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(71) Applicant *(for all designated States except US)*: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 12th Floor, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RAZ, Eyal** [US/US]; 13658 Mango Drive, Del Mar, CA 92014 (US). **REDECKE, Vanessa, Doreen** [US/US]; 8571 Villa La Jolla Drive #D, La Jolla, CA 92037 (US). **HORNER, Anthony, A.** [US/US]; 321 Union Street, Leucadia, CA 92024 (US).

(74) Agent: **BORDEN, Paula, A.**; Bozicevic, Field & Francis LLP, 1900 University Avenue, Suite 200, East Palo Alto, CA 94303 (US).

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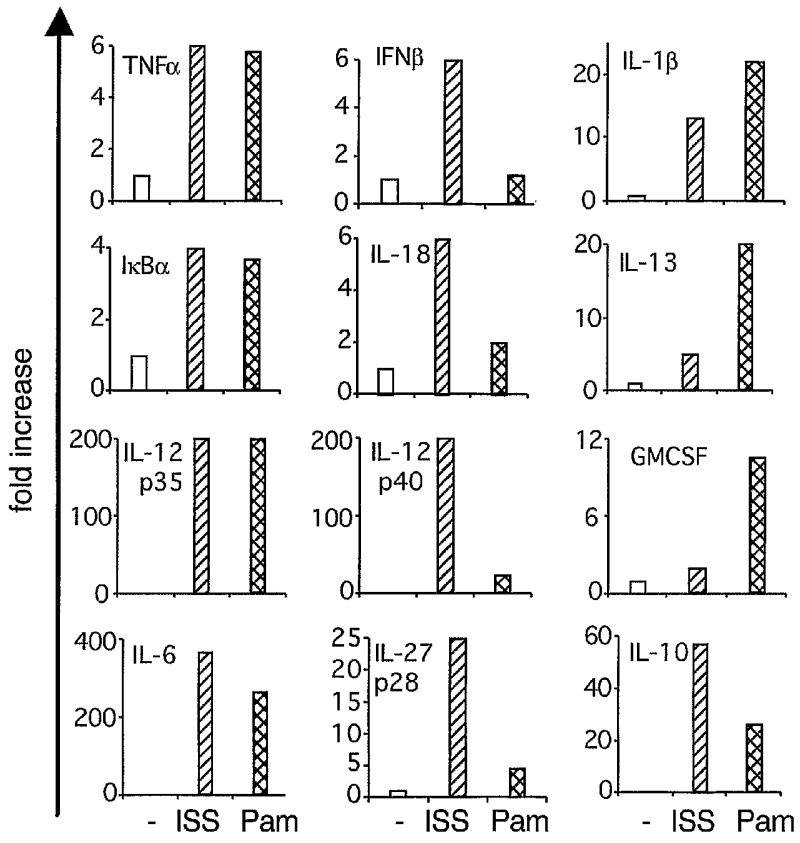
(54) Title: METHODS OF TREATING IMMUNOPATHOLOGICAL DISORDERS



(57) Abstract: The present invention provides methods of treating immunopathological disorders having a Th1 component, e.g., autoimmune disorders and allograft rejection, and/or an M1 macrophage component, e.g., an M1-mediated inflammatory disorder. The methods generally involve administering to an individual in need thereof an effective amount of a toll-like receptor-2 (TLR2) agonist. The present invention provides combination therapies for treating an immunopathology having a Th1 and/or an M1 macrophage component, generally involving administering a TLR2 agonist and at least one additional therapeutic agent.

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METHODS OF TREATING IMMUNOPATHOLOGICAL DISORDERS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/545,353 filed February 17, 2004, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] The U.S. government may have certain rights in this invention, pursuant to grant no. AI40682 awarded by the National Institutes of Health.

FIELD OF THE INVENTION

[0003] The present invention is in the field of autoimmune disorders, and in particular in the use of Toll-like receptor-2 agonists to treat immunopathologies having a Th1 component, e.g., autoimmune disorders and allograft rejection, as well as to treat M1-mediated inflammatory disorders.

BACKGROUND OF THE INVENTION

[0004] Efficient immune responses depend on the interaction between the innate and adaptive immune system. Immune responses against invading pathogens are initiated by Toll like receptors (TLR) that recognize distinct structurally conserved components of pathogens. Probably with the exception of TLR3, the receptor for dsRNA, cell activation by all TLR family members is largely dependent on the adaptor molecule MyD88. Stimulation of the TLR leads to recruitment of MyD88, engagement of IRAK and TRAF6 and activation of transcription factors such as NF-κB and AP-1. This ultimately results in upregulation of costimulatory molecules, secretion of cytokines and enhanced uptake and presentation of antigen. Both TLR-dependent activation of antigen presenting cells (APC) and processing and presentation of antigen are necessary for the induction of adaptive T- and B-cell responses. Polarization towards a Th1 or Th2 phenotype is crucial for the defense against pathogens, but can also be associated with the induction of autoimmune disease (Th1) or asthma (Th2).

[0005] Current treatment of autoimmune diseases involves administering immunosuppressive agents such as corticosteroids (e.g., cortisone), non-steroidal anti-inflammatory drugs, immunomodulating agents, antimalarial drugs, and immunosuppressants. Despite the

availability of agents to treat autoimmune disorders, there is an ongoing need for effective treatments. The present invention addresses this need.

Literature

[0006] Sabroe et al. (2003) *J. Immunol.* 171:1630-1635; Zekki et al. (2002) *Brain Pathol.* 12:308-319; Lawton and Ghosh (2003) *Curr. Opin. Chem. Biol.* 7:446-451; Takeda et al. (2003) *Annu. Rev. Immunol.* 21, 335-376; U.S. Patent Publication No. 2005/0004144; U.S. Patent Publication No 2004/0141950.

SUMMARY OF THE INVENTION

[0007] The present invention provides methods of treating immunopathologies having a Th1 component, e.g., autoimmune disorders and allograft rejection, and/or an M1 macrophage component, e.g., an M1-mediated inflammatory disorder. The methods generally involve administering to an individual in need thereof an effective amount of a toll-like receptor-2 (TLR2) agonist. The present invention provides combination therapies for treating an autoimmune disorder, generally involving administering a TLR2 agonist and at least one additional therapeutic agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Figures 1A-J depict differential immune responses induced by ISS-ODN- and Pam3Cys-based immunizations.

[0009] Figures 2A-2D depict activation of BMDC by ISS-ODN and Pam_3Cys.

DEFINITIONS

[0010] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse affect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) reducing the incidence and/or risk of relapse (remission, "flare-up") of the disease during a symptom-free period; (b) relieving or reducing a symptom of the disease; (c) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (d) inhibiting the disease, i.e., arresting its development (e.g., reducing the rate of disease progression); (e)

reducing the frequency of episodes of the disease; and (f) relieving the disease, i.e., causing regression of the disease.

[0011] The terms "individual," "host," "subject," and "patient," used interchangeably herein, refer to a mammal, particularly a human.

[0012] "Treatment failure patients" as used herein generally refers to patients who have been diagnosed as having an autoimmune disease, and who failed to respond to previous therapy for the autoimmune disease (referred to as "non-responders"), or who initially responded to previous therapy, but in whom the therapeutic response was not maintained (referred to as "relapsers").

[0013] The term "biological sample" encompasses a variety of sample types obtained from an organism and can be used in a diagnostic or monitoring assay. The term encompasses blood and other liquid samples of biological origin, solid tissue samples, such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The term encompasses samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components. The term encompasses a clinical sample, and also includes cells in cell culture, cell supernatants, cell lysates, serum, plasma, biological fluids, and tissue samples.

[0014] As used herein, the terms "M1-mediated inflammatory disorder" and "M1-mediated immunopathological disorder," used interchangeably herein, refer to a disorder that is caused by, that results from, or that is associated with, M1-type macrophages. M1 macrophages are activated by IFN-γ, and are characterized by having high inducible nitric oxide synthase (iNOS) levels and producing large amounts of nitric oxide (NO). Delayed type hypersensitivity (DTH) reaction is an example of an inflammatory reaction induced by M1 macrophages. M1 macrophages are distinguished from M2 macrophages. M2 macrophages are activated by IL-4/IL-13, and are characterized by having high arginase levels, and producing substantially no NO (or producing significantly less NO than M1 macrophages). See, e.g., Mills et al. (2000) *J. Immunol.* 164:6166-6173; Rauh et al. (2004) *Biochem. Soc. Transact.* 32:785-788; and Gordon (2003) *Nature Reviews Immunology*3:23-35. M1 macrophages generally accompany Th1-mediated diseases. Accordingly, an M1 macrophage-mediated disorder is any Th1-mediated disease.

[0015] The terms "antigen" and "epitope" are well understood in the art and refer to the portion of a macromolecule which is specifically recognized by a component of the immune system, e.g., an antibody or a T-cell antigen receptor. As used herein, the term "antigen" encompasses antigenic epitopes, e.g., fragments of an antigen which are antigenic epitopes.

Epitopes are recognized by antibodies in solution, e.g. free from other molecules. Epitopes are recognized by T-cell antigen receptor when the epitope is associated with a class I or class II major histocompatibility complex molecule.

[0016] As used herein, "pharmaceutically acceptable carrier" 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. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline or normal (0.9%) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, Chapter 43, 14th Ed. or latest edition, Mack Publishing Co., Easton PA 18042, USA; A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

[0017] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0018] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0019] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein

can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0020] It must be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a TLR2 agonist" includes a plurality of such agonists and reference to "the therapeutic agent" includes reference to one or more therapeutic agents and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0021] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION OF THE INVENTION

[0022] The present invention provides methods of treating an immunopathological disorder having a Th1 component and/or an M1 macrophage component. The present invention provides methods of treating an M1-mediated inflammatory disorder. The methods generally involve administering to an individual in need thereof an effective amount of a toll-like receptor-2 (TLR2) agonist. Immunopathologies that are treated with a subject method include any immunopathology in which an M1 macrophage inflammatory response and/or a Th1 immune response plays a primary or a secondary role, Th1-mediated immunopathologies, M1 macrophage-mediated inflammatory disorders, and any immunopathology having a Th1 and/or an M1 macrophage component. Immunopathologies that are treated with a subject method include, but are not limited to, an autoimmune disorder, Th1-mediated inflammation, allorejection, M1-mediated inflammatory disorders, and the like.

[0023] A subject method involves administration of a therapeutically effective amount of a TLR2 ligand, generally a TLR2 agonist. A TLR2 agonist is any compound or substance that functions to activate a TLR2, e.g., to induce a signaling event mediated by a TLR2 signal transduction pathway.

[0024] A subject treatment method induces a Th2 type immune response in favor of a Th1 type immune response. Thus, a subject treatment method induces or increases production of Th2 cytokines such as IL-13, GM-CSF, IL-4, IL-5, and IL-1β. In some embodiments, an effective amount of a TLR2 agonist, when administered to an individual in a subject method, results in an increase of at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, in the serum level of a Th2 cytokine, compared to the serum level before treatment with the TLR2 agonist, or compared to the serum level in the absence of treatment with the TLR2 agonist.

[0025] M1 macrophage-mediated disorders that can be treated using a subject method include, but are not limited to, a delayed type hypersensitivity reaction; post-transplant organ rejection; Crohn's disease; multiple sclerosis; rheumatoid arthritis; and giant cell arteritis.

Autoimmune disorders

[0026] The present invention provides methods of treating an autoimmune disorder, the methods generally involving administering to an individual in need thereof an effective amount of a TLR2 agonist. In some embodiments, the methods further involve administering at least one additional therapeutic agent that treats an autoimmune disorder.

[0027] In some embodiments, an effective amount of a TLR2 agonist is an amount that is effective to reduce the frequency and/or level and/or severity of at least one symptom of an autoimmune disease by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the frequency and/or level and/or severity of the symptom in the absence of treatment with the TLR2 agonist.

[0028] In some embodiments, an effective amount of a TLR2 agonist is an amount that is effective to reduce the level of autoantibody in an individual by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the level of autoantibody in the absence of treatment with the TLR2 agonist. The level of autoantibody in an individual is readily determined by any of a number of well-established immunological assays, including, but not limited to, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and the like. The level of autoantibody is typically determined in a biological sample obtained from the individual, where suitable biological samples include, but are not limited to, serum, plasma, blood, cerebrospinal fluid, and the like.

[0029] In some embodiments, an effective amount of a TLR2 agonist is an amount that is effective to reduce the level of autoreactive T lymphocytes in an individual by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the level of autoreactive T lymphocytes in the absence of treatment with the TLR2 agonist. The level of autoreactive T lymphocytes in an individual is readily determined using any of a number of well-established assays, including, but not limited to, an *in vitro* cytotoxic T lymphocyte assay (e.g., where target cells displaying a self antigen on their surface are detectably labeled, e.g., with a radiolabel or a fluorescent label), and a biological sample obtained from the individual, which biological sample contains lymphocytes, is contacted with the target cells; killing of target cells is determined by detecting release of the detectable label); fluorescence activated cell sorting (FACS) analysis; and the like. In some embodiments, an effective amount of a TLR2 agonist decreases the level of autoantigen-specific cytotoxic T lymphocytes (CTL) in an individual. Whether an autoantigen-specific CTL response is decreased can be determined using any of a number of assays known in the art, including, but not limited to, measuring specific lysis by CTL of target cells expressing antigen on their surface, which target cells have incorporated a detectable label which is released from target cells upon lysis, and can be measured, using, e.g., a ^{51}Cr-release assay, a lanthanide fluorescence-based cytolysis assay, and the like.

[0030] For example, where a subject method is used to treat systemic lupus erythematosus (SLE) in an individual having SLE, an effective amount of a TLR2 agonist is an amount that is effective to reduce the frequency and/or level and/or severity of one or more of rash, joint pain, joint swelling, muscle ache, fatigue, fever, and headache by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the frequency and/or level and/or severity of the symptom in the absence of treatment with the TLR2 agonist.

[0031] Where a subject method is used to treat systemic lupus erythematosus (SLE) in an individual having SLE, an effective amount of a TLR2 agonist is an amount that is effective to reduce the level of serum autoantibody (e.g., anti-nuclear antibody, anti-double-stranded DNA antibody, anti-Sm, anti-histone antibody, anti-RNP antibody, etc.) by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the level of serum autoantibody in the absence of treatment with the TLR2

agonist. The level of serum autoantibody is measured using standard immunological assays (e.g., ELISA, RIA, etc.)

[0032] As another non-limiting example, where a subject method is used to treat multiple sclerosis (MS) in an individual having MS, an effective amount of a TLR2 agonist is an amount that is effective to reduce the frequency and/or level and/or severity of one or more of optic neuritis, diplopia, disarthria, spasticity, paresis, monoparesis, paraparesis, hemiparesis, quadraparesis, myoclonus, paraesthesia, ataxia, and vertigo by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the frequency and/or level and/or severity of the symptom in the absence of treatment with the TLR2 agonist.

[0033] As another non-limiting example, where a subject method is used to treat rheumatoid arthritis (RA) in an individual having RA, an effective amount of a TLR2 agonist is an amount that is effective to reduce the frequency and/or level and/or severity of joint swelling and/or joint pain by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the frequency and/or level and/or severity of the symptom in the absence of treatment with the TLR2 agonist.

[0034] Where a subject method is used to treat RA in an individual having RA, an effective amount of a TLR2 agonist is an amount that is effective to reduce the level of serum rheumatoid factor by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the level of serum rheumatoid factor in the absence of treatment with the TLR2 agonist. The level of rheumatoid factor is measured using standard immunological assays (e.g., enzyme-linked immunosorbent assay, radioimmunoassay, etc.).

[0035] Where a subject method is used to treat Type 1 diabetes mellitus (DM) in an individual having Type 1 DM, an effective amount of a TLR2 agonist is an amount that is effective to reduce the level of serum glucose following a meal (e.g., the serum glucose level 1 minute to 2 hours following a meal) by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the level of serum glucose in the absence of treatment with the TLR2 agonist. The level of serum glucose is measured using any standard method and/or device.

[0036] A subject method is useful for the treatment or amelioration of autoimmune disorders including, but not limited to, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, juvenile arthritis, lichen planus, Meniere's disease, mixed connective tissue disease, multiple sclerosis, Type 1 (immune-mediated) diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychrondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynauld's phenomenon, Reiter's syndrome, Rheumatoid arthritis, sarcoidosis, scleroderma, progressive systemic sclerosis, Sjogren's syndrome, Good pasture's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, takayasu arteritis, temporal arteristis/giant cell arteritis, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis.

[0037] Whether a given TLR2 agonist is effective in treating an autoimmune disorder can be determined using an experimental animal model of the disorder. For example, whether a TLR2 agonist is effective in treating multiple sclerosis (MS) can be determined by administering the TLR2 agonist to the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. The nonobese diabetic (NOD) mouse is a well-recognized animal model of spontaneous autoimmune insulin-dependent diabetes mellitus, and as such can be used to determine the effectiveness of a TLR2 agonist in treating Type 1 diabetes mellitus. Three common animal models of rheumatoid arthritis (RA) are the streptococcal cell wall, adjuvant, and collagen arthritis rat models, any of which can be used to determine the effectiveness of a TLR2 agonist in treating RA. NZB/NZW mice are an animal model of severe systemic lupus erythematosus (SLE), and can be used to determine the effectiveness of a TLR2 agonist in treating SLE. Animal models of various autoimmune diseases are known in the art. See, e.g., Burkhardt and Kalden (1997) *Rheumatol. Int.* 17:91-99; Dustin (2003) *Arthritis Res. Ther.* 5:165-171

(discussing animal models of RA); Infante and Kraig (1999) *Int. Rev. Immunol.* 18:83-109 (discussing animal models of myasthenia gravis).

[0038] Whether a given TLR2 agonist is effective in treating an autoimmune disorder can also be determined using any of a variety of well-established assays. For example, whether a given TLR2 agonist is effective to treat SLE can be determined by measuring an autoantibody level in an individual. Whether a given TLR2 agonist is effective to treat RA can be determined by measuring an RF level in the individual. Whether a given TLR2 agonist is effective to treat MS can be determined by conducting tests for vision, muscle function, cognition, and the like. Whether a given TLR2 agonist is effective to treat Type 1 diabetes can be determined by measuring blood glucose levels, e.g., postprandial blood glucose levels, in the individual.

M1 macrophage-mediated inflammatory disorders

[0039] The present invention provides methods of treating an M1 macrophage-mediated inflammatory disorder, the methods generally involving administering to an individual in need thereof an effective amount of a TLR2 agonist. In some embodiments, the methods further involve administering at least one additional therapeutic agent that treats an M1 macrophage-mediated inflammatory disorder.

[0040] In some embodiments, an effective amount of a TLR2 agonist is an amount that is effective to reduce the frequency and/or level and/or severity of at least one symptom of an M1 macrophage-mediated inflammatory disorder by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or at least about 60%, or more, compared to the frequency and/or level and/or severity of the symptom in the absence of treatment with the TLR2 agonist.

Allograft rejection

[0041] The present invention provides methods of reducing allorejection ("allograft rejection"). The methods generally involve administering a TLR2 agonist to an individual in need thereof. Individuals in need of treatment with a subject method include individuals who are the recipients of tissue graft, e.g., bone marrow transplantation recipients, organ transplant recipients, and the like. Rejection of any allogeneic transplanted cells, tissue, or organ is referred to herein as "allograft rejection." Allografts include cells, tissues, and organs from an individual of the same species as the recipient (e.g., a non-HLA matched individual; an HLA matched individuals), as well as cells, tissues, and organs from individuals of a different species from the recipient (where a graft from an individual of a different species is also referred to as a xenograft).

[0042] Allograft cells, tissues, and organs include, but are not limited to, cells such as pancreatic β-islet cells, bone marrow cells (and sub-populations of bone marrow cells), immature allogeneic or xenogeneic hematopoietic cells (including stem cells) which can be derived, for example, from bone marrow, mobilized peripheral blood (by for example leukapheresis), fetal liver, yolk sac and/or cord blood, myoblasts, renal epithelial cells, cardiac muscle cells, neuronal cells, etc.; tissues such as bone, skin, synovial tissue, heart valve, etc; and organs such as kidney, heart, liver, lung, etc.

[0043] In some embodiments, an effective amount of a TLR2 agonist reduces graft rejection by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more, compared to the graft rejection in the absence of treatment with the TLR2 agonist.

[0044] In some embodiments, an effective amount of a TLR2 agonist reduces tissue damage associated with graft rejection by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more, compared to the allograft-associated tissue damage in the absence of treatment with the TLR2 agonist.

[0045] In some embodiments, an effective amount of a TLR2 agonist reduces the risk of graft rejection by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more, compared to the risk of graft rejection in the absence of treatment with the TLR2 agonist.

[0046] Organ transplantation is used to treat acute or chronic organ failure, e.g, acute renal failure, chronic renal failure, heart failure, liver failure, etc. A subject method is therefore useful in reducing the risk of organ rejection following organ transplantation. Tissue transplantation is used to treat various disorders. For example, skin allografts are used to treat burn patients. Pancreatic tissues are used to treat Type 1 diabetes mellitus. Cell transplantation is used to treat various disorders. For example, pancreatic β islet cells are used to treat Type 1 diabetes mellitus. Neuronal cells are used to treat various neurological disorders.

[0047] Various disorders can be treated by transplanting immature allogeneic or xenogeneic hematopoietic cells (including stem cells) which can be derived, for example, from bone marrow, mobilized peripheral blood (by for example leukapheresis), fetal liver, yolk sac and/or cord blood of the donor and which are may be T-cell depleted $CD34^+$ immature hematopoietic cells, can be transplanted to a recipient suffering from a malignant disease. Diseases that can be treated with such allografted cells include, but are not limited to, leukemia such as acute lymphoblastic leukemia (ALL), acute nonlymphoblastic leukemia (ANLL), acute myelocytic

leukemia (AML) or chronic myelocytic leukemia (CML), and severe combined immunodeficiency syndromes (SCID), including adenosine deaminase (ADA), osteopetrosis, aplastic anemia, Gaucher's disease, thalassemia and other congenital or genetically-determined hematopoietic abnormalities.

TLR2 agonists

[0048] TLR2 agonists include, but are not limited to, bacterial or synthetic lipopetides, lipoproteins (including naturally-occurring lipoproteins; derivatives of naturally-occurring lipoproteins; synthetic lipoproteins); lipopeptides (Takeuchi et al. (2000) *J. Immunol.* 164:554-557), e.g., lipopeptides from *Mycobacteria tuberculosis*, *Borrelia burgdorferi*, *Treponema pallidum*, etc.; whole bacteria, e.g., heat-killed *Acholeplasma laidlawii*, heat-killed *Listeria monocytogenes* (Flo et al. (2000) *J. Immunol.* 164:2064-2069), and the like; lipoteichoic acids (Schwandner et al. (1999) *J. Biol. Chem.* 274:17406-17409); peptidoglycans (Takeuchi et al. (1999) *Immunity* 11:443-451), e.g., peptidoglycans from *Staphylococcus aureus*, etc.; mannuronic acids; *Neisseria* porins; bacterial fimbriae, Yersinia virulence factors, cytomegalovirus virions, measles haemagglutinin; yeast cell wall extracts; yeast particle zymosan; glycosyl phosphatidyl inositol (GPI) anchor from *Trypanosoma cruzi*; and the like.

[0049] Suitable TLR2 agonists include isolated, naturally-occurring TLR2 agonists; and synthetic TLR2 agonists. TLR2 agonists isolated from a naturally-occurring source of TLR2 agonist are generally purified, e.g., the purified TLR2 agonist is at least about 80% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, at least about 99% pure, or more than 99% pure. Synthetic TLR2 agonists are prepared by standard means, and are generally at least about 80% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, at least about 99% pure, or more than 99% pure.

[0050] Suitable TLR2 agonists include synthetic triacylated and diacylated lipopeptides. An exemplary, non-limiting TLR2 ligand is Pam_3Cys (tripalmitoyl-S-glyceryl cysteine) or S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-N- palmitoyl-(R)-cysteine, where "Pam_3" is "tripalmitoyl-*S*-glyceryl"). Aliprantis et al. (1999) *Science* 285:736-739. Derivatives of Pam_3Cys are also suitable TLR2 agonists, where derivatives include, but are not limited to, *S*-[2,3-bis(palmitoyloxy)-(2-*R*,*S*)-propyl]-*N*-palmitoyl-(*R*)-Cys-(*S*)-Ser-Lys_4-hydroxytrihydrochloride; Pam_3Cys-Ser-Ser-Asn-Ala; Pam_3Cys-Ser-$(Lys)_4$; Pam_3Cys-Ala-Gly; Pam_3Cys-Ser-Gly; Pam_3Cys-Ser; Pam_3Cys-OMe; Pam_3Cys-OH; PamCAG, palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ala-Gly-OH; and the like. Another non-limiting example of a suitable TLR2 agonist is PAM_2CSK_4. PAM_2CSK_4 (dipalmitoyl-S-glyceryl cysteine-serine-$(lysine)_4$; or Pam_2Cys-Ser-$(Lys)_4$) is a synthetic diacylated lipopeptide. Synthetic TLRs

agonists have been described in the literature. See, e.g., Kellner et al. (1992) *Biol Chem Hoppe Seyler* 373:1:51-5; Seifer et al. (1990) *Biochem. J.* 26:795–802; Lee et al. (2003) *Journal of Lipid Research* 44:479-486.

[0051] In some embodiments, a suitable TLR2 agonist is a selective TLR2 agonist, e.g., a TLR2 agonist selectively activates TLR2, but does not substantially activate any other Toll-like receptor, such as TLR1, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, or TLR10. In other embodiments, a suitable TLR2 agonist activates a TLR2, and may also activate one or more other Toll-like receptors. Such agonists are "relatively" selective, e.g., such agonists may activate two or more other TLR in addition to TLR2, but do not activate receptors other than TLR.

FORMULATIONS, DOSAGES, AND ROUTES OF ADMINISTRATION

Formulations

[0052] In general, an active agent (e.g., a TLR2 agonist) is prepared in a pharmaceutically acceptable composition for delivery to a host. The present invention provides compositions comprising a TLR2 agonist; and a pharmaceutically acceptable carrier or a pharmaceutically acceptable excipient. Pharmaceutically acceptable carriers and excipients suitable for use with a TLR2 agonist include sterile aqueous of non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/ aqueous solutions, emulsions or suspensions, and microparticles, 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. A composition comprising a TLR2 agonist may also be lyophilized using means well known in the art, for subsequent reconstitution and use according to the invention.

[0053] In general, the pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions comprising the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as

auxiliary agents. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.

[0054] A TLR2 agonist can be administered in the absence of agents or compounds that might facilitate uptake by target cells. A TLR2 agonist can be administered with compounds that facilitate uptake of such an agonist by target cells (*e.g.*, by macrophages, bronchial smooth muscle cells, airway epithelial cells, etc.) or otherwise enhance transport of a TLR2 agonist to a treatment site for action.

[0055] Absorption promoters, detergents and chemical irritants (e.g., keratinolytic agents) can enhance transmission of TLR2 agonist composition into a target tissue (*e.g.*, through the skin). For general principles regarding absorption promoters and detergents which have been used with success in mucosal delivery of organic and peptide-based drugs, see, *e.g.*, Chien, *Novel Drug Delivery Systems,* Ch. 4 (Marcel Dekker, 1992). Examples of suitable nasal absorption promoters in particular are set forth at Chien, *supra* at Ch. 5, Tables 2 and 3; milder agents are preferred. Suitable agents for use in the method of this invention for mucosal/nasal delivery are also described in Chang, *et al., Nasal Drug Delivery,* "Treatise on Controlled Drug Delivery", Ch. 9 and Tables 3-4B thereof, (Marcel Dekker, 1992). Suitable agents which are known to enhance absorption of drugs through skin are described in Sloan, Use of Solubility Parameters from Regular Solution Theory to Describe Partitioning-Driven Processes, Ch. 5, "Prodrugs: Topical and Ocular Drug Delivery" (Marcel Dekker, 1992), and at places elsewhere in the text. All of these references are incorporated herein for the sole purpose of illustrating the level of knowledge and skill in the art concerning drug delivery techniques.

[0056] A colloidal dispersion system may be used for targeted delivery of TLR2 agonist to specific tissue. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

[0057] Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo.* It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 Fm can encapsulate a substantial percentage of an aqueous buffer comprising large macromolecules. A TLR2 agonist can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al.,* (1981) *Trends Biochem. Sci.,* 6:77). The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine. Exemplary liposome compositions suitable for use in a subject method are described in Louria-Hayon et al. (2002) *Vaccine* 20:3342.

[0058] Where desired, targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

[0059] The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various well known linking groups can be used for joining the lipid chains to the targeting ligand (see, e.g., Yanagawa, *et al.,* (1988) *Nuc. Acids Symp. Ser.,* 19:189; Grabarek, *et al.,* (1990) *Anal. Biochem.,* 185:131; Staros, *et al.,* (1986) *Anal. Biochem.* 156:220 and Boujrad, *et al.,* (1993) *Proc. Natl. Acad. Sci. USA,* 90:5728). Targeted delivery of a TLR2 agonist can also be achieved by conjugation of the TLR2 agonist to the surface of viral and non-viral recombinant expression vectors, to an antigen or other ligand, to a monoclonal antibody or to any molecule which has the desired binding specificity.

Routes of administration

[0060] A TLR2 agonist is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic, mucosal, and localized routes of administration.

[0061] Conventional and pharmaceutically acceptable routes of administration include inhalational routes, intranasal, intramuscular, intratracheal, subcutaneous, intradermal, topical

application, intravenous, rectal, nasal, oral, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the TLR2 agonist and/or the desired effect on the autoimmune disorder being treated. The TLR2 agonist composition can be administered in a single dose or in multiple doses, and may encompass administration of booster doses, to elicit and/or maintain the desired effect.

[0062] A TLR2 agonist can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes. In some embodiments, administration is to the respiratory tract. Inhalational routes may be suitable for treatment of certain autoimmune disorders.

[0063] The route of administration depends, in part, on the nature of the autoimmune disorder, the severity of the disease, etc. In the treatment of certain autoimmune disorders, local administration at or near the site of a lesion or other pathological manifestation of the disorder will be carried out. Systemic routes of administration, e.g., oral, subcutaneous, intramuscular, and intravenous routes of administration are suitable for the treatment of many autoimmune disorders with a subject method.

[0064] Inhalational routes of administration (*e.g.*, intranasal, intrapulmonary, and the like) may be particularly useful in some instances. Such means include inhalation of aerosol suspensions or insufflation of a TLR2 agonist composition. Nebulizer devices, metered dose inhalers, and the like suitable for delivery of polynucleotide compositions to the nasal mucosa, trachea and bronchioli are well-known in the art and will therefore not be described in detail here. For general review in regard to intranasal drug delivery, see, *e.g.*, Chien, *Novel Drug Delivery Systems,* Ch. 5 (Marcel Dekker, 1992).

[0065] Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intraspinal, intrasternal, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of a TLR2 agonist.

[0066] Systemic administration typically involves intravenous, intradermal, subcutaneous, oral, or intramuscular administration or systemically absorbed topical or mucosal administration of pharmaceutical preparations. Mucosal administration includes administration to the respiratory tissue, e.g., by inhalation, nasal drops, and the like.

[0067] A TLR2 agonist can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (*e.g.*, using a suppository) delivery.

[0068] Methods of administration of a TLR2 agonist through the skin or mucosa include, but are not necessarily limited to, topical application of a suitable pharmaceutical preparation, transdermal transmission, injection and epidermal administration. For transdermal transmission, absorption promoters or iontophoresis are suitable methods. For review regarding such methods, those of ordinary skill in the art may wish to consult Chien, *supra* at Ch. 7. Iontophoretic transmission may be accomplished using commercially available "patches" which deliver their product continuously via electric pulses through unbroken skin for periods of several days or more. An exemplary patch product for use in this method is the LECTRO PATCH™ (manufactured by General Medical Company, Los Angeles, CA) which electronically maintains reservoir electrodes at neutral pH and can be adapted to provide dosages of differing concentrations, to dose continuously and/or to dose periodically.

Formulations suitable for inhalation

[0069] Delivery of a TLR2 agonist is, in some embodiments, via insufflation of an flowable formulation comprising the TLR2 agonist, where the flowable formulation is one that is suitable for delivery by inhalation, e.g., an aerosolized formulation. The present invention thus provides compositions comprising a TLR2 agonist and a formulation suitable for delivery by inhalation, e.g., an aerosolized formulation or other flowable formulation suitable for delivery by inhalation. As used herein, the term "aerosol" is used in its conventional sense as referring to very fine liquid or solid particles carries by a propellant gas under pressure to a site of therapeutic application. The term "liquid formulation for delivery to respiratory tissue" and the like, as used herein, describe compositions comprising a TLR2 agonist with a pharmaceutically acceptable carrier in flowable liquid form. Such formulations, when used for delivery to a respiratory tissue, are generally solutions, e.g. aqueous solutions, ethanolic solutions, aqueous/ethanolic solutions, saline solutions and colloidal suspensions.

[0070] In general, aerosolized particles for respiratory delivery must have a diameter of 12 microns or less. Typically, the particle size varies with the site targeted (e.g, delivery targeted to the bronchi, bronchia, bronchioles, alveoli, or circulatory system). For example, topical lung treatment can be accomplished with particles having a diameter in the range of 1.0 to 12.0 microns. Effective systemic treatment requires particles having a smaller diameter, generally in the range of 0.5 to 6.0 microns. Thus, in some embodiments, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about

90%, or more, of an aerosolized formulation comprising a TLR2 agonist for delivery to a respiratory tissue is composed of particles in the range of from about 0.5 to about 12 micrometers, from about 0.5 to about 6 micrometers, or from about 1.0 to about 12 micrometers.

[0071] The formulation for delivery to a respiratory tissue may be provided in a container suitable for delivery of aerosolized formulations. Thus, the present invention provides a container suitable for delivery of an aerosolized formulation, the container comprising a subject formulation comprising a TLR2 agonist and a formulation suitable for delivery by inhalation. U.S. Patents 5,544,646; 5,709,202; 5,497,763; 5,544,646; 5,718,222; 5,660,166; 5,823,178; 5,829,435; and 5,906,202 describe devices and methods useful in the generation of aerosols suitable for drug delivery, any of which can be used in the present invention for delivering a formulation comprising a TLR2 agonist to a respiratory tissue.

[0072] In some embodiments, the invention provides a container, which may be a disposable container, having at least one wall that is collapsible or movable upon application of a force, wherein at least one wall has an opening. A porous membrane having pores in a range of from about 0.25 microns (micrometers) to about 6 microns covers the opening. The container comprises a flowable liquid formulation comprising a TLR2 agonist. Upon application of a force, the flowable liquid formulation is forced through the pores in the membrane and is aerosolized. The container may be provided in any known configuration, e.g., a blister pack. The container may be provided together with an aerosol delivery device, such that the aerosolized formulation exits the container and proceeds through a channel in an aerosol delivery device and into the respiratory tract of an individual.

[0073] When a pharmaceutical aerosol is employed in this invention, the aerosol contains a TLR2 agonist, which can be dissolved, suspended, or emulsified in a mixture of a fluid carrier and a propellant. The aerosol can be in the form of a solution, suspension, emulsion, powder, or semi-solid preparation. Aerosols employed in the present invention are intended for administration as fine, solid particles or as liquid mists via the respiratory tract of a patient. Various types of propellants known to one of skill in the art can be utilized. Examples of suitable propellants include, but are not limited to, hydrocarbons or other suitable gas. In the case of the pressurized aerosol, the dosage unit may be determined by providing a value to deliver a metered amount.

[0074] Administration of formulation comprising a TLR2 agonist can also be carried out with a nebulizer, which is an instrument that generates very fine liquid particles of substantially uniform size in a gas. For example, a liquid containing a TLR2 agonist is dispersed as

droplets. The small droplets can be carried by a current of air through an outlet tube of the nebulizer. The resulting mist penetrates into the respiratory tract of the patient.

[0075] A powder composition containing a TLR2 agonist, with or without a lubricant, carrier, or propellant, can be administered to a mammal in need of therapy. This embodiment of the invention can be carried out with a conventional device for administering a powder pharmaceutical composition by inhalation. For example, a powder mixture of the compound and a suitable powder base such as lactose or starch may be presented in unit dosage form in for example capsular or cartridges, *e.g.* gelatin, or blister packs, from which the powder may be administered with the aid of an inhaler.

[0076] The present invention is intended to encompass the free acids, free bases, salts, amines and various hydrate forms including semi-hydrate forms of such respiratory drugs and is particularly directed towards pharmaceutically acceptable formulations of such drugs which are formulated in combination with pharmaceutically acceptable excipient materials generally known to those skilled in the art—in some embodiments without other additives such as preservatives. In some embodiments, drug formulations do not include additional components which have a significant effect on the overall formulation such as preservatives. Thus certain formulations consist essentially of pharmaceutically active drug and a pharmaceutically acceptable carrier (e.g., water and/or ethanol). However, if a drug is liquid without an excipient the formulation may consist essentially of the drug which has a sufficiently low viscosity that it can be aerosolized using a dispenser.

[0077] Administration by inhalation will be carried out in some embodiments of the invention, because smaller doses can be delivered locally to the specific cells (e.g., cells of respiratory tissue, bronchial smooth muscle cells, airway epithelial cells, airway macrophages, etc.) which are most in need of treatment. By delivering smaller doses, any adverse side effects are eliminated or substantially reduced. By delivering directly to the cells which are most in need of treatment, the effect of the treatment will be realized more quickly.

[0078] There are several different types of inhalation methodologies which can be employed in connection with the present invention. A TLR2 agonist can be formulated in basically three different types of formulations for inhalation. First, a TLR2 agonist can be formulated with low boiling point propellants. Such formulations are generally administered by conventional meter dose inhalers (MDI's). However, conventional MDI's can be modified so as to increase the ability to obtain repeatable dosing by utilizing technology which measures the inspiratory volume and flow rate of the patient as discussed within U.S. Patents 5,404,871 and 5,542,410.

[0079] Alternatively, a TLR2 agonist can be formulated in aqueous or ethanolic solutions and delivered by conventional nebulizers. In many instances, such solution formulations are aerosolized using devices and systems such as disclosed within U.S. Patent 5,497,763; 5,544,646; 5,718,222; and 5,660,166.

[0080] In addition, a TLR2 agonist can be formulated into dry powder formulations. Such formulations can be administered by simply inhaling the dry powder formulation after creating an aerosol mist of the powder. Technology for carrying such out is described within U.S. Patent 5,775,320 and U.S. Patent 5,740,794.

[0081] With respect to each of the patents recited above, applicants point out that these patents cite other publications in intrapulmonary drug delivery and such publications can be referred to for specific methodology, devices and formulations which could be used in connection with the delivery of a TLR2 agonist. Further, each of the patents are incorporated herein by reference in their entirety for purposes of disclosing formulations, devices, packaging and methodology for the delivery of TLR2 agonist formulations.

Dosages

[0082] Although the dosage used will vary depending on the clinical goals to be achieved, a suitable dose range is one which provides up to about 1 μg to about 1,000 μg, from about 1,000 μg to about 10,000 μg, from about 1 mg to about 500 mg, or from about 10 mg to about 100 mg of a TLR2 agonist can be administered in a single dose. Alternatively, a target dose of a TLR2 agonist can be considered to be about 1-10 μM in a sample of host blood drawn within the first 24-48 hours after administration of a TLR2 agonist.

[0083] The therapeutic activity of a TLR2 agonist is generally dose-dependent. Therefore, to increase a TLR2 agonist's potency by a magnitude of two, each single dose is doubled in concentration. Increased dosages may be needed to achieve the desired therapeutic goal. The invention thus contemplates administration of multiple doses.

[0084] In many embodiments, multiple doses of a TLR2 agonist are administered. For example, a TLR2 agonist is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (bid), or three times a day (tid), substantially continuously, or continuously, over a period of time ranging from about one day to about one week, from about two weeks to about four weeks, from about one month to about two months, from about two months to about four months, from about four months to about six months, from about six

months to about eight months, from about eight months to about 1 year, from about 1 year to about 2 years, or from about 2 years to about 4 years, or more.

[0085] In some embodiments, e.g., where the autoimmune disorder is characterized by intermittent flare-ups or other episode or appearance of a symptom of an autoimmune disorder (e.g., appearance of a rash; occurrence of a seizure; occurrence of slurred speech; occurrence of lack of muscle tone and/or coordination; occurrence of joint pain; occurrence of joint swelling; etc.), a TLR2 agonist is administered immediately following a flare-up or other episode or appearance of symptoms, e.g., within 2 hours after the appearance of the symptom, e.g., from about 1 minute to about 2 hours after appearance of the symptom. In other embodiments, a TLR2 agonist is administered as needed to reduce the frequency and/or severity of a symptom associated with an autoimmune disorder, e.g., a TLR2 agonist is administered within about 1 minute to about 30 minutes following an episode or appearance of a symptom. In other embodiments, a TLR2 agonist is administered continuously.

[0086] In some embodiments, e.g., where the autoimmune disorder is Type 1 diabetes mellitus, a TLR2 agonist is administered following a meal, e.g., within 2 hours after a meal, e.g., from about 1 minute to about 2 hours after a meal. In other embodiments, TLR2 agonist is administered before a meal, e.g., from about 1 minute to about 30 minutes before a meal. In other embodiments, a TLR2 agonist is administered as needed to lower blood glucose levels, e.g., a TLR2 agonist is administered within about 1 minute to about 30 minutes following a blood glucose measurement that indicates that the blood glucose level exceeds the normal range. In other embodiments, a TLR2 agonist is administered continuously.

[0087] In some embodiments, e.g., where the disorder being treated is an allograft rejection, a TLR2 agonist is administered to an allograft recipient after the individual has received the allograft, e.g., within 2 hours after the allograft has been introduced into the individual, e.g., from about 1 minute to about 2 hours after the allograft has been introduced into the individual. In other embodiments, a TLR2 agonist is administered on an ongoing basis following introduction of the allograft into the individual e.g., a TLR2 agonist is administered daily for a period of from about 1 week to several years following introduction of the allograft into the individual. In other embodiments, a TLR2 agonist is administered continuously.

Combination therapies

[0088] In some embodiments, a TLR2s agonist is administered in combination therapy with one or more additional therapeutic agents that are used to treat an autoimmune disorder. Suitable additional therapeutic agents include, but are not limited to, immunomodulatory

agents, immunosuppressive agents, antimalarial agents, non-steroidal anti-inflammatory agents (NSAIDs), corticosteroids, agents that reduce serum glucose levels, and the like.

[0089] In some embodiments, a TLR2s agonist is administered in combination therapy with one or more additional therapeutic agents that are used to treat, prevent, or reduce the risk of allograft rejection. Suitable additional therapeutic agents include immunosuppressive agents; an antibody specific for an IL-2 receptor; and the like.

[0090] In some embodiments, at least one additional therapeutic agent is administered during the entire course of treatment with the TLR2 agonist. In other embodiments, the at least one additional therapeutic agent is administered for a period of time that is overlapping with the course of treatment with the TLR2 agonist, e.g., the at least one additional therapeutic agent treatment can begin before the treatment with the TLR2 agonist begins and end before treatment with the TLR2 agonist ends; the at least one additional therapeutic agent treatment can begin after the treatment with the TLR2 agonist begins and end after the treatment with the TLR2 agonist ends; the at least one additional therapeutic agent treatment can begin after the treatment with the TLR2 agonist begins and end before the treatment with the TLR2 agonist ends; or the at least one additional therapeutic agent treatment can begin before the treatment with the TLR2 agonist begins and end after the treatment with the TLR2 agonist ends.

NSAIDs

[0091] Suitable NSAIDs include, but are not limited to, acetylsalicylic acid, ibuprofen, diclofenac (Voltaren™), etodolac (Lodine™), fenoprofen (Nalfon™), indomethacin (Indocin™), ketoralac (Toradol™), oxaprozin (Daypro™), nabumentone (Relafen™), sulindac (Clinoml™), tolmentin (Tolectin™), naproxen (Aleve™, Naprosyn™), ketoprofen (Actron™), cyclooxygenase (cox) inhibitors, selective cyclooxygenase-2 (cox-2) inhibitors (e.g., celecoxib (Celebrex™), rofecoxib (Vioxx™), valdecoxib (Bextra™), and the like.

Corticosteroids

[0092] Suitable corticosteroids include, but are not limited to, prednisolone, dexamethasone (Decadron™), methylprednisolone (Medrol®; SoluMedrol®), corticotropin (Acthar®), cortisone, hydrocortisone (Hydrocortone®), prednisone (Deltasone®; Orasone®), triamcinolone, and the like.

Antimalarial agents

[0093] Suitable antimalarial agents include, but are not limited to, Plaquenil (hydroxychloroquine), Aralen (chloroquine), Atabrine (quinacrine), and the like.

Immunosuppressants

[0094] Suitable immunosuppressive agents include, but are not limited to, Imuran (azathioprine), Cytoxan (cyclophosphamide), cyclosporine (Sandimmune®), rapamycin (sirolimus; Rapamune), tacrolimus (FK506), mycophenolate mofetil (CellCept®), 6-mercaptopurine, 15-deoxyspergualin, mizoribine, chlorambucil (Leukeran®), and the like.

Antineoplastic agents

[0095] Suitable antineoplastic agents include, but are not limited to, Novantrone® (mitoxantrone HCl), methotrexate (Rheumatrex), brequinar sodium (DuP 785, NSC 368390), Arava® (leflunomide), and the like.

Immunomodulators

[0096] Suitable immunomodulatory agents include, but are not limited to, Betaseron® (Interferon-β1b), Avonex® (Interferon-β1a), Rebif® (Interferon-β1a), and the like. Suitable immunomodulators further include T cell receptor modulators including, but not limited to, anti-T cell receptor antibodies (e.g., anti-CD4 antibodies (e.g., cM-T412 (Boeringer), IDEC-CE9.1™ (IDEC and SKB), mAB 4162W94, Orthoclone and OKTcdr4a (Janssen-Cilag)), anti-CD3 antibodies (e.g., Nuvion (Product Design Labs), OKT3 (Johnson & Johnson), or Rituxan (IDEC)), anti-CD5 antibodies (e.g., an anti-CD5 ricin-linked immunoconjugate), anti-CD7 antibodies (e.g., CHH-380 (Novartis)), anti-CD8 antibodies, anti-CD40 ligand monoclonal antibodies (e.g., IDEC-131 (IDEC)), anti-CD52 antibodies (e.g., CAMPATH 1H (Ilex)), anti-CD2 antibodies, anti-CD11a antibodies (e.g., Xanelim (Genentech)), and anti-B7 antibodies (e.g., IDEC-114) (IDEC))) and CTLA4-immunoglobulin. Also suitable for use as a T cell receptor modulator is a CD2 antagonist. Also suitable for use as a T cell receptor modulator is a CD2 binding molecule, e.g., MEDI-507.

[0097] Also suitable for use in a subject combination therapy are tumor necrosis factor-α (TNF-α) antagonists (also referred to herein as "TNF antagonists"). Suitable TNF antagonists include, but are not limited to, antibodies to TNF-α, soluble TNF receptor (TNFR), and the like.

[0098] The terms "TNF receptor polypeptide" and "TNFR polypeptide" refer to polypeptides derived from TNFR (from any species) which are capable of binding TNF. Two distinct cell-surface TNFRs have described: Type II TNFR (or p75 TNFR or TNFRII) and Type I TNFR (or p55 TNFR or TNFRI). The mature full-length human p75 TNFR is a glycoprotein having a molecular weight of about 75-80 kilodaltons (kD). The mature full-length human p55 TNFR is a glycoprotein having a molecular weight of about 55-60 kD. Exemplary TNFR polypeptides are derived from TNFR Type I and/or TNFR type II. Soluble TNFR includes p75 TNFR

polypeptide; fusions of p75 TNFR with heterologous fusion partners, e.g., the Fc portion of an immunoglobulin.

[0099] TNFR polypeptide may be an intact TNFR or a suitable fragment of TNFR. U.S. Pat. No. 5,605,690 provides examples of TNFR polypeptides, including soluble TNFR polypeptides, appropriate for use in the present invention. In many embodiments, the TNFR polypeptide comprises an extracellular domain of TNFR. In some embodiments, the TNFR polypeptide is a fusion polypeptide comprising an extracellular domain of TNFR linked to a constant domain of an immunoglobulin molecule. In other embodiments, the TNFR polypeptide is a fusion polypeptide comprising an extracellular domain of the p75 TNFR linked to a constant domain of an IgG1 molecule. In some embodiments, when administration to humans is contemplated, an Ig used for fusion proteins is human, e.g., human IgG1.

[00100] Monovalent and multivalent forms of TNFR polypeptides may be used in the present invention. Multivalent forms of TNFR polypeptides possess more than one TNF binding site. In some embodiments, the TNFR is a bivalent, or dimeric, form of TNFR. For example, as described in U.S. Pat. No. 5,605,690 and in Mohler et al., 1993, J. Immunol., 151:1548-1561, a chimeric antibody polypeptide with TNFR extracellular domains substituted for the variable domains of either or both of the immunoglobulin heavy or light chains would provide a TNFR polypeptide for the present invention. Generally, when such a chimeric TNFR:antibody polypeptide is produced by cells, it forms a bivalent molecule through disulfide linkages between the immunoglobulin domains. Such a chimeric TNFR:antibody polypeptide is referred to as TNFR:Fc.

[00101] One non-limiting example of a suitable TNF antagonist is the soluble TNFR ENBREL® etanercept. ENBREL® is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) TNFR linked to the Fc portion of human IgG1. The Fc component of ENBREL® contains the CH2 domain, the CH3 domain and hinge region, but not the CH1 domain of IgG1. ENBREL® is produced in a Chinese hamster ovary (CHO) mammalian cell expression system. It consists of 934 amino acids and has an apparent molecular weight of approximately 150 kilodaltons. Smith et al. (1990) *Science* 248:1019-1023; Mohler et al. (1993) *J. Immunol.* 151:1548-1561; U.S. Pat. No. 5,395,760; and U.S. Pat. No. 5,605,690.

[00102] Also suitable for use are monoclonal antibodies that bind TNF-α. Monoclonal antibodies include "humanized" mouse monoclonal antibodies; chimeric antibodies; monoclonal antibodies that are at least about 80%, at least about 90%, at least about 95%, or 100% human in amino acid sequence; and the like. See, e.g., WO 90/10077; WO 90/04036;

and WO 92/02190. Suitable monoclonal antibodies include antibody fragments, such as Fv, $F(ab')_2$ and Fab; synthetic antibodies; artificial antibodies; phage display antibodies; and the like.

[00103] Examples of suitable monoclonal antibodies include infliximab (REMICADE®, Centocor); and adalimumab (HUMIRA™, Abbott) REMICADE® is a chimeric monoclonal anti-TNF-α antibody that includes about 25% mouse amino acid sequence and about 75% human amino acid sequence. REMICADE® comprises a variable region of a mouse monoclonal anti-TNF-α antibody fused to the constant region of a human IgG1. Elliott et al. (1993) *Arthritis Rheum.* 36:1681-1690; Elliott et al. (1994) *Lancet* 344:1105-1110; Baert et al. (1999) *Gastroenterology* 116:22-28. HUMIRA™ is a human, full-length IgG1 monoclonal antibody that was identified using phage display technology. Piascik (2003) *J. Am. Pharm. Assoc.* 43:327-328.

[00104] Antibodies that interfere with or block the interactions necessary for the activation of B cells by TH (T helper) cells, and thus block the production of neutralizing antibodies, are also useful as immunomodulatory agents in a subject combination therapy. Examples of such agents include antibody to CD40 ligand (anti-CD40L) (Bristol-Myers Squibb Co; see, e.g., European patent application 555,880); a soluble CD40 molecule; rituximab (anti-CD20 antibody); and the like.

Agents for treating Type 1 diabetes mellitus

[00105] Agents for treating Type 1 diabetes mellitus that are suitable for use in a subject combination therapy include any form of insulin, as long as the insulin is biologically active, i.e., the insulin is effective in reducing blood glucose levels in an individual who is responsive to insulin. In some embodiments, recombinant human insulin ("regular" insulin) or a recombinant human insulin analog is used. In a particular embodiment, the insulin analog is a monomeric form of insulin, *e.g.,* human lispro. In some instances, other forms of insulin are used alone or in combination with recombination human insulin or each other. Insulin that is suitable for use herein includes, but is not limited to, regular insulin (Humulin R, Novlin R, etc.), semilente, NPH (isophane insulin suspension; Humulin N, Novolin N, Novolin N PenFill, NPH Ilentin II, NPH-N), lente (insulin zinc suspension; Humulin-L, Lente Ilentin II, Lent L, Novolin L), protamine zinc insulin (PZI), ultralente (insulin zinc suspension, extended; Humulin U Ultralente), insuline glargine (Lantus), insulin aspart (Novolog), acylated insulin, monomeric insulin, superactive insulin, hepatoselective insulin, lispro (Humalog™), and any other insulin analog or derivative, and mixtures of any of the foregoing. Commonly used mixtures include mixtures NPH and regular insulin containing the following percentages of

NPH and regular insulin: 70%/30%, 50%/50%, 90%/10%, 80%/20%, 60%/40%, and the like. Insulin that is suitable for use herein includes, but is not limited to, the insulin forms disclosed in U.S. Patent Nos. 4,992,417; 4,992,418; 5,474,978; 5,514,646; 5,504,188; 5,547,929; 5,650,486; 5,693,609; 5,700,662; 5,747,642; 5,922,675; 5,952,297; and 6,034,054; and published PCT applications WO 00/121197; WO 09/010645; and WO 90/12814. Insulin analogs include, but are not limited to, superactive insulin analogs, monomeric insulins, and hepatospecific insulin analogs.

[00106] Superactive insulin analogs have increased activity over natural human insulin. Accordingly, such insulin can be administered in substantially smaller amounts while obtaining substantially the same effect with respect to reducing serum glucose levels. Superactive insulin analogs include, e.g., 10-Aspartic Acid-B human insulin; des-pentapeptide (B26-B30)→Asp^{B10}, Tyr^{B25} –α-carboxamide human insulin; (B26-B30)→glu^{B10}, Tyr^{B25}-α-carboxamide human insulin; destripeptide B28-30 insulin; an insulin with γ-aminobutyric acid substituted for A13Leu-A14Tyr; and further insulin analogs of the formula des(B26-B30)→X^{B10}, Tyr^{B25} –α-carboxamide human insulin, in which X is a residue substituted at position 10 of the B chain. These insulin analogs have potencies anywhere from 11 to 20 times that of natural human insulin. All of the above-described insulin analogs involve amino acid substitutions along the A or B chains of natural human insulin, which increase the potency of the compound or change other properties of the compound. Monomeric insulin includes, but is not limited to, lispro.

[00107] Insulin derivatives include, but are not limited to, acylated insulin, glycosylated insulin, and the like. Examples of acylated insulin include those disclosed in U.S. Patent No. 5,922,675, e.g., insulin derivatized with a C_6-C_{21} fatty acid (e.g., myristic, pentadecylic, palmitic, heptadecylic, or stearic acid) at an α- or ε-amino acid of glycine, phenylalanine, or lysine.

Additional therapeutic agents

[00108] Other suitable agents include Copaxone® (Glatiramer acetate); Azulfidine® (sulfasalazine); an antibody specific for an IL-2 receptor (e.g., Basiliximab, Daclizumab, and the like); etc.

Side effect management agents; palliative agents

[00109] In some embodiments, a subject therapy further comprises administering a palliative agent (e.g., an agent that reduces patient discomfort caused by a therapeutic agent, or patient discomfort caused by the disorder), or other agent for the avoidance, treatment, or reduction of

a side effect of a therapeutic agent. Such agents are also referred to as "side effect management agents" or "palliative agents."

[00110] Analgesics that can be used to alleviate pain in the methods of the invention include non-narcotic analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) acetaminophen, salicylate, acetyl-salicylic acid (aspirin, diflunisal), ibuprofen, Motrin, Naprosyn, Nalfon, and Trilisate, indomethacin, glucametacine, acemetacin, sulindac, naproxen, piroxicam, diclofenac, benoxaprofen, ketoprofen, oxaprozin, etodolac, ketorolac tromethamine, ketorolac, nabumetone, and the like, and mixtures of two or more of the foregoing. Also suitable for use are pain relievers such as Tegretol® (carbamazepine), Neurontin® (Gabapentin), Dilantin® (Phenytoin), Elavil® (Amitriptyline), and the like.

[00111] Agents that can be used to treat fatigue associated with certain autoimmune disorders include, but are notlimited to, Symmetrel® (amantadine), Cylert® (pemoline), Provigil® (modafinil), and the like.

[00112] Agents that can be used to treat depression associated with certain autoimmune disorders include, but are not limited to, Zoloft® (sertaline), Effexor® (venlafaxine), Paxil® (paroxetine), Luvox® (fluvoxamine), Celexa® (citalopram), Serzone® (nefazodone), and the like.

[00113] Agents that reduce gastrointestinal discomfort such as nausea, diarrhea, gastrointestinal cramping, and the like are suitable palliative agents for use in a subject therapy. Suitable agents include, but are not limited to, antiemetics, anti-diarrheal agents, H2 blockers, antacids, and the like. Agents that are used to treat constipation include Metamucil® (psyllium), Dulcolax® (biscodyl), Colace® (docusate), and the like.

[00114] Suitable H2 blockers (histamine type 2 receptor antagonists) that are suitable for use as a palliative agent in a subject therapy include, but are not limited to, Cimetidine (e.g., Tagamet, Peptol, Nu-cimet, apo-cimetidine, non-cimetidine); Ranitidine (e.g., Zantac, Nu-ranit, Novo-randine, and apo-ranitidine); and Famotidine (Pepcid, Apo-Famotidine, and Novo-Famotidine).

[00115] Suitable antacids include, but are not limited to, aluminum and magnesium hydroxide (Maalox®, Mylanta®); aluminum carbonate gel (Basajel®); aluminum hydroxide (Amphojel®, AlternaGEL®); calcium carbonate (Tums®, Titralac®); magnesium hydroxide; and sodium bicarbonate.

[00116] Anti-diarrheal agents include, but are not limited to, Rolgamidine, Diphenoxylate hydrochloride (Lomotil), Metronidazole (Flagyl), Methylprednisolone (Medrol), Sulfasalazine (Azulfidine), and the like.

Combination regimens

[00117] The instant invention provides methods for treating an autoimmune disease, generally involving administering combined effective amounts of a TLR2 agonist and a second therapeutic agent for a desired treatment duration. The following examples are provided for the purposes of illustration only, and are not meant to be limiting.

[00118] In some embodiments, the instant invention provides a method for treating SLE in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Plaquenil® (hydroxychloroquine), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Plaquenil® containing an amount of 400 mg orally once every 7 days for the desired treatment duration.

[00119] In some embodiments, the instant invention provides a method for treating SLE in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Rheumatrex® (methotrexate), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Rheumatrex® containing an amount of from about 2.5 mg to about 10 mg orally once per week for the desired treatment duration.

[00120] In some embodiments, the instant invention provides a method for treating SLE in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and oral prednisone, the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of oral prednisone containing an amount of from about 0.5 mg/kg to about 1.5 mg/kg orally daily for the desired treatment duration.

[00121] In some embodiments, the instant invention provides a method for treating SLE in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Imuran® (azathioprine), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Imuran® containing an amount of from about 2 mg/kg to about 3 mg/kg orally daily for the desired treatment duration.

[00122] In some embodiments, the instant invention provides a method for treating SLE in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Cytoxan® (cyclophosphamide), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of

Cytoxan® containing an amount of from about 1 mg/kg to about 3 mg/kg orally daily for the desired treatment duration.

[00123] In some embodiments, the instant invention provides a method for treating SLE in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Sandimmune® (cyclosporine), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Sandimmune® containing an amount of from about 2.5 mg/kg to about 19 mg/kg orally daily for the desired treatment duration.

[00124] In some embodiments, the instant invention provides a method for treating RA in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Arava® (leflunomide), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Arava® containing an amount of about 100mg orally daily for the first three days, followed by 10 mg or 20 mg orally daily for the desired treatment duration.

[00125] In some embodiments, the instant invention provides a method for treating RA in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Azulfidine® (sulfasalazine), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Azulfidine® containing an amount of from about 500 mg to about 2000 mg orally every 6 hours or every 12 hours for the desired treatment duration.

[00126] In some embodiments, the instant invention provides a method for treating RA in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Rheumatrex® (methotrexate), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Rheumatrex® containing an amount of from about 2.5 mg to about 5 mg every twelve hours for three doses once a week, or 7.5 mg once a week orally every 6 hours or every 12 hours, for the desired treatment duration.

[00127] In some embodiments, the instant invention provides a method for treating MS in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Betaseron® (IFN-β1b), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Betaseron® containing an amount of 0.25 mg administered subcutaneously tiw, qd, or qod for the desired treatment duration.

[00128] In some embodiments, the instant invention provides a method for treating MS in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Avonex® (IFN-β1a), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Avonex® containing an amount of 30 μg administered intramuscularly once per week for the desired treatment duration.

[00129] In some embodiments, the instant invention provides a method for treating MS in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Copaxone® (glatiramer acetate), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Copaxone® containing an amount of 20 mg administered subcutaneously qd for the desired treatment duration.

[00130] In some embodiments, the instant invention provides a method for treating MS in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Rebif® (IFN-β1a), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Rebif® containing an amount of 44 μg administered subcutaneously tiw for the desired treatment duration.

[00131] In some embodiments, the instant invention provides a method for treating MS in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and Novantrone® (mitoxantrone HCl), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of Novantrone® containing an amount of 12 mg/m^2 administered by a short (e.g., 5 minutes to 15 minutes) intravenous infusion once every 3 months for the desired treatment duration.

[00132] In some embodiments, the instant invention provides a method for treating myasthenia gravis in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and CellCept® (mycophenolate mofetil), the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of CellCept® containing an amount of 1 g orally for the desired treatment duration.

[00133] In some embodiments, the instant invention provides a method for treating Type 1 diabetes mellitus in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and an insulin, the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of an

insulin containing an amount of about 0.5 units/kg/day to about 1 unit/kg/day subcutaneously for the desired treatment duration.

[00134] In some embodiments, the instant invention provides a method for treating allograft rejection in an individual by administering to an individual in need thereof an effective amount of a TLR2 agonist and an immunosuppressive agent, the method comprising administering a dosage of a TLR2 agonist containing an amount of from about 1 mg to about 500 mg; and a dosage of an immunosuppressive agent containing an amount of about 2 mg to about 5 mg daily for the desired treatment duration, where the immunosuppressive agent is selected from cyclosporine, rapamycin, azathioprine, mycophenolate mofetil, and tacrolimus. In some embodiments, the method further comprises administering an effective amount of Basiliximab and/or Daclizumab.

[00135] Any of the above-described therapeutic regimens can be modified to include administration of a side effect management agent.

KITS

[00136] Kits with unit doses of the active agent (e.g., a TLR2 agonist), e.g. in oral or injectable doses, are provided. In such kits, in addition to the containers containing the unit doses will be an informational package insert describing the use and attendant benefits of the drugs in treating pathological condition of interest. Preferred compounds and unit doses are those described herein above.

[00137] In such kits, in addition to the containers containing the unit doses will be an informational package insert describing the use agent(s) in treating an autoimmune disorder. These instructions may be present in the subject kits in a variety of forms, one or more of which may be present in the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a package insert, etc. Yet another means would be a computer readable medium, e.g., diskette, compact disc (CD), etc., on which the information has been recorded. Other suitable media include audiovisual media, e.g., digital versatile disk (DVD), videotape, and the like. Yet another means that may be present is a website address which may be used via the Internet to access the information at a removed site. Any convenient means may be present in the kits.

[00138] The present invention provides a medication delivery device pre-loaded with a therapeutically effective amount of a TLR2 agonist, e.g., a sufficient amount for one bolus injection of the TLR2 agonist, in the treatment of a patient suffering from an autoimmune

disorder. In some embodiments, the medication delivery device is a syringe and needle, pre-loaded with a dosage of a TLR2 agonist.

[00139] In other embodiments, the medication delivery device is a pen injector (e.g., a medication delivery pen), a number of which are known in the art. Exemplary devices which can be adapted for use in the present methods are any of a variety of pen injectors from Becton Dickinson, e.g., BD™ Pen, BD™ Pen II, BD™ Auto-Injector; a pen injector from Innoject, Inc.; any of the medication delivery pen devices discussed in U.S. Patent Nos. 5,728,074, 6,096,010, 6,146,361, 6,248,095, 6,277,099, and 6,221,053; and the like. The medication delivery pen can be disposable, or reusable and refillable.

[00140] In other embodiments, the medication delivery device is an implantable drug delivery system, preferably a system that is programmable to provide for subcutaneous administration of a TLR2 agonist. Exemplary programmable, implantable systems include implantable infusion pumps. Exemplary implantable infusion pumps, or devices useful in connection with such pumps, are described in, for example, U.S. Pat. Nos. 4,350,155; 5,443,450; 5,814,019; 5,976,109; 6,017,328; 6,171,276; 6,241,704; 6,464,687; 6,475,180; and 6,512,954. A further exemplary device that can be adapted for the present invention is the Synchromed infusion pump (Medtronic).

SUBJECTS SUITABLE FOR TREATMENT

[00141] Subjects suitable for treatment according to a subject method to treat an autoimmune disorder include any individual who has been diagnosed as having an autoimmune disorder. Also suitable for treatment with a subject method are individuals who have been previously treated with a therapeutic agent to treat an autoimmune disorder, but who are intolerant to treatment with the therapeutic agent. Also suitable for treatment with a subject method are treatment failure patients. For example, individuals suitable for treatment with a subject method include individuals who have been previously treated with a therapeutic agent to treat an autoimmune disorder, but who did not respond to treatment with the therapeutic agent. In addition, individuals suitable for treatment with a subject method include individuals who have been previously treated with a therapeutic agent to treat an autoimmune disorder, which individuals responded to treatment with the therapeutic agent, but who subsequently relapsed. In many embodiments, the individual is a human.

[00142] Subjects suitable for treatment with a subject method to treat or reduce allograft rejection include individuals who are recipients of an allograft. Subjects suitable for treatment with a subject method to treat or reduce allograft rejection include individuals who are prospective allograft recipients. Such individuals include, but are not limited to, individuals

with acute or chronic organ failure; individuals with a defective heart valve; burn patients; individuals with Type 1 diabetes mellitus; and individuals who have a disorder that is amenable to treatment by bone marrow transplantation, hematopoietic stem cell transplantation, and the like. In many embodiments, the individual is a human.

[00143] Subjects suitable for treatment with a subject method to treat an M1 macrophage-mediated inflammatory disorder include any individual suffering from an M1 macrophage-mediated inflammatory disorder. In many embodiments, the individual is a human.

EXAMPLES

[00144] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); s.c., subcutaneous; i.m., intramuscular; i.v., intravenous; and the like.

Example 1: Activation of TLR2 induces a Th2 immune response

Materials and Methods

Materials

[00145] *Mice*. C57Bl/6 (Jackson Laboratories, Ber Harbor, ME) and 129/SvEv (B&K Universal LTD., East Yorkshire, U.K.) mice were 6-8 weeks old.

[00146] *Reagents*. Immunostimulatory oligodeoxynucleotide (ISS-ODN) (1668: TCCATGACGTTCCTGATGCT; SEQ ID NO:1) was synthesized by TIB MOLBIOL (Adelphia, NJ). OVA was purchased from Worthington (Lakewood, NJ) and the synthetic lipopeptide Pam3Cys was obtained from EMC Micocollections GmbH (Tübingen, Germany).

[00147] *Tissue culture*. Cells were cultured in RPMI (Cellgro, Mediatech, Inc., Herndon, VA) supplemented with 10% FCS (Life Technologies, Gaithersburg, MD), 2mM L- Glutamine (Cellgro,), and 100U/ml penicillin- 100μg/ml streptomycin (Pen/ Strep, Cellgro). Mouse bone-

marrow derived dendritic cells (BMDCs) were cultured as previously described. Lutz et al. (1999) *J Immunol Methods* 223:77.

Immunization protocols

[00148] C57Bl/6 mice were immunized with 50 μg OVA alone or in combination with ISS-ODN (50μg) or Pam3Cys (5-500 μg) s.c. on day 0 and 14. On day 39 mice received an intravenous boost of 20 μg OVA. At day 42 mice were sacrificed and total splenocytes were restimulated for secondary CTL and cytokine assays as described. Cho et al. (2000) *Nat Biotechnol* 18:509; Takabayashi et al. (2003) *J Immunol* 170:3898. Proliferation of splenocytes was determined by [^{3}H]thymidine uptake.

Experimental Asthma

[00149] 129/SvEv were immunized s.c. with 50 μg OVA alone or in combination with ISS-ODN (50 μg) or Pam3Cys (50 μg) on day 0 and 7. Mice were intranasally (i.n.) challenged with 5 μg OVA seven days and one day prior to sacrifice. At day 21, the mice were tested for airway responsiveness to methacholine (3–24 mg/ml, Sigma) and a bronchoalveolar lavage for the differential lung cell count was performed as previously described. Broide et al. (1998) *J Immunol* 161:7054; Hamelmann et al. (1997) *Am J Respir Crit Care Med* 156:766. Mediastinal lymph nodes (LN) were digested with Dnase I/ collagenase VII (Boehringer Mannheim/Roche, Indianapolis, IN / Sigma) and restimulated with Ova for T-cell cytokine analysis and used for fluorescence activated cell sorting (FACS) stains.

ELISA

[00150] OVA-specific IgG2a, IgG1 and IgE levels were measured from serum samples collected by retroorbital eye bleeds. Roman et al. (1997) *Nat Med* 3:849. IFNγ, IL-5 (BD PharMingen, San Diego, CA), and IL-13 (RD Biosystems, Minneapolis, MN) were determined from supernatants of splenocytes that were restimulated with OVA *in vitro*. Takabayashi et al. (2003) *J Immunol* 170:3898. The levels of IL-12p40, IL-12p70, IL-10 and IL-6 (PharMingen) and IL-13 (RD Biosystems,) were determined by enzyme-linked immunosorbent assay (ELISA).

Bioassay (Type I IFN)

[00151] Type I interferon (IFN) levels in supernatants from BMDC 16 hours after stimulation were measured using an antiviral protection assay as described. Cheung et al. (1991) *J Immunol* 146:121.

Flow Cytometry

[00152] BMDC and mediastinal LN were stained with antibodies purchased from eBioscience (San Diego, CA). Surface marker expression was analyzed on a FACSCalibur flow cytometer

using CellQuest (BD Biosciences, Franklin Lakes, NJ) and FlowJo software (Tree Star, San Carlos, CA).

Real time PCR

[00153] Quantitative real- time PCR was performed using the Abi Prism 7700 (Applied Biosystems, Foster City, CA). Primers were generated using the Primer3 software. Rozen, S., and H. J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. In *Bioinformatics Methods and Protocols: Methods in Molecular Biology.* K. S, and M. S, eds. Humana Press, Totowa, NJ, p. 365.

RESULTS

TLR2 ligands bias the adaptive immune response towards a Th2 phenotype

[00154] To determine the role of particular Toll-like receptors (TLRs) in the generation of adaptive immune responses, mice were immunized with different TLR ligands in combination with ovalbumin (OVA) as a model antigen. Production of immunoglobulin (Ig) subclasses (IgG2a, IgG1 and IgE), secretion of cytokines from *in vitro* restimulated splenocytes, cytotoxic T lymphocyte (CTL) response and the effect on a murine experimental model of asthma were analyzed. Preliminary results indicated that the TLR2 ligand (Pam3Cys) and TLR9 ligand (ISS-ODN) lead to the most distinctive immune responses; and these two ligands were the focus of current investigations.

[00155] As shown in Fig. 1A-C, ISS-ODN and Pam3Cys induced different antibody profiles. Immunization with ISS-ODN/OVA resulted in an antigen-specific IgG2a response, whereas immunization with Pam3Cys/OVA resulted in a pronounced IgG1 response and induction of IgE (Fig 1C). While ISS-ODN primed CD4 T-cells to produce IFNγ, Pam3Cys induced IL-13 production (Fig. 1 D-E). Restimulation with media alone did not induce any production of IFNγ or IL-13. The IgG isotype bias and the production of IgE and IL-13 induced by Pam3Cys/OVA were abrogated in TLR2- deficient mice, proving that TLR2 is a critical receptor for Pam3Cys. Immunization with peptidoglycan (100 μg) as an adjuvant in combination with OVA was less potent than immunizations with Pam3Cys/OVA in regards to cytokine production and induction of antibody response, but also showed a preferential induction of IgG1 (26547±14455 U/ml for peptidoglycan/OVA vs. 299082±73142 U/ml for Pam3Cys/OVA, 3 weeks after immunization) over IgG2a (not detectable for peptidoglycan/OVA vs. 1473±925 U/ml for Pam3Cys/OVA, 3 weeks after immunization).

[00156] Both stimuli, ISS and Pam3Cys, led to induction of CTL activity (Fig 1F); however, the induction of CTL activity by ISS-ODN was significantly more prominent than that induced by Pam3Cys. Titrating the amount of Pam3Cys over three orders of magnitude did not

significantly change this low CTL activity, nor did it change the Th2 bias in regard to cytokine and antibody production.

Immunization with ISS-ODN/OVA and Pam3Cys/OVA induced a similar proliferative response in OVA-restimulated splenocyte cultures.

[00157] To test whether the Th1 (by ISS-ODN) and Th2 (by Pam3Cys) polarization observed above alters the propensity of the immunized animals to develop experimental asthma, Pam3Cys/OVA- or ISS-ODN/OVA-immunized mice were re-challenged with OVA intranasally at two occasions and airway hyperreactivity (AHR), recruitment of eosinophils to the lung and cytokine release of in vitro restimulated bronchial LN cells were evaluated. Priming with ISS-ODN/OVA improved AHR, decreased the number of eosinophils in the lung and induced IFNγ, whereas priming with Pam3Cys/OVA aggravated the AHR, increased the number of eosinophils and led to the production of Th2 cytokines (Fig. 1H-J).

[00158] Taken together, these data show that both ISS-ODN and Pam3Cys induce significant antigen-dependent immune responses. However, ISS-ODN polarizes the immune response towards a Th1 phenotype, whereas Pam3Cys leads to Th2-specific cytokine and immunoglobulin production and only a modest CTL response. The data further suggest that the opposing Th1/Th2 polarization induced by ISS-ODN and Pam3Cys can have an effect on the development of Th2-associated diseases such as experimental asthma.

[00159] Figures 1A-J depict differential immune responses induced by ISS-ODN- and Pam3Cys-based immunizations. (Figures 1A-G) Mice were immunized s.c. with OVA (50 μg) either alone or in combination with ISS-ODN (50 μg) or Pam3Cys (500 μg, 50 μg or 5 μg) at day 0 and 14 and rechallenged i.v. with 20 μg OVA three days before sacrifice. (Figures 1A-B) Antibody isotypes (IgG1, IgG2a) were determined by ELISA from serum samples taken before immunization and at week 2, 4 and 6. (Figure 1C) IgE was determined by ELISA from serum samples at week 4. (Figures 1D- F) Total splenocytes were analyzed for the secondary CTL activity and OVA-specific cytokine secretion. (Figure 1G) Proliferative response of Ova-restimulated splenocytes was determined by [^{3}H]thymidine uptake. (Figures 1H-J) Mice were immunized s.c. with OVA (50 μg) either alone or in combination with ISS-ODN (50 μg) or Pam3Cys (50 μg) twice and re-challenged intranasally with 5 μg OVA seven days and one day prior to testing. (Figure 1H) Airway responsiveness to aerosolized methacholine was tested (I) Percentage of macrophages (macro.), lymphocytes (lympho.), neutrophils (neutro.) and eosinophils (eos.) from the BAL was determined. (Figure 1J) OVA-specific cytokine secretion of mediastinal LN cells was measured. Data are shown as mean ±SEM, n=5 per group (n=8 per

group for the AHR and eosinophil count), ISS= ISS-ODN, Pam= Pam3Cys, n.d.= not detectable.

TLR2 ligands differentially induce Th2 associated cytokines and B7RP-1

[00160] Costimulatory molecules and the cytokine production by DC play a crucial role in the differentiation of naïve CD4 T cells. To explore whether these factors may explain the differential Th-polarization induced by ISS-ODN and Pam3Cys, BMDC were stimulated with ISS-ODN or Pam3Cys and the expression of costimulatory molecules and the production of cytokines were determined. As shown in Fig. 2A, both ISS-ODN and Pam3Cys induced upregulation of the costimulatory molecules CD40, B7-1 and B7-2, whereas only Pam3Cys led to an upregulation of B7RP-1. The upregulation of B7RP-1 was even more pronounced in mature DC from mediastinal LN after immunization with OVA and Pam3Cys (Fig. 2B), whereas ISS-ODN showed less effect.

[00161] Production of IFN α/β, IL-12 p40, IL-12 p70, IL-6, IL-10 and IL-13 (Fig. 2C), and a panel of mRNAs known to be involved in Th1/Th2 polarization (Fig. 2D), by BMDC was analyzed. There was a clear difference in the pattern of cytokines induced. Whereas ISS-ODN induced primarily cytokines associated with a Th1 phenotype like IL-12, IL-18, IL-27 and IFN α/β, Pam3Cys preferentially induced Th2-associated cytokines like IL-13, GM-CSF and IL-1β. Other genes like TNFα and IκBα were comparably induced. A similar pattern of cytokine production with Pam3Cys inducing Th2 associated cytokines was observed in primary CD11c+ DC isolated from the spleen. Again, the cytokine response and gene induction by Pam3Cys in BMDC was dependent on TLR2.

[00162] Figures 2A-2D depict activation of BMDC by ISS-ODN and Pam3Cys. (Figure 2A) Expression of costimulatory molecules in vitro. BMDC were cultured in the absence or presence of ISS-ODN (1 μM) or Pam3Cys (5 μg/ml) for 16 hours. Surface expression of CD40, B7-1, B7-2 and B7RP-1 were measured by flow cytometry. Data shown are from one representative experiment out of 6 experiments and are gated on the live CD11c+ population. Grey lines: Isotype control, dashed lines: unstimulated control, solid lines: ISS-ODN or Pam3Cys (Pam) stimulated cells. (Figure 2B) Expression of B7RP-1 on DC from mediastinal LN cells in vivo. Mice were immunized as described in Figs. 1G-H. Mediastinal LN were harvested and surface expression of B7RP-1 determined by flow cytometry. Data shown are pooled cell preparations from 3 mice per group and are gated and the live CD11c+/ MHC class II high population. (Figure 2C) Concentration of IL-12p40, IL-12-p70, IL-13, IL-10 and IL-6 were measured by ELISA from the culture supernatant of BMDC after stimulation with ISS-ODN (1 μM) or Pam3Cys (5 μg/ml) for 16 hours. IFNα was determined by bioassay. Data

from one representative experiment out of 4 experiments are shown. (Figure 2D) Expression of TNFα, IκBα, IL-12p35, IL-6, IFNβ, IL-18, IL-12p40, IL-27p28, IL-1β, IL-13, GMCSF and IL-10 mRNA were determined by quantitative real-time PCR. BMDC were cultured in the absence (control) or presence of ISS-ODN (1μM) and Pam3Cys (5 μg/ml) for 6 hours. Data from one representative experiment out of 5 experiments normalized to the expression of CPH are shown and expressed as fold increase over control. ISS= ISS-ODN, Pam= Pam3Cys.

[00163] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A method for treating an immunopathological disorder having a Th1 component and/or an M1 macrophage component in an individual, the method comprising administering to an individual in need thereof an effective amount of a toll-like receptor-2 (TLR2) agonist.

2. The method of claim 1, wherein the immunopathological disorder is an M1 macrophage-mediated inflammatory disorder.

3. The method of claim 2, wherein the disorder is a delayed type hypersensitivity.

4. The method of claim 1, wherein the disorder is an autoimmune disorder.

5. The method of claim 1, further comprising administering an effective amount of at least one additional agent selected from a corticosteroid, an anti-malarial agent, a non-steroidal anti-inflammatory agent, an immunosuppressive agent, an anti-neoplastic agent, and an immunomodulatory agent.

6. The method of claim 5, wherein the corticosteroid is selected from dexamethasone, prednisolone, prednisone, methylprednisolone, cortisone, hydrocortisone, and triamcinolone.

7. The method of claim 5, wherein the non-steroidal anti-inflammatory agent is selected from ibuprofen, acetylsalicylic acid, indomethacin, ketorolac, and naproxen.

8. The method of claim 5, wherein the antimalarial agent is selected from hydroxychloroquine, chloroquine, and quinacrine.

9. The method of claim 5, wherein the immunosuppressant is selected from azathioprine, cyclophosphamide, cyclosporine, mycophenolate mofetil, and chlorambucil.

10. The method of claim 5, wherein the anti-neoplastic agent is selected from mitoxantrone HCl, methotrexate, and leflunomide.

11. The method of claim 5, wherein the immunomodulatory agent is selected from interferon-β1a, interferon-β1b, an antibody specific for CD20, and an antibody specific for CD2.

12. The method of claim 4, wherein the individual failed to respond to previous treatment with a therapeutic agent to treat the autoimmune disorder.

13. The method of claim 4, wherein the individual responded to previous treatment with a therapeutic agent to treat the autoimmune disorder and subsequently relapsed.

14. The method of claim 4, wherein the autoimmune disorder is multiple sclerosis, and the method further comprises administering an effective amount of an agent selected from Avonex® interferon-β1a, Betaseron® interferon-β1b, Copaxone™ glatiramer acetate, Novantrone® mitoxantrone, Rebif® interferon-β1a, SoluMedrol methylprednisolone, Acthar® corticotropin, and Deltasone® prednisone.

15. The method of claim 4, wherein the autoimmune disorder is systemic lupus erythematosus, and the method further comprises administering an effective amount of an agent selected from prednisone, Imran® azathioprine, Cytoxan® cyclophosphamide, and Sandimmune® cyclosporine.

16. The method of claim 4, wherein the autoimmune disorder is rheumatoid arthritis, and the method further comprises administering an effective amount of an agent selected from Rheumatrex® methotrexate, Arava® leflunomide, and Azulfidine® sulfasalazine.

17. A method of reducing allograft rejection in an individual, the method comprising administering to an individual in need thereof an effective amount of a toll-like receptor-2 (TLR2) agonist.

18. The method of claim 17, further comprising at least one additional agent selected from mycophenolate mofetil, azathioprine, cyclophosphamide, cyclosporine, rapamycin, tacrolimus, an antibody specific for an IL-2 receptor, and a corticosteroid.

19. The method of claim 17, wherein the individual is a bone marrow transplantation recipient.

20. The method of claim 17, wherein the individual is a β-islet cell recipient.

21. The method of claim 17, wherein the individual is an organ transplant recipient.

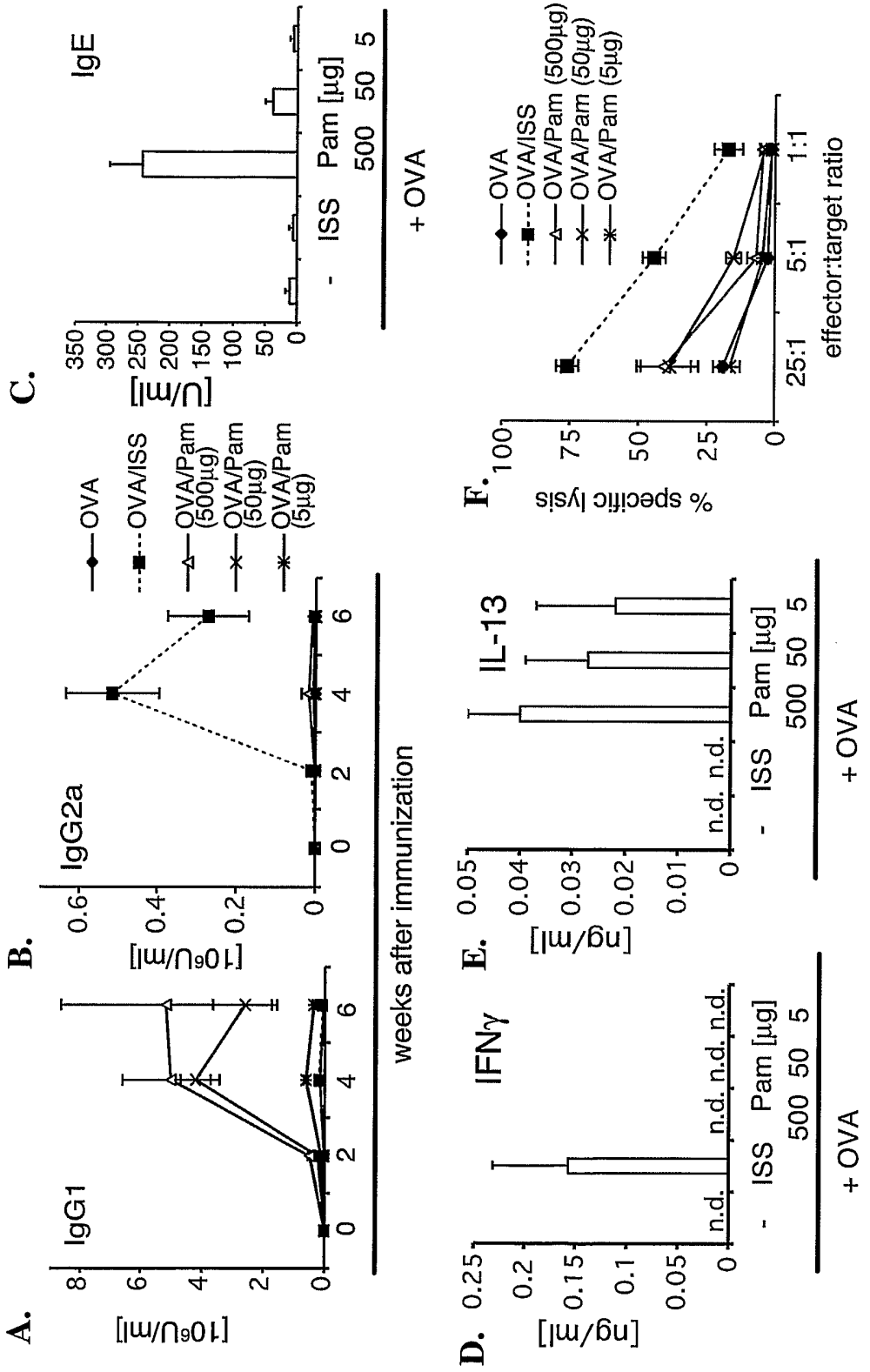
22. A composition comprising:

a) a toll-like receptor-2 agonist in an amount effective to treat an autoimmune disorder; and

b) a pharmaceutically acceptable excipient.

23. The composition according to claim 22, further comprising at least one additional agent selected from a corticosteroid, an anti-malarial agent, a non-steroidal anti-inflammatory agent, an immunosuppressive agent, an anti-neoplastic agent, and an immunomodulatory agent, wherein the at least one additional agent is present in an amount effective to treat an autoimmune disorder.

FIG. 1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B.
IgG2a
[10⁶U/ml]
OVA
OVA/ISS
OVA/Pam (500µg)
OVA/Pam (50µg)
OVA/Pam (5µg)
weeks after immunization
C.
IgE
[U/ml]
- ISS Pam [µg]
500 50 5
+ OVA
D.
IFNγ
[ng/ml]
n.d. n.d. n.d. n.d.
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500 50 5
+ OVA
E.
IL-13
[ng/ml]
n.d. n.d.
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500 50 5
+ OVA
F.
% specific lysis
OVA
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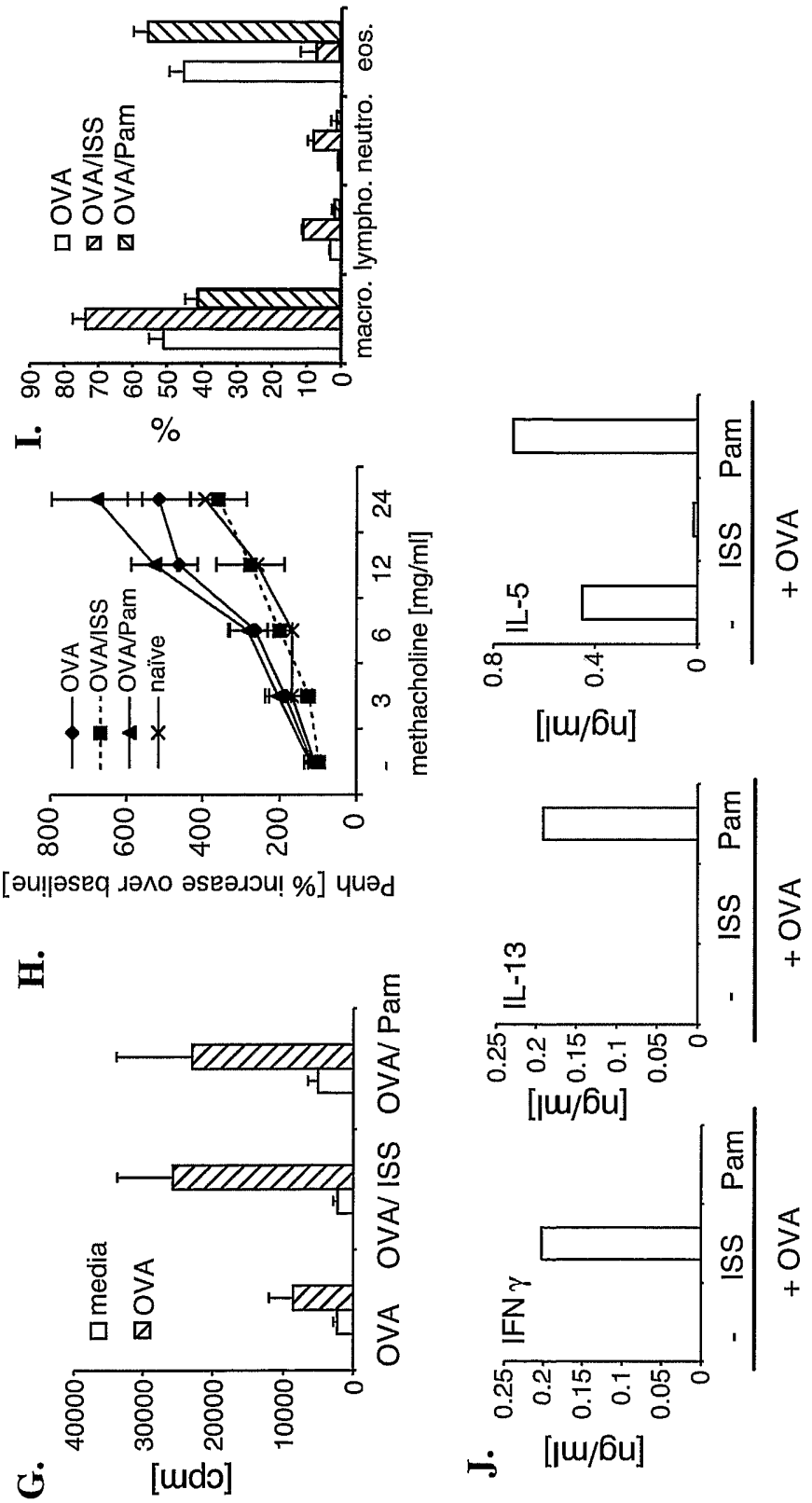
effector:target ratio

FIG. 1 (CONT.)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[cpm]
media
OVA
OVA
OVA/ ISS
OVA/ Pam
H.
Penh [% increase over baseline]
OVA
OVA/ISS
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methacholine [mg/ml]
I.
%
OVA
OVA/ISS
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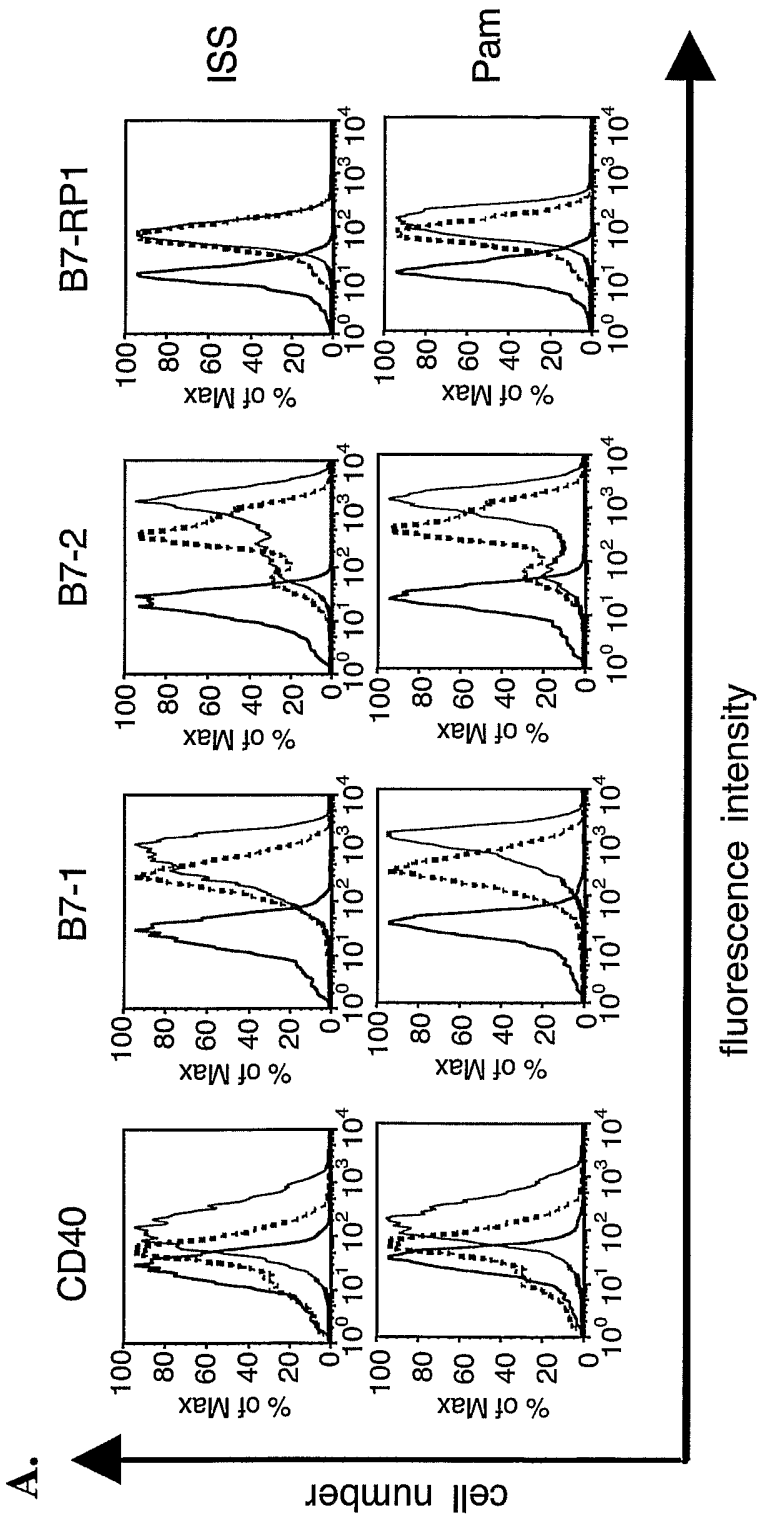
+ OVA

FIG. 2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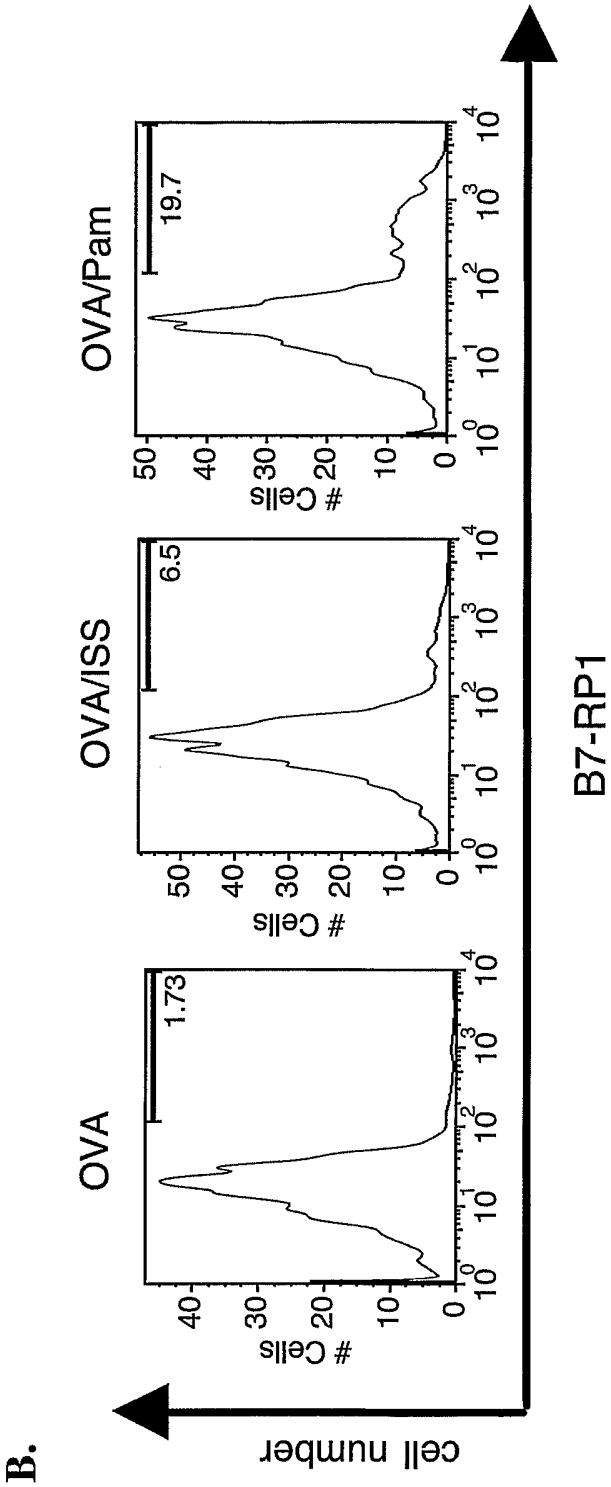

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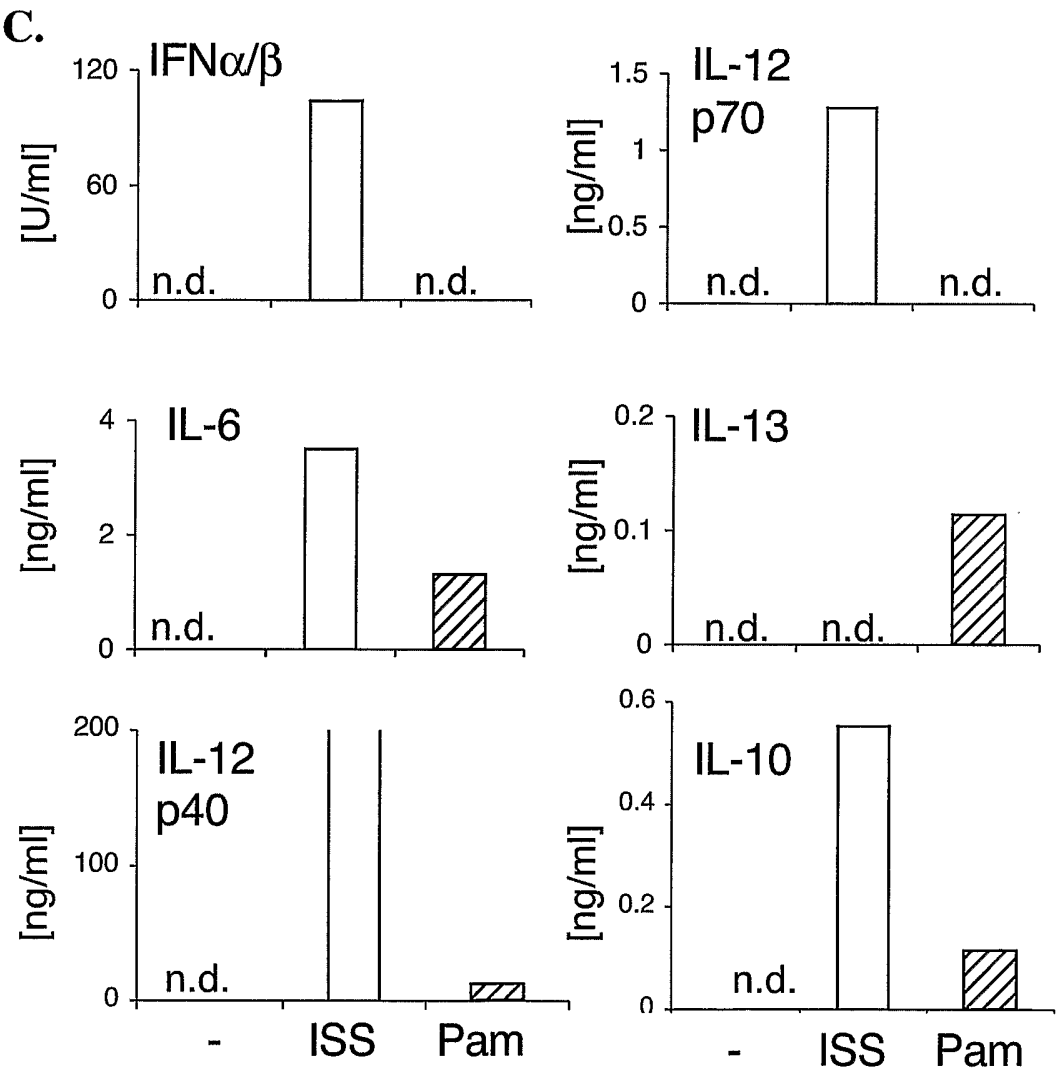
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0
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[ng/ml]
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p40
[ng/ml]
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100
0
n.d.
IL-10
[ng/ml]
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0
n.d.
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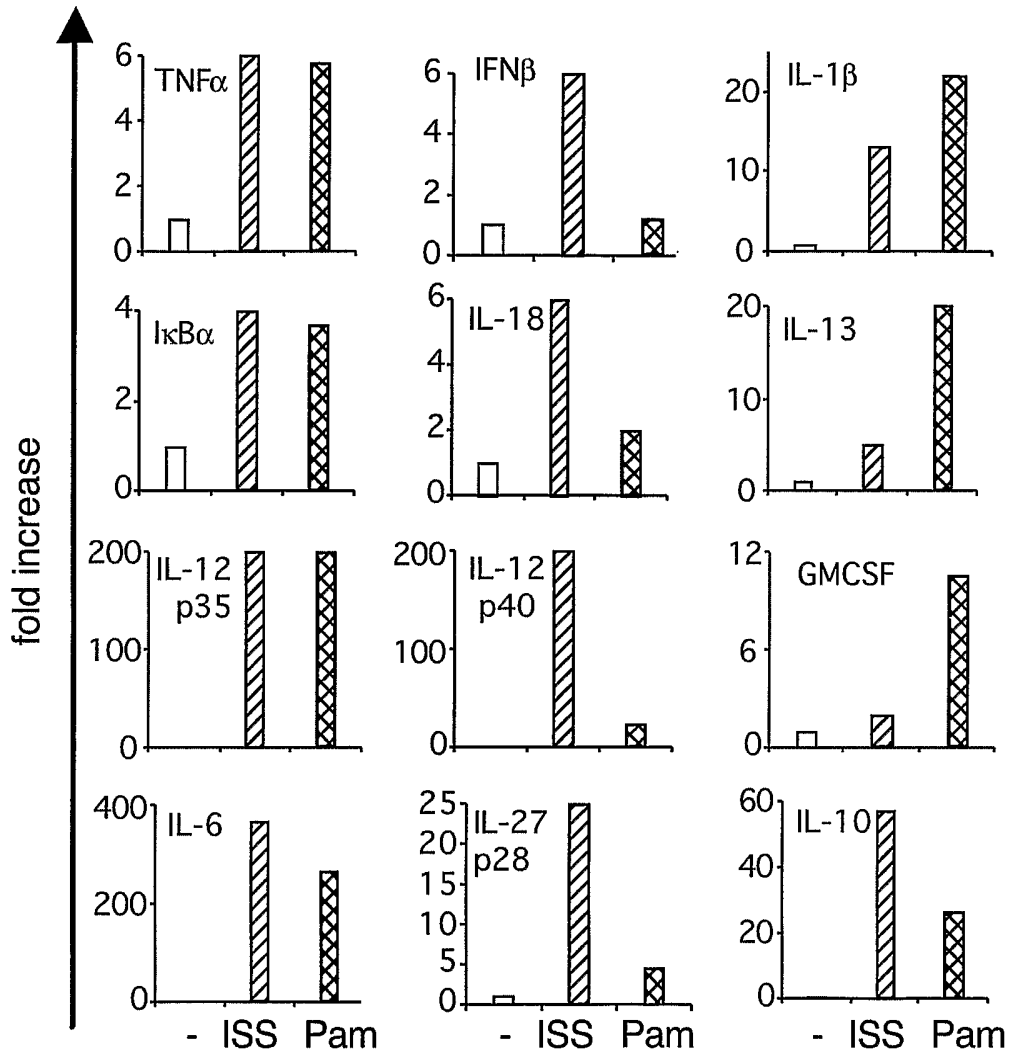
ISS
Pam
-
ISS
Pam

FIG. 2 (CONT.)

D.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IκBα
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