

Variants of the nucleotide sequences described herein which encode a polypeptide which retains biological activity are also embraced by the invention. For example, nucleic acid molecules which hybridize under high stringency conditions with the disclosed nucleic acid molecule. As used herein, the term "hybridizes under high stringency conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences having substantial homology (*e.g.*, typically greater

than 70% homology) to each other remain stably hybridized to each other. A preferred, non-limiting example of high stringency conditions are hybridization in a hybridization buffer that contains 6X sodium chloride/ sodium citrate (SSC) at a temperature of about 45°C for several hours to overnight, followed by one or more washes in a washing buffer containing 0.2 X SSC, 0.1% SDS at a temperature of about 50-65°C.

Another aspect of the invention features biologically active portions (*i.e.*, bioactive fragments) of a protein kinase C theta pathway component, including polypeptide fragments suitable for use in making fusion proteins.

In one embodiment, a protein kinase C theta pathway component or a bioactive fragment thereof can be obtained from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, a pathway component immunogen or bioactive fragment is produced by recombinant DNA techniques. Alternative to recombinant expression, a pathway component or bioactive fragment can be synthesized chemically using standard peptide synthesis techniques.

The polypeptide, bioactive fragment or fusion protein, as used herein is preferably "isolated" or "purified". The terms "isolated" and "purified" are used interchangeably herein. "Isolated" or "purified" means that the polypeptide, bioactive fragment or fusion protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the polypeptide is derived, substantially free of other protein fragments, for example, non-desired fragments in a digestion mixture, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations in which the polypeptide is separated from other components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of polypeptide having less than about 30% (by dry weight) of contaminating protein, more preferably less than about 20% of contaminating protein, still more preferably less than about 10% of contaminating protein, and most preferably less than about 5% contaminating protein. When polypeptide is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the polypeptide preparation. When polypeptide is produced by, for

example, chemical or enzymatic processing from isolated or purified protein, the preparation is preferably free of enzyme reaction components or chemical reaction components and is free of non-desired fragments, *i.e.*, the desired polypeptide represents at least 75% (by dry weight) of the preparation, preferably at least 80%, more preferably at least 85%, and even more preferably at least 90%, 95%, 99% or more or the preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of polypeptide in which the polypeptide is separated from chemical precursors or other chemicals which are involved in the synthesis of the polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations having less than about 30% (by dry weight) of chemical precursors or reagents, more preferably less than about 20% chemical precursors or reagents, still more preferably less than about 10% chemical precursors or reagents, and most preferably less than about 5% chemical precursors or reagents.

Bioactive fragments of polypeptides of a protein kinase C theta pathway component include polypeptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the polypeptide of a pathway component which include less amino acids than the full length protein, and exhibit at least one biological activity of the full-length protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the full-length protein. A biologically active portion of a polypeptide of the invention can be a polypeptide which is, for example, 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more amino acids in length. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native protein. Mutants can also be utilized as assay reagents, for example, mutants having reduced, enhanced or otherwise altered biological properties identified according to one of the activity assays described herein.

Variants of a polypeptide molecule of a protein kinase C theta pathway component which retain biological activity are also embraced by the invention. In one embodiment, such a variant polypeptide has at least about 80%, 85%, 90%, 95%, 98% identity.

To determine the percent identity of two amino acid sequences (or of two nucleotide or amino acid sequences), the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the first sequence or second sequence for optimal alignment). 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 residue 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.*, % homology = # of identical positions/total # of positions x 100), optionally penalizing the score for the number of gaps introduced and/or length of gaps introduced.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In one embodiment, the alignment generated over a certain portion of the sequence aligned having sufficient identity but not over portions having low degree of identity (*i.e.*, a local alignment). A preferred, non-limiting example of a local alignment algorithm utilized for the comparison of sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-68, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-77. Such an algorithm is incorporated into the BLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST alignments can be generated and percent identity calculated using BLAST protein searches (*e.g.*, the XBLAST program) using the sequence of a polypeptide of a pathway component or a portion thereof as a query, score = 50, wordlength = 3.

In another embodiment, the alignment is optimized by introducing appropriate gaps and percent identity is determined over the length of the aligned sequences (*i.e.*, a gapped alignment). To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Research* 25(17):3389-3402. In another embodiment, the alignment is optimized by introducing appropriate gaps and percent identity is determined over the entire length of the sequences aligned (*i.e.*, a global alignment). A preferred, non-limiting example of a mathematical algorithm utilized for the global comparison of sequences is the algorithm of Myers and Miller, *CABIOS* (1989). Such an algorithm is incorporated into 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.

The invention also provides chimeric or fusion proteins of a protein kinase C theta pathway component. As used herein, a "chimeric protein" or "fusion protein" comprises a polypeptide of a pathway component operatively linked to a different polypeptide. Within a fusion protein, the entire polypeptide of a pathway component can be present or a bioactive portion of the polypeptide can be present. Such fusion proteins can be used to modify the activity of a protein kinase C theta pathway component.

Preferably, a chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety. A nucleic acid molecule encoding a polypeptide of a pathway component can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the polypeptide of a pathway component.

Other stimulatory agents that can be used to stimulate the activity of a protein kinase C theta pathway component protein are chemical compounds that stimulate expression or activity of a pathway component in cells, such as compounds that directly stimulate the protein product of a pathway component and compounds that promote the interaction between a protein product of a pathway component and substrates or target DNA binding sites. Such compounds can be identified using screening assays that select for such compounds, as described in detail below.

B. Inhibitory Agents

Inhibitory agents of the invention can be, for example, intracellular binding molecules that act to inhibit the expression or activity of a PKCθ pathway component. For molecules that are expressed intracellularly, intracellular binding molecules can be used to modulate expression and/or activity. As used herein, the term "intracellular binding molecule" is intended to include molecules that act intracellularly to inhibit the expression or activity of a protein by binding to the protein itself, to a nucleic acid (*e.g.*, an mRNA molecule) that encodes the protein or to a target with which the protein normally interacts (*e.g.*, to a DNA target sequence to which the marker binds). Examples of intracellular binding molecules, described in further detail below, include antisense marker nucleic acid molecules (*e.g.*, to inhibit translation of mRNA), intracellular antibodies (*e.g.*, to inhibit the activity of protein) and dominant negative mutants of the pathway component proteins. In the case of molecules that are secreted or expressed on the cell surface, in addition to inhibition by intracellular binding molecules (*e.g*, antisense nucleic acid molecules or molecules which mediate RNAi) the activity of such molecules can be inhibited using agents which act outside the cell, *e.g.*, to disrupt the binding between a ligand and its receptor such as antibodies.

In one embodiment, an inhibitory agent of the invention is an antisense nucleic acid molecule that is complementary to a gene encoding a protein kinase C theta pathway component or to a portion of said gene, or a recombinant expression vector encoding said antisense nucleic acid molecule. The use of antisense nucleic acids to downmodulate the expression of a particular protein in a cell is well known in the art (see *e.g.*, Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986; Askari, F.K. and McDonnell, W.M. (1996) *N. Eng. J. Med.* 334:316-318; Bennett, M.R. and Schwartz, S.M. (1995) *Circulation* 92:1981-1993; Mercola, D. and Cohen, J.S. (1995) *Cancer Gene Ther.* 2:47-59; Rossi, J.J. (1995) *Br. Med. Bull.* 51:217-225; Wagner, R.W. (1994) *Nature* 372:333-335). An antisense nucleic acid molecule comprises a nucleotide sequence that is complementary to the coding strand of another nucleic acid molecule (*e.g.*, an mRNA sequence) and accordingly is capable of hydrogen bonding to the coding strand of the other nucleic acid molecule. Antisense sequences complementary to a sequence of an mRNA can be complementary to a sequence found in the coding region of the mRNA, the 5' or 3' untranslated region of the mRNA or a region bridging the coding region and

an untranslated region (*e.g.*, at the junction of the 5' untranslated region and the coding region). Furthermore, an antisense nucleic acid can be complementary in sequence to a regulatory region of the gene encoding the mRNA, for instance a transcription initiation sequence or regulatory element. Preferably, an antisense nucleic acid is designed so as to be complementary to a region preceding or spanning the initiation codon on the coding strand or in the 3' untranslated region of an mRNA. An antisense nucleic acid molecule for inhibiting the expression of protein in a cell can be designed based upon the nucleotide sequence encoding the protein constructed according to the rules of Watson and Crick base pairing.

An antisense nucleic acid molecule can exist in a variety of different forms. For example, the antisense nucleic acid can be an oligonucleotide that is complementary to only a portion of a gene. An antisense oligonucleotide can be constructed using chemical synthesis procedures known in the art. An antisense oligonucleotide can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.* phosphorothioate derivatives and acridine substituted nucleotides can be used. To inhibit expression in cells in culture, one or more antisense oligonucleotides can be added to cells in culture media, typically at about 200 μg oligonucleotide/ml.

Alternatively, an antisense nucleic acid molecule can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, nucleic acid transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest). Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the expression of the antisense RNA molecule in a cell of interest, for instance promoters and/or enhancers or other regulatory sequences can be chosen which direct constitutive, tissue specific or inducible expression of antisense RNA. For example, for inducible expression of antisense RNA, an inducible eukaryotic regulatory system, such as the Tet system (*e.g.*, as described in Gossen, M. and Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Gossen, M. *et al.* (1995) *Science* 268:1766-1769; PCT Publication No. WO 94/29442; and PCT Publication No. WO 96/01313) can be used. The antisense expression vector is prepared as described below for

recombinant expression vectors, except that the cDNA (or portion thereof) is cloned into the vector in the antisense orientation. The antisense expression vector can be in the form of, for example, a recombinant plasmid, phagemid or attenuated virus. The antisense expression vector is introduced into cells using a standard transfection technique, as described herein for recombinant expression vectors.

In another embodiment, a compound that mediates RNAi can be used to inhibit a protein kinase C theta pathway component. RNA interference is a post-transcriptional, targeted gene-silencing technique that uses double-stranded RNA (dsRNA) to degrade messenger RNA (mRNA) containing the same sequence as the dsRNA (Sharp, P.A. and Zamore, P.D. 287, 2431-2432 (2000); Zamore, P.D., *et al. Cell* 101, 25-33 (2000). Tuschl, T. *et al. Genes Dev.* 13, 3191-3197 (1999)). The process occurs when an endogenous ribonuclease cleaves the longer dsRNA into shorter, 21- or 22-nucleotide-long RNAs, termed small interfering RNAs or siRNAs. The smaller RNA segments then mediate the degradation of the target mRNA. Kits for synthesis of RNAi are commercially available from, *e.g.* New England Biolabs and Ambion. In one embodiment one or more of the chemistries described above for use in antisense RNA can be employed.

In another embodiment, an antisense nucleic acid for use as an inhibitory agent is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region (for reviews on ribozymes see *e.g.*, Ohkawa, J. *et al.* (1995) *J. Biochem.* 118:251-258; Sigurdsson, S.T. and Eckstein, F. (1995) *Trends Biotechnol.* 13:286-289; Rossi, J.J. (1995) *Trends Biotechnol.* 13:301-306; Kiehntopf, M. *et al.* (1995) *J. Mol. Med.* 73:65-71). A ribozyme having specificity for the mRNA of a pathway component can be designed based upon the nucleotide sequence of a protein kinase C theta pathway component cDNA sequence. For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the base sequence of the active site is complementary to the base sequence to be cleaved in the mRNA of a pathway component. See for example U.S. Patent Nos. 4,987,071 and 5,116,742, both by Cech *et al.* Alternatively, a pathway component mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See for example Bartel, D. and Szostak, J.W. (1993) *Science* 261: 1411-1418.

A polypeptide molecule of a protein kinase C theta pathway component or a portion or fragment of a protein kinase C theta pathway component, can also be used as an immunogen to generate antibodies that bind a pathway component or that block pathway component binding using standard techniques for polyclonal and monoclonal antibody preparation.

To make antibodies a full-length polypeptide can be used or, alternatively, the invention provides antigenic peptide fragments for use as immunogens. Preferably, an antigenic fragment comprises at least 8 amino acid residues of the amino acid sequence of a polypeptide of a protein kinase C theta pathway component and encompasses an epitope of the polypeptide such that an antibody raised against the peptide forms a specific immune complex with the polypeptide of a pathway component. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of polypeptides that are located on the surface of the protein, *e.g.*, hydrophilic regions. Such regions can be readily identified using art recognized methods.

An immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed polypeptide or a chemically synthesized polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic preparation induces a polyclonal antibody response, respectively.

In one embodiment, inhibitory compounds of the invention are antibodies or modified antibody molecules. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin as well as VH and VL domains that can be cloned from antibody molecules and used to generate modified antigen binding molecules, such as minibodies or diabodies.

The invention provides polyclonal and monoclonal antibodies. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of an antigen. A monoclonal antibody composition thus typically displays a single binding affinity for a particular antigen or polypeptide with which it immunoreacts.

Polyclonal antibodies can be prepared as described above by immunizing a suitable subject with an immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized antigen. If desired, the antibody molecules can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem* .255:4980-83; Yeh *et al.* (1976) *PNAS* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.,* 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds to the antigen.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al. Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal*

Antibodies, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of a protein kinase C theta pathway are detected by screening the hybridoma culture supernatants for antibodies that bind to the antigen, *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with an antigen to thereby isolate immunoglobulin library members that bind the antigen. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum.*

Antibod. Hybridomas 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *PNAS* 88:7978-7982; and McCafferty *et al. Nature* (1990) 348:552-554.

Another type of inhibitory agent that can be used to inhibit the expression and/or activity of a protein kinase C theta pathway in a cell is an intracellular antibody specific for a protein kinase C theta pathway, preferably an intracellular molecule of the invention. The use of intracellular antibodies to inhibit protein function in a cell is known in the art (see *e.g.*, Carlson, J. R. (1988) *Mol. Cell. Biol.* 8:2638-2646; Biocca, S. *et al.* (1990) *EMBO J.* 9:101-108; Werge, T.M. *et al.* (1990) *FEBS Letters* 274:193-198; Carlson, J.R. (1993) *Proc. Natl. Acad. Sci. USA* 90:7427-7428; Marasco, W.A. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:7889-7893; Biocca, S. *et al.* (1994) *Bio/Technology* 12:396-399; Chen, S-Y. *et al.* (1994) *Human Gene Therapy* 5:595-601; Duan, L *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:5075-5079; Chen, S-Y. *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:5932-5936; Beerli, R.R. *et al.* (1994) *J. Biol. Chem.* 269:23931-23936; Beerli, R.R. *et al.* (1994) *Biochem. Biophys. Res. Commun.* 204:666-672; Mhashilkar, A.M. *et al.* (1995) *EMBO J.* 14:1542-1551; Richardson, J.H. *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:3137-3141; PCT Publication No. WO 94/02610 by Marasco *et al.*; and PCT Publication No. WO 95/03832 by Duan *et al.*).

To inhibit activity using an intracellular antibody, a recombinant expression vector is prepared which encodes the antibody chains in a form such that, upon introduction of the vector into a cell, the antibody chains are expressed as a functional antibody in an intracellular compartment of the cell. For inhibition of the activity of a protein kinase C theta pathway according to the inhibitory methods of the invention, an intracellular antibody that specifically binds the protein product of a protein kinase C theta pathway is expressed in the cytoplasm of the cell. To prepare an intracellular antibody expression vector, antibody light and heavy chain cDNAs encoding antibody chains specific for the target protein of interest are isolated, typically from a hybridoma that secretes a monoclonal antibody specific for the protein kinase C theta pathway. Hybridomas secreting anti-protein kinase C theta pathway monoclonal antibodies, or recombinant monoclonal antibodies, can be prepared as described below.

Once a monoclonal antibody specific for the marker protein has been identified (*e.g.*, either a hybridoma-derived monoclonal antibody or a recombinant antibody from a combinatorial library), DNAs encoding the light and heavy chains of the monoclonal antibody are isolated by standard molecular biology techniques. For hybridoma derived antibodies, light and heavy chain cDNAs can be obtained, for example, by PCR amplification or cDNA library screening. For recombinant antibodies, such as from a phage display library, cDNA encoding the light and heavy chains can be recovered from the display package (*e.g.*, phage) isolated during the library screening process. Nucleotide sequences of antibody light and heavy chain genes from which PCR primers or cDNA library probes can be prepared are known in the art. For example, many such sequences are disclosed in Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 and in the "Vbase" human germline sequence database.

Once obtained, the antibody light and heavy chain sequences are cloned into a recombinant expression vector using standard methods. To allow for cytoplasmic expression of the light and heavy chains, the nucleotide sequences encoding the hydrophobic leaders of the light and heavy chains are removed. An intracellular antibody expression vector can encode an intracellular antibody in one of several different forms. For example, in one embodiment, the vector encodes full-length antibody light and heavy chains such that a full-length antibody is expressed intracellularly. In another embodiment, the vector encodes a full-length light chain but only the VH/CH1 region of the heavy chain such that a Fab fragment is expressed intracellularly. In the most preferred embodiment, the vector encodes a single chain antibody (scFv) wherein the variable regions of the light and heavy chains are linked by a flexible peptide linker (*e.g.*, $(Gly_4Ser)_3$) and expressed as a single chain molecule. To inhibit the activity of a protein kinase C theta pathway in a cell, the expression vector encoding the intracellular antibody is introduced into the cell by standard transfection methods, as discussed herein.

Yet another form of an inhibitory agent of the invention is an inhibitory form of a polypeptide of a protein kinase C theta pathway, *e.g*, a dominant negative inhibitor. For example, in one embodiment, an active site (*e.g.*, an enzyme active site or a DNA binding domain) can be mutated. Such dominant negative proteins can be

expressed in cells using a recombinant expression vector encoding the protein, which is introduced into the cell by standard transfection methods.

Other inhibitory agents that can be used to inhibit the activity of a marker protein are chemical compounds that directly inhibit marker activity or inhibit the interaction between the marker and target DNA or another protein. Such compounds can be identified using screening assays that select for such compounds, as described in detail below.

III. *Screening Assays*

The invention provides methods (also referred to herein as "screening assays") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that have a modulatory effect on a protein kinase C theta pathway component, in effector T cells relative to regulatory T cells.

A. *Cell Free Assays*

In one embodiment, the screening assay can be done in a cell-free format. A protein kinase C theta pathway component, *e.g.*, PKC theta or a non-PKC theta polypeptide which acts upstream or downstream of PKC theta in a pathway involving PKC theta, *e.g.*, a PKC pathway component, *e.g.*, CARMA1, vav or Bcl 10, is expressed by recombinant methods in host cells and the polypeptide can be isolated from the host cell culture medium using standard methods for purifying polypeptides, for example, by ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, and/or immunoaffinity purification with antibodies specific for a protein kinase C theta pathway component to produce protein that can be used in a cell free composition. Alternatively, an extract of a pathway component or cells expressing a pathway component can be prepared for use as a cell-free composition.

In one embodiment, the protein kinase C theta pathway component is then contacted with a test compound and the ability of the test compound to bind to the pathway component or bioactive fragment thereof, is determined. Binding of the test compound to a pathway component can be accomplished, for example, by coupling the test compound or the pathway component (*e.g.*, polypeptide or fragment thereof) with an enzymatic or radioisotopic label such that binding of the test compound to the pathway

component can be determined by detecting the labeled compound or the pathway component in a complex. For example, test compounds or a pathway component (*e.g.*,polypeptides) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test compounds or a pathway component (*e.g.*,polypeptides) can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

Binding of the test compound to a protein kinase C theta pathway component can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules. In a preferred embodiment, the assay includes contacting a polypeptide pathway component or biologically active portion thereof with a target molecule of a pathway component, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a polypeptide pathway component, wherein determining the ability of the test compound to interact with a pathway component comprises determining the ability of the test compound to preferentially bind to a pathway component or the bioactive portion thereof as compared to a control molecule. In another embodiment, the assay includes contacting a polypeptide pathway component or biologically active portion thereof with a target molecule of a pathway component, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to modulate binding between a polypeptide protein kinase C theta pathway component and a known modulator of the polypeptide.

In another embodiment, when a binding partner of the molecule of the invention is known, *e.g.,* vav, CARMA1, and Bcl 10, that binding partner can be used in a screening assay to identify modulator compounds.

In another embodiment, the assay is a cell-free assay in which a polypeptide pathway component or bioactive portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the polypeptide pathway component or biologically active portion thereof is determined. This embodiment of the invention is particularly useful when the pathway component is an intracellular molecule and its activity can be measured in a cell-free system.

In yet another embodiment, the cell-free assay involves contacting a polypeptide protein kinase C theta pathway component or biologically active portion thereof with a molecule to which a protein kinase C theta pathway component binds (*e.g.*, a known binding partner) to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to modulate the activity of the pathway component, as compared to a control compound. The activity of the target molecule can be determined by, for example, detecting the phosphorylation of an appropriate substrate, *e.g.*, vav or Bcl 10, and the like, detecting catalytic/enzymatic activity of the target using an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response.

In one embodiment, the amount of binding of a protein kinase C theta pathway component to the target molecule in the presence of the test compound is greater than the amount of binding of a protein kinase C theta pathway component to the target molecule in the absence of the test compound, in which case the test compound is identified as a compound that enhances binding of a protein kinase C theta pathway component. In another embodiment, the amount of binding of a protein kinase C theta pathway component to the target molecule in the presence of the test compound is less than the amount of binding of a protein kinase C theta pathway component to the target molecule in the absence of the test compound, in which case the test compound is identified as a compound that inhibits binding of a protein kinase C theta pathway component.

Binding of the test compound to a polypeptide protein kinase C theta pathway component can be determined either directly or indirectly as described above.

In the methods of the invention for identifying test compounds that modulate an interaction between a polypeptide pathway component and a target molecule, the full-length polypeptide pathway component may be used in the method, or, alternatively, only portions of a pathway component may be used. The degree of interaction between a polypeptide pathway component and the target molecule can be determined, for example, by labeling one of the polypeptides with a detectable substance (*e.g.*, a radiolabel), isolating the non-labeled polypeptide and quantitating the amount of detectable substance that has become associated with the non-labeled polypeptide. The assay can be used to identify test compounds that either stimulate or inhibit the interaction between a pathway component protein and a target molecule. A test compound that stimulates the interaction between a polypeptide pathway component and a target molecule, *e.g.*, an agonist, is identified based upon its ability to increase the degree of interaction between a polypeptide pathway component and a target molecule as compared to the degree of interaction in the absence of the test compound. A test compound that inhibits the interaction between a polypeptide pathway component and a target molecule, *e.g.*, an antagonist, is identified based upon its ability to decrease the degree of interaction between a polypeptide pathway component and a target molecule as compared to the degree of interaction in the absence of the compound.

In more than one embodiment of the assays of the present invention it may be desirable to immobilize either a protein kinase C theta pathway component or a pathway component target molecule, for example, to facilitate separation of complexed from uncomplexed forms of one or both of the polypeptides, or to accommodate automation of the assay. Binding of a test compound to a polypeptide pathway component, or interaction of a polypeptide pathway component with a pathway component target molecule in the presence and absence of a test compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the polypeptides to be bound to a matrix. For example, glutathione-S-transferase/ pathway component fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St.

Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target polypeptide or a polypeptide pathway component, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix is immobilized in the case of beads, and complex formation is determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of pathway component binding or activity determined using standard techniques.

Other techniques for immobilizing polypeptides on matrices can also be used in the screening assays of the invention. For example, either a polypeptide pathway component or a pathway component target molecule can be immobilized utilizing conjugation of biotin and streptavidin. A biotinylated polypeptide pathway component or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which are reactive with a pathway component or target molecules but which do not interfere with binding of a pathway component to its target molecule can be derivatized to the wells of the plate, and unbound target or a pathway component is trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with a pathway component or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with a polypeptide pathway component or target molecule.

B. ***Cell-Based Assays***

In one embodiment, a cell that naturally expresses or, more preferably, a cell that has been engineered to express a protein kinase C theta pathway component, for example, by introducing into the cell an expression vector encoding the polypeptide is used in the screening methods of the invention. Alternatively, a polypeptide pathway component (*e.g.*, a cell extract from a protein kinase C theta pathway component

expressing cell or a composition that includes a purified molecule of a protein kinase C theta pathway component, either natural or recombinant) can be used.

Compounds that modulate expression and/or activity of a protein kinase C theta pathway component (or a molecule that acts upstream or downstream of a protein kinase C theta pathway component) can be identified using various "read-outs." Methods for detecting alterations in the expression of and/or an expression profile of a pathway component are known in the art and include, for example, a differential display methodology, Northern blot analysis, quantitative RT-PCR, and Western blot analysis.

An example of a "read-out" is the use of an indicator cell which can be transfected with an expression vector, incubated in the presence and in the absence of a test compound, and the effect of the compound on the expression of the pathway component or on a biological response regulated by a pathway component can be determined. The biological activities include activities determined *in vivo*, or *in vitro*, according to standard techniques for each protein kinase C theta pathway component. A biological activity can be a direct activity or an indirect activity. Examples of such activities include the migration of PKC theta to the cell membrane, detecting the phosphorylation of an appropriate substrate, *e.g.,* Bcl 10, or detecting activation of NFkB or its translocation to the nucleus, or detecting transcription of a gene whose transcription is modulated by NFkB (*e.g.,* where the mRNA is measured, the gene product is measured, or transcription of a reporter gene is measured).

In one embodiment one biological activity of a molecule of the invention is modulated, *e.g.,* phosphorylation of Bcl 10, activation of NFkB or its translocation to the nucleus, or cytokine production. In another embodiment, two biological activities of a molecule of the invention are modulated, *e.g.,* cytokine production and phosphorylation of Bcl 10.

The ability of a test compound to modulate binding of a protein kinase C theta pathway component to a target molecule or to bind to itself can also be determined. Determining the ability of the test compound to modulate binding of a protein kinase C theta pathway component to a target molecule (*e.g.*, a binding partner, *e.g.,* vav or CARMA1) can be accomplished as described above, by, coupling a target molecule of a pathway component with a radioisotope, enzymatic or fluorescent label such that binding of the test compound to a pathway component is determined by detecting the labeled pathway component-target molecule in a complex.

In another embodiment, a different molecule (*i.e.*, a molecule which is not a pathway component) acting upstream or downstream in a pathway involving a pathway component can be included in an indicator composition for use in a screening assay. Non-limiting examples of molecules that may be used as upstream or downstream indicators include, members of the NF-kappa B and NFAT signaling pathways. Compounds identified in a screening assay employing such a molecule would also be useful in modulating a molecule of the invention activity, albeit indirectly.

The cells used in the instant assays can be eukaryotic or prokaryotic in origin.

Recombinant expression vectors that can be used for expression of a polypeptide or a non-polypeptide pathway component acting upstream or downstream of the pathway component in the indicator cell are known in the art. In one embodiment, within the expression vector coding sequences are operatively linked to regulatory sequences that allow for inducible or constitutive expression of the polypeptide in the indicator cell (*e.g.*, viral regulatory sequences, such as a cytomegalovirus promoter/enhancer, can be used). Use of a recombinant expression vector that allows for inducible or constitutive expression of the polypeptide in the indicator cell is preferred for identification of compounds that enhance or inhibit the activity of protein kinase C theta pathway components. In an alternative embodiment, within the expression vector the coding sequences are operatively linked to regulatory sequences of the endogenous gene (*i.e.*, the promoter regulatory region derived from the endogenous pathway component gene). Use of a recombinant expression vector in which expression is controlled by the endogenous regulatory sequences is preferred for identification of compounds that enhance or inhibit the transcriptional expression of a protein kinase C theta pathway component.

In one embodiment, an assay is a cell-based assay in which a cell expressing a protein kinase C theta pathway component is contacted with a test compound and the ability of the test compound to modulate the activity of the pathway component(s) is determined. The cell, for example, can be of mammalian origin or a yeast cell. The component (*e.g.*, a polypeptide pathway component, or biologically active portion thereof), for example, can be expressed heterologously or native to the cell. Determining the ability of the test compound to modulate the activity of the

component can be accomplished by assaying for any of the activities of a protein kinase C theta pathway component as described herein.

For example, determining the ability of the test compound to modulate the activity of a polypeptide pathway component can be accomplished by assaying for the activity of, for example, a protein kinase C theta pathway component or a target molecule thereof. In another embodiment, determining the ability of the test compound to modulate the activity of a polypeptide, or biologically active portion thereof, is accomplished by assaying for the ability to bind a target molecule or a bioactive portion thereof. In a preferred embodiment, the cell which expresses a polypeptide, or biologically active portion thereof, further expresses a target molecule, or biologically active portion thereof. In another preferred embodiment, the cell expresses more than two protein kinase C theta pathway components or biologically active portions thereof.

According to the cell-based assays for the present invention, determining the ability of the test compound to modulate the activity of a polypeptide or biologically active portion thereof, can be determined by assaying for any of the native activities of a molecule of a polypeptide or by assaying for an indirect activity which is coincident with the activity of a polypeptide, as described herein, for example, assaying for cytokine production or differentiation of naïve T cells into effector T cells, or by assaying the activity of a protein encoded by a gene having a response element.

Furthermore, determining the ability of the test compound to modulate the activity of a polypeptide or biologically active portion thereof can be determined by assaying for an activity which is not native to the polypeptide, but for which the cell has been recombinantly engineered. For example, the cell can be engineered to express a reporter gene construct that includes DNA encoding a reporter protein operably linked to a gene regulated by a polypeptide of the invention. It is also intended that in preferred embodiments, the cell-based assays of the present invention comprise a final step of identifying the compound as a modulator of a pathway component activity.

As used interchangeably herein, the terms "operably linked" and "operatively linked" are intended to mean that the nucleotide sequence is linked to a regulatory sequence in a manner which allows expression of the nucleotide sequence in a host cell (or by a cell extract). Regulatory sequences are art-recognized and can be selected to direct expression of the desired polypeptide in an appropriate host cell. The term regulatory sequence is intended to include promoters, enhancers, polyadenylation

signals and other expression control elements. Such regulatory sequences are known to those skilled in the art and are described in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transfected and/or the type and/or amount of polypeptide desired to be expressed.

A variety of reporter genes are known in the art and are suitable for use in the screening assays of the invention. Examples of suitable reporter genes include those which encode chloramphenicol acetyltransferase, beta-galactosidase, alkaline phosphatase or luciferase. Standard methods for measuring the activity of these gene products are known in the art.

In yet another aspect of the invention, a polypeptide pathway component can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins which bind to or interact with a PKC theta pathway component and are involved in the activity of the pathway component. Such pathway component-target molecules are also likely to be involved in the regulation of cellular activities modulated by a polypeptide pathway component.

At least one exemplary two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a polypeptide pathway component is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encode an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming a pathway component-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription

factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with a polypeptide pathway component.

Another exemplary two-hybrid system, referred to in the art as the CytoTrap™ system, is based in the modular nature of molecules of the Ras signal transduction cascade. Briefly, the assay features a fusion protein comprising the "bait" protein and Son-of-Sevenless (SOS) and the cDNAs for unidentified proteins (the "prey") in a vector that encodes myristylated target proteins. Expression of an appropriate bait-prey combination results in translocation of SOS to the cell membrane where it activates Ras. Cytoplasmic reconstitution of the Ras signaling pathway allows identification of proteins that interact with the bait protein of interest, for example, a PKC theta pathway component protein. Additional mammalian two hybrid systems are also known in the art and can be utilized to identify proteins that interact with a pathway component.

In another aspect, the invention pertains to a combination of two or more assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity and/or expression of a pathway component protein can be confirmed in an *in vitro* system, *e.g.*, in cell culture, or *in vivo*, *e.g.*, in an animal such as an animal model of inflammation, using art recognized techniques, or as described herein.

In an embodiment of a screening assay of the invention, once a test compound is identified as modulating a PKC theta pathway component, the effect of the test compound can be assayed for an ability to modulate effector T cell function relative to T regulatory cell function and can be confirmed as an effector T cell modulator, for example, based on measurements of the effects in immune cells, either *in vitro* (*e.g.*, using cell lines or cells derived from a subject) or *in vivo* (*e.g.*, using an animal model). Accordingly, the screening methods of the invention can further comprise determining the effect of the compound on at least one T effector cell activity and/or at least one T regulatory activity to thereby confirm that a compound has the desired effect.

In one embodiment, a compound is further assayed for the ability to modulate an activity associated with a T effector cell, *e.g.*, proliferation or cytokine production or cytotoxicity by a T effector cell. In a further embodiment, the ability of a compound is further assayed for the ability to modulate an activity associated with a T regulatory cell, *e.g.*, proliferation or cytokine production by regulatory T cells, the

ability to downregulate T effector cells or induce tolerance. For example, determining the ability of a test compound to modulate tolerance can be determined by assaying secondary T cell responses. If the T cells are unresponsive to the subsequent activation attempts, as determined by IL-2 synthesis and/or T cell proliferation, a state of tolerance has been induced, *e.g.*, T regulatory cells have been activated. Alternatively, if IL-2 synthesis is stimulated and T cells proliferate, T effector cells have been activated. See, *e.g.*, Gimmi, C.D. et al. (1993) *Proc. Natl. Acad. Sci.* USA 90, 6586-6590; and Schwartz (1990) *Science*, 248, 1349-1356, for example assay systems that can used as the basis for an assay in accordance with the present invention. T cell proliferation can be measured, for example, by assaying [^{3}H] thymidine incorporation and methods to measure protein levels of members of the MAP kinase cascade or activation of the AP-1 complex. Cytokine levels can be assayed by any number of commercially available kits for immunoassays, including but not limited to, Stratagene, Inc., La Jolla, CA. Tolerized T cells will have decreased IL-2 production when compared with stimulated T cells. Other methods for measuring the diminished activity of tolerized T cells include, without limitation, measuring intracellular calcium mobilization, measuring protein levels of members of the MAP kinase cascade, members of the NFAT cascade, and/or by measuring the activity of the AP-1 complex of transcription factors in a T cell upon engagement of its T cell receptors.

In another embodiment, an assay for the expansion of a population of T regulatory and/or T effector cells by detecting cells expressing markers associated with one or the other cell population using techniques described herein or known in the art.

Alternatively, a modulator of a protein kinase C theta pathway component identified as described herein can be used in an animal model to determine the mechanism of action of such a modulator. For example, an agent can be tested in art recognized animal models of human diseases (*e.g.*, EAE as a model of multiple sclerosis and the NOD mice as a model for diabetes) or other well characterized animal models of human autoimmune diseases. Such animal models include the *mrl/lpr/lpr* mouse as a model for lupus erythematosus, murine collagen-induced arthritis as a model for rheumatoid arthritis, and murine experimental myasthenia gravis (see Paul ed., *Fundamental Immunology*, Raven Press, New York, 1989, pp. 840-856). A modulatory (*i.e.*, stimulatory or inhibitory) agent of the invention can be administered to test animals and the course of the disease in the test animals can then be monitored using standard

methods for the particular model being used. Effectiveness of the modulatory agent is evidenced by amelioration of the disease condition in animals treated with the agent as compared to untreated animals (or animals treated with a control agent).

It will be understood that it may be desirable to formulate such compound(s) as pharmaceutical compositions (described supra) prior to contacting them with cells.

In one aspect, cell-based systems, as described herein, may be used to identify agents that may act to modulate effector T cell function relative to T regulatory cell function, for example. For example, such cell systems may be exposed to an agent, suspected of exhibiting an ability to modulate effector T cell function relative to T regulatory cell function, at a sufficient concentration and for a time sufficient to elicit response in the exposed cells. After exposure, the cells are examined to determine whether one or more responses have been altered.

In addition, in one embodiment, the ability of a compound to modulate effector T cell markers and/or effector T cell markers can be measured.

In addition, animal-based disease systems, such as those described herein, may be used to identify agents capable of modulating effector T cell function relative to T regulatory cell function, for example. Such animal models may be used as test substrates for the identification of drugs, pharmaceuticals, therapies and interventions which may be effective in modulating effector T cell function relative to T regulatory cell function. In addition, an agent identified as described herein (*e.g.*, a modulating agent of a molecule of the invention) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent.

Additionally, gene expression patterns may be utilized to assess the ability of an agent to modulate effector T cell function relative to T regulatory cell function. For example, the expression pattern of one or more genes may form part of "an expression profile" or "transcriptional profile" which may be then used in such an assessment. "Gene expression profile" or "transcriptional profile", as used herein, includes the pattern of mRNA expression obtained for a given tissue or cell type under a given set of conditions. Gene expression profiles may be generated, for example, by utilizing a differential display procedure, Northern analysis and/or RT-PCR.

In one embodiment, the sequences of a molecule of the invention may be used as probes and/or PCR primers for the generation and corroboration of such gene expression profiles.

Gene expression profiles may be characterized for known states within the cell or animal-based model systems. Subsequently, these known gene expression profiles may be compared to ascertain the effect a test agent has to modify such gene expression profiles and to cause the profile to more closely resemble that of a more desirable profile.

Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

IV. *Test Compounds*

The test compounds or agents of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K. S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci., USA* 91:11422; Zuckermann *et al.* (1994) *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds can be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), 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); (Felici (1991) *J.*

Mol. Biol. 222:301-310); (Ladner *supra.*). In a preferred embodiment, the library is a natural product library.

Non limiting exemplary compounds which can be screened for activity include, but are not limited to, peptides, nucleic acids, carbohydrates, small organic molecules, and natural product extract libraries.

Candidate/test compounds or agents include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries (see, *e.g.*, Lam, K.S. *et al.* (1991) *Nature* 354:82-84; Houghten, R. *et al.* (1991) *Nature* 354:84-86) and combinatorial chemistry-derived molecular libraries made of D- and/or L- configuration amino acids; 2) phosphopeptides (*e.g.*, members of random and partially degenerate, directed phosphopeptide libraries, see, *e.g.*, Songyang, Z. *et al.* (1993) *Cell* 72:767-778); 3) antibodies (*e.g.*, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, $F(ab')_2$, Fab expression library fragments, and epitope-binding fragments of antibodies); 4) small organic and inorganic molecules (*e.g.*, molecules obtained from combinatorial and natural product libraries); 5) enzymes (*e.g.*, endoribonucleases, hydrolases, nucleases, proteases, synthatases, isomerases, polymerases, kinases, phosphatases, oxido-reductases and ATPases), 6) mutant forms of protein kinase C theta pathway components, *e.g.*, dominant negative mutant forms of protein kinase C theta pathway components, and 7) antisense RNA molecules or molecules that mediate RNAi.

Art recognized techniques of structure based drug design can also be used to identify compounds that modulate the expression or activity of one or more protein kinase C theta pathway components.

V. *Diagnostic Assays*

The present invention also features diagnostic assays, for determining expression of a protein kinase C theta pathway component, within the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing such a disease or disorder, or for use as a monitoring method to assess treatment efficacy and/or disease remission. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing such a disorder (*e.g.*, a disorder associated with expression or activity of a protein kinase C theta pathway

component) or as a method to prevent relapse of a disease or disorder. Such assays can be used for prognostic or predictive purpose to thereby phophylactically treat an individual prior to the onset of a disease or disorder. A preferred agent for detecting a protein kinase C theta pathway component protein is an antibody capable of binding to a pathway component protein, preferably an antibody with a detectable label or primers for amplifying a gene encoding a pathway component. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. The invention also encompasses kits for the detection of expression or activity of a pathway component in a biological sample in order to assess the balance between T effector cells and T regulatory cells to a particular antigen in the subject. For example, the kit can comprise a labeled compound or agent capable of detecting a pathway component or its activity in a biological sample; means for determining the amount of a pathway component in the sample; and/or means for comparing the amount of a pathway component in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit.

VI. *Recombinant Expression Vectors*

Another aspect of the invention pertains to vectors, preferably expression vectors, for producing protein reagents (*e.g.*, fusion proteins reagents) of the instant invention or for causing a protein kinase C theta pathway component to be expressed in a cell, *e.g.*, a patient's cell, *e.g., in vitro* or *in vivo*. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. A preferred vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. Preferred protein reagents include polypeptides or bioactive fragments thereof of protein kinase C theta pathway components.

The recombinant expression vectors of the invention comprise a nucleic acid that encodes a polypeptide of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed.

Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). The expression vectors can be introduced into host cells to thereby produce proteins, including fusion proteins or peptides. Alternatively, retroviral expression vectors and/or adenoviral expression vectors can be utilized to express the proteins of the present invention.

The recombinant expression vectors of the invention can be designed for expression of polypeptides in prokaryotic or eukaryotic cells. For example, polypeptides can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990).

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Purified fusion proteins are particularly useful in the cell-free assay methodologies of the present invention.

In yet another embodiment, a nucleic acid molecule encoding a polypeptide of a protein kinase C theta pathway component is expressed in mammalian cells, for example, for use in the cell-based assays described herein. When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. In another embodiment, the recombinant mammalian expression

vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid).

Another aspect of the invention pertains to assay cells into which a recombinant expression vector has been introduced. An assay cell can be prokaryotic or eukaryotic, but preferably is eukaryotic. A preferred assay cell is a T cell, for example, a human T cell. T cells can be derived from human blood and expanded *ex vivo* prior to use in the assays of the present invention. Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

VII. *Methods of the Invention*

A. Methods of Use

The modulatory methods of the invention can be performed *in vitro* (*e.g.*, by culturing the cell with the agent or by introducing the agent into cells in culture) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject or by introducing the agent into cells of a subject, such as by gene therapy).

In one embodiment, a subject is identified as one that would benefit from modulation of the balance between T effector and T regulatory cells prior to treatment to modulate a PKC theta pathway component. For example, in one embodiment, the relative activity of T regulatory and T effector cells can be measured. In another embodiment, the relative numbers of T effector cells and T regulatory cells can be calculated. In another embodiment, the presence of T effector and T regulatory cells can be detected at a particular site, *e.g.*, the site of a transplant.

In one embodiment, a subject's cells are assayed for the activity and/or expression of one or more of the pathway components prior to treatment with a modulator of a pathway component (identified as described herein) in order to identify the subject as one that would benefit from the modulation of T effector or T regulatory cells.

In another embodiment, a subject can be monitored after treatment with a conventional immunomodulatory reagent to determine whether the patient would benefit from modulation of the balance between T effector and T regulatory cells.

In another embodiment, a modulator of a pathway component is administered to a subject *in vivo* or *in vitro* prior to exposure to an antigen or simultaneously with exposure to an antigen. In one embodiment, the therapy is a therapeutic protein for repeated administration, *e.g.,* Factor VIII treatment.

For practicing the modulatory method *in vitro*, cells can be obtained from a subject by standard methods and incubated (*i.e.*, cultured) *in vitro* with a modulatory agent of the invention in order to modulate the activity of a pathway component in the cells. For example, peripheral blood mononuclear cells (PBMCs) can be obtained from a subject and isolated by density gradient centrifugation, *e.g.*, with Ficoll/Hypaque. Specific cell populations can be depleted or enriched using standard methods. For example, T cells can be enriched, for example, by positive selection using antibodies to T cell surface markers, for example, by incubating cells with a specific primary monoclonal antibody (mAb), followed by isolation of cells that bind the mAb using magnetic beads coated with a secondary antibody that binds the primary mAb. Specific cell populations can also be isolated by fluorescence activated cell sorting according to standard methods. If desired, cells treated *in vitro* with a modulatory agent of the invention can be re-administered to the subject. For administration to a subject, it may be preferable to first remove residual agents in the culture from the cells before administering them to the subject. This can be done for example by a Ficoll/Hypaque gradient centrifugation of the cells. For further discussion of *ex vivo* genetic modification of cells followed by re-administration to a subject, see also U.S. Patent No. 5,399,346 by W.F. Anderson *et al.*

For practicing the modulatory method *in vivo* in a subject, the modulatory agent can be administered to the subject such that activity of a pathway component in cells of the subject is modulated. The term "subject" is intended to include living organisms in which an immune response can be elicited. Preferred subjects are mammals. Examples of subjects include humans, monkeys, dogs, cats, mice, rats, cows, horses, goats and sheep.

For stimulatory or inhibitory agents that comprise nucleic acids (including recombinant expression vectors encoding marker protein, antisense RNA, intracellular antibodies or dominant negative inhibitors), the agents can be introduced into cells of the subject using methods known in the art for introducing nucleic acid (*e.g.*, DNA) into cells *in vivo*. Examples of such methods encompass both non-viral and viral methods, including:

Direct Injection: Naked DNA can be introduced into cells *in vivo* by directly injecting the DNA into the cells (see *e.g.*, Acsadi *et al.* (1991) *Nature* 332:815-818; Wolff, *et al.* (1990) *Science* 247:1465-1468). For example, a delivery apparatus (*e.g.*, a "gene gun") for injecting DNA into cells *in vivo* can be used. Such an apparatus is commercially available (*e.g.*, from BioRad).

Cationic Lipids: Naked DNA can be introduced into cells *in vivo* by complexing the DNA with cationic lipids or encapsulating the DNA in cationic liposomes. Examples of suitable cationic lipid formulations include N-[-1-(2,3-dioleoyloxy)propyl]N,N,N-triethylammonium chloride (DOTMA) and a 1:1 molar ratio of 1,2-dimyristyloxy-propyl-3-dimethylhydroxyethylammonium bromide (DMRIE) and dioleoyl phosphatidylethanolamine (DOPE) (see *e.g.*, Logan, J.J. *et al.* (1995) *Gene Therapy* 2:38-49; San, H. *et al.* (1993) *Human Gene Therapy* 4:781-788).

Receptor-Mediated DNA Uptake: Naked DNA can also be introduced into cells *in vivo* by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor (see for example Wu, G. and Wu, C.H. (1988) *J. Biol. Chem.* 263:14621; Wilson, *et al.* (1992) *J. Biol. Chem.* 267:963-967; and U.S. Patent No. 5,166,320). Binding of the DNA-ligand complex to the receptor facilitates uptake of the DNA by receptor-mediated endocytosis. A DNA-ligand complex linked to adenovirus capsids which naturally disrupt endosomes, thereby releasing material into the cytoplasm can be used to avoid degradation of the complex by intracellular lysosomes (see for example Curiel, *et al.* (1991) *Proc. Natl. Acad. Sci., USA* 88:8850; Cristiano, *et al.* (1993) *Proc. Natl. Acad. Sci., USA* 90:2122-2126).

Retroviruses: Defective retroviruses are well characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A.D. (1990) *Blood* 76:271). A recombinant retrovirus can be constructed having a nucleotide sequences of interest incorporated into the retroviral genome. Additionally, portions of the retroviral genome can be removed to render the retrovirus replication defective. The replication

defective retrovirus is then packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in *Current Protocols in Molecular Biology*, Ausubel, F.M. *et al.* (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines include ψCrip, ψCre, ψ2 and ψAm. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, endothelial cells, lymphocytes, myoblasts, hepatocytes, bone marrow cells, *in vitro* and/or *in vivo* (see for example Eglitis, *et al.* (1985) *Science* 230:1395-1398; Danos and Mulligan (1988) *Proc. Natl. Acad. Sci., USA* 85:6460-6464; Wilson *et al.* (1988) *Proc. Natl. Acad. Sci., USA* 85:3014-3018; Armentano *et al.* (1990) *Proc. Natl. Acad. Sci., USA* 87:6141-6145; Huber *et al.* (1991) *Proc. Natl. Acad. Sci., USA* 88:8039-8043; Ferry, *et al.* (1991) *Proc. Natl. Acad. Sci., USA* 88:8377-8381; Chowdhury, *et al.* (1991) *Science* 254:1802-1805; van Beusechem, *et al.* (1992) *Proc. Natl. Acad. Sci., USA* 89:7640-7644; Kay, *et al.* (1992) *Human Gene Therapy* 3:641-647; Dai, *et al.* (1992) *Proc. Natl. Acad. Sci., USA* 89:10892-10895; Hwu, *et al.* (1993) *J. Immunol.* 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). Retroviral vectors require target cell division in order for the retroviral genome (and foreign nucleic acid inserted into it) to be integrated into the host genome to stably introduce nucleic acid into the cell. Thus, it may be necessary to stimulate replication of the target cell.

Adenoviruses: The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See for example Berkner, *et al.* (1988) *BioTechniques* 6:616; Rosenfeld, *et al.* (1991) *Science* 252:431-434; and Rosenfeld *et al.* (1992) *Cell* 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (*e.g.*, Ad2, Ad3, and Ad7 *etc.*) are well known to those skilled in the art. Recombinant adenoviruses are advantageous in that they do not require dividing cells to be effective gene delivery vehicles and can be used to infect a wide variety of cell types, including airway

epithelium (Rosenfeld, *et al.* (1992) cited *supra*), endothelial cells (Lemarchand, *et al.* (1992) *Proc. Natl. Acad. Sci., USA* 89:6482-6486), hepatocytes (Herz and Gerard (1993) *Proc. Natl. Acad. Sci., USA* 90:2812-2816) and muscle cells (Quantin, *et al.* (1992) *Proc. Natl. Acad. Sci., USA* 89:2581-2584). Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (*e.g.*, retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner, *et al.* cited *supra*; Haj-Ahmand and Graham (1986) *J. Virol.* 57:267). Most replication-defective adenoviral vectors currently in use are deleted for all or parts of the viral E1 and E3 genes but retain as much as 80 % of the adenoviral genetic material.

Adeno-Associated Viruses: Adeno-associated virus (AAV) is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka, *et al. Curr. Topics in Micro. Immunol.* (1992) 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte, *et al.* (1992) *Am. J. Respir. Cell. Mol. Biol.* 7:349-356; Samulski *et al.* (1989) *J. Virol.* 63:3822-3828; and McLaughlin, *et al.* (1989) *J. Virol.* 62:1963-1973). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such as that described in Tratschin, *et al.* (1985) *Mol. Cell. Biol.* 5:3251-3260 can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for example Hermonat, *et al.* (1984) *Proc. Natl. Acad. Sci., USA* 81:6466-6470; Tratschin, *et al.* (1985) *Mol. Cell. Biol.* 4:2072-2081; Wondisford, *et al.* (1988) *Mol. Endocrinol.* 2:32-39; Tratschin, *et al.* (1984) *J. Virol.* 51:611-619; and Flotte, *et al.* (1993) *J. Biol. Chem.* 268:3781-3790).

The efficacy of a particular expression vector system and method of introducing nucleic acid into a cell can be assessed by standard approaches routinely used in the art. For example, DNA introduced into a cell can be detected by a filter hybridization technique (*e.g.*, Southern blotting) and RNA produced by transcription of

introduced DNA can be detected, for example, by Northern blotting, RNase protection or reverse transcriptase-polymerase chain reaction (RT-PCR). The gene product can be detected by an appropriate assay, for example by immunological detection of a produced protein, such as with a specific antibody, or by a functional assay to detect a functional activity of the gene product.

In one embodiment, a retroviral expression vector encoding a marker is used to express marker protein in cells *in vivo*, to thereby stimulate marker protein expression or activity *in vivo*. Such retroviral vectors can be prepared according to standard methods known in the art (*e.g.*, as discussed above).

A modulatory agent, such as a chemical compound, can be administered to a subject as a pharmaceutical composition. Such compositions typically comprise the modulatory agent and a pharmaceutically acceptable carrier. As used herein the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions. Pharmaceutical compositions can be prepared as described below.

B. *Methods of Treatment*

Numerous disease conditions associated with a predominant effector T cell function are known and could benefit from modulation of the type of response mounted in the individual suffering from the disease condition. The methods can involve either direct administration of a modulatory agent to a subject in need of such treatment or *ex vivo* treatment of cells obtained from the subject with an agent followed by re-administration of the cells to the subject. The treatment may be further enhanced by administering other immunomodulatory agents. Application of the immunomodulatory methods of the invention to such diseases is described in further detail below.

Many autoimmune disorders are the result of inappropriate or unwanted activation of T effector cells resulting in the production of cytokines and autoantibodies involved in the pathology of the diseases. In addition, T effector cell function is associated with graft rejection. Allergies are also mediated by T effector cells. Accordingly, when a reduced effector T cell or antibody response is desired, the methods of the invention can be used to downmodulate the expression and/or activity a molecule preferentially associated with T effector cells, *e.g.*, such that at least one T effector cell function is downmodulated relative to at least one T regulatory cell function. In another embodiment, such disorders can be ameliorated by upmodulating the expression and/or activity of a molecule preferentially associated with T regulatory cells, e.g., such that at least one T regulatory cell function is upmodulated relative to at least one T effector cell function.

In contrast, there are conditions that would benefit from enhancing at least one activity of T effector cells and/or downmodulating at least one activity of T regulatory cells. For example, immune effector cells often fail to react effectively with cancer cells. Accordingly, when a enhanced effector T cell or antibody response is desired, the methods of the invention can be used to upmodulate the expression and/or activity a molecule preferentially associated with T effector cells, *e.g.*, such that at least one T effector cell function is upmodulated relative to at least one T regulatory cell function.

In one embodiment, these modulatory methods can be used in combination with an antigen to either enhance or reduce the immune response to the antigen. For example, T effector cell responses can be enhanced in a vaccine preparation or reduced in order to reduce effector cell responses to a therapeutic protein which much be chronically administered to the subject, *e.g.*, factor VIII.

More specifically, preferentially downregulating at least one activity of the effector T cells relative to modulating at least one activity of regulatory T cell function in a subject is useful, *e.g.*, in situations of tissue, skin and organ transplantation, in graft-versus-host disease (GVHD), or in autoimmune diseases such as systemic lupus erythematosus, and multiple sclerosis. For example, preferentially promoting regulatory T cell function and/or reducing effector T cell function results in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by immune cells, followed by an

immune reaction that destroys the transplant. The administration of an agent or modulator as described herein, alone or in conjunction with another immunoregulatory agent prior to or at the time of transplantation can modulate effector T cell function as well as regulatory T cell function in a subject.

Many autoimmune disorders are the result of inappropriate activation of immune cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive immune cells may reduce or eliminate disease symptoms. The efficacy of reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/*lpr/lpr* mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., *Fundamental Immunology*, Raven Press, New York, 1989, pp. 840-856).

As used herein, the term "autoimmunity" refers to the condition in which a subject's immune system (*e.g.*, T and B cells) starts reacting against his or her own tissues. Non-limiting examples of autoimmune diseases and disorders having an autoimmune component that may be treated according to the invention include type 1 diabetes, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis), multiple sclerosis, myasthenia gravis, systemic lupus erythematosis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, including keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Crohn's disease, Graves ophthalmopathy, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis.

Preferably, inhibition of effector cell function is useful therapeutically in the treatment of allergy and allergic reactions, *e.g.*, by inhibiting IgE production. Inhibition of effector T cell function and/or promotion of regulatory T cell function can be accompanied by exposure to allergen in conjunction with appropriate MHC molecules. Allergic reactions can be systemic or local in nature, depending on the route of entry of the allergen and the pattern of deposition of IgE on mast cells or basophils. Thus, inhibition of effector T cell mediated allergic responses can occur locally or systemically by administration of an agent or inhibitor.

Preferably, inhibition of at lest one effector T cell function may also be important therapeutically in viral infections of immune cells. For example, in the acquired immune deficiency syndrome (AIDS), viral replication is stimulated by immune cell activation. Inhibition of effector T cell function may result in inhibition of viral replication and thereby ameliorate the course of AIDS.

Upregulating T effector cells is also useful in therapy. Upregulation of at least one T effector activity can be useful in enhancing an existing immune response or eliciting an initial immune response. For example, preferably increasing at least one T effector cell activity using agents which stimulate a molecule of the invention in effector T cells is useful in cases of infections with microbes, *e.g.,* bacteria, viruses, or parasites. These would include viral skin diseases such as Herpes or shingles, in which case such an agent can be delivered topically to the skin. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of such agents systemically. In another embodiment, expression and/or activity of at least one molecule of the invention associated with T regulatory cells can be downmodulated.

Immunity against a pathogen, *e.g.*, a virus, can be induced by vaccinating with a viral protein along with an agent that activates effector T cell function in an appropriate adjuvant. Nucleic acid vaccines can be administered by a variety of means, for example, by injection (*e.g.*, intramuscular, intradermal, or the biolistic injection of DNA-coated gold particles into the epidermis with a gene gun that uses a particle accelerator or a compressed gas to inject the particles into the skin (Haynes *et al.* 1996. *J. Biotechnol.* 44:37)). Alternatively, nucleic acid vaccines can be administered by non-invasive means. For example, pure or lipid-formulated DNA can be delivered to the respiratory system or targeted elsewhere, *e.g.,* Peyers patches by oral delivery of DNA

(Schubbert. 1997. *Proc. Natl. Acad. Sci., USA* 94:961). Attenuated microorganisms can be used for delivery to mucosal surfaces. (Sizemore *et al.* (1995) *Science.* 270:29). Pathogens for which vaccines are useful include hepatitis B, hepatitis C, Epstein-Barr virus, cytomegalovirus, HIV-1, HIV-2, tuberculosis, malaria and schistosomiasis.

In another application, preferential upregulation or enhancement of at least one effector T cell function is useful in the induction of tumor immunity. In another embodiment, the immune response can be stimulated by the transmission of activating signal. For example, immune responses against antigens to which a subject cannot mount a significant immune response, *e.g.*, to an autologous antigen, such as a tumor specific antigens can be induced in this fashion.

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disease, disorder or condition that would benefit from preferentially modulating at least one effector T cell function while having little effect on a T regulatory response and vice versa. Administration of a prophylactic agent can occur prior to the manifestation of symptoms, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

These agents can be administered *in vitro* (*e.g.*, by contacting the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder that would benefit from up- or downmodulation of T effector cells while not affecting regulatory T cells.

The modulatory agents of the invention can be administered alone or in combination with one or more additional agents. For example, in one embodiment, two agents described herein can be administered to a subject. In another embodiment, an agent described herein can be administered in combination with other immunomodulating agents. Examples of other immunomodulating reagents include antibodies that block a costimulatory signal, (*e.g.*, against CD28, ICOS), antibodies that activate an inhibitory signal via CTLA4, and/or antibodies against other immune cell markers (*e.g.*, against CD40, against CD40 ligand, or against cytokines), fusion proteins (*e.g.*, CTLA4-Fc, PD-1-Fc), and immunosuppressive drugs, (*e.g.*, rapamycin, cyclosporine A or FK506). In certain instances, it may be desirable to further administer

other agents that upregulate immune responses, for example, agents which deliver T cell activation signals, in order elicit or augment an immune response.

Unlike current immunosuppressives, agents or inhibitors as described herein, because they would foster development of a homeostatic immunoregulatory mechanism, would require short term administration (*e.g.*, for a period of several weeks to months), rather than prolonged treatment, to control unwanted immune responses. Prolonged treatment with the agent or inhibitor or with a general immunosuppressant is unnecessary as the subject develops a robust regulatory T cell response to antigens (*e.g.*, donor antigens, self antigens) associated with the condition. Because the resulting immunoregulation is mediated by natural T cell mechanisms, no drugs would be needed to maintain immunoregulation once the dominant regulatory T cell response is established. Elimination of life-long treatment with immunosuppressants would eliminate many, if not all, side effects currently associated with treatment of autoimmunity and organ grafts.

In one embodiment, immune responses can be enhanced in an infected patient by removing immune cells from the patient, contacting immune cells *in vitro* an agent that activates effector T cell function, and reintroducing the *in vitro* stimulated immune cells into the patient.

VIII. *Pharmaceutical Compositions*

Modulatory agents, *e.g.,* inhibitory or stimulatory agents as described herein, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the agent and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, intramuscular, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought

about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be

accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, modulatory agents are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations should be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (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 LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a

delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 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 compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which 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 can be measured, for example, by high performance liquid chromatography.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

IX. ***Administration of Modulating Agents***

Modulating agents of the invention are administered to subjects in a biologically compatible form suitable for pharmaceutical administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the agent to be administered in which any toxic effects are outweighed by the therapeutic effects of the agent.

Administration of a therapeutically active amount of the therapeutic compositions of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of agent may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of agent to elicit a desired response in the individual. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses can be administered daily or the dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The agent can be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active compound can be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound. For example, to administer the agent by other than parenteral administration, it may be desirable to coat, or co-administer the agent with, a material to prevent its inactivation.

Agent can be co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Adjuvant is used in its broadest sense and includes any immune stimulating compound such as interferon. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEEP) and trasylol. Liposomes include water-in-oil-in-water emulsions as well as conventional liposomes (Sterna *et al.* (1984) *J. Neuroimmunol.* 7:27).

The active compound may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

When the active compound is suitably protected, as described above, the agent can be orally administered, for example, with an inert diluent or an assimilable edible carrier. As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and attached Appendices, are incorporated herein by reference.

EXAMPLES

EXAMPLE 1: *Identification of Genes Preferentially Expressed in T Effector Cells or T Regulatory Cells Using Affymetrix™ Gene Chips*

This example describes the identification of genes which are present in certain T cell types and absent from other T cell types. In particular, genes used differentially between effector T cells (Th1 and Th2) and regulatory T cells are identified.

Methods

Culture of T cell lines

Differentiated cell lines were produced from cells prepared from human cord blood or peripheral blood CD4+CD45RA+ naïve T cells by a variety of methods, including flow cytometry and magnetic bead separations. Purity of the starting populations was >95%. Cells were then stimulated by CD3 and CD28 antibodies in RPMI 1640 with 10%FCS and 1% Human AB serum with defined mixtures of cytokines and neutralizing antibodies to cytokines to produce the differentiated cell types. Th1 cells were produced by culture with IL12 (62U/ml) and anti-IL4 (0.2ug/ml); Th2 cells were produced by culture in IL4(145U/ml) and anti-IL12 (10ug/ml) and anti-IFNγ (10ug/ml); and regulatory T cells were produced by culture in TGFβ (32U/ml), IL9 (42U/ml), anti-IL4 (10ug/ml) and anti-IL12 (10ug/ml) and anti-IFNγ(10ug/ml). (Note: anti-IL12 was not used in all experiments). All cultures were supplemented with IL2 (65U/ml) and IL15 (4500U/ml). Cells were split into larger culture dishes as warranted by cell division. At the conclusion of one round of cell differentiation (7-12 days), cells were harvested for preparation of total RNA for use in the gene chip experiments.

Affymetrix™ Gene Chip experiment

RNA from each cell type was prepared using the Qiagen™ RNeasy kit as described by the manufacturer. After isolation of high quality total RNA from each cell type, the RNA was biotin labeled and fragmented for use in the Affymetrix™ Gene chip as recommended by Affymetrix™. Briefly, RNA was copied into cDNA using Superscript™ II polymerase and a T7 primer. The complementary strand was then

synthesized using *E. coli* DNA Polymerase I. The product, dsDNA, was phenol/chloroform extracted and ethanol precipitated. In vitro transcription using Biotinylated nucleosides was then performed to amplify and label the RNA using the ENZO™ Bioarray High Yield RNA transcript labeling kit. The labeled product was cleaned up using the clean-up procedure described with the Qiagen RNeasy kit. Labeled RNA was fragmented by incubation in 200mM Tris acetate, 500mM potassium acetate and 150mM magnesium acetate and the recommended amount was loaded onto the Affymetrix™ Hu133 gene array, chips A and B. Affymetrix™ chips were hybridized as recommended by the manufacturer and washed as recommended in the Affymetrix™ automated chip washer. Following washing and tagging of Biotinylated RNA fragments with fluorochromes, the chips were read in the Affymetrix™ chip reader.

For each cell type and each chip all probesets, representing a total of approximately 34,000 human genes, was scored as "present" or "absent" based on statistical analysis of the fluorescent signals on sense and nonsense portions of the chip using Affymetrix™ Microarray Suite software. These "present" and "absent" calls for each probeset, along with the signal strength were imported into Microsoft™ Access databases. Using queries, datafiles of all genes scored present for each cell type were created. Genes which scored present on all cell types were removed from further study using queries. Datafiles of genes which were unique to a cell type or preferentially expressed in one cell type relative to another were created using queries to select genes which only scored present on Th1, Th2 or regulatory T cells. In addition, datafiles of genes which were only present in the effector (Th1 and Th2) cells but absent in the regulatory T cells or present only in the regulatory T cells but absent in the effector T cells were created.

Among the genes which appeared to be preferentially used in the activated effector T cells relative to the regulatory T cells, were genes for a series of proteins known to be required for signal transduction in activated T cells through Protein Kinase C theta. Examination of the results obtained regarding the presence of genes associated with the PKC theta signaling pathway revealed that while effector T cells appeared to be actively transcribing messages for molecules utilized in this pathway, the regulatory T cells did not. Figure 2 shows the genechip expression data for the relevant probesets.

EXAMPLE 2: ***PKC theta is not required to activate regulatory T cells***

Two experiments were performed to verify that PKC theta signaling was preferentially utilized by effector T cells versus regulatory T cells and was required for effector T cell activation but not for regulatory cell activation. The first experiment verified the decrease in expression of the PKC theta protein in regulatory T cells. Populations of Th1, Th2 and regulatory T cells were prepared as described above. These cells were centrifuged onto microscope slides and stained using antibodies specific for the TCR and for PKC theta. Examination of the different cell types (Figure 3) revealed that while all of the cell types expressed the TCR, PKC theta expression was only strongly expressed in peripheral blood T cells and Th2 cells, while it was diffusely expressed throughout the cytoplasm of the Th1 cells. Little to no expression was evident in the regulatory T cells.

The lack of requirement for functional PKC theta by regulatory T cells was demonstrated by treating Th1, Th2 and regulatory T cells with a commercially available inhibitor of the novel protein kinase C enzymes (PKCθ and PKCδ), Rottlerin. Differentiated cells, prepared as above, were re-stimulated using CD3 and CD28 in the presence of a range of concentrations of the commercial inhibitor Rottlerin. In three of three experiments, Rottlerin inhibited cell division by Th1 and Th2 cells at 5uM but did not inhibit the proliferation of regulatory T cells (Figure 4).

EXAMPLE 3: ***Inhibition of PKCθ selectively inhibits Th1 and Th2 cell proliferation.***

The chemical inhibitor of protein kinase C theta, Rottlerlin, has been shown to have additional inhibitory effects on other cellular enzymes critical for cell division at higher concentrations (Davies, SP, *et al.* (2000) *Biochem. J.* 351:95-105). Therefore, in order to demonstrate that PKCθ inhibitors have the ability to block proliferation of Th1 and Th2 cells more completely than TGFβ-derived Treg cells, a more selective molecule was utilized.

In vitro, peptides derived from a PDPK1 (SEQ ID NO:13 and SEQ ID NO:14) interacting portion of PKCθ have been shown to be capable of specifically inhibiting PKCθ activity (Ghosh, S. and,D'Acquisto F., WO 03/004612). The specificity of these peptides is much greater than the specificity demonstrated for any

available small molecule inhibitors, and these peptides have been shown to specifically inhibit PKCθ compared to other PKC family members.

However, peptide inhibitors of intracellular enzymes do not cross the cell membrane and therefore are not be effective in *in vitro* assays using whole cells. To circumvent this problem the PKCθ inhibitory peptide can be synthesized attached at its N-terminus to the third helix of the antennapedia homeodomain; a peptide known to permit entry of peptides and proteins through biological membranes with no apparent damage to the cells (Fenton, M., *et al.* (1998) *J. Immunol. Methods* 212:41-48; Dostmann, WRG, *et al.* (2000) *Proc. Natl. Acad. Sci., USA* 97:14772-14777). The sequence of the peptide used in these studies was:

NH_2-RQIKIWFQNRRMKWKKMDQNMFRNFSFNMP-COOH (SEQ ID NO:15)

In order to test the ability of this antennapedia-PKCθ peptide to selectively inhibit the proliferation of Th1 and Th2 but not TGFβ-derived Treg cells, differentiated cells were cultured in wells coated with CD3 and CD28 following protocols well known in the art, in the presence or absence of the peptide inhibitor. Three days after initiation of the cultures, three replicate tissue culture wells for each condition for each cell type were fed with media containing ^{3}H-thymidine to monitor cell division. Wells were harvested 18 hr later and incorporated ^{3}H was measured by scintillation counting. Replicate wells were averaged and when comparisons of proliferation were made for each cell type between cells with no inhibitor or increasing concentrations of inhibitor it was found that the antenapedia-PKCθ inhibitory peptide had inhibited proliferation of the Th1 and Th2 cells to 16 % of control levels but TGFβ-derived Treg cells proliferated between 50 and 80% of their control level (Figure 5).

EQUIVALENTS

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.

Claims:

1. A method for treating a condition in a subject in need of such treatment, comprising administering an agent that modulates the expression or activity of a protein kinase C theta pathway component, wherein the effect of such treatment is to modulate the balance of effector T cell function relative to regulatory T cell function in the subject.

2. The method of claim 1, wherein the component is a nucleic acid selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, and 11.

3. The method of claim 1, wherein the component is a polypeptide selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, and 12.

4. The method of claim 1, wherein the agent is a protein, peptide, small molecule or nucleic acid.

5. The method of any one of claims 1, 2, 3 or 4, wherein the condition is a transplant, an allergic disorder, an autoimmune disorder, a viral infection, a microbial infection, a parasitic infection or cancer.

6. A method for modulating the expression or activity of a protein kinase C theta pathway component, comprising:

contacting a population of cells, the population of cells comprising one or more of the following:

T cells;

naïve T cells;

regulatory T cells;

effector T cells; or

peripheral blood leukocytes,

with an agent that modulates the expression or activity of a PKC theta pathway component, wherein the effect of such contacting is to modulate the balance of effector T cell function relative to regulatory T cell function in the population of cells.

7. The method of claim 6, further comprising administering the population of cells that have been contacted with an agent to a subject suffering from a condition, the effect of which is treat the condition.

8. The method of claim 6, wherein the agent is protein, peptide, small molecule or nucleic acid.

9. The method of any one of claims 6, 7 or 8, wherein the condition is a transplant, an allergic disorder, an autoimmune disorder, a viral infection, a microbial infection, a parasitic infection or cancer.

10. An assay for identifying agents modulating the expression or activity of a protein kinase C theta pathway component, comprising:

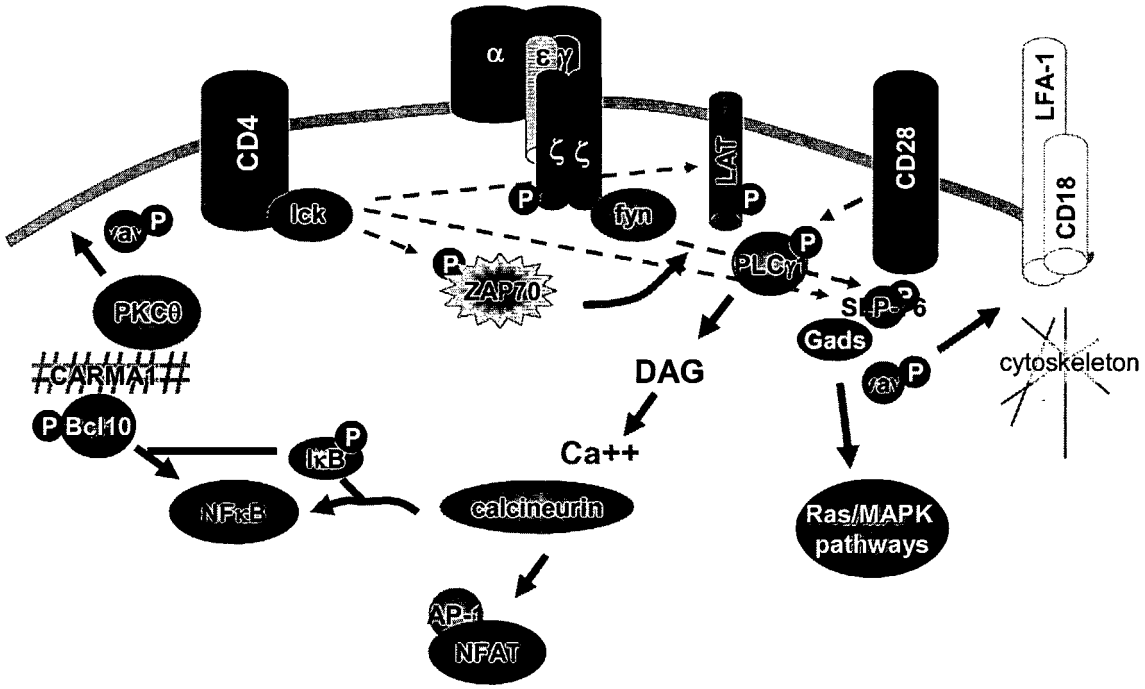
contacting an indicator composition comprising a protein kinase C theta pathway component with a plurality of test agents; and,

determining the ability of a test agent to modulate the expression or activity of a protein kinase C theta component,

wherein the agent identified is able to modulate the balance of effector T cell function relative to regulatory T cell function.

11. The assay of claim 10, wherein the agent is a protein, peptide, small molecule or nucleic acid.

12. The assay of claim 10, wherein the indicator composition is a cell expressing the PKC theta pathway component.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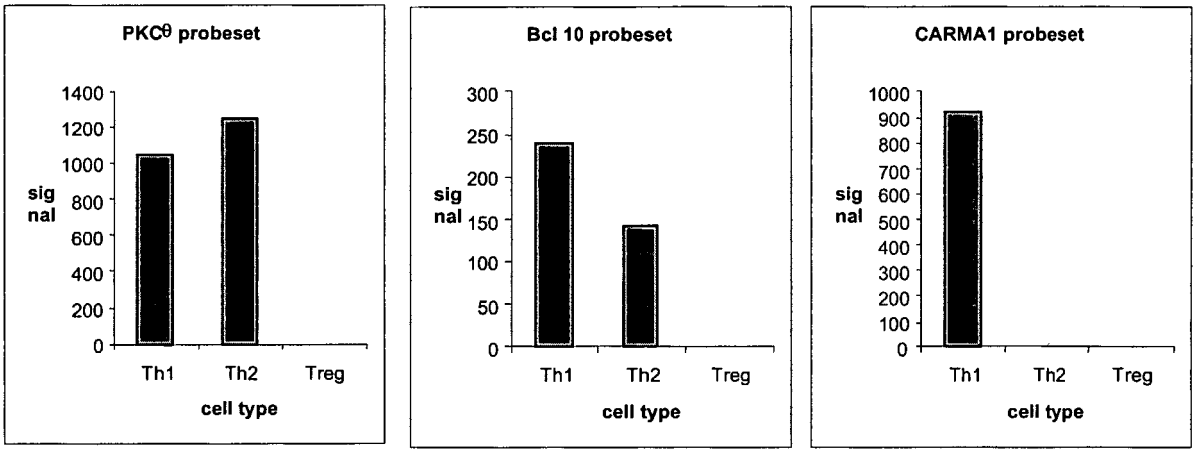
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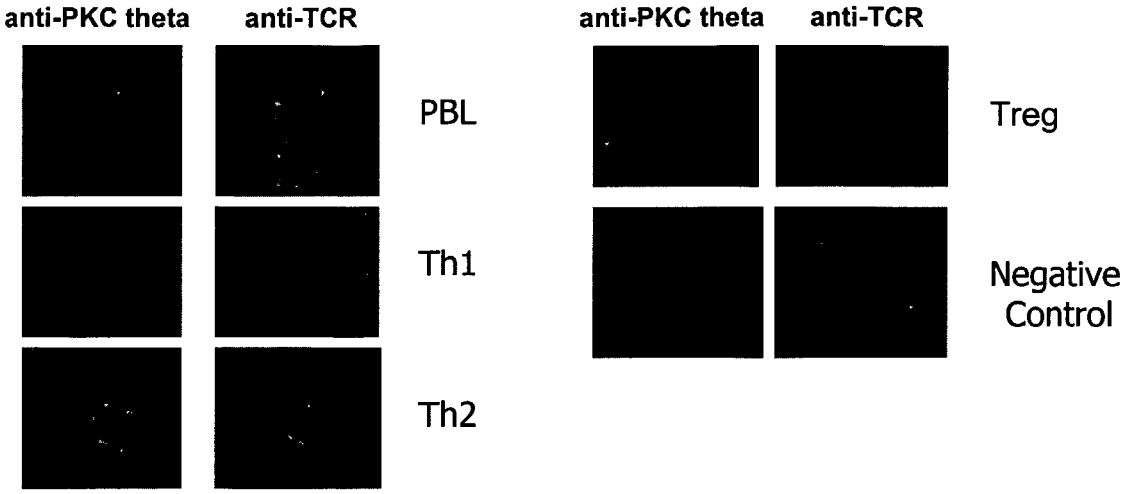
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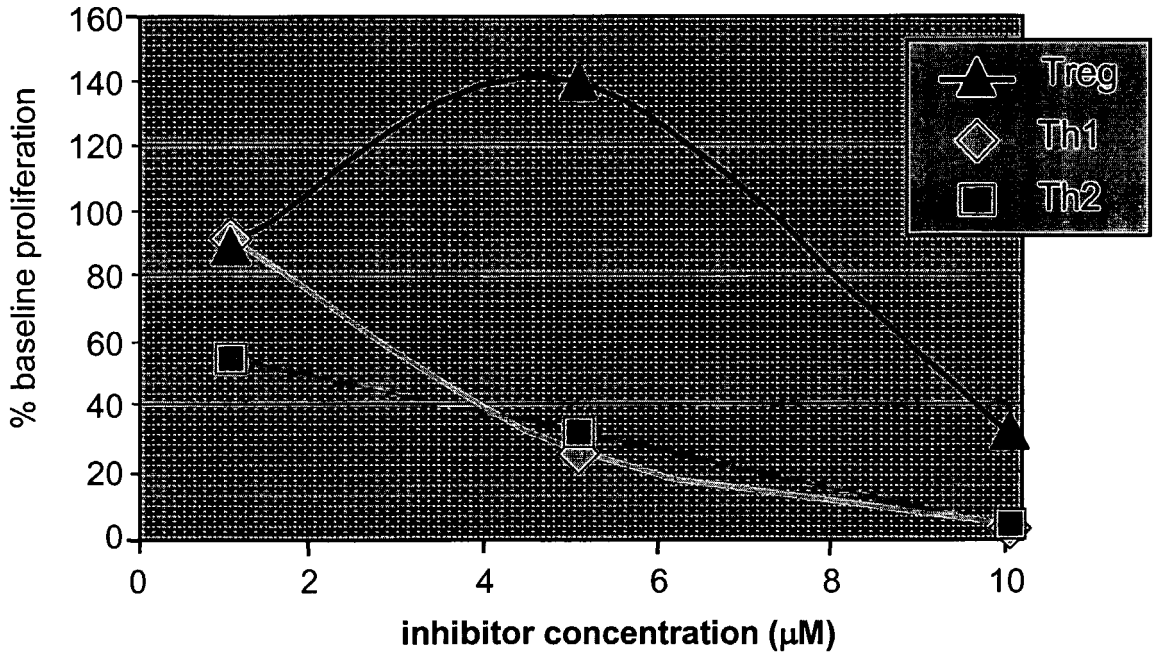
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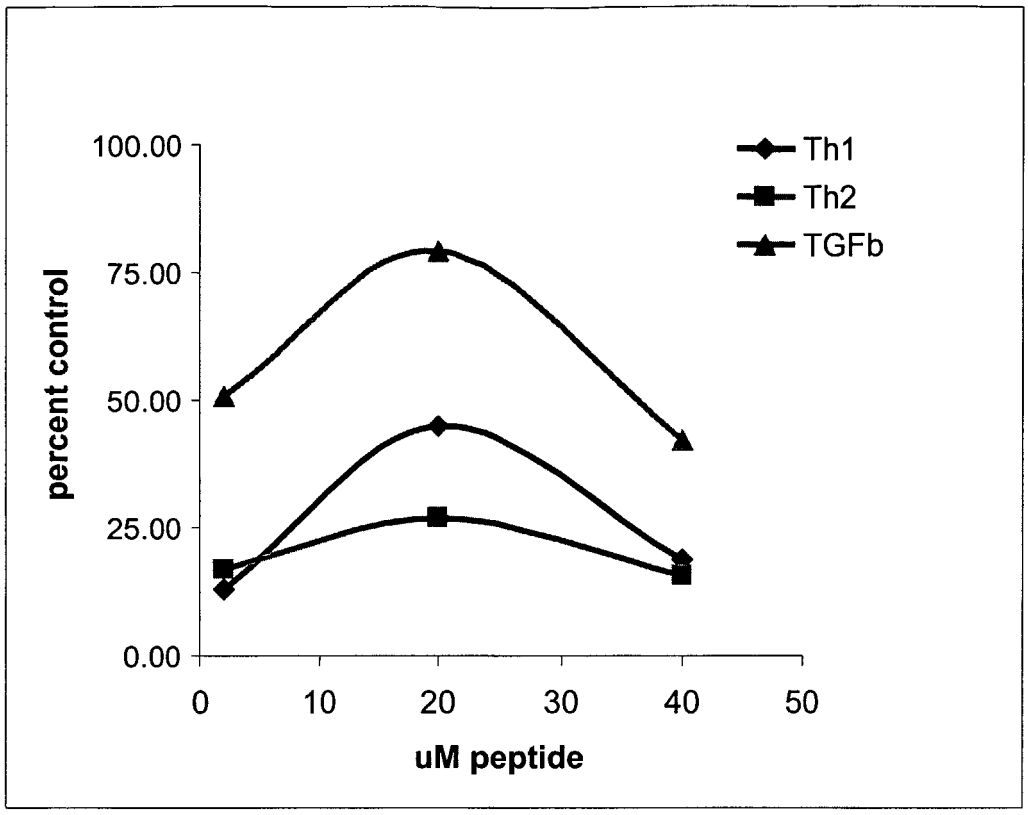
FIGURE 5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<211> 845
<212> PRT
<213> Homo sapiens

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            20   25   30
Leu Ala Gln Ala Leu Arg Asp Gly Val Leu Leu Cys Gln Leu Leu Asn
        35   40   45
Asn Leu Leu Pro His Ala Ile Asn Leu Arg Glu Val Asn Leu Arg Pro
    50   55   60
Gln Met Ser Gln Phe Leu Cys Leu Lys Asn Ile Arg Thr Phe Leu Ser
65   70   75   80
Thr Cys Cys Glu Lys Phe Gly Leu Lys Arg Ser Glu Leu Phe Glu Ala
                85   90   95

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Phe Asp Leu Phe Asp Val Gln Asp Phe Gly Lys Val Ile Tyr Thr Leu
            100                 105                 110
Ser Ala Leu Ser Trp Thr Pro Ile Ala Gln Asn Arg Gly Ile Met Pro
        115                 120                 125
Phe Pro Thr Glu Glu Glu Ser Val Gly Asp Glu Asp Ile Tyr Ser Gly
    130                 135                 140
Leu Ser Asp Gln Ile Asp Asp Thr Val Glu Glu Asp Glu Asp Leu Tyr
145                 150                 155                 160
Asp Cys Val Glu Asn Glu Glu Ala Glu Gly Asp Glu Ile Tyr Glu Asp
                165                 170                 175
Leu Met Arg Ser Glu Pro Val Ser Met Pro Pro Lys Met Thr Glu Tyr
            180                 185                 190
Asp Lys Arg Cys Cys Cys Leu Arg Glu Ile Gln Gln Thr Glu Glu Lys
        195                 200                 205
Tyr Thr Asp Thr Leu Gly Ser Ile Gln Gln His Phe Leu Lys Pro Leu
    210                 215                 220
Gln Arg Phe Leu Lys Pro Gln Asp Ile Glu Ile Ile Phe Ile Asn Ile
225                 230                 235                 240
Glu Asp Leu Leu Arg Val His Thr His Phe Leu Lys Glu Met Lys Glu
                245                 250                 255
Ala Leu Gly Thr Pro Gly Ala Ala Asn Leu Tyr Gln Val Phe Ile Lys
            260                 265                 270
Tyr Lys Glu Arg Phe Leu Val Tyr Gly Arg Tyr Cys Ser Gln Val Glu
        275                 280                 285
Ser Ala Ser Lys His Leu Asp Arg Val Ala Ala Ala Arg Glu Asp Val
    290                 295                 300
Gln Met Lys Leu Glu Glu Cys Ser Gln Arg Ala Asn Asn Gly Arg Phe
305                 310                 315                 320
Thr Leu Arg Asp Leu Leu Met Val Pro Met Gln Arg Val Leu Lys Tyr
                325                 330                 335
His Leu Leu Leu Gln Glu Leu Val Lys His Thr Gln Glu Ala Met Glu
            340                 345                 350
Lys Glu Asn Leu Arg Leu Ala Leu Asp Ala Met Arg Asp Leu Ala Gln
        355                 360                 365
Cys Val Asn Glu Val Lys Arg Asp Asn Glu Thr Leu Arg Gln Ile Thr
    370                 375                 380
Asn Phe Gln Leu Ser Ile Glu Asn Leu Asp Gln Ser Leu Ala His Tyr
385                 390                 395                 400
Gly Arg Pro Lys Ile Asp Gly Glu Leu Lys Ile Thr Ser Val Glu Arg
                405                 410                 415
Arg Ser Lys Met Asp Arg Tyr Ala Phe Leu Leu Asp Lys Ala Leu Leu
            420                 425                 430
Ile Cys Lys Arg Arg Gly Asp Ser Tyr Asp Leu Lys Asp Phe Val Asn
        435                 440                 445
Leu His Ser Phe Gln Val Arg Asp Asp Ser Ser Gly Asp Arg Asp Asn
    450                 455                 460
Lys Lys Trp Ser His Met Phe Leu Leu Ile Glu Asp Gln Gly Ala Gln
465                 470                 475                 480
Gly Tyr Glu Leu Phe Phe Lys Thr Arg Glu Leu Lys Lys Lys Trp Met
                485                 490                 495
Glu Gln Phe Glu Met Ala Ile Ser Asn Ile Tyr Pro Glu Asn Ala Thr
            500                 505                 510
Ala Asn Gly His Asp Phe Gln Met Phe Ser Phe Glu Glu Thr Thr Ser
        515                 520                 525
Cys Lys Ala Cys Gln Met Leu Leu Arg Gly Thr Phe Tyr Gln Gly Tyr
    530                 535                 540
Arg Cys His Arg Cys Arg Ala Ser Ala His Lys Glu Cys Leu Gly Arg
545                 550                 555                 560
Val Pro Pro Cys Gly Arg His Gly Gln Asp Phe Pro Gly Thr Met Lys
                565                 570                 575
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Lys Asp Lys Leu His Arg Arg Ala Gln Asp Lys Lys Arg Asn Glu Leu
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Gly Leu Pro Lys Met Glu Val Phe Gln Glu Tyr Tyr Gly Leu Pro Pro
        595                 600                 605
Pro Pro Gly Ala Ile Gly Pro Phe Leu Arg Leu Asn Pro Gly Asp Ile
    610                 615                 620
Val Glu Leu Thr Lys Ala Glu Ala Glu Gln Asn Trp Trp Glu Gly Arg
625                 630                 635                 640
Asn Thr Ser Thr Asn Glu Ile Gly Trp Phe Pro Cys Asn Arg Val Lys
                645                 650                 655
Pro Tyr Val His Gly Pro Pro Gln Asp Leu Ser Val His Leu Trp Tyr
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Ala Gly Pro Met Glu Arg Ala Gly Ala Glu Ser Ile Leu Ala Asn Arg
        675                 680                 685
Ser Asp Gly Thr Phe Leu Val Arg Gln Arg Val Lys Asp Ala Ala Glu
    690                 695                 700
Phe Ala Ile Ser Ile Lys Tyr Asn Val Glu Val Lys His Ile Lys Ile
705                 710                 715                 720
Met Thr Ala Glu Gly Leu Tyr Arg Ile Thr Glu Lys Lys Ala Phe Arg
                725                 730                 735
Gly Leu Thr Glu Leu Val Glu Phe Tyr Gln Gln Asn Ser Leu Lys Asp
            740                 745                 750
Cys Phe Lys Ser Leu Asp Thr Thr Leu Gln Phe Pro Phe Lys Glu Pro
        755                 760                 765
Glu Lys Arg Thr Ile Ser Arg Pro Ala Val Gly Ser Thr Lys Tyr Phe
    770                 775                 780
Gly Thr Ala Lys Ala Arg Tyr Asp Phe Cys Ala Arg Asp Arg Ser Glu
785                 790                 795                 800
Leu Ser Leu Lys Glu Gly Asp Ile Ile Lys Ile Leu Asn Lys Lys Gly
                805                 810                 815
Gln Gln Gly Trp Trp Arg Gly Glu Ile Tyr Gly Arg Val Gly Trp Phe
            820                 825                 830
Pro Ala Asn Tyr Val Glu Glu Asp Tyr Ser Glu Tyr Cys
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<210> 5
<211> 4276
<212> DNA
<213> Homo sapiens

<400> 5

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<210> 6
<211> 1147
<212> PRT
<213> Homo sapiens

<400> 6

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Ala Lys Leu Thr Pro Tyr Leu Arg Gln Cys Lys Val Ile Asp Glu Gln
        35                  40                  45
Asp Glu Asp Glu Val Leu Asn Ala Pro Met Leu Pro Ser Lys Ile Asn
    50                  55                  60
Arg Ala Gly Arg Leu Leu Asp Ile Leu His Thr Lys Gly Gln Arg Gly
65                  70                  75                  80
Tyr Val Val Phe Leu Glu Ser Leu Glu Phe Tyr Tyr Pro Glu Leu Tyr
                85                  90                  95
Lys Leu Val Thr Gly Lys Glu Pro Thr Arg Arg Phe Ser Thr Ile Val
            100                 105                 110
Val Glu Glu Gly His Glu Gly Leu Thr His Phe Leu Met Asn Glu Val
        115                 120                 125
Ile Lys Leu Gln Gln Gln Met Lys Ala Lys Asp Leu Gln Arg Cys Glu
    130                 135                 140
Leu Leu Ala Arg Leu Arg Gln Leu Glu Asp Glu Lys Lys Gln Met Thr
145                 150                 155                 160
Leu Thr Arg Val Glu Leu Leu Thr Phe Gln Glu Arg Tyr Tyr Lys Met
                165                 170                 175
Lys Glu Glu Arg Asp Ser Tyr Asn Asp Glu Leu Val Lys Val Lys Asp
            180                 185                 190
Asp Asn Tyr Asn Leu Ala Met Arg Tyr Ala Gln Leu Ser Glu Glu Lys
        195                 200                 205
Asn Met Ala Val Met Arg Ser Arg Asp Leu Gln Leu Glu Ile Asp Gln
    210                 215                 220
Leu Lys His Arg Leu Asn Lys Met Glu Glu Glu Cys Lys Leu Glu Arg
225                 230                 235                 240
Asn Gln Ser Leu Lys Leu Lys Asn Asp Ile Glu Asn Arg Pro Lys Lys
                245                 250                 255
Glu Gln Val Leu Glu Leu Glu Arg Glu Asn Glu Met Leu Lys Thr Lys
            260                 265                 270
Asn Gln Glu Leu Gln Ser Ile Ile Gln Ala Gly Lys Arg Ser Leu Pro
        275                 280                 285
Asp Ser Asp Lys Ala Ile Leu Asp Ile Leu Glu His Asp Arg Lys Glu
    290                 295                 300
Ala Leu Glu Asp Arg Gln Glu Leu Val Asn Arg Ile Tyr Asn Leu Gln
305                 310                 315                 320
Glu Glu Ala Arg Gln Ala Glu Glu Leu Arg Asp Lys Tyr Leu Glu Glu
                325                 330                 335
Lys Glu Asp Leu Glu Leu Lys Cys Ser Thr Leu Gly Lys Asp Cys Glu
            340                 345                 350
Met Tyr Lys His Arg Met Asn Thr Val Met Leu Gln Leu Glu Glu Val
        355                 360                 365
Glu Arg Glu Arg Asp Gln Ala Phe His Ser Arg Asp Glu Ala Gln Thr
    370                 375                 380
Gln Tyr Ser Gln Cys Leu Ile Glu Lys Asp Lys Tyr Arg Lys Gln Ile
385                 390                 395                 400
Arg Glu Leu Glu Glu Lys Asn Asp Glu Met Arg Ile Glu Met Val Arg
                405                 410                 415
Arg Glu Ala Cys Ile Val Asn Leu Glu Ser Lys Leu Arg Arg Leu Ser
            420                 425                 430
Lys Asp Ser Asn Asn Leu Asp Gln Ser Leu Pro Arg Asn Leu Pro Val
        435                 440                 445
Thr Ile Ile Ser Gln Asp Phe Gly Asp Ala Ser Pro Arg Thr Asn Gly
    450                 455                 460
Gln Glu Ala Asp Asp Ser Ser Thr Ser Glu Glu Ser Pro Glu Asp Ser
465                 470                 475                 480
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Lys Tyr Phe Leu Pro Tyr His Pro Pro Gln Arg Arg Met Asn Leu Lys
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Gly Ile Gln Leu Gln Arg Ala Lys Ser Pro Ile Ser Leu Lys Arg Thr
            500                 505                 510
Ser Asp Phe Gln Ala Lys Gly His Glu Glu Glu Gly Thr Asp Ala Ser
        515                 520                 525
Pro Ser Ser Cys Gly Ser Leu Pro Ile Thr Asn Ser Phe Thr Lys Met
    530                 535                 540
Gln Pro Pro Arg Ser Arg Ser Ser Ile Met Ser Ile Thr Ala Glu Pro
545                 550                 555                 560
Pro Gly Asn Asp Ser Ile Val Arg Arg Tyr Lys Glu Asp Ala Pro His
                565                 570                 575
Arg Ser Thr Val Glu Glu Asp Asn Asp Ser Gly Gly Phe Asp Ala Leu
            580                 585                 590
Asp Leu Asp Asp Asp Ser His Glu Arg Tyr Ser Phe Gly Pro Ser Ser
        595                 600                 605
Ile His Ser Ser Ser Ser Ser His Gln Ser Glu Gly Leu Asp Ala Tyr
    610                 615                 620
Asp Leu Glu Gln Val Asn Leu Met Phe Arg Lys Phe Ser Leu Glu Arg
625                 630                 635                 640
Pro Phe Arg Pro Ser Val Thr Ser Val Gly His Val Arg Gly Pro Gly
                645                 650                 655
Pro Ser Val Gln His Thr Thr Leu Asn Gly Asp Ser Leu Thr Ser Gln
            660                 665                 670
Leu Thr Leu Leu Gly Gly Asn Ala Arg Gly Ser Phe Val His Ser Val
        675                 680                 685
Lys Pro Gly Ser Leu Ala Glu Lys Ala Gly Leu Arg Glu Gly His Gln
    690                 695                 700
Leu Leu Leu Leu Glu Gly Cys Ile Arg Gly Glu Arg Gln Ser Val Pro
705                 710                 715                 720
Leu Asp Thr Cys Thr Lys Glu Glu Ala His Trp Thr Ile Gln Arg Cys
                725                 730                 735
Ser Gly Pro Val Thr Leu His Tyr Lys Val Asn His Glu Gly Tyr Arg
            740                 745                 750
Lys Leu Val Lys Asp Met Glu Asp Gly Leu Ile Thr Ser Gly Asp Ser
        755                 760                 765
Phe Tyr Ile Arg Leu Asn Leu Asn Ile Ser Ser Gln Leu Asp Ala Cys
    770                 775                 780
Thr Met Ser Leu Lys Cys Asp Asp Val Val His Val Arg Asp Thr Met
785                 790                 795                 800
Tyr Gln Asp Arg His Glu Trp Leu Cys Ala Arg Val Asp Pro Phe Thr
                805                 810                 815
Asp His Asp Leu Asp Met Gly Thr Ile Pro Ser Tyr Ser Arg Ala Gln
            820                 825                 830
Gln Leu Leu Leu Val Lys Leu Gln Arg Leu Met His Arg Gly Ser Arg
        835                 840                 845
Glu Glu Val Asp Gly Thr His His Thr Leu Arg Ala Leu Arg Asn Thr
    850                 855                 860
Leu Gln Pro Glu Glu Ala Leu Ser Thr Ser Asp Pro Arg Val Ser Pro
865                 870                 875                 880
Arg Leu Ser Arg Ala Ser Phe Leu Phe Gly Gln Leu Leu Gln Phe Val
                885                 890                 895
Ser Arg Ser Glu Asn Lys Tyr Lys Arg Met Asn Ser Asn Glu Arg Val
            900                 905                 910
Arg Ile Ile Ser Gly Ser Pro Leu Gly Ser Leu Ala Arg Ser Ser Leu
        915                 920                 925
Asp Ala Thr Lys Leu Leu Thr Glu Lys Gln Glu Glu Leu Asp Pro Glu
    930                 935                 940
Ser Glu Leu Gly Lys Asn Leu Ser Leu Ile Pro Tyr Ser Leu Val Arg
945                 950                 955                 960
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Ala Phe Tyr Cys Glu Arg Arg Arg Pro Val Leu Phe Thr Pro Thr Val
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Leu Ala Lys Thr Leu Val Gln Arg Leu Leu Asn Ser Gly Gly Ala Met
            980                 985                 990
Glu Phe Thr Ile Cys Lys Ser Asp Ile Val Thr Arg Asp Glu Phe Leu
        995                 1000                1005
Arg Arg Gln Lys Thr Glu Thr Ile Ile Tyr Ser Arg Glu Lys Asn Pro
    1010                1015                1020
Asn Ala Phe Glu Cys Ile Ala Pro Ala Asn Ile Glu Ala Val Ala Ala
1025                1030                1035                1040
Lys Asn Lys His Cys Leu Leu Glu Ala Gly Ile Gly Cys Thr Arg Asp
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Leu Ile Lys Ser Asn Ile Tyr Pro Ile Val Leu Phe Ile Arg Val Cys
            1060                1065                1070
Glu Lys Asn Ile Lys Arg Phe Arg Lys Leu Leu Pro Arg Pro Glu Thr
        1075                1080                1085
Glu Glu Glu Phe Leu Arg Val Cys Arg Leu Lys Glu Lys Glu Leu Glu
    1090                1095                1100
Ala Leu Pro Cys Leu Tyr Ala Thr Val Glu Pro Asp Met Trp Gly Ser
1105                1110                1115                1120
Val Glu Glu Leu Leu Arg Val Val Lys Asp Lys Ile Gly Glu Glu Gln
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Arg Lys Thr Ile Trp Val Asp Glu Asp Gln Leu
            1140                1145
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<210> 7
<211> 2809
<212> DNA
<213> Homo sapiens

<400> 7

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<210> 8
<211> 233
<212> PRT
<213> Homo sapiens

<400> 8

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Ile	Ala	Glu	Arg	His	Phe	Asp	His	Leu	Arg	Ala	Lys	Lys	Ile	Leu	Ser
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Arg	Glu	Asp	Thr	Glu	Glu	Ile	Ser	Cys	Arg	Thr	Ser	Ser	Arg	Lys	Arg
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Ala	Gly	Lys	Leu	Leu	Asp	Tyr	Leu	Gln	Glu	Asn	Pro	Lys	Gly	Leu	Asp
65					70					75					80
Thr	Leu	Val	Glu	Ser	Ile	Arg	Arg	Glu	Lys	Thr	Gln	Asn	Phe	Leu	Ile
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Gln	Lys	Ile	Thr	Asp	Glu	Val	Leu	Lys	Leu	Arg	Asn	Ile	Lys	Leu	Glu
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His	Leu	Lys	Gly	Leu	Lys	Cys	Ser	Ser	Cys	Glu	Pro	Phe	Pro	Asp	Gly
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Ala	Thr	Asn	Asn	Leu	Ser	Arg	Ser	Asn	Ser	Asp	Glu	Ser	Asn	Phe	Ser
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Glu	Lys	Leu	Arg	Ala	Ser	Thr	Val	Met	Tyr	His	Pro	Glu	Gly	Glu	Ser
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Ser	Thr	Thr	Pro	Phe	Phe	Ser	Thr	Asn	Ser	Ser	Leu	Asn	Leu	Pro	Val
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Leu	Glu	Val	Gly	Arg	Thr	Glu	Asn	Thr	Ile	Phe	Ser	Ser	Thr	Thr	Leu
			180					185					190		
Pro	Arg	Pro	Gly	Asp	Pro	Gly	Ala	Pro	Pro	Leu	Pro	Pro	Asp	Leu	Gln
		195					200					205			
Leu	Glu	Glu	Glu	Gly	Thr	Cys	Ala	Asn	Ser	Ser	Glu	Met	Phe	Leu	Pro
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225					230										

<210> 9
<211> 1550
<212> DNA
<213> Homo sapiens

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<210> 10
<211> 317
<212> PRT
<213> Homo sapiens

<400> 10
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Gly Leu Asp Ser Met Lys Asp Glu Glu Tyr Glu Gln Met Val Lys Glu
        35   40   45
Leu Gln Glu Ile Arg Leu Glu Pro Gln Glu Val Pro Arg Gly Ser Glu
    50   55   60
Pro Trp Lys Gln Gln Leu Thr Glu Asp Gly Asp Ser Phe Leu His Leu
65   70   75   80
Ala Ile Ile His Glu Glu Lys Ala Leu Thr Met Glu Val Ile Arg Gln
                85   90   95
Val Lys Gly Asp Leu Ala Phe Leu Asn Phe Gln Asn Asn Leu Gln Gln
            100   105   110
Thr Pro Leu His Leu Ala Val Ile Thr Asn Gln Pro Glu Ile Ala Glu
        115   120   125
Ala Leu Leu Gly Ala Gly Cys Asp Pro Glu Leu Arg Asp Phe Arg Gly
    130   135   140
Asn Thr Pro Leu His Leu Ala Cys Glu Gln Gly Cys Leu Ala Ser Val
145   150   155   160
Gly Val Leu Thr Gln Ser Cys Thr Thr Pro His Leu His Ser Ile Leu
                165   170   175
Lys Ala Thr Asn Tyr Asn Gly His Thr Cys Leu His Leu Ala Ser Ile
            180   185   190
His Gly Tyr Leu Gly Ile Val Glu Leu Leu Val Ser Leu Gly Ala Asp
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Val Asp Leu Gln Asn Pro Asp Leu Val Ser Leu Leu Leu Lys Cys Gly
225                 230                 235                 240
Ala Asp Val Asn Arg Val Thr Tyr Gln Gly Tyr Ser Pro Tyr Gln Leu
                245                 250                 255
Thr Trp Gly Arg Pro Ser Thr Arg Ile Gln Gln Gln Leu Gly Gln Leu
            260                 265                 270
Thr Leu Glu Asn Leu Gln Met Leu Pro Glu Ser Glu Asp Glu Glu Ser
        275                 280                 285
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Tyr Asp Asp Cys Val Phe Gly Gly Gln Arg Leu Thr Leu
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<210> 11
<211> 3625
<212> DNA
<213> Homo sapiens

<400> 11

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<210> 12
<211> 968
<212> PRT
<213> Homo sapiens

<400> 12

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Pro Gln Met Ala Leu Pro Thr Asp Gly Pro Tyr Leu Gln Ile Leu Glu
        35                  40                  45
Gln Pro Lys Gln Arg Gly Phe Arg Phe Arg Tyr Val Cys Glu Gly Pro
    50                  55                  60
Ser His Gly Gly Leu Pro Gly Ala Ser Ser Glu Lys Asn Lys Lys Ser
65                  70                  75                  80
Tyr Pro Gln Val Lys Ile Cys Asn Tyr Val Gly Pro Ala Lys Val Ile
                85                  90                  95
Val Gln Leu Val Thr Asn Gly Lys Asn Ile His Leu His Ala His Ser
            100                 105                 110
Leu Val Gly Lys His Cys Glu Asp Gly Ile Cys Thr Val Thr Ala Gly
        115                 120                 125
Pro Lys Asp Met Val Val Gly Phe Ala Asn Leu Gly Ile Leu His Val
    130                 135                 140
Thr Lys Lys Lys Val Phe Glu Thr Leu Glu Ala Arg Met Thr Glu Ala
145                 150                 155                 160
Cys Ile Arg Gly Tyr Asn Pro Gly Leu Leu Val His Pro Asp Leu Ala
                165                 170                 175
Tyr Leu Gln Ala Glu Gly Gly Gly Asp Arg Gln Leu Gly Asp Arg Glu
            180                 185                 190
Lys Glu Leu Ile Arg Gln Ala Ala Leu Gln Gln Thr Lys Glu Met Asp
        195                 200                 205
Leu Ser Val Val Arg Leu Met Phe Thr Ala Phe Leu Pro Asp Ser Thr
    210                 215                 220
Gly Ser Phe Thr Arg Arg Leu Glu Pro Val Val Ser Asp Ala Ile Tyr
225                 230                 235                 240
Asp Ser Lys Ala Pro Asn Ala Ser Asn Leu Lys Ile Val Arg Met Asp
                245                 250                 255
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Asp Lys Val Gln Lys Asp Asp Ile Gln Ile Arg Phe Tyr Glu Glu Glu
        275                 280                 285
Glu Asn Gly Gly Val Trp Glu Gly Phe Gly Asp Phe Ser Pro Thr Asp
    290                 295                 300
Val His Arg Gln Phe Ala Ile Val Phe Lys Thr Pro Lys Tyr Lys Asp
305                 310                 315                 320
Ile Asn Ile Thr Lys Pro Ala Ser Val Phe Val Gln Leu Arg Arg Lys
                325                 330                 335
Ser Asp Leu Glu Thr Ser Glu Pro Lys Pro Phe Leu Tyr Tyr Pro Glu
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Ile Lys Asp Lys Glu Glu Val Gln Arg Lys Arg Gln Lys Leu Met Pro
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Gly Gly Met Phe Gly Ser Gly Gly Gly Gly Gly Gly Thr Gly Ser Thr
385                 390                 395                 400
Gly Pro Gly Tyr Ser Phe Pro His Tyr Gly Phe Pro Thr Tyr Gly Gly
                405                 410                 415
Ile Thr Phe His Pro Gly Thr Thr Lys Ser Asn Ala Gly Met Lys His
            420                 425                 430
Gly Thr Met Asp Thr Glu Ser Lys Lys Asp Pro Glu Gly Cys Asp Lys
        435                 440                 445
Ser Asp Asp Lys Asn Thr Val Asn Leu Phe Gly Lys Val Ile Glu Thr
    450                 455                 460
Thr Glu Gln Asp Gln Glu Pro Ser Glu Ala Thr Val Gly Asn Gly Glu
465                 470                 475                 480
Val Thr Leu Thr Tyr Ala Thr Gly Thr Lys Glu Glu Ser Ala Gly Val
                485                 490                 495
Gln Asp Asn Leu Phe Leu Glu Lys Ala Met Gln Leu Ala Lys Arg His
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Ala Asn Ala Leu Phe Asp Tyr Ala Val Thr Gly Asp Val Lys Met Leu
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Leu Ala Val Gln Arg His Leu Thr Ala Val Gln Asp Glu Asn Gly Asp
    530                 535                 540
Ser Val Leu His Leu Ala Ile Ile His Leu His Ser Gln Leu Val Arg
545                 550                 555                 560
Asp Leu Leu Glu Val Thr Ser Gly Leu Ile Ser Asp Asp Ile Ile Asn
                565                 570                 575
Met Arg Asn Asp Leu Tyr Gln Thr Pro Leu His Leu Ala Val Ile Thr
            580                 585                 590
Lys Gln Glu Asp Val Val Glu Asp Leu Leu Arg Ala Gly Ala Asp Leu
        595                 600                 605
Ser Leu Leu Asp Arg Leu Gly Asn Ser Val Leu His Leu Ala Ala Lys
    610                 615                 620
Glu Gly His Asp Lys Val Leu Ser Ile Leu Leu Lys His Lys Lys Ala
625                 630                 635                 640
Ala Leu Leu Leu Asp His Pro Asn Gly Asp Gly Leu Asn Ala Ile His
                645                 650                 655
Leu Ala Met Met Ser Asn Ser Leu Pro Cys Leu Leu Leu Leu Val Ala
            660                 665                 670
Ala Gly Ala Asp Val Asn Ala Gln Glu Gln Lys Ser Gly Arg Thr Ala
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Leu His Leu Ala Val Glu His Asp Asn Ile Ser Leu Ala Gly Cys Leu
    690                 695                 700
Leu Leu Glu Gly Asp Ala His Val Asp Ser Thr Thr Tyr Asp Gly Thr
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Thr Pro Leu His Ile Ala Ala Gly Arg Gly Ser Thr Arg Leu Ala Ala
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Val Val Pro Gly Thr Thr Pro Leu Asp Met Ala Thr Ser Trp Gln Val
    770                 775                 780
Phe Asp Ile Leu Asn Gly Lys Pro Tyr Glu Pro Glu Phe Thr Ser Asp
785                 790                 795                 800
Asp Leu Leu Ala Gln Gly Asp Met Lys Gln Leu Ala Glu Asp Val Lys
                805                 810                 815
Leu Gln Leu Tyr Lys Leu Leu Glu Ile Pro Asp Pro Asp Lys Asn Trp
            820                 825                 830
Ala Thr Leu Ala Gln Lys Leu Gly Leu Gly Ile Leu Asn Asn Ala Phe
        835                 840                 845
Arg Leu Ser Pro Ala Pro Ser Lys Thr Leu Met Asp Asn Tyr Glu Val
    850                 855                 860
Ser Gly Gly Thr Val Arg Glu Leu Val Glu Ala Leu Arg Gln Met Gly
865                 870                 875                 880
Tyr Thr Glu Ala Ile Glu Val Ile Gln Ala Ala Ser Ser Pro Val Lys
                885                 890                 895
Thr Thr Ser Gln Ala His Ser Leu Pro Leu Ser Pro Ala Ser Thr Arg
            900                 905                 910
Gln Gln Ile Asp Glu Leu Arg Asp Ser Asp Ser Val Cys Asp Thr Gly
        915                 920                 925
Val Glu Thr Ser Phe Arg Lys Leu Ser Phe Thr Glu Ser Leu Thr Ser
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Gly Ala Ser Leu Leu Thr Leu Asn Lys Met Pro His Asp Tyr Gly Gln
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<212> DNA
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Pro Ala Met Asp Gly Thr Ala Ala Glu Pro Arg Pro Gly Ala Gly Ser
    50                  55                  60
Leu Gln His Ala Gln Pro Pro Pro Gln Pro Arg Lys Lys Arg Pro Glu
65                  70                  75                  80
Asp Phe Lys Phe Gly Lys Ile Leu Gly Glu Gly Ser Phe Ser Thr Val
                85                  90                  95
Val Leu Ala Arg Glu Leu Ala Thr Ser Arg Glu Tyr Ala Ile Lys Ile
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Leu Glu Lys Arg His Ile Ile Lys Glu Asn Lys Val Pro Tyr Val Thr
        115                 120                 125
Arg Glu Arg Asp Val Met Ser Arg Leu Asp His Pro Phe Phe Val Lys
    130                 135                 140
Leu Tyr Phe Thr Phe Gln Asp Asp Glu Lys Leu Tyr Phe Gly Leu Ser
145                 150                 155                 160
Tyr Ala Lys Asn Gly Glu Leu Leu Lys Tyr Ile Arg Lys Ile Gly Ser
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Phe Asp Glu Thr Cys Thr Arg Phe Tyr Thr Ala Glu Ile Val Ser Ala
            180                 185                 190
Leu Glu Tyr Leu His Gly Lys Gly Ile Ile His Arg Asp Leu Lys Pro
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Glu Asn Ile Leu Leu Asn Glu Asp Met His Ile Gln Ile Thr Asp Phe
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Gly Thr Ala Lys Val Leu Ser Pro Glu Ser Lys Gln Ala Arg Ala Asn
225                 230                 235                 240
Ser Phe Val Gly Thr Ala Gln Tyr Val Ser Pro Glu Leu Leu Thr Glu
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Lys Ser Ala Cys Lys Ser Ser Asp Leu Trp Ala Leu Gly Cys Ile Ile
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Tyr Gln Leu Val Ala Gly Leu Pro Pro Phe Arg Ala Gly Asn Glu Tyr
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Leu Ile Phe Gln Lys Ile Ile Lys Leu Glu Tyr Asp Phe Pro Glu Lys
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Phe Phe Pro Lys Ala Arg Asp Leu Val Glu Lys Leu Leu Val Leu Asp
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Ala Thr Lys Arg Leu Gly Cys Glu Glu Met Glu Gly Tyr Gly Pro Leu
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Lys Ala His Pro Phe Phe Glu Ser Val Thr Trp Glu Asn Leu His Gln
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Cys Met Gln Val Ser Ser Ser Ser Ser Ser His Ser Leu Ser Ala Ser
385                 390                 395                 400
Asp Thr Gly Leu Pro Gln Arg Ser Gly Ser Asn Ile Glu Gln Tyr Ile
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His Asp Leu Asp Ser Asn Ser Phe Glu Leu Asp Leu Gln Phe Ser Glu
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Asp Glu Lys Arg Leu Leu Leu Glu Lys Gln Ala Gly Gly Asn Pro Trp
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His Gln Phe Val Glu Asn Asn Leu Ile Leu Lys Met Gly Pro Val Asp
    450                 455                 460
Lys Arg Lys Gly Leu Phe Ala Arg Arg Arg Gln Leu Leu Leu Thr Glu
465                 470                 475                 480
Gly Pro His Leu Tyr Tyr Val Asp Pro Val Asn Lys Val Leu Lys Gly
                485                 490                 495
Glu Ile Pro Trp Ser Gln Glu Leu Arg Pro Glu Ala Lys Asn Phe Lys
            500                 505                 510
Thr Phe Phe Val His Thr Pro Asn Arg Thr Tyr Tyr Leu Met Asp Pro
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<212> PRT
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<220>
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            20                  25                  30
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专利名称(译)	分子优先与效应细胞及其使用方法相关		
公开(公告)号	EP1565218A2	公开(公告)日	2005-08-24
申请号	EP2003783270	申请日	2003-11-10
[标]申请(专利权)人(译)	托勒克斯股份有限公司		
申请(专利权)人(译)	TOLERRX公司		
当前申请(专利权)人(译)	TOLERRX公司		
[标]发明人	RAO PATRICIA		
发明人	RAO, PATRICIA		
IPC分类号	A61K A61K48/00 C07K1/00 C07K17/00 C12N5/00 C12N15/00 C12Q1/70 G01N33/53		
CPC分类号	A61P31/04 A61P31/12 A61P33/00 A61P35/00 A61P37/00 A61P37/08 A61P43/00 C07K2319/10 C12N9/1205 G01N2333/9121 G01N2500/02		
代理机构(译)	赫尔比希，CHRISTIAN		
优先权	60/424777 2002-11-08 US 60/467477 2003-05-02 US		
外部链接	Espacenet		

摘要(译)

本发明至少部分基于发现T调节细胞中不存在并存在于效应T细胞（Th1和Th2）上的某些基因，例如蛋白激酶Cθ（PKCθ）。此外，调节性T细胞不利用炎性细胞因子产生所必需的途径和炎性效应T细胞的细胞增殖。因此，在一个方面，本发明提供了相对于效应T细胞功能促进免疫细胞中调节性T细胞功能的方法，包括使免疫细胞与抑制免疫细胞中蛋白激酶Cθ途径的试剂接触。另一方面，本发明提供了治疗患有该病症的受试者的方法将受益于相对于受试者中的效应T细胞功能促进调节性T细胞功能，包括施用抑制受试者免疫细胞中蛋白激酶Cθ途径的试剂。在另一方面，本发明提供了用于筛选特异性调节效应T细胞功能而不调节调节性T细胞功能的化合物的测定法，包括使蛋白激酶Cθ途径分子与测试化合物接触并测定测试化合物调节该测试化合物的能力。蛋白激酶Cθ途径分子活性，其中蛋白激酶Cθ途径分子活性的调节表明测试化合物是效应T细胞的特异性调节剂。功能。