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(54) Title: USE OF SOLUBLE COMPLEXES TO FACILITATE CELL ACTIVATION

(57) Abstract: The present invention provides methods and compositions which use soluble complexes for stimulation of a biological effect in target cells.

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USE OF SOLUBLE COMPLEXES TO FACILITATE CELL ACTIVATION

TECHNICAL FIELD

[0001] The present invention relates to the use of soluble complexes comprising target cell directed moieties for stimulation of a biological effect in target cells. It also relates to the administration of the soluble complexes to stimulate a biological effect in a target cell.

BACKGROUND ART

[0002] Induction of a biological effect in a cell can be stimulated or activated in a variety of ways. For example, interactions of cell surface receptors and ligands can generate intracellular signals that result in cell activation or stimulation. The particular biological effect that can result from such activation or stimulation depends on, among other things, the cell receptor(s), the ligand(s), their interaction(s) and the type of cell involved.

[0003] Generally, cell surface receptors transmit signals received on the outside of a cell to the inside through ligand-induced allosteric conformational change and/or through ligand-induced association. Receptors can also be stimulated to induce cell activation through interaction with non-ligand molecules, such as antibodies or ligand mimics.

[0004] Studies on the allosterically activated receptor class have yielded many pharmacologically agents that act as antagonists or agonists. Antagonists block the binding of the natural ligand without inducing the conformational change in the receptor thereby blocking a signal transduction pathway. Agonists bind to the receptor in a manner which mimics the natural ligand closely enough to induce the same conformational change as natural ligand thereby initiating a signal transduction pathway.

[0005] In some cases, activation of cell signaling occurs when multiple receptors on the cell surface are brought into close proximity in a process referred to as receptor clustering. Receptor clustering as a means for receptor activation has been well documented, especially for receptor kinases (Ullrich *et al.* (1990) *Cell* 61:203-212; Kolanus *et al.* (1993) *Cell* 74:171-183).

[0006] Receptors activated by a ligand-induced association or clustering, such as multimerization, including dimerization, include, for example, those for cell growth and differentiation factors. Factors which serve as ligands for these receptors are typically large polypeptide hormone and cytokines such as erythropoietin, granulocyte colony stimulating factor (G-CSF), or granulocyte macrophage colony stimulating factor (GM-CSF), and human

growth hormone (hGH). Many of the multimerization-activated receptors have cytoplasmic tails that contain protein kinase domains or docking sites. Ligand-induced multimerization of the extracellular domains of these receptors results in the juxtaposition of their cytoplasmic tails. In some cases, they then presumably phosphorylate each other in trans and thereby initiate the cytosolic signaling pathway. In some cases the cytoplasmic domains of multimerization-activated receptors do not have kinase domains themselves, but function the same as if they did because they associate with protein kinases via docking sites.

[0007] Receptors activated by multimerization or aggregation are frequently found in the immune system. They include, for example, the T cell surface receptors such as CD4, CD8, CD28, CD26, CD45, CD10, and CD3/TCR (T cell antigen receptor). The ligands for these T cell receptors are most often cell surface proteins themselves, and can be found on antigen presenting cells. Aggregation-activated receptors frequently have short cytoplasmic domains which act to bind and thereby recruit other cell surface and/or cytosolic factors following the aggregation of their extracellular domains. Generally, receptor aggregation or clustering is important in stimulating the signaling pathway in the cell.

[0008] Important in this aggregation process, whether it be dimerization or multimerization, is for the receptors to have some mobility on the cell surface so that they can move or be moved into appropriate proximity for aggregation to occur.

[0009] Non-ligand molecules which interact with receptors, such as antibodies or ligand mimics, can also induce cell stimulation or activation. Such molecules can activate receptors to initiate cytosolic signaling through inducing receptor aggregation and/or through inducing allosteric conformational change. Because antibodies are naturally multivalent, they are often able to mimic the natural ligand in inducing association or clustering of the receptors to induce a biological effect such as, for example, an agonistic effect.

[0010] Interactions of receptors or ligands on cell surfaces with their respective ligand or receptor (*i.e.*, cognate molecule) or with non-cognate molecules can lead to generation of intracellular signaling that ultimately results in the activation or stimulation of the cell.

[0011] Thus, molecular interactions at the cell surface can stimulate biological effects in cells which can be of use in treating diseases and/or disorders and/or in generating useful biological reagents. For example, such interactions can lead to stimulation of proliferation in a particular cell population or to induction of programmed cell death in a cancer cell. There remains a need for improved ways to stimulate or activate cells to induce a desired biological effect.

[0012] All publications and patent applications cited herein are hereby incorporated by reference in their entirety.

DISCLOSURE OF THE INVENTION

[0013] The invention, in one aspect, is directed to methods and compositions for stimulating a biological effect in a target cell comprising contacting a target cell with a soluble complex comprising a first moiety, wherein the first moiety interacts with a receptor on the surface of the target cell and wherein the interaction of the complex comprising the first moiety with the receptor stimulates a biological effect in the target cell. Optionally, the method further includes contacting the target cell with a second soluble complex comprising a second moiety, wherein the second moiety interacts with a second receptor on the surface of the target cell.

[0014] In another embodiment, the invention is directed to a method for stimulating a biological effect in a target cell comprising contacting a target cell with a first soluble complex comprising a first target cell directed moiety and contacting the target cell with a second soluble complex comprising a second target cell directed moiety, wherein the first target cell directed moiety interacts with a first receptor on the surface of the target cell and the second target cell directed moiety interacts with a second receptor on the surface of the target cell, and wherein the interaction of the moieties with the receptors stimulates a biological effect in the target cell.

[0015] In the invention, the soluble complexes comprise two or more target cell directed moieties. In some embodiments, the complex comprises more than one copy of the same target cell directed moiety. In some embodiments, the complex comprises different target cell directed moieties.

[0016] In one embodiment, the method stimulates T cell proliferation and/or T cell differentiation.

[0017] In another embodiment, the method stimulates apoptosis. Optionally, the method and compositions further include the soluble complexes coupled to an agent which stimulates a biological effect.

MODES FOR CARRYING OUT THE INVENTION

[0018] We have discovered that presenting a target cell directed moiety in the form of a soluble complex to the target cell is particularly effective in stimulating a biological effect in

the target cell. The target cell directed moiety interacts with a receptor on the target cell and a biological effect is stimulated in the target cell. The moiety is in a complex with at least two or more target cell directed moieties. In some instances, the complex comprises target cell directed moieties which are the same as each other. In some instances, the complex comprises target cell directed moieties which are different from each other. In some methods, target cells are contacted with a first soluble complex comprising a first target cell directed moiety. In some instances, the target cells are contacted with a first soluble complex and with a second soluble complex comprising a second target cell directed moiety. When contacted with two soluble complexes, the target cells can be contacted with the first soluble complex and the second soluble complex at the same time or can be contacted with each soluble complex at different times. In some embodiments, the target cells are contacted with a soluble complex comprising both a first target cell directed moiety and a second target cell directed moiety.

[0019] The use of a soluble complex as a component of a target cell stimulating complex offers distinct benefits for and advantages to stimulating a target cell. Presentation of a target cell directed moiety in a soluble complex generally provides a local concentration of the moiety to the target cell through the presence of a number of moieties in the complex. In some instances, presenting the target cell directed moiety in a soluble complex to a target cell allows for some mobility of the target cell receptor after contact with the moiety and, accordingly, for aggregation of the target cell receptor. For some cell receptors, the ability to aggregate and/or move on the cell surface is important for effective cell signaling. In some instances, the soluble complexes can also be used to deliver agents (e.g., drugs, antigens, cytokines, chemokines, hormones) to particular cells and/or tissues.

General Techniques

[0020] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook *et al.*, 1989); *Oligonucleotide Synthesis* (Gait, ed., 1984); *Animal Cell Culture* (Freshney, ed., 1987); *Methods in Enzymology* (Academic Press, Inc.); *Handbook of Experimental Immunology* (Weir *et al.*, eds.); *Gene Transfer Vectors for Mammalian Cells* (Miller *et al.*, eds., 1987); *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., 1987); *Antibodies: A Laboratory Manual* (Harlow *et al.*, eds., 1988), Cold Spring

Harbor Laboratory Press; *PCR: The Polymerase Chain Reaction*, (Mullis *et al.*, eds., 1994); *Current Protocols in Immunology* (Coligan *et al.*, eds., 1991); *The Immunoassay Handbook* (Wild, ed., Stockton Press NY, 1994); and *Methods of Immunological Analysis* (Masseyeff *et al.*, eds., Weinheim: VCH Verlags gesellschaft mbH, 1993)

Definitions

[0021] As used herein, a "target cell directed moiety" is a moiety that interacts with a receptor on a target cell. The target cell directed moiety can be an entire molecule or a portion of a molecule. The target cell directed moiety can be included within or attached to another molecule as long as the target cell directed moiety is capable of interacting with the receptor on the target cell.

[0022] As used herein, a "target cell receptor" is a molecule on the surface of a target cell that, upon interaction with a soluble target cell directed moiety complex, participates in and/or contributes to the stimulation a of biological effect in the target cell.

[0023] As used herein, entities that are "coupled" are joined, linked, attached, or connected, either directly or indirectly.

[0024] As used herein, "T cells" are CD4-positive or CD8-positive lymphocytes that express the CD3 antigen.

[0025] As used herein, "activated T cells" are T cells that have undergone differentiation to a particular subset of T cell. Activated T cells include, but are not limited to, Th1, Th2, Th0, Tc1 and Tc2 subsets. Activated T cells include any T cell subtype and are not limited to any particular defined cytokine profile. As used herein, activated T cells may refer to either polyclonal or monoclonal populations of T cells.

[0026] As used herein, the term "antibody" refers to a polypeptide or group of polypeptides which are comprised of at least one antibody combining site. An "antibody combining site" or "binding domain" is formed from the folding of variable domains of an antibody molecule(s) to form three-dimensional binding spaces with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows an immunological reaction with the antigen. An antibody combining site may be formed from a heavy and/or a light chain domain (V_H and V_L, respectively), which form hypervariable loops which contribute to antigen binding. The term "antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, altered antibodies, univalent antibodies, the Fab proteins, and single domain antibodies.

[0027] An "individual" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, humans, farm animals, sport animals, rodents and pets.

[0028] An "effective amount" or a "sufficient amount" of a substance is that amount sufficient to effect beneficial or desired results, including clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. An effective amount can be administered in one or more administrations.

[0029] As used herein, the singular form "a", "an", and "the" includes plural references unless indicated otherwise. For example, "a" target cell includes one or more target cells.

Methods of the Invention

[0030] The invention relates to the use of soluble complexes comprising target cell directed moieties in methods of stimulating a biological effect in target cells. As described herein, the complexes comprise two or more target cell directed moieties. In the methods, when the complex contacts the target cell, at least one moiety interacts with a receptor on the target cell and the interaction stimulates a biological effect in the target cell.

[0031] The degree and/or type(s) of biological effect stimulated in a target cell depends on a number of factors, including, for example, the target cell type, the target cell receptor, the target cell directed moiety and the presentation of the moiety to the target cell receptor. In some cases, interaction of a target cell directed moiety alone with a target cell receptor may stimulate a biological effect in the target cell. In other cases, presentation of the target cell directed moiety in the form of a soluble complex to the target cell is necessary to stimulate a measurable biological effect in the target cell. In either case, presentation of a target cell directed moiety in the form of a soluble complex to the target cell receptor is an effective way to stimulate a biological effect in the target cell. In some instances, contacting a target cell with a soluble target cell directed moiety complex is more effective in stimulating a biological response than contacting the target cell with the target cell directed moiety alone (*i.e.*, not in a complex) or with the target cell directed moiety coupled to a bead.

[0032] In methods of the invention, stimulation of a biological effect in the target cell results from contacting the target cell with at least one target cell directed moiety in a soluble complex. In some embodiments, stimulation of a biological effect in the target cell requires interaction of more than one different target cell directed moieties in soluble complexes with one or more target cell receptors. In some embodiments, stimulation of a biological effect in

the target cell requires interaction of more than two different target cell directed moieties with one or more target cells receptors.

[0033] Examples of target cells for the invention include, but are not limited to, cells of the immune system, bone marrow cells, cells of hematopoietic origin, hematopoietic and other stem cells, infected cells, hyperplastic cells and tumor and/or cancer cells. Exemplary target cells include T cells, natural killer (NK) cells, tumor infiltrating lymphocytes (TIL), lymphokine-activated killer (LAK) cells, B cells, monocytes, granulocytes, macrophages, immature and mature dendritic cells. The target cell may also be any non-cancerous cell that could provide a direct or indirect therapeutic response, for example, fibroblasts and neuronal cells.

[0034] Examples of biological responses that can be stimulated in cells of the immune system, bone marrow cells, and/or stem cells, include, but are not limited to, activation, proliferation, differentiation, and/or induction of cytokine production. Examples of biological responses that can be stimulated in non-immune system cells include, but are not limited to, production of hormones, neurotransmitters and/or other biological response molecules. Examples of target cell directed moieties that can stimulate such biological effects in such cells are listed herein.

[0035] Examples of biological responses that can be stimulated in infected cells, hyperplastic cells, tumor cells and/or cancer cells include, but are not limited to, anti-proliferative responses, cytotoxic effects, apoptosis, and necrosis. Examples of target cell directed moieties that can stimulate such biological effects in such cells are listed herein.

[0036] Methods of the invention are appropriate for use *in vitro* and/or *in vivo*. For example, target cells in culture can be contacted with the complexes according to the methods and, once the biological effect is stimulated, the cells and/or culture media can be harvested for further use. In some embodiments, methods of the invention can be used for *ex vivo* purposes, for example, where cells are collected from an individual and put in culture conditions as needed, the biological effect is stimulated according to the methods of the invention and the resultant cells and/or cell products are administered to an individual in need thereof.

[0037] For example, target cells can be contacted in culture with a soluble target cell directed moiety complex(es) to stimulate an increased level of production and/or secretion of a variety of cytokines. The cytokine(s) in the cell culture supernatant can be separated from the target cells and used for a variety of purposes including administration to a subject in need thereof. Soluble complexes of the invention can be used to stimulate cytokine production from

a homogeneous cell population (e.g., a population enriched for a particular subset of cells, e.g., CD4+ T cells) or from a heterogeneous cell population. The particular cytokine(s) stimulated by the soluble target cell directed moiety complexes depends on the target cell population and on the target cell directed moiety used for the stimulation. For example, cells from the immune system can be stimulated to produce cytokines including, but not limited to, IL-2, IL-4, IL-5, IL-10, IL-15, IL-18, IL-27, TRAIL, FasL, IFN-γ, TNF-α and TNF-β. Target cell directed moieties for stimulation of cytokine production include those described herein, such as a lectin (e.g., PHA) or an anti-target cell receptor antibody (e.g., anti-CD3 and anti-CD28 antibodies).

[0038] In stimulating cytokine production or secretion from the cells in culture, the soluble target cell directed moiety complexes may be added to the cells once or repeatedly. Separation of the cytokine-containing culture supernatant from the cells can be done using separation technologies including filtration, precipitation, fractionation and sedimentation. Preferably, the culture supernatant containing the desired cytokine(s) is removed from the cells prior to substantial cell lysis and without causing substantial cell lysis. If the soluble complex is streptavidinated (*i.e.*, coupled with streptavidin), biotinylated magnetic particles and an application of a magnetic field can be used to remove any cells attached to the complexes, from the culture supernatant. This can be accomplished in a batch mode (e.g., using a permanent magnet) or in a continuous mode by flowing the mixture of complexes, target cells and cell culture supernatant over a permanent magnet. Where contact with the soluble target cell directed moiety complex causes the target cells to proliferate, the target cell culture can be saturated by the addition of excess soluble complex prior to removal of the cells and complexes from the supernatant. Cytokines of the cell culture supernatant can be further purified using techniques known in the art, including, for example, using cytokine-specific affinity columns.

[0039] In another example of *ex vivo* stimulation, T cells can be isolated from peripheral blood mononuclear cells (PBMCs) and stimulated to proliferate and/or differentiate into, for example, Th1, Th2, Th0, Tc1, Tc2 or any activated T cell subtype not limited to a particular defined cytokine producing profile. For example, target T cells can include those of any antigen specificity, including non-antigen specific, and include T cell populations that are monoclonal or polyclonal. T cells that result from the methods of the invention include those of any antigen specificity, including non-antigen specific, and monoclonal or polyclonal T cell populations. Also, methods of the invention can be used to generate T cells of any effector profile including any surface marker profile or any cytokine profile.

[0040] In the methods described herein, the target cells can be stimulated with the soluble target cell directed moiety complex once or repeatedly until the desired effect is obtained. Thus, in some embodiments, the methods comprise contacting the target cells with soluble complexes formed with streptavidin and biotinylated target cell directed moieties, once or repeatedly. In some embodiments, following stimulation of the target cells with the soluble target cell directed moiety complex, the cells can be stimulated with other agents that serve to further result in the desired effect.

[0041] In some embodiments, methods of the invention are performed *in vivo*. In such methods, the target cell is contacted after the soluble target cell directed moiety complex(es) is administered to an individual. The administered complexes contact the target cell and stimulate a biological effect in the individual. Many of the complexes described herein are appropriate for use *in vivo*, including, but not limited to, those that are particularly selective for the target cell and that stimulate target cell growth arrest or apoptosis, that stimulate target cell proliferation and that stimulate target cell differentiation.

[0042] In one aspect of the invention, methods are provided for modulating immune system function. The complexes and/or compositions of the invention are administered to subjects in need of immune system modulation in amounts effective to modulate immune system function. Modulation of immune system function includes, but is not limited to, increasing immune function such as by specifically stimulating T cells (including cytotoxic T lymphocytes (CTL)), B cells, NK cells, bone marrow cells, monocytes, macrophage, immature dendritic cells, mature dendritic cells, stem cells and/or early lineage progenitor cells to produce a prophylactic or therapeutic result relating to infectious disease, cancer, and the like. Specifically included is the use of particular complexes of the invention for the treatment of disorders characterized by reduced T cell levels *in vivo*, *e.g.*, HIV and other disorders associated with a compromised immune system. Modulation of immune system function also includes, but is not limited to, decreasing immune function such as by suppressing specifically the immune system to treat autoimmune disease, allergy and the like. In one embodiment, the complexes of the invention are used to shift a Th2-type immune response toward a Th1-type immune response through the stimulation of Th1 cell production. In another embodiment, the compositions of the invention are used to stimulate blood cell proliferation and/or differentiation.

[0043] The invention provides methods and compositions for increasing the size of a subpopulation of T cells. In such methods, "increasing size of a subpopulation of T cells"

refers to stimulating the expansion of a T cell subpopulation by contacting the T cells with at least one soluble complex comprising a T cell directed moiety where the interaction of the T cell directed moiety with a T cell receptor stimulates proliferation or expansion of the T cell subpopulation of cells. Preferably, the number of T cells belonging to the subpopulation that are present after this contacting is at least 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 70, 90, 150, 500, 5000, 50,000 or 100,000 fold greater than the number of these cells present without administration of the complexes or after the corresponding control incubation in the absence of the complexes. More preferably, the number of T cells belonging to the subpopulation that are present after this contacting is at least 2, 3, 4, 5, 10, 15, 20, 50, 100, 1000, 10,000 or 100,000 fold greater than the number of these cells present after the corresponding *in vivo* contact or *in vitro* incubation in the presence of the same T cell directed moiety attached to the surface of a bead. It is also contemplated that the percentage may remain the same but the actual numbers of the relevant subset may increase if the total number of T cells increases. In some instances, the change in the percentage of cells that belong to the subpopulation of T cells is at least 2, 3, 4, 5, 10, 20, 50, 100, 1000, 10,000 or 100,000 fold greater than corresponding change in the percentage of cells that belong to the subpopulation of T cells in absence of administration of the complexes, in a control sample that has not be incubated with the complexes or after the corresponding incubation in the presence of the same T cell directed moiety attached to the surface of a bead.

[0044] For example, methods are provided for stimulating production of Th1 or Th2 cells, subsets of T helper cells. The Th1 subset is responsible for classical cell-mediated functions such as delayed-type hypersensitivity and activation of CTLs. Thus, the Th1 subset may be particularly suited to respond to viral infections, intracellular pathogens, and tumor cells because it secretes IFN-γ and other cytokines, which activate other components of the immune system, such as CTLs. The Th2 subset suppresses the cellular immune response and functions more effectively as a helper for B-cell activation and eosinophilic inflammation. The Th2 subset may be more suited to respond to free-living bacteria and helminthic parasites and may mediate allergic reactions, since IL-4 and IL-5 are known to induce IgE production and eosinophil activation, respectively.

[0045] Differences in the cytokines secreted by Th1 and Th2 cells are believed to reflect different biological functions of these two subsets. See, for example, Romagnani (2000) *Ann. Allergy Asthma Immunol.* 85:9-18. In general, since distinct patterns of cytokines are secreted by Th1 and Th2 cells, one type of response can moderate the activity of the other type of

response. A shift in the Th1/Th2 balance can result in an allergic response, for example, or, alternatively, in an increased CTL response. Methods of the invention can also be used to redirect a Th2 immune response.

[0046] In one embodiment, for example, the invention provides methods for producing a population of Th1 cells from a blood sample in the absence of exogenous growth or differentiation factors, such as IL-2 or IFN-gamma. Mononuclear cells collected from a blood sample, for example, by leukapheresis, serve as a source material for production of Th1 cells in culture. In one embodiment, CD4+ T cells are first purified from the source material. Such a purification can be accomplished by, for example, positive selection. The starting population of T cells are then contacted with the complexes of the invention to stimulate the desired biological effect in the T cells. In one embodiment, the cells are activated by simultaneous contact with a soluble first moiety complex that interacts with the CD3 receptor complex on the T cells and a soluble second moiety complex which interacts with the CD28 receptor on the T cells. In one embodiment, the activation is accomplished by incubating the starting population of T cells with a soluble complex comprising anti-CD3 antibodies and a soluble complex comprising anti-CD28 antibodies. In some embodiments, these soluble complexes are biotinylated antibodies linked through molecules of streptavidin. In these embodiments, the anti-CD3 and/or anti-CD28 antibodies may be found together in the same complex or may be formed in distinct complexes. In some embodiments, one target cell directed moiety may be directly or indirectly coupled to another target cell directed moiety in the soluble complex.

[0047] The T cells are stimulated with the T cell directed-soluble complexes one or more times, typically two or more, three or more, four or more, five or more times. For example, T cells may be stimulated three times with the soluble anti-CD3/anti-CD28 complexes over the course of 9 days in culture. Cells resulting from such as expansion would have a Th1 phenotype as can be demonstrated by their production of IFN-gamma, their lack of production of IL-4 and their cell surface markers. Thus, the invention provides methods for increasing the size of a subpopulation of activated T cells. Methods are also provided for producing large numbers of activated T cells.

[0048] Activated T cells, such as Th1 cells, would be of use in treating symptoms of individuals with cancers, infectious diseases, allergic diseases and diseases or disorders that are associated with overactive humoral immunity. Individuals with cancer and tumor-bearing animals have been shown to exhibit suppressed cellular immune responses as evidenced by decreased DTH, CTL function and NK activity (Broder *et al.* (1978) *N. Engl. J. Med.*

299:1335-1341) apparently due to a lack of Th1 cells. Excess production of Th2 cytokines and/or depressed production of Th1 cytokines resulting in a Th1/Th2 cytokine imbalance has also been reported in virtually all types of cancer tested. As with asthma and allergies, enhanced Th2 responses are found in a variety of infectious diseases, such as chronic hepatitis C virus infection (Fan *et al.* (1998) *Mediators Inflamm.* 7:295), leprosy (Yamamura (1992) · *Science* 255:12), toxoplasmosis (Sher *et al.* (1992) *Immunol. Rev.* 127:183) and AIDS (Clerici *et al.* (1993) *Immunol. Today* 14:107-111), and autoimmune conditions, such as lupus (Funauchi *et al.* (1998) *Scand. J. Rheumatol.* 27:219).

[0049] Therapies that increase Th1 cells and/or shift the balance from Th2 to Th1 have been shown to have therapeutic utility in treating cancer and infection conditions. For example, down-regulation of the Th2 response in tumor-bearing mice by treatment with anti-IL-4 mAb significantly suppresses growth of murine renal cell carcinoma tumors (Takeuchi *et al.* (1997) *Cancer Immunol. Immunother.* 43:375-381), while IL-2 gene transfected murine renal cell carcinoma cells mediate tumor rejection (Hara *et al.* (1996) *Jpn. J. Cancer Res.* 87:724-729). IL-2 is a Th1 associated cytokine. Adoptive immunotherapy involving transfer of influenza-specific Th1 cells was protective against influenza infection, while Th2 infusion failed to induce protection (Graham *et al.* (1994) *J. Exp. Med.* 180:1273).

[0050] Accordingly, methods of the present invention are for use in the production of Th1 cells that can be used in adoptive immunotherapy for a variety of conditions in which an increase in the population of Th1 cells would be of beneficial, such as in treatment of a variety of diseases, including cancer, infectious disease, allergy and diseases characterized by overactive humoral immunity, such as systemic lupus erythematosus. Methods of the invention in which complexes that stimulate differentiation of T cells to Th1 cells can be used to shift a Th2 immune response toward a Th1 immune response in an individual in need thereof. Methods of the invention can also be used to stimulate production of Th1 cells in an individual in need thereof.

[0051] In another embodiment, methods of the present invention involve the use of soluble compositions further comprising antigen to stimulate T cells to respond a particular antigen, such as a tumor antigen, an antigen associated with infectious disease or a self-antigen peptide. Such compositions include those which have a specific antigen, or fragment thereof, coupled to the soluble complex. Such compositions may also include soluble complexes having coupled major histocompatability complex (MHC) molecules (such as, class I, class II or class IB

molecules) loaded with antigen peptide. Such compositions may be used, for example, in methods to prime T cells *in vitro*.

[0052] In some embodiments, methods involve the use of soluble complexes to deliver antigens to cells or to a subject in need thereof, in particular, to deliver antigen to particular cells and/or organs of the immune system, such as lymph nodes and spleen. In these methods, the antigen of interest can be coupled to the soluble complex and the antigen-soluble complex administered parenterally, such as by intravenous delivery. The soluble complexes can be directed to a particular site through using a target cell directed moiety that will preferentially direct the complexes to the desired cells and/or organ. For example, using LFA-1 and CD62L together with an antigen in a soluble complex would result in the delivery of the soluble complex to the lymph nodes. Antigen-soluble complexes directed to the lymph nodes may further include an antigen linked to a Tat polypeptide of HIV or any other appropriate signaling peptide which facilitates processing of the antigen. Given the directed aspect of this form of antigen administration, the spread of antigen in the individual would be restricted to the particular desired sites and lower doses of antigen can be delivered since it is preferentially directed to sites where it would be most useful.

[0053] In another aspect of the invention, methods are provided for suppressing proliferation of target cells and/or for inducing cell death in target cells. The complexes and/or compositions of the invention are administered to subjects in need of suppression of cell proliferation and/or induction of cell death in amounts effective to suppress target cell proliferation and/or to induce cell death in the target cell. Such individuals include those with cancer, tumor cells, infected cells and/or diseases or disorders characterized by cell proliferation. Suppressing proliferation (including, for example, through slowing or arresting cell division) and/or inducing cell death (including, for example, through stimulating apoptosis) in target cancer cells, tumor cells, and/or infected cells produces a prophylactic or therapeutic result relating to cancer, infectious disease, and the like.

[0054] In some embodiments, methods for suppressing cell proliferation involve the use of soluble complexes with a target cell directed moiety that interacts with a receptor on the target cell that stimulates suppression of proliferation and/or induction of cell death in the target cell. Such a receptor on the target cell is herein referred to as a "negative signaling" receptor. As used herein, "negative signaling" refers to the inhibition of cell growth, for example, by cell cycle arrest or the induction of apoptosis (programmed cell death).

[0055] Negative signaling receptors and their ligands are known in the art and include, for example, the tumor necrosis factor (TNF) receptor family, such as TNF receptor (TNF-R), TNF-like receptors, lymphotoxin-β receptor (LT-β-R), Fas receptor, and ligands, such as TNF, lymphotoxin-α (LT-α, formerly called TNF-β), lymphotoxin-β (LT-β), TNF-related apoptosis inducing ligand (TRAIL or Apo-2L) and Fas ligand (FasL). TNF-R signaling is cytotoxic to cells with transformed phenotypes or to tumor cells and can lead to selective lysis of tumor cells and virus-infected cells. Like TNF-R, signaling by LT-β-R can activate pathways that lead to cytotoxicity and cell death in tumor cells. Fas receptor (Fas-R) can stimulate cytotoxicity by programmed cell death in a variety of both tumor and non-tumor cells.

[0056] The ligands TNF and LT-α bind to and activate TNF receptors p60 and p80, herein referred to as TNF-R. LT-α1/β2 heterodimeric complex binds the LT-β–R and induces cytotoxic effects on cells bearing the LT-β–R in the presence of an LT-β–R activating agent, such as IFN-gamma. See, for example, U.S. Pat. 6,312,691. Fas ligands are capable of inducing apoptosis in cells that express a Fas receptor. The human and mouse Fas ligand genes and cDNAs have been isolated and sequenced (Genbank Accession No. U08137; Takahashi *et al.* (1994) *Intl. Immunol.* 6:1567-1574; Takahashi *et al.* (1994) *Cell* 76:969-976).

[0057] In addition to stimulation through interaction with specific ligands, antibody binding can also activate negative signaling receptors to signal growth arrest and/or apoptosis. Antibodies that have negative signaling properties include, but are not limited to, anti-Fas, anti-LT-β–R, anti-CD40, anti-Class II MHC, anti-Her-2, anti-CD19, anti-Le^y, anti-idiotype, anti-IgM, anti-CD20, anti-CD21 and anti-CD22 as reported, for example, in Trauth *et al.* (1989) *Science* 245:301-305; Funakoshi *et al.* (1994) *Blood* 83:2787-2794; Bridges *et al.* (1987) *J. Immunol.* 139:4242-4249; Scott *et al.* (1991) *J. Biol. Chem.* 266:14300-14305); Ghetie *et al.* (1992) *Blood* 80:2315-2320; Ghetie *et al.* (1994) *Blood* 83:1329-1336; Schreiber *et al.* (1992) *Cancer Res.* 52:3262-3266; Levy *et al.* (1990) *J. Natl. Cancer Inst. Monographs* 10:61-68; Vitetta *et al.* (1994) *Cancer Res.* 54:5301-5309; Page *et al.* (1988) *J. Immunol.* 140:3717-3726; Beckwith *et al.* (1991) *J. Immunol.* 147:2411-2418; U.S. Pat. Nos. 6,312,691 and 6,368,596. Furthermore, negative signaling can sometimes be optimized by hypercrosslinking with secondary antibodies or by using "cocktails" of primary antibodies (Marches *et al.* (1996) *Therap. Immunol.* 2:125-136).

[0058] In addition to the target cell directed moiety that interacts with the target cell receptor to stimulate a biological effect, the soluble complex can also include a cell targeting

molecule that directs the complex to the target cell. Such targeting molecules are components of the complex that enhance the accumulation of the complex at certain tissue or cellular sites in preference to other tissue or cellular sites when administered to an intact individual, organ or cell culture. Such a targeting moiety can be *inter alia* a peptide, a region of a larger peptide, an antibody specific for a target cell surface molecule or marker, or antigen binding fragment thereof, a nucleic acid, a carbohydrate, a region of a complex carbohydrate, a special lipid, or a small molecule such as a drug, hormone, or hapten, attached to any of the aforementioned molecules. Antibodies with specificity toward cell type-specific cell surface markers are known in the art and are readily prepared by methods known in the art. The complexes can be targeted to any cell type in which a stimulation of the biological effect is desired, *e.g.*, a cell type in which proliferation is to be stimulated or a cell type in which growth arrest is to be induced.

[0059] The methods of the invention may further include delivery of an agent (*e.g.*, a drug) to cells (*e.g.*, in culture or in an individual) or to an individual using the soluble complexes of the invention as a delivery vehicle for the agent. In this embodiment, the soluble complexes of the invention are coupled to an agent that will work along with the target cell directed molecule to result in the desired effect. For example, the soluble complex with a target cell directed moiety designed to stimulate T cell proliferation coupled to its surface can be coupled to a cytokine that further stimulates T cell growth (*e.g.*, IL-2, IL-15, IL-18, IL-27). Thus, the soluble complex provides an additional stimulatory component to support T cell proliferation. In another example, the soluble complex is formed with an anti-apoptosis agent and a target cell directed moiety designed to send an anti-apoptosis signal to the target cell.

[0060] In such methods, the soluble complexes can be coupled to one or more agents. Agents for the invention are described elsewhere herein. Such agents can be coupled to the soluble complex before, during and/or after the target cell directed moiety is made into the complex.

Compositions of the invention

[0061] A complex for use in the present invention comprises a soluble complex with at least two moieties that interact with a receptor on a target cell (*i.e.*, "a target cell directed moiety"). For the purposes of the invention, the receptor on the target cell is a molecule that, upon interaction with the soluble target cell directed moiety complex, stimulates or contributes to stimulation of a biological effect in the target cell.

[0062] The choice of the target cell directed moiety and the target cell receptor to which the moiety is directed depends on the target cell and the desired biological effect to be stimulated. Target cell receptors for use in stimulation of a biological effect include, but are not limited to, CD3, CD28, CD2, MHC class I complex (including dimer, tetramer, multimer) loaded with peptide, MHC class II complex (including dimer, tetramer, multimer) loaded with peptide, MHC class IB complex (including dimer, tetramer, multimer) loaded with peptide, T cell receptor complexes (including alpha-beta and gamma-delta), CD16, CD45, CD25, CD27, ICOS, CD40, CD40L, CTLA-4, OX-40, OX40L, CD30, CD30L, CD137, 4-1-BBL, B7.1, B7.2, FasR, FasL, TRAIL, DR4, DR5, DR3, TNFR1, TNFR2, chemokine receptors, receptors of cytokines (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL12P35, IL12P40, IL12P70, IL-13, IL-15, IL-18, IL-23, IL-27, TNF-alpha, TNF-beta, TGF-beta, IFN-gamma, GM-CSF), common gamma chain of IL-2 receptor, and any associated components of cytokine receptors. Additional examples of target cell receptors for use in stimulation of a biological effect include TNF-R, LT-βR, Her-2, CD19, IgM, CD20, CD21 and CD22. In some instances, target cell receptors for use in stimulation of a biological effect include those that signal the target cell through a tyrosine kinase, such as a src-family tyrosine kinase or a JAK family kinase, through a phosphatidylinositol 3-OH kinase.

[0063] Accordingly, target cell directed moieties that interact with a receptor on the surface of a target cell include, but are not limited to, natural or non-natural ligands of the receptor and antibodies that bind the receptor. Target cell directed moieties of the present invention include, but are not limited to, those that interact with CD3, CD28, CD2, MHC class I complex (including dimer, tetramer, multimer) loaded with peptide, MHC class II complex (including dimer, tetramer, multimer) loaded with peptide, MHC class IB complex (including dimer, tetramer, multimer) loaded with peptide, T cell receptor complexes (including alpha-beta and gamma-delta), CD16, CD45, CD25, CD27, ICOS, CD40, CD40L, CTLA-4, OX-40, OX40L, CD30, CD30L, CD137, 4-1-BBL, B7.1, B7.2, Fas, FasL, TRAIL, DR4, DR5, DR3, TNFR1, TNFR2, chemokine receptors, receptors of cytokines (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL12P35, IL12P40, IL12P70, IL-13, IL-15, IL-18, IL-23, IL-27, TNF-alpha, TNF-beta, TGF-beta, IFN-gamma, GM-CSF), common gamma chain of IL-2 receptor and any associated components of cytokine receptors. Additional examples of target cell directed moieties for use in stimulation of a biological effect include those that interact with TNF-R, LT-βR, Her-2, CD19, IgM, CD20, CD21 and CD22. In some instances, target cell directed moieties of the invention include those that upon interaction with the target cell receptor result

in target cell stimulation through a tyrosine kinase, such as a src-family tyrosine kinase or a JAK family kinase, through a phosphatidylinositol 3-OH kinase. In some instances, target cell directed moieties include a specific antigen, or fragment thereof, including tumor antigen and antigen associated with an infectious disease, such as a viral antigen.

[0064] In some embodiments, the soluble complexes may further comprise a nonclassical MHC molecule or a nonclassical MHC molecule ligand, for example, HLA nonclassical class I molecules and their ligands.

[0065] Target cell directed moieties also include lectins, including lectins which can function as mitogens. In some embodiments, lectins which bind particular cell surface receptors, for example, through interaction with glycosylated moieties on the particular receptor, can be used to induce aggregation of the receptor. Thus, the lectin is a target cell directed moiety that can contribute to stimulation of a biological effect in the target cell. Generally, lectins are glycoproteins that can be extracted from plants, seeds and other sources, and many are commercially available. In some cases, the lectins are biotinylated. Examples of lectins for use in the soluble complexes of the invention include, but are not limited to, *Aleuria aurantia* lectin, *Amaranthus caudatus* lectin, *Bauhinia purpurea* lectin, Concanavalin A (Con A), Succinylated Con A, *Datura stramonium* lectin, *Dolichos biflorus* agglutinin, *Erythrina cristagalli* lectin, *Euonymus europaeus* lectin, *Galanthus nivalis* lectin, *Griffonia (Bandeiraea) simplicifolia* lectin I (GSL I, BSL I), GSL I- isolectin B_4, *Griffonia (Bandeiraea) simplicifolia* lectin II (GSL II, BSL II), *Hippeastrum* hybrid lectin, Jacalin, *Lens culinaris* agglutinin, *Lotus tetragonolobus* lectin, *Lycopersicon esculentum* lectin, *Maackia amurensis* lectin I (MAL I), *Maackia amurensis* lectin II (MAL II), *Maclura pomifera* lectin, *Narcissus pseudonarcissus* lectin, Peanut agglutinin, *Phaseolus vulgaris* agglutinin (PHA-E+L), *Phaseolus vulgaris* erythroagglutinin (PHA-E), *Phaseolus vulgaris* leucoagglutinin (PHA-L), *Pisum sativum* agglutinin, *Psophocarpus tetragonolobus* lectin I (PTL I), *Psophocarpus tetragonolobus* lectin II (PTL II), *Ricinus communis* agglutinin I (RCA_{120}), *Ricinus communis* agglutinin II (ricin, RCA_{60}), *Sambucus nigra* lectin, *Solanum tuberosum* lectin, *Sophora japonica* agglutinin, Soybean agglutinin, *Ulex europaeus* agglutinin I (UEA I), *Vicia villos* lectin, Wheat germ agglutinin (WGA), Succinylated WGA, and *Wisteria floribunda* lectin.

[0066] As described herein, the target cell directed moiety can be included within or attached to another molecule as long as the target cell directed moiety portion of such a hybrid molecule is capable of interacting with the receptor on the target cell. Such molecules include target cell directed moiety - immunoglobulin (Ig) fusion proteins, for example, hybrid

molecules containing a target cell directed moiety linked to an Fc fragment of an Ig. Ig fusion proteins are known in the art, including those that contain an Fc fragment that comprises the hinge, CH2 and CH3 regions of human IgG molecules. See, for example, U.S. Pat. No. 5,116,964; Linsley et al. (1991) *J. Exp. Med.* 173:721-730; Linsley et al. (1991) *J. Exp. Med.* 174:561-569. Fusion proteins within the scope of the invention can be prepared by expression of a nucleic acid encoding the fusion protein in a variety of different systems known in the art and by other means known in the art.

[0067] In the complex, the target cell directed moiety is coupled, either directly or indirectly, to a coupling moiety(ies). When the target cell directed moiety is coupled directly to the coupling moiety, the molecule comprising the target cell directed moiety is either covalently or noncovalently attached to the coupling moiety. The coupling of the molecule comprising the target cell directed moiety to the coupling moiety can be accomplished using techniques described herein and known in the art, including, but not limited to, direct covalent linkage, covalent conjugation via a crosslinker moiety and noncovalent conjugation (*e.g.*, via a specific binding pair, via electrostatic bonding or via hydrophobic bonding).

[0068] When the target cell directed moiety is indirectly coupled to the coupling moiety, the target cell directed moiety or the molecule comprising the moiety is attached to a linker and the linker is attached, either directly or indirectly, to a coupling moiety. The target cell directed moiety or the molecule comprising the moiety is either covalently or noncovalently attached to the linker by techniques described herein and known in the art, including, but not limited to, direct covalent linkage, covalent conjugation via a crosslinker moiety (which may include a spacer arm) and noncovalent conjugation (*e.g.*, via a specific binding pair (*e.g.*, biotin and avidin), via electrostatic bonding or via hydrophobic bonding). The linker is either directly or indirectly and either covalently or noncovalently attached to a coupling moiety by techniques described herein and known in the art, including, but not limited to, direct covalent linkage, covalent conjugation via a crosslinker moiety (which may include a spacer arm), noncovalent conjugation via a specific binding pair (*e.g.*, via a specific binding pair (*e.g.*, biotin and avidin), via electrostatic bonding or via hydrophobic bonding).

[0069] Accordingly, in some embodiments, the target cell directed moiety or the molecule comprising the moiety is attached to the complex through a coupling moiety or linker comprised of a specific binding pair such as biotin or an analogue of biotin, e.g., iminobiotin, and avidin or streptavidin. A biotin group can be used, for example, to join a target cell directed moiety with an avidin or streptavidin incorporated into or attached onto the molecule

comprising the target cell directed moiety to another avidinated molecule. Alternatively, a biotin group can be attached to the molecule comprising the target cell directed moiety and avidin or streptavidin used to form a complex with the biotinylated moieties. In either case, labeling one component with biotin and the other component with avidin or streptavidin allows for the formation of a non-covalently bound complex in which the target cell directed moiety is coupled to a biotin-(strept)avidin linker in a soluble complex. Methods and techniques for attaching biotin, avidin and streptavidin to molecules and cells are well known in the art. See, for example, O'Shannessey et al. (1984) *Immunol. Lett.* 8:273-277; O'Shannessey et al. (1985) *J. Appl. Biochem.* 7:347-355; Wade et al. (1985) *Biochem. J.* 229:785-790; Rosenberg et al. (1986) *J. Neurochem.* 46:641-648 O'Shannessey et al. (1987) *Anal. Biochem.* 163:204-209; O'Shannessey et al. (1987) *J. Immunol. Meth.* 99:153-161; Reisfield et al. (1987) *Biochem. Biophys. Res. Com.* 142:519-526; Bayer et al. (1988) *Anal. Biochem.* 170:271-281; Green (1965). *Biochem. J.* 94:23c-24c.; Green (1975) *Avidin. Adv. Protein. Chemistry*, Academic Press, New York (Anfinsen et al. eds.) 29:85-133.

[0070] Soluble complexes of the present invention can also be made using phenylboronic acid (PBA), or derivatives thereof, complexed with boronic compound complexing reagents, for example, those derived from salicylhydroxamic acid (SHA), or derivatives thereof. Ortho-substituted acetamidophenylboronic acids have been proposed as potential linkers for selective bioconjugation via the vicinal diol moieties of the carbohydrate residues associated with glycoproteins (Cai et al. (1991) *Bioconjugate Chem.* 2:317-322). See, for example, U.S. Pat. Nos. 5,872,224 and 6,462,179.

[0071] In some embodiments, the linker can comprise at least one antibody, or the antigen binding portion thereof. An antibody that serves as a linker can bind the target cell directed moieties, or the molecule comprising the moieties, into a soluble complex. For example, a first antibody that binds a target cell receptor serves as the target cell receptor moiety is complexed with a second antibody that binds a target cell directed moiety and a third antibody that binds to both the first and second antibodies.

[0072] Non-covalent associations can also occur through ionic interactions involving a target cell directed moiety and residues within a coupling moiety or linker. Non-covalent associations can also occur through ionic interactions involving a target cell directed moiety and residues within a linker, such as charged amino acids, or through the use of a linker portion comprising charged residues that can interact with the at least two target cell directed moieties for the complex. For example, non-covalent conjugation can occur between a generally

negatively-charged target cell directed moiety and positively-charged amino acid residues of a coupling moiety or linker, *e.g.*, polylysine, polyarginine and polyhistidine residues.

[0073] Covalent conjugation of the target cell directed moiety to the coupling moiety or linker molecule may be effected in any number of ways, typically involving one or more crosslinking agents and functional groups on the target cell directed moiety, linker molecule and/or both.

[0074] Various cross-linking reagents that can be used to covalently link target cell directed moieties into soluble complexes are known in the art and generally, commercially available. For example, heterobifunctional cross-linkers that can be used include, but are not limited to, Sulfosuccinimidyl 6-[3-(2-pyridyldithio)-proprionamido]-hexanoate (Sulfo-LC-SPDP), 4-Succinimidyloxycarbonyl-methyl-α-[2-pyridyldithio]-toluene (SMPT), Sulfosuccinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (Sulfo-SMCC), Succinimidyl 4-(ρ-maleimidophenyl)-butyrate (SMPB), *m*-Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), 4-[-N-Maleimidophenyl]butric acid hydrazide-HCL (MPBH) and 3-[2-Pyridyldithio]propionyl hydrazide (PDPH). The heterobifunctional cross-linking reagents MPBH and PDPH are reactive towards carbohydrate and sulfhydryl groups. The heterobifunctional cross-linking reagents Sulfo-LC-SPDP, SMPT, Sulfo-SMCC, SMPB and MBS are reactive towards amino and sulfhydryl groups. Examples of photoactivatable cross-linkers include, but is not limited to, Sulfosuccinimidyl-[4-azidosalicylamido]-hexanoate (Sulfo-NHS-LC-ASA), *N*-Succinimidyl [4-azidophenyl]-1,3'-dithioproprionate (SADP), *N*-Succinimidyl 6-[4--azido-2'-nitrophenylamino]-hexanoate (SANPAH) and ρ-nitrophenyl-2-diazo-3,3,3-trifluoro-propionate (PNP-DTP). The photoactivatable cross-linking reagents Sulfo-NHS-LC-ASA, SADP, SANPAH and PNP-DTP are reactive towards amino groups.

[0075] Target cell directed moieties or molecules containing target cell directed moieties that are polypeptides will contain amino acid side chain moieties containing functional groups such as amino, carboxyl, or sulfhydryl groups that will serve as sites for coupling the target cell directed moiety to the linker. Residues that have such functional groups may be added to the target cell directed moiety if the target cell directed moiety does not already contain these groups. Such residues may be incorporated by solid phase synthesis techniques or recombinant techniques, both of which are well known in the peptide synthesis arts. In the case of target cell directed moieties or molecules containing target cell directed moieties that are carbohydrate or lipid, functional amino and sulfhydryl groups may be incorporated therein by conventional chemistry. For instance, primary amino groups may be incorporated by reaction

with ethylenediamine in the presence of sodium cyanoborohydride and sulfhydryls may be introduced by reaction of cystamine dihydrochloride followed by reduction with a standard disulfide reducing agent. In a similar fashion, the linker molecule may also be derivatized to contain functional groups if it does not already possess appropriate functional groups.

[0076] Hydrophilic linkers of variable lengths are useful for connecting peptides or other bioactive molecules to linker molecules. Suitable linkers include linear oligomers or polymers of ethyleneglycol. Such linkers include linkers with the formula $R_1S(CH_2CH_2O)_nCH_2CH_2O(CH_2)_mCO_2R_2$ wherein n=0-200, m=1 or 2, R_1 =H or a protecting group such as trityl, R_2 =H or alkyl or aryl, *e.g.*, 4-nitrophenyl ester. These linkers are useful in connecting a molecule containing a thiol reactive group such as haloaceyl, maleiamide, etc., via a thioether to a second molecule which contains an amino group via an amide bond. These linkers are generally flexible with regard to the order of attachment, *i.e.*, the thioether can be formed first or last.

[0077] In addition or alternatively, a ligand can be coupled to the soluble complex which serves to preferentially direct the complex to a particular cell, organ, tissue and/or site within an individual. Such a ligand may serve to increase up-take of the complex by a particular organ or tissue. Directing ligands can be coupled to the soluble complex through any of the means described herein for the target cell directed moiety. Soluble complexes with coupled directing ligands may or may not have a target cell directed moiety also coupled. Soluble complexes preferentially directed to cells and/tissue of the immune system include those containing antigen(s) to which an immune response is desired. Examples of ligands which direct the soluble complexes to the lymph nodes are CD62L and LFA-1. Soluble complexes directed to the lymph nodes may further include an antigen linked to a Tat polypeptide of HIV to facilitate processing of the antigen.

[0078] In some embodiments, the compositions and/or methods of the invention involve soluble complexes further coupled to an agent (e.g., a drug or antigen) and can serve as a delivery vehicle for the agent. As used herein and in this context, an "agent" includes but is not limited to an atom or molecule, wherein a molecule may be inorganic or organic, a biological effector molecule and/or a nucleic acid encoding an agent such as a biological effector molecule, a protein, a polypeptide, a peptide, a nucleic acid, a peptide nucleic acid (PNA), a virus, a virus-like particle, a nucleotide, a ribonucleotide, a synthetic analogue of a nucleotide, a synthetic analogue of a ribonucleotide, a modified nucleotide, a modified

ribonucleotide, an amino acid, an amino acid analogue, a modified amino acid, a modified amino acid analogue, a steroid, a proteoglycan, a lipid, a fatty acid and a carbohydrate.

[0079] As used herein, the term "biological effector molecule" or "biologically active molecule" refers to an agent that has activity in a biological system, including, but not limited to, a protein, polypeptide or peptide including, but not limited to, a structural protein, an enzyme, a cytokine (such as an interferon and/or an interleukin), a growth factor, an anti-apoptosis agent, an antigen, an antibiotic, a polyclonal or monoclonal antibody, or an effective part thereof, such as an Fv fragment, which antibody or part thereof may be natural, synthetic or humanized, a peptide hormone, a receptor, and a signaling molecule. As described herein, included within the term "immunoglobulin" are intact immunoglobulins as well as antibody fragments such as Fv, a single chain Fv (scFv), a Fab or a $F(ab')_2$.

[0080] In some embodiments, the soluble complexes are coupled to agents that promote Th1/Th2 cell growth, including, for example, IL-2, IL-7, IL-15, IL-18, IL-23, IL-27 and the like. In some embodiments, the soluble complexes are coupled to agents that promote Th1/Th2 cell differentiation, including, for example, IL-4, IL-12 and the like. In some embodiments, the soluble complexes are coupled to with anti-apoptosis agents including, for example, cellular FLICE (FADD-like IL-1 beta-converting enzyme) inhibitory protein (cFLIP), cIAP (inhibitor of apoptosis protein) 1 and 2.

[0081] In some embodiments, the compositions and/or methods of the invention involve soluble complexes which are coupled to a) target cell directed moieties such as MHC I or MHC II tetramers loaded with a specific peptide or antigen specific for B cells and b) an agent such as an antigen or drug, e.g., FasL, TRAIL, TNF-alpha, IL-2, IL-15, IL-18, IL-23, or IL-27. Such compositions can thus direct the agent to the targeted B cell.

[0082] As described herein, complexes of use in the invention may include an antibody that binds a receptor on the target cell, or the receptor binding portion of the antibody, as a target cell directed moiety. In such complexes, the anti-target cell antibody is coupled, either directly or indirectly, to the coupling moiety for forming the soluble complex. As an example, the antibody can be labeled with biotin or avidin and complexed with another antibody so labeled with the use of avidin or biotin, respectively, through a biotin-avidin coupling. For example, the target cell directed moiety(ies) can be biotinylated and formed into a soluble complex with avidin or streptavidin using techniques known in the art. Alternatively, a target cell directed moiety(ies) covalently labeled with avidin or streptavidin and formed into a soluble complex

with biotin using techniques known in the art. In either case, for use in the invention, the target cell directed antibody labeled with biotin or avidin/streptavidin maintains the ability to bind to the target cell receptor.

[0083] Within the context of the present invention, antibodies are understood to include various kinds of antibodies, including, but not necessarily limited to, naturally occurring antibodies, monoclonal antibodies, polyclonal antibodies, antibody fragments that retain antigen binding specificity (*e.g.*, Fab, and $F(ab')_2$) and recombinantly produced binding partners, single domain antibodies, hybrid antibodies, chimeric antibodies, single-chain antibodies, human antibodies, humanized antibodies, and the like. Generally, antibodies are understood to be reactive against a selected antigen on the surface of a cell if they bind with an affinity (association constant) of greater than or equal to 10^7 M^{-1}.

[0084] Polyclonal antibodies against selected antigens on the surface of cells may be readily generated by one of ordinary skill in the art from a variety of warm-blooded animals such as horses, cows, various fowl, rabbits, mice, or rats. In some cases, human polyclonal antibodies against selected antigens may be purified from human sources.

[0085] Preferably, monoclonal antibodies are used in the antibody compositions of the invention. Monoclonal antibodies specific for selected antigens on the surface of cells may be readily generated using conventional techniques (see, for example, Harlow *et al.*, 1988, supra, and U.S. Pat. Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with an antigen, and monoclonal antibodies can be isolated. Other techniques may also be utilized to construct monoclonal antibodies (see, for example, Huse *et al.* (1989) *Science* 246:1275-1281; Sastry *et al.* (1989) *Proc. Natl. Acad. Sci.* USA 86:5728-5732; Alting-Mees *et al.* (1990) *Strategies in Molecular Biology* 3:1-9).

[0086] Similarly, binding partners may be constructed utilizing recombinant DNA techniques. For example, the genes which encode the variable region from a hybridoma producing a monoclonal antibody of interest are amplified using nucleotide primers for the variable region. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources. The primers may be utilized to amplify heavy or light chain variable regions, which may then be inserted into appropriate expression vectors. These vectors may then be introduced into cells, for example *E. coli* cells, for expression. Utilizing these techniques, large amounts of a single-chain protein containing a fusion of the V_H and V_L domains may be produced (see, for example, Bird *et al.* (1988)

Science 242:423-426). In addition, such techniques may be utilized to change a "murine" antibody to a "human" antibody, without altering the binding specificity of the antibody.

[0087] As used herein, a "single domain antibody" (dAb) is an antibody which is comprised of a V_H domain, which reacts immunologically with a designated antigen. A dAb does not contain a V_L domain, but may contain other antigen binding domains known to exist in antibodies, for example, the kappa and lambda domains. Methods for preparing dAbs are known in the art. See, for example, Ward *et al.* (1989) *Nature* 341:544-546. Antibodies may also be comprised of V_H and V_L domains, as well as other known antigen binding domains. Examples of these types of antibodies and methods for their preparation are known in the art (see, *e.g.*, U.S. Pat. No. 4,816,467).

[0088] Further exemplary antibodies include "univalent antibodies", which are aggregates comprised of a heavy chain/light chain dimer bound to the Fc (*i.e.*, constant) region of a second heavy chain. This type of antibody generally escapes antigenic modulation. See, *e.g.*, Glennie *et al.* (1982) *Nature* 295:712-714.

[0089] Antibodies can be fragmented using conventional techniques and the fragments (including "Fab" fragments) screened for utility in the same manner as described above for whole antibodies. The "Fab" region refers to those portions of the heavy and light chains which are roughly equivalent, or analogous, to the sequences which comprise the branch portion of the heavy and light chains, and which have been shown to exhibit immunological binding to a specified antigen, but which lack the effector Fc portion. "Fab" includes aggregates of one heavy and one light chain (commonly known as Fab'), as well as tetramers containing the 2H and 2L chains (referred to as $F(ab)_2$), which are capable of selectively reacting with a designated antigen or antigen family. Methods of producing Fab fragments of antibodies are known within the art and include, for example, proteolysis, and synthesis by recombinant techniques. For example, $F(ab')_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments. "Fab" antibodies may be divided into subsets analogous to those described herein, *i.e.*, "hybrid Fab", "chimeric Fab", and "altered Fab".

[0090] "Hybrid antibodies" are antibodies wherein one pair of heavy and light chains is homologous to those in a first antibody, while the other pair of heavy and light chains is homologous to those in a different second antibody. Typically, each of these two pairs will bind different epitopes, particularly on different antigens. This results in the property of

"divalence", *i.e.*, the ability to bind two antigens simultaneously. Such hybrids may also be formed using chimeric chains, as set forth herein.

[0091] The invention also encompasses "altered antibodies", which refers to antibodies in which the naturally occurring amino acid sequence in a vertebrate antibody has been varied. Utilizing recombinant DNA techniques, antibodies can be redesigned to obtain desired characteristics. The possible variations are many, and range from the changing of one or more amino acids to the complete redesign of a region, for example, the constant region. Changes in the constant region, in general, to attain desired cellular process characteristics. Changes in the variable region may be made to alter antigen binding characteristics. The antibody may also be engineered to aid the specific delivery of a molecule or substance to a specific cell or tissue site. The desired alterations may be made by known techniques in molecular biology, *e.g.*, recombinant techniques, site directed mutagenesis, and other techniques.

[0092] By "humanized" is meant alteration of the amino acid sequence of an antibody so that fewer antibodies and/or immune responses are elicited against the humanized antibody when it is administered to a human. For the use of the antibody in a mammal other than a human, an antibody may be converted to that species format.

[0093] "Chimeric antibodies", are antibodies in which the heavy and/or light chains are fusion proteins. Typically the constant domain of the chains is from one particular species and/or class, and the variable domains are from a different species and/or class. The invention includes chimeric antibody derivatives, *i.e.*, antibody molecules that combine a non-human animal variable region and a human constant region. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. A variety of approaches for making chimeric antibodies have been described and can be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes selected antigens on the surface of differentiated cells or tumor cells. See, for example, Morrison *et al.* (1985) *Proc. Natl. Acad. Sci.* U.S.A. 81:6851; Takeda *et al.* (1985) *Nature* 314:452; U.S. Pat. Nos. 4,816,567 and 4,816,397; European Patent Publications EP171496 and EP173494; United Kingdom patent GB 2177096B.

[0094] Bispecific antibodies may contain a variable region of an anti-target cell receptor antibody and a variable region specific for a different target cell directed moiety. In other cases, bispecific antibodies may contain a variable region of an anti-target cell receptor antibody and a variable region specific for a linker molecule. Bispecific antibodies may be

obtained forming hybrid hybridomas, for example by somatic hybridization. Hybrid hybridomas may be prepared using the procedures known in the art such as those disclosed in Staerz *et al.* (1986, *Proc. Natl. Acad. Sci.* U.S.A. 83:1453) and Staerz *et al.* (1986, *Immunology Today* 7:241). Somatic hybridization includes fusion of two established hybridomas generating a quadroma (Milstein *et al.* (1983) *Nature* 305:537-540) or fusion of one established hybridoma with lymphocytes derived from a mouse immunized with a second antigen generating a trioma (Nolan *et al.* (1990) *Biochem. Biophys. Acta* 1040:1-11). Hybrid hybridomas are selected by making each hybridoma cell line resistant to a specific drug-resistant marker (De Lau *et al.* (1989) *J. Immunol. Methods* 117:1-8), or by labeling each hybridoma with a different fluorochrome and sorting out the heterofluorescent cells (Karawajew *et al.* (1987) *J. Immunol. Methods* 96:265-270).

[0095] Bispecific antibodies may also be constructed by chemical means using procedures such as those described by Staerz *et al.* (1985) *Nature* 314:628 and Perez *et al.* (1985) *Nature* 316:354. Chemical conjugation may be based, for example, on the use of homo- and heterobifunctional reagents with E-amino groups or hinge region thiol groups. Homobifunctional reagents such as 5,5'-dithiobis(2-nitrobenzoic acid) (DNTB) generate disulfide bonds between the two Fabs, and 0-phenylenedimaleimide (O-PDM) generate thioether bonds between the two Fabs (Brenner *et al.* (1985) *Cell* 40:183-190, Glennie *et al.* (1987) *J. Immunol.* 139:2367-2375). Heterobifunctional reagents such as N-succinimidyl-3-(2-pyridylditio) propionate (SPDP) combine exposed amino groups of antibodies and Fab fragments, regardless of class or isotype (Van Dijk *et al.* (1989) *Int. J. Cancer* 44:738-743).

[0096] Bifunctional antibodies may also be prepared by genetic engineering techniques. Genetic engineering involves the use of recombinant DNA based technology to ligate sequences of DNA encoding specific fragments of antibodies into plasmids, and expressing the recombinant protein. Bispecific antibodies can also be made as a single covalent structure by combining two single chains Fv (scFv) fragments using linkers (Winter *et al.* (1991) *Nature* 349:293-299); as leucine zippers coexpressing sequences derived from the transcription factors fos and jun (Kostelny *et al.* (1992) *J. Immunol.* 148:1547-1553); as helix-turn-helix coexpressing an interaction domain of p53 (Rheinnecker *et al.* (1996) *J. Immunol.* 157:2989-2997), or as diabodies (Holliger *et al.* (1993) *Proc. Natl. Acad. Sci.* U.S.A. 90:6444-6448).

[0097] Antibodies against selected antigens on the surface of target cells may also be obtained from commercial sources.

[0098] A tetrameric immunological complex may be prepared by mixing a first monoclonal antibody which is capable of binding to at least one receptor on the surface of a target cell and a second monoclonal antibody which is capable of binding to a second receptor on a target cell. The first and second monoclonal antibodies are from a first animal species. The first and second antibodies are reacted with monoclonal antibodies of a second animal species which are directed against the Fc-fragments of the antibodies of the first animal species. The first and second antibody may also be reacted with the $F(ab')_2$ fragments of monoclonal antibodies of a second animal species which are directed against the Fc-fragments of the antibodies of the first animal species. See, for example, U.S. Pat. No. 4,868,109. For example, the first and second antibody may be reacted with an about equimolar amounts of the monoclonal antibodies of the second animal species or of the $F(ab')_2$ fragments thereof.

[0099] An anti-target cell receptor antibodies may also be coupled through a biotin-(strept)avidin linkage as described herein. Many alternative indirect ways to specifically link the antibodies in the composition for use in the invention would also be apparent to those skilled in the art.

[00100] Antibodies may be selected for use in the antibody compositions of the invention based on their ability to stimulate the desired biological effect in the target cell. In some embodiments, anti-target cell antibodies include antibodies specific for the antigens CD3 and CD28 which are present on the surface of human CD4+ T cells.

[00101] In some embodiments, an anti-CD3 or an anti-CD28 antibody are coupled in the same complex. In some embodiments, a complex is formed only with anti-CD3 antibodies and another complex is formed only with anti-CD28 antibodies. Thus, in an embodiment, a composition for stimulating proliferation and differentiation of T cells from PBMCs comprises complexes comprising an anti-CD3 antibody, or CD3 binding portion thereof, coupled with an anti-CD28 antibody, or CD28 binding portion thereof. In another embodiment, compositions for stimulating proliferation and differentiation of T cells from PBMCs comprise a) complexes comprising an anti-CD3 antibody, or CD3 binding portion thereof, and b) complexes comprising an anti-CD28 antibody, or CD28 binding portion thereof. In an embodiment, a composition for stimulating proliferation and differentiation of T cells from PBMCs consists essentially of a) complexes comprising an anti-CD3 antibody, or CD3 binding portion thereof and b) complexes comprising an anti-CD28 antibody, or CD28 binding portion thereof.

[00102] Monoclonal antibodies against CD3 and CD28, in the antibody composition of the invention, are used to stimulate a biological effect in T cells. Examples of monoclonal

antibodies specific for CD3 and CD28 are OKT3 and L293, respectively, and additional examples are in the art.

[00103] For example, complexes comprising anti-CD3 and anti-CD28 an be made using biotinylated antibodies. For complex formation, streptavidin (SA) is dissolved in a saline solution at 1 mg/ml and the solution is filtered through a 0.2 μm filter. The biotinylated anti-CD3 antibody and the biotinylated anti-CD28 antibody are added to a saline solution and the solution was mixed well. The SA is then added to the antibody solution and the mixture was rotated end-to-end for 30 minutes at room temperature. The soluble SA-CD3/CD28 complexes are then added to cells, generally, within 24 hours.

Formulations and routes of administration

[00104] According to still another aspect of the invention, the compositions of the invention, including compositions comprising complexes of the invention and compositions comprising cells stimulated and/or generated using the methods of the invention, and mixtures thereof, are used in the preparation of medicaments, for treating the conditions described herein. These compositions of the invention are administered as pharmaceutically acceptable compositions. The compositions may be administered by any suitable means, including, but not limited to, intravenously, parenterally or locally. The compositions can be administered in a single dose by bolus injection or continuous infusion or in several doses over selected time intervals in order to titrate the dose.

[00105] The particular administration mode selected will depend upon the particular composition, treatment, cells involved, etc.. For the administration of cells, typically, about $10\text{-}10^{12}$ cells can be administered in a volume of 50 μl to 1 liter, 1 ml to 1 liter, 10 ml to 250 ml, 50 ml to 150, and typically 100 ml. The volume will depend upon, for example, the type of cell administered, the disorder treated and the route of administration.

[00106] As used herein, "pharmaceutically acceptable excipient" includes any material which, when combined with an active ingredient of a composition, allows the ingredient to retain biological activity and without causing disruptive reactions with the subject's immune system. Various pharmaceutically acceptable excipients are well known in the art.

[00107] Exemplary pharmaceutically acceptable excipients include sterile aqueous or non-aqueous solutions and suspensions. Examples include, but are not limited to, any of the standard pharmaceutical excipients such as a phosphate buffered saline solution, water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media.

Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Compositions comprising such excipients are formulated by well known conventional methods (see: for example, Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co.).

CLAIMS

We claim:

1. A method for stimulating a biological effect in a target cell, comprising contacting a target cell with a soluble complex comprising two or more target cell directed moieties, wherein a first target cell directed moiety interacts with a first receptor on the surface of the target cell, and wherein the interaction of the first moiety with the receptor stimulates a biological effect in the target cell.

2. The method according to claim 1, wherein the complex further comprises a second target cell directed moiety, wherein the second moiety interacts with a second receptor on the surface of the target cell.

3. A method for stimulating a biological effect in a target cell, comprising contacting a target cell with a first soluble complex comprising a first target cell directed moiety and two or more target cell directed moieties, and
contacting the target cell with a second soluble complex comprising a second target cell directed moiety and two or more target cell directed moieties,
wherein the first target cell directed moiety interacts with a first receptor on the surface of the target cell, wherein the second target cell directed moiety interacts with a second receptor on the surface of the target cell, and wherein the interaction of the moieties with the receptors stimulates a biological effect in the target cell.

4. The method according to claim 1 or 3, wherein the first moiety comprises an antibody or antigen-binding portion thereof that binds the receptor on the target cell.

5. The method according to claim 2 or 3, wherein the second moiety comprises an antibody or antigen-binding portion thereof that binds the receptor on the target cell.

6. The method according to claim 1 or 3, wherein the first moiety comprises a ligand that interacts with the receptor on the target cell.

7. The method according to claim 2 or 3, wherein the second moiety comprises a ligand that interacts with the receptor on the target cell.

8. The method according to claim 2 or 3, wherein the target cell is selected from the group consisting of T cells, B cells, NK cells, monocytes, macrophages, dendritic cells, fibroblasts, neuronal cells and stem cells.

9. The method according to claim 8, wherein the target cell is a T cell.

10. The method according to claim 9, wherein the first moiety interacts with CD3 on the T cell surface and the second moiety interacts with CD28 on the T cell surface.

11. The method according to claim 10, wherein the biological effect stimulated is T cell proliferation, or wherein the biological effect stimulated is T cell differentiation.

12. The method according to claim 1 or 3, wherein the biological effect stimulated is apoptosis.

13. The method according to claim 12, wherein the first receptor on the surface of the target cell is selected from the group consisting of a Fas receptor, a Fas receptor ligand, a TNF receptor, and a TNF receptor ligand.

14. The method according to claim 13, wherein the TNF receptor is selected from the group consisting of CD40, OX40, DR3, DR4 and DR5.

15. The method according to claim 13, wherein the TNF receptor ligand is selected from the group consisting of CD40 ligand, OX40 ligand, DR3 ligand, DR4 ligand and DR5 ligand.

16. The method according to claim 1 or 3, wherein the contacting occurs in an individual.

17. The method according to claim 1, wherein the soluble complex comprises a streptavidin-biotin linkage.

18. The method according to claim 3, wherein the soluble complexes comprise streptavidin-biotin linkages.

19. The method according to claim 1 or 3, wherein the biological effect stimulated is cytokine production.

20. The method according to claim 1, wherein the soluble complex further comprises an agent selected from the group consisting of cytokine, chemokine and hormone.

21. A composition comprising a soluble complex, wherein the complex comprises a first antibody and a second antibody,

wherein the first antibody comprises a first antibody or antigen-binding portion thereof that binds CD3,

wherein the second antibody comprises a second antibody or antigen-binding portion thereof that binds CD28, and.

wherein the first antibody is associated with the second antibody through a biotin-streptavidin linkage.

22. The composition according to claim 21, wherein the soluble complex further comprises an agent selected from the group consisting of cytokine, chemokine and hormone.

23. The composition according to claim 22, wherein the agent is a cytokine selected from the group consisting of IL-2, IL-4, IL-5, IL-15, IL-18, IL-27, TNF-alpha, FasL and TRAIL.

24. The composition according to claim 21, wherein the soluble complex further comprises an antigen peptide, wherein the antigen peptide is associated with major histocompatability complex (MHC) molecule and wherein the MHC molecule associated with the complex.

25. The composition according to claim 24, wherein the MHC molecule is selected from the group consisting of a MHC class I molecule, a MHC class II molecule and a MHC class IB molecule.

26. The composition according to claim 25, wherein the antigen peptide is a tumor antigen peptide, a viral antigen peptide or a self-peptide.

27. The composition according to claim 21 further comprising an HLA non-classical class I molecule.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/39122

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 39/395; C07K 16/46, 6/00
US CL : 424/133.1, 134.1, 136.1, 178.1, 179.1, 180.1, 181.1, 182.1; 530/387.3, 391.1, 391.5, 391.7
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/133.1, 134.1, 136.1, 178.1, 179.1, 180.1, 181.1, 182.1; 530/387.3, 391.1, 391.5, 391.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/0155604 A1 (LEDBETTER et al.) 24 October 2002, see entire document.	1-19,21
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Y		20,22-27
Y	US 2003/0166277 (ZAUDERER et al.) 4 September 2003, see entire document.	24-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/39122

Continuation of B. FIELDS SEARCHED Item 3:
WEST 2.1, MEDICINE/BIOTECH (compendium databases on DIALOG) search terms: inventor names, cd3, cd28, bispecific, antibod?, avidin, biotin, cd40, ox40, bifunctional, cytokine,