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(54) Title: METHODS AND REAGENTS FOR TREATING AUTOIMMUNE DISORDERS AND/OR GRAFT REJECTION

(57) Abstract: Disclosed are methods and compositions for treating autoimmune diseases, disorders, or conditions as well as for preventing and treating graft rejection. The methods comprise administering dead microbial cells, which expressed autoantigens or alloantigens, to a subject in need through mucosal route administration. Compounds that can be used as mucosal carriers are also disclosed.

METHODS AND REAGENTS FOR TREATING AUTOIMMUNE DISORDERS AND/OR GRAFT REJECTION

Related Applications

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. provisional patent application, U.S. S.N. 61/348,892, filed May 27, 2010 ("the '892 application"). The entire contents of the '892 application are incorporated herein by reference.

Sequence Listing

[0002] In accordance with 37 C.F.R. § 1.52(e)(5), a Sequence Listing in the form of a text file (entitled "Sequence_Listing," created on May 25, 201 1 and 22 kilobytes) is incorporated herein by reference in its entirety.

Background

[0003] The immune system functions to recognize "non-self entities in order to mount a protective effector response to fight off infection by microbes. The immune system also functions to recognize "self entities, but normally does not mount an immunological response against these entities, a phenomenon known as "immunological tolerance." Several mechanisms have been postulated to explain the development and maintenance of immunological tolerance. First, clonal deletion causes self-reactive immune cells to be destroyed during the development of the immune system of an individual. Clonal anergy leads to the inactivation of self-reactive immune cells, thus preventing the amplification of an immune response to autoantigens. Idiotype networking describes the process of neutralizing antibodies serving to recognize and eliminate self-reactive antibodies. These mechanisms, among others, maintain the delicate balance between protecting the host from foreign pathogens while minimizing autoreactivity of the immune system.

[0004] A shift in the balance between non-self immunoreactivity and self tolerance can lead to autoimmunity. Autoimmunity can be defined as the failure of the body's immune system to maintain immunological tolerance to selected self antigens. Autoimmune diseases caused by such aberrant immunological responses can be chronic, debilitating, and life threatening. Some examples of autoimmune diseases are Coeliac Disease, diabetes mellitus type I, systemic lupus erythematosus, Graves' Disease, and rheumatoid arthritis, among others. Autoimmune diseases may affect certain organs or particular types of tissue leading to a variety of type and severity of pathologies associated with such diseases.

[0005] Even the appropriate recognition of non-self entities can sometimes create health issues. For example, in transplant recipients, immune system activity against non-self entities can lead to severe complications such as graft rejection. Graft rejection involves an immunologic reaction in a recipient to donor antigens, in which the graft tissue is attacked and potentially damaged or destroyed. One of the important goals of immunology research is to successfully treat or prevent graft rejection in transplant patients.

[0006] Treatment of autoimmune disorders and/or graft rejection typically involves immunosuppression, which causes a global decrease in the responsiveness of the immune system. However, global immunosuppression in patients suffering from autoimmune diseases or graft rejection can lead to increased susceptibility to opportunistic infections, as well as an increased risk of developing cancer. Treatments of autoimmune disease or graft rejection that are organ and/or antigen specific would be advantageous over existing immunosuppressive therapies. There is a need in the art for methods of decreasing or eliminating the autoimmune response to self antigens in patients suffering from autoimmune disease. There is also a need in the art for methods of decreasing or eliminating immune recognition of donor tissues in recipients of transplants suffering from graft rejection.

[0007] Treatment of autoimmune disorders or graft rejection by inducing oral tolerance to autoantigens or alloantigens, respectively, has been contemplated in the art. Oral tolerance, which encompasses the immunological tolerance to antigens that access the body via the oral route, is defined as the specific suppression of cellular and/or

humoral responses to an antigen by prior administration of the antigen by the oral route (see for example, Faria, et al. Clinical and Developmental Immunology; 13(2-4); 143-157, 2006). Attempts to induce oral tolerance to autoantigens and allergens in phase III human trials have given negative results. Nasal administration of synthetic acetylcholine receptor polypeptides to decrease clinical symptoms of Experimental Myasthenia Gravis has also been unsuccessful (see, for example, Karachunski, et al. J. Clin. Invest. 100(12), 3027-3035, 1997 and Baggi, et al. Journal of Clinical Investigation, 104(9), 1999). There remains a need in the art for effective methods of tolerizing patients to autoantigens in order to treat or prevent autoimmune disease. There also remains a need in the art for effective methods of tolerizing patients to alloantigens in order to treat or prevent graft rejection.

Summary

[0008] The present invention provides methods and compositions for treatment of autoimmune diseases, disorders, or conditions and graft rejection.

[0009] In some embodiments, the present invention provides microorganisms that express (or have expressed) at least one autoallergen or alloantigen polypeptide. In some embodiments, microorganisms have been engineered to express at least one autoallergen or alloantigen polypeptide. In some embodiments, microorganisms express at least one autoallergen or alloantigen polypeptide so that the at least one autoallergen or alloantigen polypeptide is contained within the microorganism. In some embodiments, the microorganisms are dead. In some embodiments, the microorganisms are *E. coli.*

[0010] In some embodiments, microorganisms that express (or have expressed) at least one autoallergen or alloantigen polypeptide are useful in medicine, for example in the treatment and/or prevention of one or more autoimmune diseases, disorders, or conditions. In some embodiments, microorganisms that express (or have expressed) one or more autoantigen or alloantigen polypeptides are delivered mucosally to an individual suffering from or susceptible to an autoimmune disease, disorder or condition or graft rejection, respectively. In some embodiments, the autoimmune disease, disorder or condition is Myasthenia Gravis.

Definitions

[0011] *Alloantigen polypeptide:* The term "alloantigen", as used herein, is any polypeptide associated with allorecognition and/or graft rejection. Alloantigen polypeptides are generally polypeptides expressed by an individual that are genetically different from another individual of the same species (e.g., a graft recipient). The term "alloantigen polypeptide" refers to a polypeptide whose amino acid sequence includes at least one characteristic sequence of an alloantigen. A wide variety of alloantigen sequences are known in the art.

[0012] *Allograft rejection:* The terms "allograft rejection" or "graft rejection", as used herein, refer to rejection of tissue transplanted between two genetically different individuals of the same species. Rejection of a graft typically results from proper recognition of alloantigen peptides and activation of the adaptive immune system.

[0013] *Allorecognition:* The term "allorecognition", as used herein, typically refers to T cell recognition of genetically encoded polymorphisms between different members (e.g., graft donor and graft recipient) of the same species.

[0014] *Animal:* The term "animal", as used herein, refers to humans as well as non-human animals, including, for example, mammals, birds, reptiles, amphibians and fish. Preferably, a non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a primate, or a pig). An animal may be a transgenic animal.

[0015] *Antigen:* The term "antigen", as used herein, refers to an entity that elicits production of an antibody (i.e., a humoral response) and/or an antigen-specific reaction with T-cells (i.e., a cellular response) in an animal.

[0016] *Antigen presenting cell:* An "antigen presenting cell" or "APC", as defined herein, is a cell which processes and presents antigens to T-cells to elicit an antigen-specific response, e.g., macrophages and dendritic cells.

[0017] *Associated with:* As used herein, a polypeptide that is "associated with" an autoimmune disease, disorder or condition and/or graft rejection as described herein, is a polypeptide whose presence, level and/or form is correlated with an autoimmune response and/or allorecognition, respectively. In some embodiments, a polypeptide is "associated

with" an autoimmune disease, disorder or condition, wherein the presence of an immune reaction to (e.g., autoantibodies to and/or activated T cells that recognize) the polypeptide correlates with the presence of the autoimmune disease, disorder or condition. In some embodiments, a polypeptide is "associated with" graft rejection, wherein the presence of an immune reaction to (e.g., alloreactive antibodies to and/or activated T cells that recognize) the polypeptide correlates with the presence of the graft rejection.

[0018] *Autoantigen polypeptide:* As used herein, an "autoantigen polypeptide" or an "autoallergen polypeptide" is any polypeptide associated with an autoimmune disease, disorder or condition. Autoantigen polypeptides are generally polypeptides expressed by an organism that are recognized by the immune system of the organism. The term "autoantigen polypeptide" or "autoallergen polypeptide" refers to a polypeptide whose amino acid sequence includes at least one characteristic sequence of an autoantigen. A wide variety of autoantigen sequences are known in the art. Exemplary autoimmune diseases and associated candidate autoantigen polypeptides include, but are not limited to, those presented in Table 2.

[0019] *Characteristic portion:* As used herein, the phrase a "characteristic portion" of a protein or polypeptide is one that contains a continuous stretch of amino acids, or a collection of continuous stretches of amino acids, that together are characteristic of a protein or polypeptide. Each such continuous stretch generally will contain at least two amino acids. Furthermore, those of ordinary skill in the art will appreciate that typically at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids are required to be characteristic of a protein. In general, a characteristic portion is one that imparts at least one functional characteristic with an intact protein.

[0020] *Characteristic sequence:* A "characteristic sequence" is a sequence that is found in all members of a family of polypeptides or nucleic acids, and is absent from polypeptides or nucleic acids that are not in the family, and therefore can be used by those of ordinary skill in the art to define members of the family.

[0021] *Combination Therapy:* The term "combination therapy", as used herein, refers to those situations in which two or more different pharmaceutical agents are administered in overlapping regimens so that the subject is simultaneously exposed to both agents.

[0022] *Epitope:* The term "epitope", as used herein, refers to a binding site including an amino acid motif of between approximately six and fifteen amino acids which can be bound by an immunoglobulin (e.g., IgE, IgG, etc.) or recognized by a T-cell receptor when presented by an APC in conjunction with the major histocompatibility complex (MHC). A linear epitope is one where the amino acids are recognized in the context of a simple linear sequence. Such linear epitopes are also commonly referred to as sequential epitopes in the art. A conformational epitope is one where the amino acids are recognized in the context of a particular three dimensional structure. It is known in the art that antigenic epitopes are commonly found to be parts of a polypeptide chain which are rich in hydrophilic amino acids. Computer programs which are used to identify hydrophilic regions and/or predict antigenic epitopes are known in the art. Exemplary such computer programs include, but are not limited to, ADEPT, Preditop and JaMBW antigenicity plot, among others.

[0023] *Nicotinic Acetylcholine Receptor Polypeptides:* As used herein, a "nicotinic acetylcholine receptor polypeptide" is any polypeptide whose amino acid sequence contains a characteristic portion of a nicotinic acetylcholine receptor sequence. It is known in the art that the nicotinic acetylcholine receptor is composed of five subunits arranged symmetrically around a central pore. The embryonic form of nAChR is composed of a l, βï, δ, and γ subunits in a 2:1:1:1 ratio. The adult form of nAChR is composed of a l, βï, δ, and ε subunits in a 2:1:1:1 ratio. The a subunit of the nAChR is composed of four transmembrane regions, Ml, M2, M3, and M4, with extramembrane surfaces above and below. The two acetylcholine binding sites are located between the a- and γ- subunits and a- and δ- subunits (Karlin, Nature Reviews Neuroscience, 3:102-114, 2002). It will be appreciated that a nicotinic acetylcholine receptor polypeptide can encompass any one or combination of subunits.

[0024] *Polypeptide:* The term "polypeptide", as used herein, generally has its art-recognized meaning of a polymer of at least three amino acids. However, the term is also used to refer to specific functional classes of polypeptides, such as, for example, autoantigen polypeptides, nicotinic acetylcholine receptor polypeptides, alloantigen polypeptides, etc. For each such class, the present specification provides several examples of known sequences of such polypeptides. Those of ordinary skill in the art will

appreciate, that the term "polypeptide" is intended to be sufficiently general as to encompass not only polypeptides having a complete sequence explicitly recited herein (or in a reference or database specifically mentioned herein), but also to encompass polypeptides that represent functional fragments (i.e., fragments retaining at least one activity and/or one characteristic sequence or portion) of such complete polypeptides. Moreover, those of ordinary skill in the art understand that protein sequences generally tolerate some substitution without destroying activity. Thus, any polypeptide that retains activity and shares at least about 30-40% overall sequence identity, often greater than about 50%, 60%, 70%>, or 80%>, and further usually including at least one region of much higher identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99% in one or more highly conserved regions, usually encompassing at least 3-4 and often up to 20 or more amino acids, with another polypeptide of the same class, is encompassed within the relevant term "polypeptide" as used herein. Other regions of similarity and/or identity can be determined by those of ordinary skill in the art by analysis of the sequences of various polypeptides presented in the Tables herein.

[0025] *Population:* The term "population", as used herein, refers to human as well as non-human populations, including, for example, populations of mammals, birds, reptiles, amphibians and fish. Preferably, the non-humans are mammals (e.g., rodents, mice, rats, rabbits, monkeys, dogs, cats, primates, or pigs). As used herein the terms "individual" or "subject" encompass any member of these populations.

[0026] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" means an amount of a substance *{e.g.*, a therapeutic agent, composition, and/or formulation) that elicits a desired biological response when administered as part of a therapeutic regimen. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat the disease, disorder, and/or condition. As will be appreciated by those of ordinary skill in this art, the effective amount of a substance may vary depending on such factors as the desired biological endpoint, the substance to be delivered, the target cell or tissue, *etc.* For example, the effective amount of compound in a formulation to treat a disease, disorder, and/or condition is the amount that alleviates, ameliorates, relieves, inhibits, prevents, delays

onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is administered in a single dose; in some embodiments, multiple unit doses are required to deliver a therapeutically effective amount.

[0027] *Treat or Treating:* The terms "treat" or "treating," as used herein, refer to partially or completely alleviating, inhibiting, delaying onset of, reducing the incidence of, ameliorating and/or relieving a disorder or condition, or one or more symptoms of the disorder, disease or condition.

[0028] *Unit Dose:* The expression "unit dose" as used herein refers to a physically discrete unit of a formulation appropriate for a subject to be treated. It will be understood, however, that the total daily usage of a formulation of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective dose level for any particular subject or organism may depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of specific active compound employed; specific composition employed; age, body weight, general health, sex and diet of the subject; time of administration, and rate of excretion of the specific active compound employed; duration of the treatment; drugs and/or additional therapies used in combination or coincidental with specific compound(s) employed, and like factors well known in the medical arts. A particular unit dose may or may not contain a therapeutically effective amount of a therapeutic agent.

Description of the Drawing

[0029] *Figure 1:* Schematic representation of normal mechanism of synaptic transmission at the neuromuscular junction.

[0030] *Figure 2:* Schematic representation of the nicotinic acetylcholine receptor.

[0031] *Figure 3:* Schematic representation of the nicotinic acetylcholine receptor.

[0032] *Figure 4:* Schematic representation of the nicotinic acetylcholine receptor.

[0033] *Figure 5:* Schematic representation of impaired synaptic transmission in myasthenia gravis.

[0034] *Figure 6:* More than 50% of autoantibodies in myasthenia gravis bind to a 12 amino acid conformationally defined epitope called the "main immunogenic region."

[0035] *Figure 7:* More than 50% of autoantibodies in myasthenia gravis bind to a 12 amino acid conformationally defined epitope called the "main immunogenic region."

Detailed Description of Certain Embodiments

[0036] The present application references various patents, patent applications and published references. The contents of each such reference are hereby incorporated by reference.

[0037] The present invention provides methods and compositions for treatment of autoimmune diseases, disorders, or conditions as well as allorecognition and/or graft rejection. The present invention encompasses the recognition that some autoimmune diseases and graft rejections are associated with increased immunoreactivity to autoantigens and alloantigens, respectively, which leads to a loss of immunological tolerance. The present invention encompasses the recognition that a patient may be treated with a composition comprising an autoantigen or alloantigen in order to increase immunological tolerance of the autoantigen or alloantigen in the patient. The present invention provides methods of administering autoantigens or alloantigens to a patient in need thereof for treatment of autoimmune disorders or graft rejection.

Autoimmune Diseases, Disorders or Conditions

[0038] Autoimmunity is the failure of an organism to recognize its own constituent parts as "self," which results in an immune response against the organism's own tissues, cells, and entities. Any disease that results from such an aberrant immune response is termed an "autoimmune disease" or "autoimmune disorder." Exemplary autoimmune diseases and/or suspected autoimmune diseases include, but are not limited to, diseases presented in Table 1:

Table 1: Exemplary Autoimmune Diseases and Suspected Autoimmune Diseases

Disease	Characteristics
Acute disseminated encephalomyelitis (ADEM)	form of encephalitis caused by an autoimmune reaction and typically occurring a few days or weeks after a viral infection or a vaccination
Addison's disease	often caused by autoimmune destruction of the adrenal cortex
Alopecia universalis	body's white blood cells attack hair and result in total baldness
Ankylosing spondylitisis	chronic, painful, progressive inflammatory arthritis affecting primarily spine and sacroiliac joints, causing eventual fusion of the spine
Antiphospholipid antibody syndrome (APS)	affects the blood-clotting process; causes blood clots to form in veins and/or arteries
Aplastic anemia	often caused by an autoimmune attack on the bone marrow
Autoimmune hemolytic anemia	disorder characterized by IgM attack against red blood cells
Autoimmune hepatitis	liver is the target of the body's own immune system
Autoimmune inner ear disease (AIED)	progressive non-age-related sensorineural hearing loss and sometimes vertigo
Autoimmune lymphoproliferative syndrome (ALPS)	autoimmunity and lymphoma may be related; mutation in one of the genes that regulates the death of lymphocytes
Autoimmune oophoritis	immune system attacks the female reproductive organs
Balo disease	rare form of multiple sclerosis; also known as "concentric sclerosis," "encephalitis periaxialsis concentrica," or "leukoencephalitis periaxalis concentric"
Behcet's disease	exact cause is unknown in this multi-system condition, where the immune system, predominantly overactive, produces inflammation in bodily tissues, primarily causing vasculitis
Bullous pemphigoid	chronic, autoimmune disease that primarily affects the skin
Cardiomyopathy	refers to a number of diseases that weaken the heart muscle
Chagas' disease	in the chronic phase, believed to result from homology of a *T. cruzi* antigen to body tissue, resulting in a delayed autoimmune reaction leading to Chagasic cardiopathy (cardiomegaly), vovulus, or constipation, and ultimately death
Chronic fatigue immune dysfunction syndrome (CFIDS)	disorder whose primary symptom is usually intense fatigue; though the syndrome likely has multiple causes, some maintain that autoimmune damage to the brain stem is the principal mechanism in a significant subset of cases
Chronic inflammatory demyelinating polyneuropathy	rare autoimmune disorder in which there is swelling of nerve roots and destruction of the covering (myelin sheath) over the nerves

Crohn's disease	form of inflammatory bowel disease characterized by chronic inflammation of the intestinal tract; major symptoms include abdominal pain and diarrhea
Cicatrical pemphigoid	also known as "mucous membrane pemphigoid" or "benign pemphigoid"; chronic autoimmune disease of the mucosal membranes and/or skin
Coeliac sprue-dermatitis herpetiformis	characterized by chronic inflammation of the proximal portion of the small intestine caused by exposure to certain dietary gluten proteins
Cold agglutinin disease	acquired autoimmune hemolytic anemia due to an IgM autoantibody usually directed against the I antigen on red blood cells
CREST syndrome	acronym for calcinosis, Raynaud phenomenon, esophageal dysfunction, sclerodactyly, and telangiectasia; variant of the two groups of scleroderma, localized and systemic and is a relatively stable and slow-moving form of scleroderma
Degos disease	rare systemic disorder that affects small and medium-sized arteries, causing occlusive arteriopathy
Diabetes mellitus	consequence of an autoimmune attack on the insulin-producing beta cells in the islets of Langerhans of the pancreas
Discoid lupus	benign, distinctive disc-shaped skin eruption
Dysautonomia	malfunction of the autonomic nervous system, including such disorders as postural orthostatic tachycardia syndrome (POTS); though dysautonomia appears to have multiple causes, post-viral autoimmune damage appears to be a frequent cause
Endometriosis	common medical condition wherein the tissue lining the uterusis found outside of the uterus, typically affecting other organs in the pelvis; can lead to serious health problems, primarily pain and infertility
Essential mixed cryoglobulinemia	rare autoimmune disorder that may involve the blood and various other tissues and organs
Fibromyalgia-fibromyositis	widespread pain and tenderness, fatigue, and exhaustion after minimal effort
Goodpasture's syndrome	characterized by rapid destruction of the kidneys and hemorrhaging of the lungs through autoimmune reaction against an antigen found in both organs
Grave's disease	the most common form of hyperthyroidism; caused by anti-thyroid antibodies that have the effect of stimulating the thyroid into overproduction of thyroid hormone
Guillain-Barre syndrome (GBS)	acquired immune-mediated inflammatory disorder of the peripheral nervous system; also called acute inflammatory demyelinating polyneuropathy, acute idiopathic polyradiculoneuritis, acute idiopathic polyneuritis, and Landry's ascending paralysis

Hashimoto's thyroiditis	common form of hypothyroidism, characterized by initial inflammation of the thyroid, dysfunction, and goiter
Hidradenitis suppurativa	rare skin disease in which apocrine sweat glands become severely inflamed
Idiopathic and/or acute thrombocytopenic purpura	body produces anti-platelet antibodies resulting in a low platelet count
Idiopathic pulmonary fibrosis	disease of inflammation that results in scarring, or fibrosis, of the lungs
IgA neuropathy	kidney disease marked by IgA glomerulonephritis due to the glomerular immune deposit formation in the kidney
Interstitial cytisis	urinary bladder disease characterized by any of the following symptoms, though symptoms vary greatly from patient to patient: pelvic pain, urinary frequency (as often as every 30 minutes, or even fewer), urgency, pain with sexual intercourse, and pain with urination
Juvenile arthritis	rheumatic autoimmune disease characterized by chronic inflammation of the synovial tissue found in joints; onset in a child under the age of 16 years
Kawasaki's disease	autoimmune attack on the arteries around the heart
Lichen planus	inflammatory autoimmune skin disease which can affect the eyes, the skin, and the mucosa lining of the mouth and genitalia
Lupus erythematosus	chronic autoimmune disease wherein the immune system, for unknown reasons, becomes hyperactive and attacks normal tissue; attack results in inflammation and brings about symptoms
Lyme disease	caused by a bacterium; after several months, approximately 60% of patients with untreated infection will begin to have intermittent bouts of arthritis, with severe joint pain and swelling; up to 5% of untreated patients may develop chronic neurological complaints months to years after infection, including shooting pains, numbness or tingling in the hands or feet, and problems with concentration and short term memory
Meniere disease	recurrent and usually progressive group of symptoms, including tinnitus (ringing in the ears), vertigo (dizziness), and a sensation of fullness or pressure in the ears
Mixed connective tissue disease (MCTD)	used to describe overlapping groups of connective tissue disorders that cannot be diagnosed in more precise terms
Multiple sclerosis	disorder of the central nervous system characterized by decreased nerve function due to myelin loss and secondary axonal damage
Myasthenia gravis	disorder of neuromuscular transmission leading to fluctuating weakness and fatigue; weakness is caused by circulating antibodies that block acetylcholine receptors at the neuromuscular junction

Neuromyotonia	spontaneous muscular activity resulting from repetitive motor unit action potentials of peripheral origin; develops as a result of both acquired and hereditary diseases; the acquired form is more frequent and is usually caused by antibodies against neuromuscular junction
Opsoclonus myoclonus syndrome (OMS)	neurological disorder that results from an autoimmune attack on the nervous system; symptoms include opsoclonus, myoclonus, ataxia, intention tremor, dysphasia, dysarthria, mutism, hypotonia, lethargy, irritability, and malaise
Optic neuritis	inflammation of the optic nerve that may cause a complete or partial loss of vision
Ord's thyroiditis	similar to Hashimoto's disease, except that the thyroid is reduced in size
Pemphigus vulgaris	autoimmune disorder that causes blistering and raw sores on skin and mucous membranes
Pernicious anemia	autoimmune disorder characterized by anemia due to malabsorption of vitamin B12
Polyarthritis (in dogs)	immune reaction severely affecting the joints of dogs
Polychondritis	rare degenerative autoimmune disease characterized by recurrent inflammation of the cartilage in the body
Polymyositis and dermatomyositis	autoimmune neuromuscular and/or connective tissue diseases
Primary biliary cirrhosis	autoimmune disease that affects the biliary epithelial cells (BECs) of the small bile duct in the liver
Psoriasis	skin disorder in which rapidly-multiplying skin cells produce itchy, scaly inflamed patches on the skin
Polyarteritis nodosa	inflammation of the arteries resulting in damage to the walls of the arteries, thus creating a narrowing of the vessels
Polyglandular syndromes	group of symptoms and signs of disordered function related to one another by some anatomic, physiologic, or biochemical peculiarity affecting many glands
Polymyalgia rheumatica	inflammatory syndrome
Primary agammaglobulinemia	immune disorder related to antibody deficiency (hypogammaglobulinemia)
Raynaud phenomenon	patients usually report "cold fingers" accompanied by color changes of the skin (white, blue or red); most persons with RP note cold-induced numbness of the fingers and toes and occasional discomfort with a sense of hand clumsiness.
Rheumatoid arthritis	autoimmune disorder that causes the body's immune system to attack the bone joints
Reiter's syndrome	autoimmune attack on various body systems in response to a bacterial infection and the body's confusion over the HLA-B27 marker

Rheumatic fever	hypersensitive reaction of the immune system to group A beta-hemolytic streptococcal infection
Sarcoidosis	disease wherein granulomas can form anywhere in the body but particularly in the lungs
Schizophrenia	mental disease characterized by impairments in the perception or expression of reality and by significant social or occupational dysfunction
Scleroderma	chronic disease characterized by excessive deposits of collagen; progressive systemic scleroderma can be fatal
Sjogren's syndrome	autoimmune disorder in which immune cells attack and destroy the exocrine glands that produce tears and saliva
Stiff person syndrome	also referred to as "Moersch-Woltmann syndrome"; rare, severe autoimmune neurologic disease involving the central nervous system
Takayasu's arteritis	disorder that results in the narrowing of the lumen of arteries
Temporal arteritis (also known as "giant cell arteritis")	inflammation of blood vessels, most commonly the large and medium arteries of the head; untreated, the disorder can lead to significant vision loss
Ulcerative colitis	inflammatory disease of the bowel that usually affects the distal end of the large intestine and rectum; some medical authorities classify colitis as an autoimmune disease
Uveitis	uvea refers to the layer between sclera and retina; uveitis refers to inflammation of uvea
Vasculitis	result of chronic inflammation of the blood vessel walls
Vitiligo	spontaneous loss of pigment from areas of skin; pigment-free areas have few or no melanocytes; anti-melanocyte antibodies detected in some cases
Vulvodynia ("vulvar vestibulitis")	pain in the vulva, often severe
Wegener's granulomatosis	form of vasculitis that affects the lungs, kidneys and other organs

[0039] One of ordinary skill in the art will recognize that Table 1 presents an exemplary, not comprehensive, list of known or suspected autoimmune diseases, disorders or conditions. Any disease, disorder or condition that is characterized by failure of an organism to recognize its own constituent parts as "self," resulting in an immune response against an organism's own tissues, cells and entities, can be classified as an autoimmune disease, disorder or condition.

[0040] Autoimmune diseases, disorders or conditions may be caused by a variety of factors. In some embodiments, autoimmune diseases, disorders or conditions may be initiated by a genetic predisposition. In some embodiments, autoimmune diseases, disorders or conditions may be initiated by certain exogenous agents *(e.g.,* viruses, bacteria, chemical agents, *etc.).*

[0041] Some forms of autoimmunity arise as a result of trauma to an area usually not exposed to lymphocytes *(e.g.,* neural tissue, lens of the eye, *etc.).* When tissues in these areas become exposed to lymphocytes, their surface proteins can act as antigens and trigger production of antibodies and cellular immune responses which then begin to destroy those tissues.

[0042] In some embodiments, autoimmune diseases, disorders or conditions develop after exposure of a subject to antigens which are antigenically similar *(i.e.,* cross-reactive with) the subject's own tissue. For example, in rheumatic fever, an antigen of the streptococcal bacterium (which causes rheumatic fever) is cross-reactive with parts of the human heart. Antibodies cannot differentiate between bacterial components and heart muscle entities; consequently cells with either of those antigens can be destroyed.

[0043] In some embodiments, autoimmune diseases, disorders or conditions *(e.g.,* type I diabetes, multiple sclerosis, rheumatoid arthritis, *etc.)* are characterized as being a result of mostly cell-mediated autoimmune response and appear to be primarily due to activity of T cells (Sinha *et al.,* 1990, *Science,* 248:1380; incorporated herein by reference). In some embodiments, autoimmune diseases, disorders or conditions *(e.g.,* myasthenia gravis, lupus erythematosus, *etc.)* are characterized as being a result of primarily a humoral immune response.

[0044] Without wishing to be bound by any particular theory, it is proposed that one can treat autoimmunity by, for example, increasing the production of cytokines such as IL-10 that may lead to the production of Treg cells. It is thought that immune responses to certain antigens can be regulated by IL-10, which acts an immune suppressive cytokine. In some embodiments, Tregs are induced in an immune response. It is thought that Tregs function in an anti-inflammatory role to limit damage that would otherwise be caused by a strong inflammatory response.

[0045] In some embodiments, the present invention provides strategies for treating autoimmunity that include administration of autoantigens encapsulated in or by a bacterium. Without wishing to be bound by any particular theory, it is proposed that bacteria are taken up by immune cells such as dendritic cells so that autoantigen polypeptides are presented in a manner that leads to increased production of IL-10. In some embodiments, increased production of IL-10 may result in increased production of Treg cells.

Autoallergen Polypeptides

[0046] As already noted, autoimmunity is the result of an immune response against an organism's own tissues, cells, and/or entities. In some circumstances, autoimmune diseases, disorders and conditions result when the body mounts an immune response against a self polypeptide. An "autoallergen polypeptide" or "autoantigen polypeptide" is any polypeptide associated with an autoimmune disease, disorder or condition. Autoallergen polypeptides are generally polypeptides expressed by an organism that are recognized by the immune system of the organism. Exemplary autoimmune diseases and associated candidate autoantigen polypeptides include, but are not limited to, those presented in Table 2:

***Table 2: Exemplary Autoantigens Involved With Autoimmune Diseases* (Mocci, *et al. Current Opinion in Immunology,* 2000).**

Disease	**Candidate Autoantigens**
IDDM	Pancreatic β-cell antigens, insulin, GAD and its isoforms
Multiple sclerosis	MBP, PLP, MOG
Rheumatoid arthritis	Collagen type II, human cartilage gp39 (HCgp39), gp130-RAPS
Scleroderma	Fibrillarin, small nucleolar protein
Myasthenia gravis	Acetylcholine receptor (AChR)
Graves' disease	Thyroid stimulating factor receptor (TSH-R)
Systemic lupus erythematosus	Nuclear antigens, DNA, histones, glycoprotein gp70, ribosomes
Primary billiary cirrhosis	PDC-E2 (mitochondrial enzyme, pyruvate dehydrogenase dehydolipoamide acetyltransferase)
Alopecia areata	Hair follicle antigens
Ulcerative colitis	Human tropomyosin isoform 5 (hTM5)

[0047] One of ordinary skill in the art will recognize that Table 2 presents an exemplary, not comprehensive, list of autoimmune disorders and corresponding candidate autoantigens. Any polypeptide that is associated with an autoimmune disease, disorder or condition can be classified as an autoantigen.

[0048] It will be appreciated that autoantigen polypeptides may have a complete sequence explicitly recited herein (or in a reference or database specifically mentioned herein), or alternatively may be polypeptides that represent functional fragments (i.e., fragments retaining at least one activity and/or one characteristic sequence or portion) of such complete polypeptides. Moreover, those of ordinary skill in the art understand that protein sequences generally tolerate some substitution without destroying activity. Thus, any polypeptide that retains activity and shares at least about 30-40% overall sequence identity, often greater than about 50%>, 60%>, 70%>, or 80%>, and further usually including at least one region of much higher identity, often greater than 90%> or even 95%, 96%, 97%, **98%o,** or 99%, in one or more highly conserved regions, usually encompassing at least 3-4 and often up to 20 or more amino acids, with another autoantigen polypeptide of the same class, is encompassed within the relevant term "autoantigen polypeptide" as used herein.

Graft Rejection

[0049] An overwhelming adaptive immune response against foreign organ(s) or tissue is a major risk factor in organ transplantation, and may cause post-transplantation complications in patients. Activation of the adaptive immune system in graft rejection is thought to be carried out by two pathways, the direct and indirect pathways. It is thought that the direct pathway generally results from allorecognition by T cell receptors of intact foreign major histocompatibility (MHC) entities, with or without bound peptides on the surface of donor cells. The indirect pathway generally results from internalization and processing of donor MHC entities for presentation by host antigen presenting cells (APC).

[0050] Graft rejection can also occur where MHC-matched organ(s) or tissues are transplanted. It is thought that additional alloantigens, such as, for example, minor histocompatibility polypeptides, costimulatory signals, among others, may play a role in graft rejection.

[0051] In some embodiments, graft rejection arises in an acute phase following transplantation of donor organs or tissues. In some embodiments, a direct pathway of immune activation leads to acute graft rejection. In some embodiments, graft rejection arises in a chronic phase following transplantation of donor organs or tissues. In some embodiments, a indirect pathway of immune activation leads to chronic graft rejection. It is to be understood that the present invention encompasses methods and compositions for treatment of acute and/or chronic graft rejection.

Alloantigen Polypeptides

[0052] As described herein, an alloantigen polypeptide is any polypeptide associated with allorecognition and/or graft rejection. Alloantigen polypeptides are generally polypeptides expressed by an individual that are genetically different from another individual of the same species. The term "alloantigen polypeptide" refers to a polypeptide whose amino acid sequence includes at least one characteristic sequence of an alloantigen. A wide variety of alloantigen sequences are known in the art.

[0053] In some embodiments, an alloantigen polypeptide for use in accordance with the present invention is an MHC polypeptide. In some embodiments, an alloantigen polypeptide for use in accordance with the present invention is a Class I MHC polypeptide. In some embodiments, an alloantigen polypeptide for use in accordance with the present invention is a Class II MHC polypeptide. In some embodiments, an alloantigen polypeptide for use in accordance with the present invention contains part of or all of an extracellular domain of an MHC polypeptide. In some embodiments, an alloantigen polypeptide for use in accordance with the present invention is a minor histocompatibility complex polypeptide. In some embodiments, an alloantigen for use in accordance with the present invention is a costimulatory entity (e.g., CD28, CD80, and CD86, among others). In some embodiments, an alloantigen polypeptide for use in accordance with the present invention is a non-MHC protein produced by or present in graft tissue and not produced by or present in a host. One of ordinary skill in the art will recognize that alloantigen polypeptides described herein are exemplary. Any polypeptide that is associated with an allorecognition and/or graft rejection can be classified as an alloantigen.

[0054] It will be appreciated that alloantigen polypeptides may have a complete sequence explicitly recited herein (or in a reference or database specifically mentioned herein), or alternatively may be polypeptides that represent functional fragments (i.e., fragments retaining at least one activity and/or one characteristic sequence or portion) of such complete polypeptides. Moreover, those of ordinary skill in the art understand that protein sequences generally tolerate some substitution without destroying activity. Thus, any polypeptide that retains activity and shares at least about 30-40% overall sequence identity, often greater than about 50%>, 60%>, 70%>, or 80%>, and further usually including at least one region of much higher identity, often greater than 90%> or even 95%, 96%, 97%, **98%o,** or **99%o** in one or more highly conserved regions, usually encompassing at least 3-4 and often up to 20 or more amino acids, with another alloantigen polypeptide of the same class, is encompassed within the relevant term "alloantigen polypeptide" as used herein.

Microbial Cells

[0055] The present invention provides microbial cells that express, have expressed, or can be caused to express, autoantigen or alloantigen polypeptides. Any microorganism capable of expressing autoantigen or alloantigen polypeptides may be used as a delivery vehicle in accordance with the present invention. Such microorganisms include but are not limited to bacteria, viruses, fungi (including yeast), algae and protozoa. Bacteria are preferred, particularly bacteria such as *E. coli* that naturally colonize within humans, e.g., in the gastrointestinal tract.

[0056] Generally, microorganisms are single cell, single spore or single virion organisms. In some embodiments, microorganisms in accordance with the present invention are amenable to genetic engineering. Microorganisms that can be genetically engineered or manipulated to produce a desired autoantigen or alloantigen polypeptide are preferred (e.g., see Ausubel et al, *Current Protocols in Molecular Biology.* Wiley and Sons, Inc. 1999, incorporated herein by reference). Genetic manipulation includes mutation of the host genome, insertion of genetic material into the host genome, deletion of genetic material from the host genome, transformation of the host with

extrachromosomal genetic material, transformation with linear plasmids, transformation with circular plasmids, insertion of genetic material into the host (e.g., injection of mRNA), insertion of transposons, and/or chemical modification of genetic material. Methods for constructing nucleic acids (including an expressible gene), and introducing such nucleic acids into an expression system to express the encoded protein are well established in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, incorporated herein by reference).

[0057] According to the present invention, some features of microorganisms as autoantigen or alloantigen polypeptide delivery systems include (i) integrity of the delivery system prior to endocytosis, (ii) known mechanisms of endocytosis (often including targeting to particular cell types), (iii) ease of production of the delivered autoantigen or alloantigen polypeptides, (iv) experimental accessibility of the organisms, including ease of genetic manipulation, (v) ability to permit release of the autoantigen or alloantigen polypeptide after endocytosis, and/or (vi) the possibility that the encapsulating organism will also act as an adjuvant (e.g., *L. monocytogenes, E. coli,* etc.) in that it can bias an immune response to the autoantigen toward a particular type of response, e.g., including production of IL-10 and/or Treg cells.

[0058] Without wishing to be bound by any particular theory, the present inventors note that many mucosal tissues are highly populated with certain microbial cells, and particularly with *E. coli.* For example, some nonpathogenic *E. coli* strains are part of the normal flora of the gut. As a result, those skilled in the art typically would not expect delivery of such microbial cells to induce a traditional immune response against the microbial cells. For example, microbial cells and/or components thereof may sometimes be used in immunological contexts to avoid adjuvant-type stimulation.

[0059] Further, without wishing to be bound by any particular theory, the present inventors note that use of microorganisms such as bacteria as therapeutic delivery vehicles in accordance with the present invention may offer advantages over delivery of autoantigen or alloantigen polypeptides that are not encapsulated inside microorganisms. In some embodiments, use of such microorganisms to deliver autoantigens and/or alloantigens may downregulate Th2-type, and/or upregulate Thl-type responses to such

autoantigen and/or alloantigen polypeptides. Alternatively or additionally, use of such microorganisms to deliver autoantigens and/or alloantigens may induce or promote Treg-type responses to such autoantigen and/or alloantigen polypeptides.

[0060] As noted, in some embodiments, bacteria are utilized as microorganisms according to the present invention. Generally, bacteria are classified as gram-negative or gram-positive depending on the structure of the cell walls. In some embodiments, gram-negative bacteria are used in accordance with the present invention.

[0061] Non-limiting examples of genera and species of gram-negative bacteria include Escherichia coli, Vibrio cholerae, Salmonella, Listeria, Legionella, Shigella, Yersenia, Citrobacter, Enterobacter, Klebsiella, Morganella, Proteus, Providencia, Serratia, Plesiomonas, and Aeromonas.

[0062] Non-limiting examples of genera and species of gram-positive bacteria include *Bacillus subtilis, Sporolactobacillus, Clostridium, Arthrobacter, Micrococcus, Mycobacterium, Peptococcus, Peptostreptococcus,* and *Lactococcus.*

[0063] Systems for expressing polypeptides in gram-negative bacterial cells are known and can be used in accordance with the present invention. For example, *E. coli* is a well-studied bacteria, and methods of protein expression in *E. coli* are well-established. Many strains of *E. coli* have the advantage of being non-pathogenic; *E. coli* is found naturally in the gut. In some embodiments, *E. coli* is utilized as a delivery vehicle in the present invention.

[0064] Expression technologies have also been developed for other bacterial systems. To give but a few examples, *Vibrio cholerae* has been used as a delivery vehicle for production of antigens for use as a live vaccine against infectious organisms (see, for example, Calderwood et al; US Patent 5,747,028). *Salmonella* has been used as delivery vehicle for production of antigens for use as a live vaccine against infectious organisms (see, for example, Miller and Mekalanos; US Patent 5,731,196). Recombinant attenuated *Salmonella* which secretes antigenic determinants of *Listeria* has been used as a live vaccine to protect against listeriosis (see, for example, Hess et al; *Proc. Natl. Acad. Sci. USA* 93:1458-1463, 1996). Attenuated *Salmonella typhimurium* has been used as a gram-negative host for secretion of polypeptides for controlling fertility (see, for example, Donner et al; WO 98/50067). Other attenuated gram-negative strains including *Yersinia*

were used to express and secrete such polypeptides (see also Donner et al.; WO 98/50067). Gram-positive bacteria have also been studied as delivery vehicles for proteins (e.g., see WO 97/14806 that describes the use of *Lactococcus).*

[0065] Expression systems have also been developed for yeast. It is well known that yeast are amenable to genetic manipulation to express a protein or proteins of choice (see, for example, Ausubel et al., *supra).* Furthermore, in general most yeast are non-pathogenic. Without limitation to these species, two well-characterized species of yeast are the budding yeast *Saccharomyces cerevisiae,* and the fission yeast, *Schizosaccharomyces pombe.* Moreover, the administration of yeast that carry compounds that alter an immune response has been studied (e.g., see US Patent No. 5,830,463).

Live and Killed Cells

[0066] Microorganisms of the present invention may be administered to a subject as live or dead microorganisms. In many embodiments, microorganisms are administered as dead organisms.

[0067] In some embodiments where live microorganisms are administered to individuals, the microorganisms are attenuated and/or are administered in suitable encapsulation materials to decrease immune responses to the microorganisms. A variety of attenuated organisms is known in the art.

[0068] As noted above, in many embodiments, microorganisms are administered to subjects after being killed. Any method of killing the microorganisms may be utilized that does not greatly alter the expressed polypeptides. In certain embodiments, a killing method is selected to minimize or avoid release of expressed polypeptides from the microorganism. Methods of killing microorganism include but are not limited to using heat, antibiotics, chemicals such as iodine, bleach, ozone, and alcohols, radioactivity (i.e., irradiation), UV light, electricity, and pressure. Preferred methods of killing microorganisms are reproducible and kill at least 99% of the microorganisms. Particularly

preferred is the use of heat above 50 °C for a period of time that kills greater than 99% of the cells and preferably 100% of the cells.

[0069] In some embodiments, microorganisms are amenable to killing such that the autoantigen or alloantigen polypeptide remains encapsulated within the microorganism. In some embodiments, autoantigen or alloantigen polypeptide is encapsulated within the cytoplasm of the microorganism. In some embodiments, autoantigen or alloantigen polypeptide is encapsulated within the periplasm of the microorganism. In certain embodiments, autoantigen or alloantigen polypeptide is encapsulated within an organelle of the microorganism. In some embodiments, autoantigen or alloantigen polypeptide is encapsulated within the cytoplasmic membrane of the microorganism. In some embodiments, autoantigen or alloantigen polypeptide is encapsulated within the outer membrane of the microorganism.

[0070] In certain embodiments of the present invention, microbial cells that express autoantigen or alloantigen polypeptides are killed by methods described herein. In some embodiments, methods selected for use in killing the microorganism minimize disruption of the cell membrane. For example, in some embodiments, at least 80%>, 85%, 90%>, 95%, **96%o, 97%o, 98%o, 99%o,** or more of the cell membranes remain intact (e.g., as monitored by trypan blue exclusion). Such methods are particularly advantageous, among other things, because they can reduce or eliminate release of autoantigen or alloantigen polypeptides that are present inside the cell prior to administration to a patient in need thereof.

Pharmaceutical Compositions

[0071] As discussed above, the present invention provides microorganisms that express or have expressed autoantigen or alloantigen polypeptides that are useful for treating autoimmune diseases, disorders, and conditions. In certain embodiments, the present invention provides microbial cells with autoantigen or alloantigen polypeptides contained therein.

[0072] In some embodiments of the present invention, pharmaceutical compositions are provided, wherein these compositions comprise microorganisms described herein and a pharmaceutically acceptable carrier. Optionally the compositions include adjuvants and/or immunomodulatory agents as discussed below.

[0073] In some embodiments, it may be desirable to include microorganisms expressing more than one autoantigen or alloantigen polypeptide in a composition of the present invention. In some embodiments, a microorganism may express more than one polypeptide. In some embodiments, a mixture of microorganisms that each express one or more polypeptides may be used in accordance with the present invention. To give but one example, at least three different autoantigen polypeptides, Pancreatic β-cell antigens, insulin and GAD are thought to contribute to IDDM. To give another non-limiting example, several different alloantigen polypeptides are thought to contribute to graft rejection, including major histocompatibility complex polypeptides, minor histocompatibility polypeptides, and costimulatory entities. Inventive compositions may include a mixture of microorganisms that express more than one or all of the autoantigen or alloantigen polypeptides. Also, it may be desirable to include autoantigen polypeptides that are associated with a variety of different kinds autoimmune disorders so that multiple autoimmune disorders are treated simultaneously.

Pharmaceutically Acceptable Carriers

[0074] As used herein, the term "pharmaceutically acceptable carrier" includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. *Remington's Pharmaceutical Sciences* Ed. by Gennaro, Mack Publishing, Easton, PA, 1995, discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the microorganisms of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as hydroxypropyl cellulose, sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as

cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator. Viscosity-enhancing carriers such as hydroxypropyl cellulose are preferred carriers of the invention for rectal administration since they facilitate retention of the pharmaceutical composition within the rectum. In addition, in embodiments that involve rectal administration the volume of carrier that is added to the pharmaceutical composition is selected in order to maximize retention of the composition. In particular, the volume should not be so large as to jeopardize retention of the administered composition at the site of administration.

Adjuvants

[0075] Administration of microbial cells that express, have expressed, or can be caused to express, autoantigen or alloantigen polypeptides in accordance with the present invention may be accompanied by one or more supplementary compounds for enhancing or modifying the activity, properties, or marketability of the composition. For example, adjuvants for enhancing the immunogenicity of antigens are known in the art. Some examples of immunogenicity enhancers include, for example, gel-type adjuvants (e.g., aluminum hydroxide/aluminum phosphate, calcium phosphate), microbial adjuvants (e.g., immunomodulatory DNA sequences that include CpG motifs; endotoxins such as monophosphoryl lipid A; exotoxins such as cholera toxin, E. coli heat labile toxin, and pertussis toxin; and muramyl dipeptide); oil-emulsion and emulsifier-based adjuvants (e.g., Freund's Incomplete Adjuvant, MF59, and SAF); particulate adjuvants (e.g., liposomes, biodegradable microspheres, and saponins); and synthetic adjuvants (e.g., nonionic block copolymers, muramyl peptide analogues, polyphosphazene, and synthetic polynucleotides). Some additional supplementary compounds include, for example,

disinfectants, preservatives, surfactants, stabilizing agents, chelating agents, and coloring agents.

[0076] Adjuvants that enhance the absorption efficiency of the mucosa are also known in the art. Some examples of such mucosa absorption enhancers include, for example, bile salts, such as sodium glycocholate, and surfactants, such as polyoxyethylene-9-lauryl ether.

[0077] In certain embodiments immunomodulatory adjuvants and/or entities are comprised or synthesized by the microorganisms of the invention. In other embodiments they may be provided as impure preparations (e.g., isolates of cells expressing a cytokine gene, either endogenous or exogenous to the cell) or purified preparations and mixed with the microorganisms. In some embodiments, an adjuvant is administered as part of the pharmaceutical composition. In some embodiments, an adjuvant in administered separately from a pharmaceutical composition. It is recognized that in some embodiments microorganisms that are utilized to synthesize and deliver the autoantigen or alloantigen polypeptides according to the present invention can act as adjuvants themselves.

Administration

[0078] After formulation with an appropriate pharmaceutically acceptable carrier in a desired dosage, pharmaceutical compositions of this invention can be administered to humans and/or other mammals by a variety of routes. In particular the compositions can be administered topically (as by powders, ointments, or drops), orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, subcutaneously, intramuscularly, intragastrically, bucally, ocularly, or nasally, depending on the severity and nature of the autoimmune disorder being treated or prevented. Preferably the compositions are delivered to mucosal tissues. The mucosa refers to the epithelial tissue that lines the internal cavities of the body. For example, the mucosa comprises the alimentary canal, including the mouth, esophagus, stomach, intestines, and anus; the respiratory tract, including the nasal passages, trachea, bronchi, and lungs; and the genitalia.

[0079] The inventors have established that rectal delivery is a particularly suitable delivery route for inventive compositions. Compositions for rectal administration are preferably suppositories which can be prepared by mixing the microorganisms of this

invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol and/or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectal vault and release the microorganisms (e.g., see Williams, *Scand. J. Gastroenterol. Suppl.* 172:60-2, 1990 and Torres-Lugo et al., *Biomaterials* 21(12): 1191-6, 2000). Retention enemas and rectal catheters can also be used as is known in the art. Viscosity-enhancing carriers such as hydroxypropyl cellulose are also preferred carriers of the invention for rectal administration since they facilitate retention of the pharmaceutical composition within the rectum. Generally, the volume of carrier that is added to the pharmaceutical composition is selected in order to maximize retention of the composition. In particular, the volume should not be so large as to jeopardize retention of the administered composition in the rectal vault.

[0080] If desired, injectable preparations (e.g., for subcutaneous administration) such as sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables. Delayed absorption of a parenterally administered composition may be accomplished by dissolving or suspending the microorganisms in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the microorganisms in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of microorganisms to polymer and the nature of the particular polymer employed, the rate of microorganism release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the microorganisms in liposomes or microemulsions which are compatible with body tissues.

[0081] Liquid dosage forms, e.g., for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the microorganisms, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

[0082] Dosage forms for topical or transdermal administration in accordance with the present invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants, or patches. In some embodiments, microorganisms are admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ointments, pastes, creams and gels utilized in accordance with the present invention may contain, in addition to the microorganisms of this invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc, zinc oxide, or mixtures thereof.

[0083] Solid dosage forms, e.g., for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, microorganisms may be mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay and i) lubricants such as

talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof.

[0084] Solid compositions may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. Solid dosage forms of tablets, dragees, capsules, pills and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms, microorganisms may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the microorganisms only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Recipients of Pharmaceutical Compositions

[0085] In yet another aspect, according to methods of treatment of the present invention, an individual who suffers from or is susceptible to an autoimmune disease, disorder or condition may be treated with a pharmaceutical composition, as described herein. It will be appreciated that an individual can be considered susceptible to an autoimmune disease, disorder or condition without having suffered symptoms of the particular disease in question. Individuals that are susceptible to an autoimmune disease but lack any relevant medical history can be identified by any methods known in the art. It is thought that for some autoimmune diseases, characteristics of an individual may cause an increased susceptibility to autoimmune disease. Exemplary characteristics of an individual include, but are not limited to sex, age, hormone levels, and ethnicity, among others. In some embodiments, females are more susceptible to autoimmune disease. In some embodiments, increased estrogen levels leads to increased risk of autoimmune disease. In some embodiments, young adult women (under 40 years of age) are

considered to be more susceptible to autoimmune disease. In some embodiments, older men (over 60 years of age) are considered to be more susceptible to autoimmune disease.

[0086] In still another aspect, according to methods of treatment of the present invention, an individual who suffers from or is susceptible to graft rejection may be treated with a pharmaceutical composition, as described herein. It will be appreciated that an individual can be considered susceptible to graft rejection without having suffered symptoms of the particular disease in question. Individuals that are susceptible to graft rejection but lack any relevant medical history can be identified by any methods known in the art. It is thought that for some cases of graft rejection, characteristics of an individual may cause an increased susceptibility to graft rejection. Exemplary characteristics of an individual include, but are not limited to sex, age, hormone levels, and ethnicity, among others. It is also thought that for some cases of graft rejection, characteristics of organ(s) or tissue to be transplanted may cause an increased susceptibility to graft rejection. For example, it is thought that the type of organ or tissue transplanted may cause increased susceptibility to graft rejection. In some cases, skin, small bowel, and lung are particularly susceptible to graft rejection. It is thought that pancreatic islets, vascularized pancreas, heart, liver and kidney may be less susceptible to graft rejection (Jones, et al. *J Immunol.* 2001 :166:2824-2830). Additional exemplary factors that may contribute to increased susceptibility to graft rejection include graft size, vascularization, the presence of tissue specific antigens, lymphatic drainage, among others.

[0087] In some embodiments, genetic testing, for example, for the presence of markers shown to be associated with autoimmune diseases, disorders, or conditions or graft rejection, can be used to identify individuals that are susceptible to autoimmune disease or graft rejection. Without wishing to be bound by any particular theory, it is thought that the presence of certain alleles of particular genes is associated with certain autoimmune diseases. As a non-limiting example, it is thought that the presence of certain alleles of Human Leukocyte Antigen B27 may be associated with increased susceptibility to seronegative spondyloarthropathy. As another non-limiting example, it is thought that polymorphisms between donor and recipient MHC may be associated with increased susceptibility to graft rejection.

[0088] In general, it is believed that inventive compositions will be clinically useful in treating autoimmune diseases or graft rejection associated with any autoantigen or alloantigen polypeptide, respectively.

[0089] It will be appreciated that therapeutic and prophylactic methods encompassed by the present invention are not limited to treating autoimmune diseases or graft rejection in humans, but may be used to treat autoimmune diseases or graft rejection in any animal including but not limited to mammals, e.g., bovine, canine, feline, caprine, ovine, porcine, murine and equine species.

Therapeutically Effective Dose

[0090] The invention provides methods for treatment of autoimmune diseases, disorders or conditions or graft rejection comprising administering a therapeutically effective amount of an inventive pharmaceutical composition comprising a microorganism expressing an autoantigen or alloantigen polypeptide to an individual in need thereof, in such amounts and for such time as is necessary to achieve the desired result. It will be appreciated that this encompasses administering an inventive pharmaceutical composition as a therapeutic measure to treat an individual who suffers from an autoimmune disease, disorder or condition or as a prophylactic measure to desensitize an individual that is susceptible to an autoimmune disease, disorder or condition.

[0091] In certain embodiments of the present invention a "therapeutically effective amount" of a pharmaceutical composition is an amount effective in ameliorating, reducing incidence and/or severity of, and/or delaying onset of one or more symptoms of an autoimmune disease, disorder or condition or graft rejection in an individual who suffers from decreased immunotolerance or an individual who is susceptible to an autoimmune disease, disorder or condition or graft rejection. Pharmaceutical compositions, according to the method of the present invention, may be administered using any amount and any route of administration effective for such treatment.

[0092] As described herein, in some embodiments, pharmaceutical compositions are delivered via mucosal administration. In some embodiments, pharmaceutical compositions are delivered orally. In some embodiments, pharmaceutical compositions are delivered rectally. In some embodiments, pharmaceutical compositions are delivered

nasally. In some embodiments, pharmaceutical compositions are delivered vaginally. As will be appreciated by those of ordinary skill in the art, exact dosage utilized in a therapeutic regimen is typically chosen by the individual physician in view of the patient to be treated and the route of administration. Dosage and administration are commonly adjusted to provide sufficient levels of the autoantigen polypeptide or to maintain the desired effect. Additional factors which may be taken into account include the severity of the autoimmune disease; age, weight and gender of the individual; diet, time and frequency of administration, therapeutic combinations, reaction sensitivities and tolerance/response to therapy. Treatment will typically be between twice a week and once a month, continuing for up to 3 months to 5 or more years, although this is highly dependent on the individual patient response. In general, therapeutically effective amounts will be in the microgram to milligram range of autoantigen or alloantigen polypeptide.

[0093] In certain embodiments dosage may be increased in steps, e.g., by doubling the dosage in a series of weekly administrations over an initial period (e.g., 4-16 weeks, preferably 6-10 weeks). For example, an initial once weekly schedule of administration is a well-established immunotherapy paradigm for escalation to "maintenance" doses of immunotherapeutic extracts. In certain embodiments this may be followed with a biweekly or monthly schedule of administration at the final "high" dosage until the subject achieves increased immunotolerance (e.g., for 2-6 months or more, preferably 3-4 months). For example, without limitation, in certain embodiments, the compositions of the invention may be administered in increasing dosage levels until they reach about 0.1 μg to about 1,000 μg, preferably from about 1 μg to about 500 μg, more preferably 10 μg to about 100 μg of the autoantigen polypeptide per kg of subject body weight. The increased spacing between administrations during the "maintenance" period may provide the immune system a sufficient period of time, with continued but not relentless exposure, to respond to the treatment and develop immunotolerance. In certain embodiments it may prove advantageous to gradually decrease the dosage over time after this "maintenance" period until the patient is fully immunotolerant (e.g., as determined by a skin prick test, serum IgE levels, a supervised challenge with the autoantigen, etc.).

[0094] In some embodiments, autoantigen or alloantigen polypeptides expressed in microbial cells in accordance with the present invention are formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of autoantigen polypeptide appropriate for the patient to be treated. It will be understood, however, that the total daily, weekly or monthly usage of the compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. For any inventive autoantigen polypeptide, a therapeutically effective dose can be estimated initially either in cell culture assays or in non-human animal models, usually mice, rabbits, dogs, or pigs. A non-human animal model may also used to achieve a desirable concentration range. Such information can then be used to determine useful doses for administration in humans.

Combination Therapy

[0095] According to the present invention, autoantigen or alloantigen polypeptides may be administered in combination with one or more other pharmaceutical agents. For example, autoantigen or alloantigen polypeptides may be administered in combination with one or more immunosuppressive agents and/or in combination with one or more other pharmaceutical agents (e.g., pain relievers, anti-inflammatories, antibiotics, antibodies, etc).

[0096] In certain embodiments, autoantigen or alloantigen polypeptides are administered in combination with one or more immunosuppressive therapies. For example, in some such embodiments, autoantigen polypeptides are administered in combination with one or more of: glucocorticoids, alkylating agents, antimetabolites, methotrexate, azathioprine, mercaptopurine, interferon, opioids, TNF binding proteins, mycophenolate, fingolimod, ciclosporin, tacrolimus, sirolimus, and combinations thereof.

Examples

[0097] Efforts have been made to induce desensitization by oral and nasal administration of intact AchR or AchR T cell epitopes. The present invention

encompasses the recognition that oral feeding of AChR could induce tolerance in EAMG animals, but could also lead to elevated antibody titers. Animal studies have shown positive results with oral and nasal administration of AChR T Cell epitopes and recombinant AChR lacking B cell epitopes. No human trials of oral or nasal therapies have been published.

[0098] Thus, the present invention contemplates experiments involving (1) producing HKE expressing a portion of the extracellular domain of the a-subunit of the AChR containing the MIR, (2) administering to normal animals *(e.g.*, mice, rats, *etc.),* (3) assessing production of anti-AChR IgG/myasthenic symptoms *(e.g.*, safety study to test possibility that HKE-AChR would induce myasthenia gravis), (4) generating EAMG animals *(e.g.*, mice, rats, *etc.)* using immunization with, *e.g.*, Torpedo AChR, and/or (5) desensitizing EAMG with HKE-AChR *(e.g.*, via rectal, oral, and/or nasal routes).

Example 1: Treatment of Myasthenia Gravis by Mucosal Administration of Microbially-Encapsulated Nicotinic Acetylcholine Receptor Polypeptide

Myasthenia Gravis

[0099] Myasthenia gravis, which is a relatively rare autoimmune disease that affects approximately 1 in 20,000 individuals, is characterized by fatigue and weakness in multiple muscle groups. It is an autoimmune disease that is caused by circulating antibodies to the nicotinic acetylcholine receptor (nAChR). Autoantibodies to the nAChR block the stimulative effect of binding of the neurotransmitter acetylcholine at the post-synaptic neuromuscular junction. In many cases, myasthenia gravis is associated with or caused by a thymoma. Currently available treatments for myasthenia gravis include thymectomy, plasmapheresis to remove the circulating antibody, and administration of cholinesterase inhibitors (e.g., physostigmine). In some embodiments, myasthenia gravis pathology can be completely recreated by passive transfer of pathogenic antibody. In some embodiments, animal models can be created by immunizing with one or more relevant antigens (EAMG).

[00100] In healthy patients, an electrical stimulus triggers the release of acetylcholine, which binds to the acetylcholine receptor at the neuromuscular junction on the muscle

cell. This process activates the entry of sodium into through the membrane, which causes a local membrane depolarization leading to muscle contraction. Ultimately, acetylcholinesterase degrades the acetylcholine neurotransmitter, thereby terminating the signal. In patients with Myasthenia gravis, antibodies to acetylcholine receptor polypeptides cause an overall decrease in the availability and number of receptors for binding to the neurotransmitter acetylcholine. This prevents the sodium flux, membrane depolaraization, and synaptic transmission at the neuromuscular junction leading to improper muscle function.

[00101] The nicotinic acetylcholine receptor is composed of five subunits arranged symmetrically around a central pore. The embryonic form of nAChR is composed of al, β1, δ, and γ subunits in a 2:1:1:1 ratio. The adult form of nAChR is composed of a 1, β1, δ, and ε subunits in a 2:1:1:1 ratio. The a subunit of the nAChR is composed of four transmembrane regions, Ml, M2, M3, and M4, with extramembrane surfaces above and below. The two acetylcholine binding sites are located between the a- and γ- subunits and a- and δ- subunits (Karlin, Nature Reviews Neuroscience, 2002).

[00102] The anti-AChR antibodies associated with Myasthenia gravis are heterogeneous. However, the main target of anti-AChR antibodies is a small region on the extracellular portion of the nAChR alpha subunit called the main immunogenic region (MIR). Residues 67-76 of the nAChR make up at least the majority of the MIR polypeptide. The N-terminal half of the MIR polypeptide plays a significant role in binding, with residues Asn68 and Asp71 playing the most important role.

Nicotinic Acetylcholine Receptor Polypeptides

[00103] The present invention encompasses use of nicotinic acetylcholine receptor polypeptides for use in treatment and/or prevention of Myasthenia Gravis. A nicotinic acetylcholine receptor polypeptide is any polypeptide whose amino acid sequence contains a characteristic portion of a nicotinic acetylcholine receptor sequence. As described above, the nicotinic acetylcholine receptor is composed of five subunits arranged symmetrically around a central pore. It will be appreciated that a nicotinic acetylcholine receptor polypeptide can encompass any one or combination of subunits. A nicotinic acetylcholine receptor polypeptide can have a complete sequence explicitly

recited herein (or in a reference or database specifically mentioned herein), but also can encompass polypeptides that represent functional fragments of nicotinic acetylcholine receptor (i.e., fragments retaining at least one activity and/or one characteristic sequence or portion) of such complete polypeptides.

[00104] Exemplary nicotinic acetylcholine receptor polypeptides include, but are not limited to, those presented in Table 3:

ACHA_HUMAN Acetylcholine receptor subunit alpha (P02708)	MEPWPLLLLFSLCSAGLVLGSEHETRLVAKLFKDYSSVVRPVEDHRQVVEVTV GLQLIQLINVDEVNQIVTTNVRLKQGDMVDLPRPSCVTLGVPLFSHLQNEQWV DYNLKWNPDDYGGVKKIHIPSEKIWRPDLVLYNNADGDFAIVKFTKVLLQYTG HITWTPPAIFKSYCEIIVTHFPFDEQNCSMKLGTWTYDGSVVAINPESDQPDL SNFMESGEWVIKESRGWKHSVTYSCCPDTPYLDITYHFVMQRLPLYFIVNVI I PCLLFSFLTGLVFYLPTDSGEKMTLSISVLLSLTVFLLVIVELIPSTSSAVPL IGKYMLFTMVFVIASI ITIVIVINTHHRSPSTHVMPNWVRKVFIDTIPNIMFF STMKRPSREKQDKKIFTEDIDISDISGKPGPPPMGFHSPLIKHPEVKSAIEGI KYIAETMKSDQE SNNAAAE WKYVAMVMDH ILLGVFMLVC IIGTLAVFAGRL IE LNQQG (SEQ ID NO: 1)
ACHA_HUMAN Acetylcholine receptor subunit beta (PI 1230)	MTPGALLMLLGALGAPLAPGVRGSEAEGRLREKLFSGYDSSVRPAREVGDRVR VSVGLILAQLISLNEKDEEMSTKVYLDLEWTDYRLSWDPAEHDGIDSLRITAE SVWLPDVVLLNNNDGNFDVALDISVVVSSDGSVRWQPPGIYRSSCSIQVTYFP FDWQNCTMVFSSYSYDSSEVSLQTGLGPDGQGHQEIHIHEGTFIENGQWEIIH KPSRLIQPPGDPRGGREGQRQEVIFYLI IRRKPLFYLVNVIAPCILITLLAIF VFYLPPDAGEKMGLS IFALLTLTVFLLLLADKVPETSLSVP IIIKYLMFTMVL VTFSVILSVVVLNLHHRSPHTHQMPLWVRQIFIHKLPLYLRLKRPKPERDLMP EPPHCSSPGSGWGRGTDEYFIRKPPSDFLFPKPNRFQPELSAPDLRRFIDGPN RAVALLPELREVVSSISYIARQLQEQEDHDALKEDWQFVAMVVDRLFLWTFI I FTSVGTLVI FLDATYHLPPPDPFP (SEQ ID NO: 2)
ACHD_HUMAN Acetylcholine receptor subunit delta (Q07001)	MEGPVLTLGLLAALAVCGSWGLNEEERLIRHLFQEKGYNKELRPVAHKEESVD VALALTLSNLISLKEVEETLTTNVWIEHGWTDNRLKWNAEEFGNISVLRLPPD MVWLPEIVLENNNDGSFQISYSCNVLVYHYGFVYWLPPAIFRSSCPISVTYFP FDWQNCSLKFSSLKYTAKEITLSLKQDAKENRTYPVEWI IIDPEGFTENGEWE IVHRPARVNVDPRAPLDSPSRQDITFYLI IRRKPLFYI INILVPCVLISFMVN LVFYLPADSGEKTSVAISVLLAQSVFLLLISKRLPATSMAIPLIGKFLLFGMV LVTMVVVICVIVLNIHFRTPSTHVLSEGVKKLFLETLPELLHMSRPAEDGPSP GALVRRSSSLGYISKAEEYFLLKSRSDLMFEKQSERHGLARRLTTARRPPASS EQAQQELFNELKPAVDGANFIVNHMRDQNNYNEEKDSWNRVARTVDRLCLFVV TPVMVVGTAWIFLQGVYNQPPPQPFPGDPYSYNVQDKRFI (SEQ ID NO: 3)
ACHG_HUMAN Acetylcholine receptor subunit gamma (P07510)	MHGGQGPLLLLLLAVCLGAQGRNQEERLLADLMQNYDPNLRPAERDSDVVNV SLKLTLTNLISLNEREEALTTNVWIEMQWCDYRLRWDPRDYEGLWVLRVPSTM VWRPD IVLENNVDGVFEVALYCNVLVSPDGC IYWLPPAI FRSACS ISVTYFPF DWQNCSLIFQSQTYSTNEIDLQLSQEDGQTIEWIFIDPEAFTENGEWAIQHRP AKMLLDPAAPAQEAGHQKVVFYLL IQRKPLFYVINI IAPCVL ISSVAI L IHFL PAKAGGQKCTVAINVLLAQTVFLFLVAKKVPETSQAVPLISKYLTFLLVVTIL IVVNAVVVLNVSLRSPHTHSMARGVRKVFLRLLPQLLRMHVRPLAPAAVQDTQ SRLQNGSSGWSITTGEEVALCLPRSELLFQQWQRQGLVAAALEKLEKGPELGL SQFCGSLKQAAPAIQACVEACNLIACARHQQSHFDNGNEEWFLVGRVLDRVCF LAMLSLFICGTAGIFLMAHYNRVPALPFPGDPRPYLPSPD (SEQ ID NO: 4)
ACHE_HUMAN Acetylcholine receptor subunit epsilon (Q04844)	MARAPLGVLLLLGLLGRGVGKNEELRLYHHLFNNYDPGSRPVREPEDTVTISL KVTLTNLISLNEKEETLTTSVWIGIDWQDYRLNYSKDDFGGIETLRVPSELVW LPEIVLENNIDGQFGVAYDANVLVYEGGSVTWLPPAIYRSVCAVEVTYFPFDW QNCSLIFRSQTYNAEEVEFTFAVDNDGKTINKIDIDTEAYTENGEWAIDFCPG VIRRHHGGATDGPGETDVIYSLI IRRKPLFYVINI IVPCVLISGLVLLAYFLP AQAGGQKCTVSINVLLAQTVFLFLIAQKIPETSLSVPLLGRFLIFVMVVATLI VMNCVIVLNVSQRTPTTHAMSPRLRHVLLELLPRLLGSPPPPEAPRAASPPRR ASSVGLLLRAEELILKKPRSELVFEGQRHRQGTWTAAFCQSLGAAAPEVRCCV DAVNFVAESTRDQEATGEEVSDWVRMGNALDNICFWAALVLFSVGSSLIFLGA YFNRVPDLPYAPCIQP (SEQ ID NO: 5)

[00105] One of ordinary skill in the art will recognize that Table 3 presents an exemplary, not comprehensive, list of nicotinic acetylcholine receptor polypeptides. Any polypeptide or characteristic portion or sequence that is associated with a nicotinic acetylcholine receptor can be classified as a nicotinic acetylcholine receptor polypeptide.

[00106] In some preferred embodiments, nicotinic acetylcholine receptor polypeptides comprise the main immunogenic region (MIR) of the alpha subunit of the nicotinic acetylcholine receptor. An exemplary MIR sequence is underlined in SEQ ID NO: 1.

Expression of Nicotinic Acetylcholine Receptor Polypeptides in Microbial Cells

[00107] The present invention provides microbial cells that express, or can be caused to express, nicotinic acetylcholine receptor polypeptides. Any microorganism capable of expressing nicotinic acetylcholine receptor polypeptides may be used in accordance with the present invention. Such microorganisms include but are not limited to bacteria, viruses, fungi (including yeast), algae and protozoa. Bacteria are preferred, particularly bacteria such as *E. coli.*

[00108] In some embodiments, microorganisms in accordance with the present invention are genetically engineered to produce a desired nicotinic acetylcholine receptor polypeptide. Genetic manipulation includes mutation of the host genome, insertion of genetic material into the host genome, deletion of genetic material from the host genome, transformation of the host with extrachromosomal genetic material, transformation with linear plasmids, transformation with circular plasmids, insertion of genetic material into the host (e.g., injection of mRNA), insertion of transposons, and/or chemical modification of genetic material. Methods for constructing nucleic acids (including an expressible gene), and introducing such nucleic acids into an expression system to express the encoded protein are well established in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, incorporated herein by reference). In some embodiments, nicotinic acetylcholine receptor polypeptides are encapsulated within the microorganism.

[00109] In some embodiments, nicotinic acetylcholine receptor polypeptides that are expressed by microorganisms in accordance with the present invention include the N-terminal extracellular region of the alpha-subunit of the nicotinic acetylcholine receptor. An exemplary, non-limiting example of an N-terminal extracellular region of the alpha subunit of the *Torpedo marmorata* nicotinic acetylcholine receptor is amino acid residues 1-209 (see, for example, Schrattenholz, et al. Journal of Biological Chemistry, 272:49, 32393-32399, 1998). In some embodiments, the nicotinic acetylcholine receptor polypeptides that are expressed include the major immunogenic epitope (e.g., the underlined region of SEQ ID NO: 1).

Animal Models

[00110] Animal models of experimental autoimmune Myasthenia Gravis (EAMG) can be induced in vertebrates by immunization with either *Torpedo californica* or electric eel AChR in adjuvants. EAMG was first induced in rabbits by immunization with AChR in complete Freund's adjuvant by Patrick and Lindstrom. Myasthenia Gravis-like disease can also be induced in rodents by passive transfer of serum from Myasthenia Gravis patients, grafting Myasthenia Gravis thymus tissue or peripheral blood lymphocytes, or by local production of **IFN-**γ at the neuromuscular junction in gamma-interferon-e transgenic mice. The pathogenesis of mouse EAMG is similar to that of human Myasthenia Gravis, making the mouse EAMG model ideal for analysis of immunotherapeutics for treatment of Myasthenia Gravis (Christadoss, et al. 2000).

[00111] EAMG mice are treated with mucosally administered killed bacteria containing nicotinic acetylcholine receptor polypeptides. Assays to determine the symptoms of Myasthenia Gravis and presence of autoantibodies to nicotinic acetylcholine receptors are performed. Assays for evaluating EAMG in mice are known in the art. In some embodiments, mice are evaluated by assaying for anti-acetylcholine receptor antibodies. In some embodiments, mice are evaluated by measuring muscle acetylcholine receptor content.

[00112] In some embodiments, neuromuscular function of EAMG mice treated with bacteria in accordance with the present invention is determined. In some embodiments, mice are evaluated by fatiguing weakness tests. In some embodiments, mice are evaluated

by tests for strength, e.g., grip strength tests, among others. In some embodiments, mice are evaluated by tests for neuronal conduction. In some embodiments, mice are evaluated by tests for muscular contractility. In some embodiments, mice are evaluated by electromyography.

[00113] Results from evaluative assays in EAMG mice treated with therapeutic microbial cells can be compared with results from the same assays performed on EAMG mice that have not been treated. It will be appreciated that results from evaluative assays may be compared in the same animal at different time points (e.g., results before treatment with therapeutic microbial cells vs. results after treatment with therapeutic microbial cells). Assays may be performed at any time, for example, before administration of microbial cells, at the time of administration of microbial cells, or after administration of microbial cells (e.g., 1 hour, 2 hours, 3 hours, 1 day, 2 days, 3 days, 4 days, etc. post-administration). In some embodiments, microbial cells that are encompassed by the present invention cause one or more symptoms of the disease, e.g., Myasthenia Gravis or EAMG, to be reduced or delayed. In some embodiments, microbial cells that are encompassed by the present invention cause one or more aspects of the immune response to be modified as compared with its state prior to or absent the administration.

Example 2: Treatment of Graft rejection by Mucosal Administration of Microbially-Encapsulated MHC Polypeptide

Major Histocompatibility Complex Polypeptides

[00114] The present invention encompasses use of alloantigen polypeptides, e.g., MHC polypeptides, for use in treatment and/or prevention of graft rejection. An MHC polypeptide is any polypeptide whose amino acid sequence contains a characteristic portion of an MHC sequence. MHC entities can be subdivided into MHC Class I and MHC Class II entities. Generally, Class I entities are composed of two polypeptide chains and are approximately 350 amino acids long and are typically post-translationally modified (e.g., glycosylated). MHC Class I polypeptides typically fold into three separate domains (al, a2 and a3). A beta-pleated sheet and two alpha helices are located between the a1 and a2 domains. This region is capable of binding a small antigen, which is

displayed to immune effector entities such as T-cells. MHC Class II polypeptides are composed of two polypeptide chains, a and β, each of which is approximately 230-240 amino acids long and are post-translationally modified (e.g., glycosylated). MHC Class II polypeptides typically fold into two separate domains, a1 and a2 for the a chain and βï and β2 for the β chain. Similar to MHC Class I entities, MHC Class II entities typically have a beta pleated sheet and two alpha helices located between the α1 and βï domains, where small peptides may bind.

[00115] In some embodiments, MHC polypeptides in accordance with the present invention comprise the extracellular domain of the polypeptide. Typically, the "extracellular domain" of class I MHC entities involves the al, a2 and a3 domains, while the extracellular domain of class II MHC entities involves the a1 and a2 domains of the a chain and the βï and β2 domains of the β chain, as described above. In some embodiments, an MHC polypeptide is a single chain or double chain MHC protein (e.g., the a or β chain of Class II entities or the heavy chain of Class I entities) which may constitute all or part of the extracellular portion of the MHC complex (i.e., that portion of the MHC that is accessible to a T cell) which is in other than its native state, for example, not associated with the cell membrane of a cell that normally expresses that MHC. In some embodiments, the extracellular domain of MHC refers both to the native extracellular domains described above as well as to modified or chemically synthesized extracellular domains. Modified extracellular domains include, but are not limited to MHC domains in which conservative substitutions are made for various amino acids, side chains of various amino acids are modified (e.g. to improve peptide presentation), or amino acids are added or eliminated (e.g. to provide the minimal entity necessary for presentation).

Expression of MHC Polypeptides in Microbial Cells

[00116] The present invention provides microbial cells that express, or can be caused to express, MHC polypeptides. Any microorganism capable of expressing MHC polypeptides may be used in accordance with the present invention. Such microorganisms include but are not limited to bacteria, viruses, fungi (including yeast), algae and protozoa. Bacteria are preferred, particularly bacteria such as *E. coli.*

[00117] In some embodiments, microorganisms in accordance with the present invention are genetically engineered to produce a desired MHC polypeptide. Genetic manipulation includes mutation of the host genome, insertion of genetic material into the host genome, deletion of genetic material from the host genome, transformation of the host with extrachromosomal genetic material, transformation with linear plasmids, transformation with circular plasmids, insertion of genetic material into the host (e.g., injection of mRNA), insertion of transposons, and/or chemical modification of genetic material. Methods for constructing nucleic acids (including an expressible gene), and introducing such nucleic acids into an expression system to express the encoded protein are well established in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, incorporated herein by reference). In some embodiments, MHC polypeptides are encapsulated within the microorganism.

Animal Models

[00118] Mouse skin transplantation is an established in vivo model used to study immune response associated with graft rejection in mice. See for example, Schwoebel, *et al. Laboratory Animals* (2005) 39:209-214. Additionally, Turgeon *et al.* have described non-obese diabetic severe combined immunodeficient β2 microglobulin null mice engrafted with human peripheral blood lymphocytes for use as an in vivo model for studying human skin graft rejection (see, for example, Turgeon *et al. Experimental Biology and Medicine* 228 (9): 1096. (2003)).

[00119] Skin transplantation from tail portions from allogenic mice or human skin is performed and animals are monitored for signs of transplant rejection (e.g., fever, visible lesions, etc.).

[00120] Mice to receive, or that have received, transplanted tissue are treated with mucosally administered killed bacteria containing MHC polypeptides. Assays to determine the symptoms of graft rejection are performed. Assays for evaluating graft rejection in mice are known in the art. In some embodiments, mice are evaluated by performing histological and/or immunohistochemical analyses.

[00121] Results from evaluative assays in transplant recipient mice treated with therapeutic microbial cells can be compared with results from the same assays performed on transplant recipient mice that have not been treated. It will be appreciated that results from evaluative assays may be compared in the same animal at different time points (e.g., results before treatment with therapeutic microbial cells vs. results after treatment with therapeutic microbial cells). Assays may be performed at any time, for example, before administration of microbial cells, at the time of administration of microbial cells, or after administration of microbial cells (e.g., 1 hour, 2 hours, 3 hours, 1 day, 2 days, 3 days, 4 days, etc. post-administration). In some embodiments, microbial cells that are encompassed by the present invention cause one or more symptoms of the disease, e.g., graft rejection, to be reduced or delayed. In some embodiments, microbial cells that are encompassed by the present invention cause one or more aspects of the immune response to be modified as compared with its state prior to or absent the administration.

Other Embodiments

[00122] Other embodiments of the invention will be apparent to those skilled in the art from a consideration of the specification or practice of the invention disclosed herein. It is intended that the specification and Examples be considered as exemplary only, with the true scope of the invention being indicated by the following Claims.

Claims

What is claimed is:

1. A method comprising steps of:

providing a composition comprising:

dead microbial cells that have expressed an autoantigen polypeptide such that the autoantigen polypeptide is contained within the dead microbial cells; and

a pharmaceutically acceptable mucosal carrier; and

administering the composition via a mucosal route to an individual suffering from or susceptible to an autoimmune disease disorder or condition associated with an immune response against the autoantigen,

the administering being performed under conditions and for a time sufficient that one or more symptoms of the disease, disorder or condition is reduced or delayed.

2. A composition comprising:

dead microbial cells that have expressed an autoantigen polypeptide such that the autoantigen polypeptide is contained within the dead microbial cells and

a pharmaceutically acceptable mucosal carrier.

3. A method comprising steps of:

providing a composition comprising:

dead microbial cells that have expressed an alloantigen polypeptide such that the alloantigen polypeptide is contained within the dead microbial cells; and

a pharmaceutically acceptable mucosal carrier; and

administering the composition via a mucosal route to an individual suffering from or susceptible to graft rejection associated with an immune response against the alloantigen,

the administering being performed under conditions and for a time sufficient that one or more symptoms of the graft rejection is reduced or delayed.

4. A composition comprising:

dead microbial cells that have expressed an alloantigen polypeptide such that the alloantigen polypeptide is contained within the dead microbial cells and

a pharmaceutically acceptable mucosal carrier.

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Figure 1

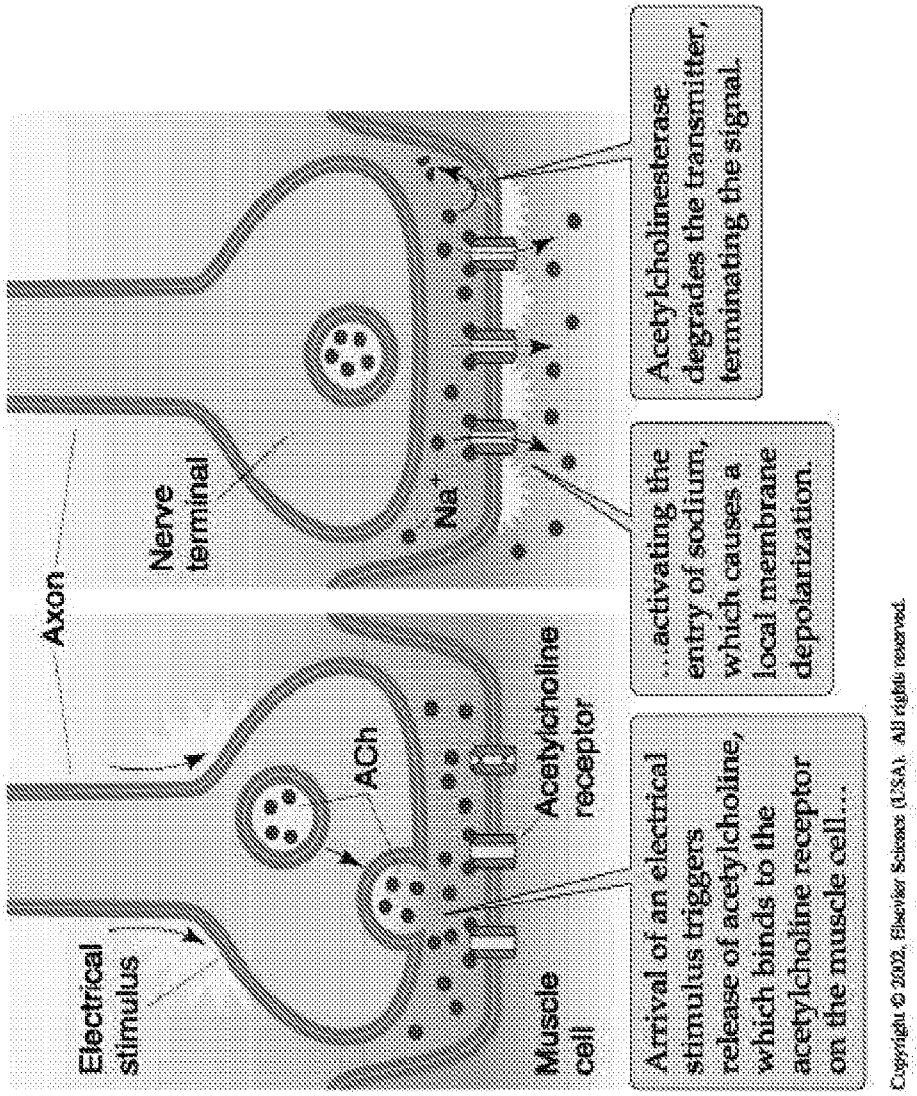
Electrical stimulus
Axon
Nerve terminal
ACh
Acetylcholine receptor
Muscle cell
Na+
Arrival of an electrical stimulus triggers release of acetylcholine, which binds to the acetylcholine receptor on the muscle cell…
…activating the entry of sodium, which causes a local membrane depolarization.
Acetylcholinesterase degrades the transmitter, terminating the signal.
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Figure 2

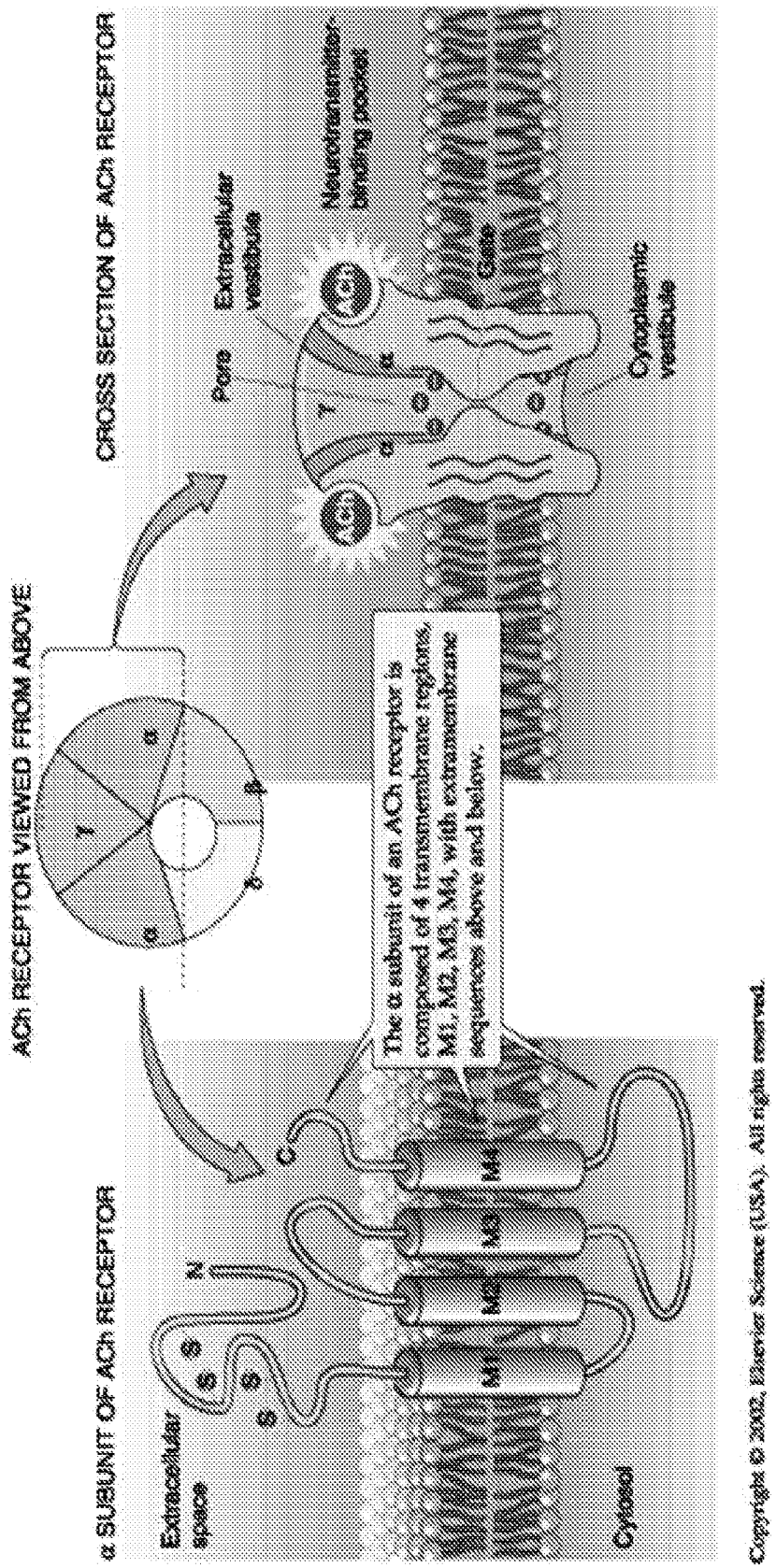
α SUBUNIT OF ACh RECEPTOR
Extracellular space
Cytosol
N
C
M1
M2
M3
M4
ACh RECEPTOR VIEWED FROM ABOVE
α
γ
δ
β
CROSS SECTION OF ACh RECEPTOR
Pore
Extracellular vestibule
Neurotransmitter-binding pocket
Gate
Cytoplasmic vestibule
The α subunit of an ACh receptor is composed of 4 transmembrane regions, M1, M2, M3, M4, with extramembrane sequences above and below.
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Figure 3

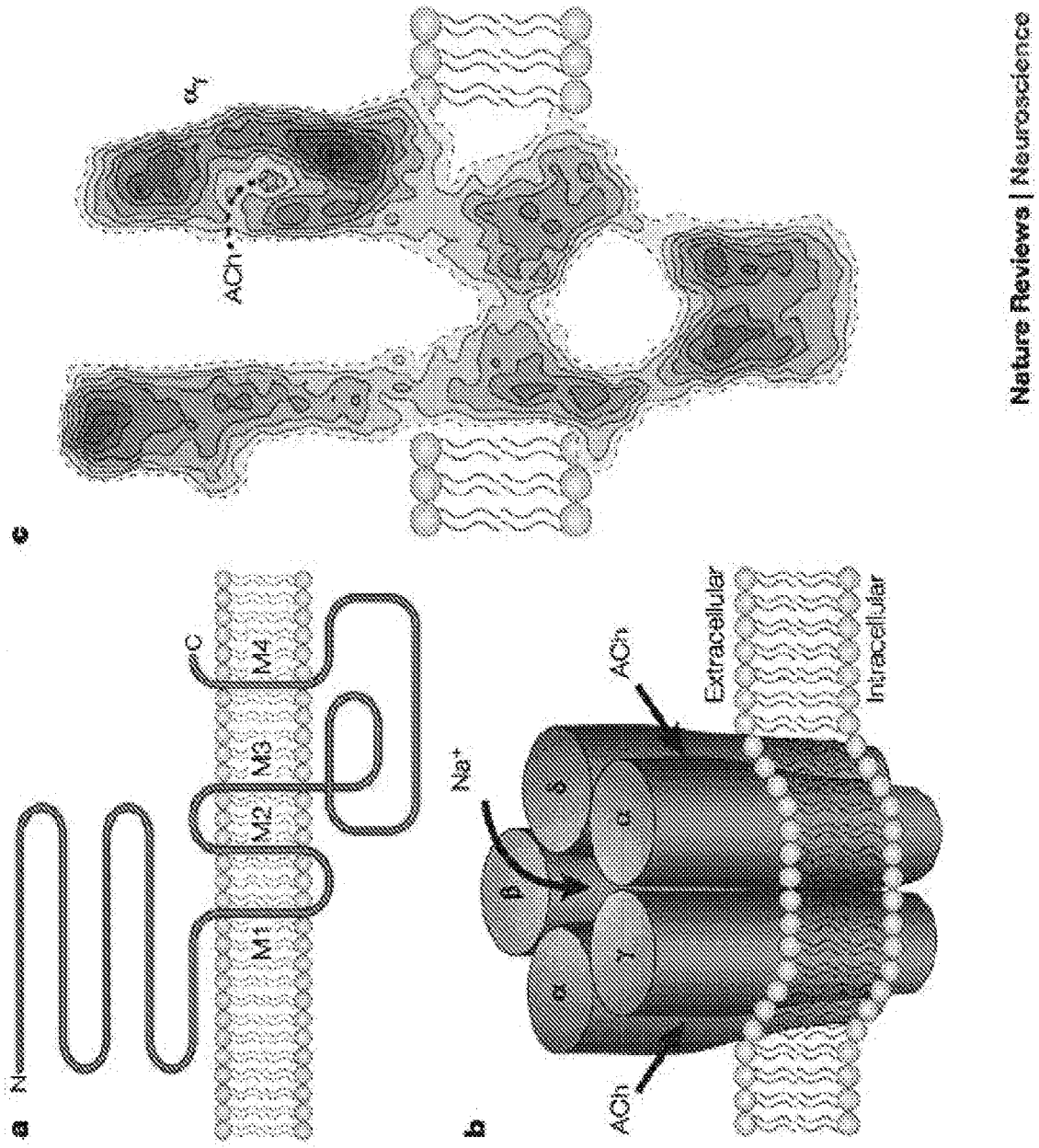
a
N
C
M1
M2
M3
M4
b
Na+
ACh
ACh
Extracellular
Intracellular
c
ACh
Nature Reviews | Neuroscience

Figure 4

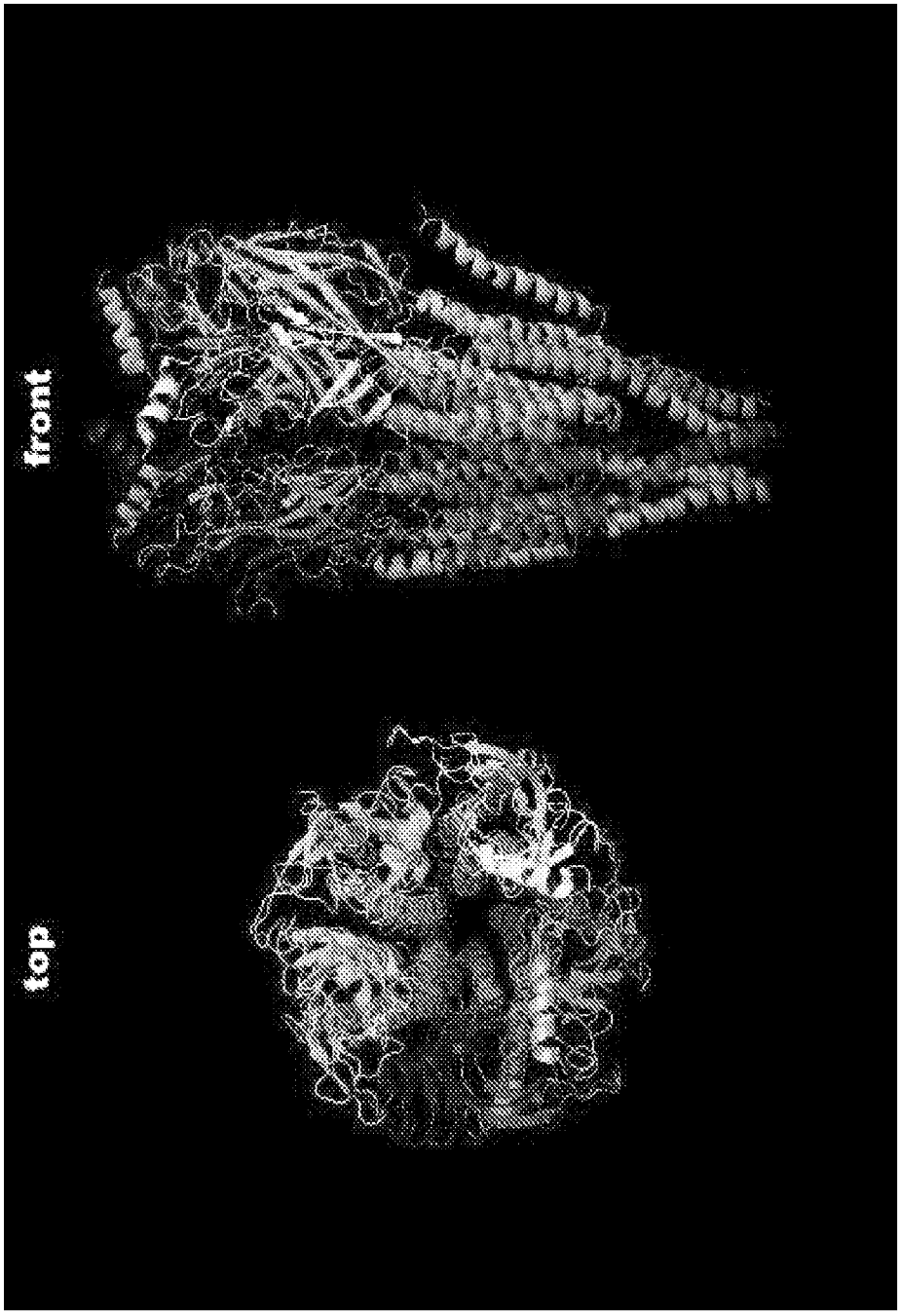

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Figure 5

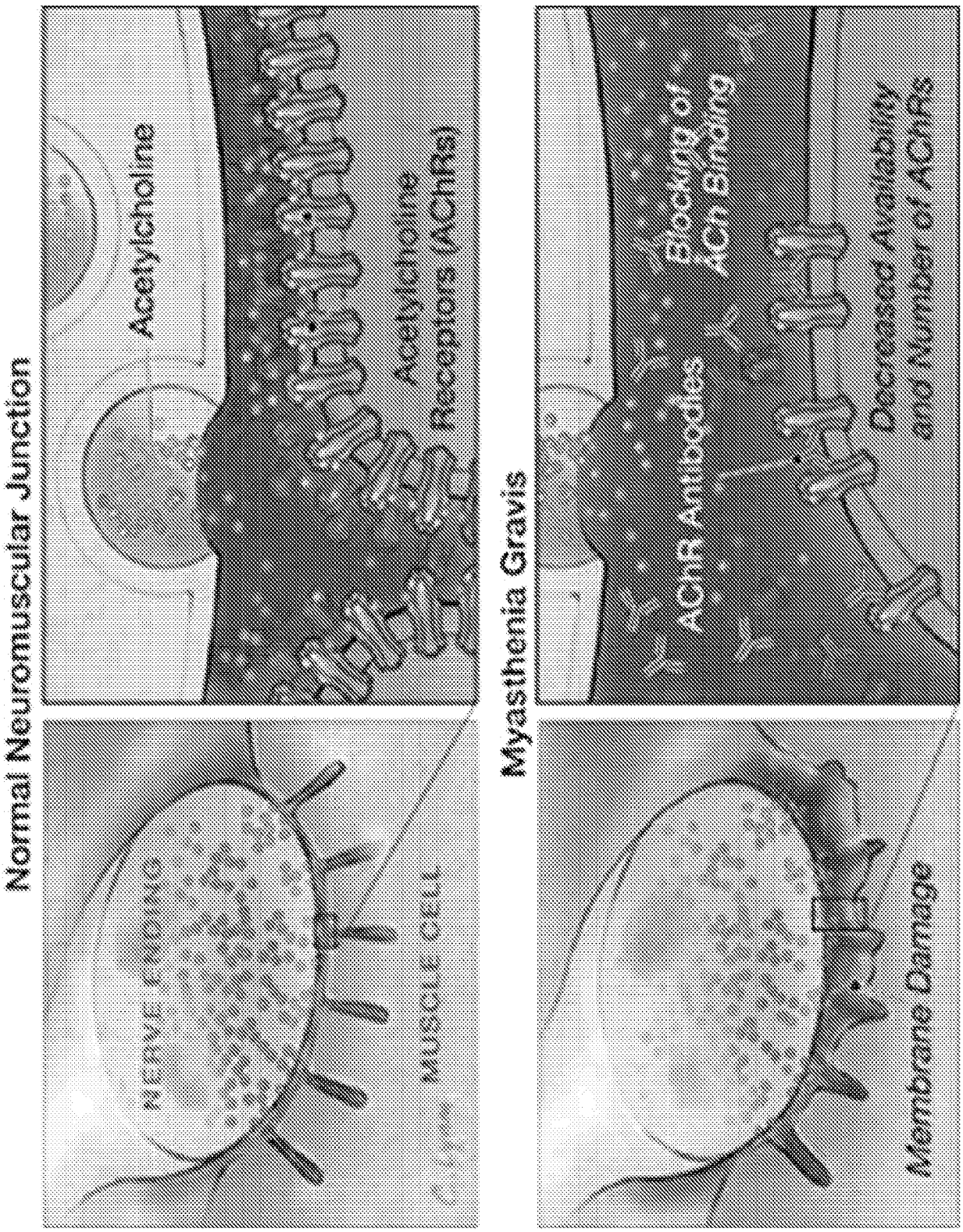
Normal Neuromuscular Junction
Acetylcholine
Acetylcholine Receptors (AChRs)
Myasthenia Gravis
AChR Antibodies Bind to AChRs
Blocking of Ach Binding
Destruction of AChRs
Decreased Availability and Number of AChRs
Membrane Damage

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Figure 6

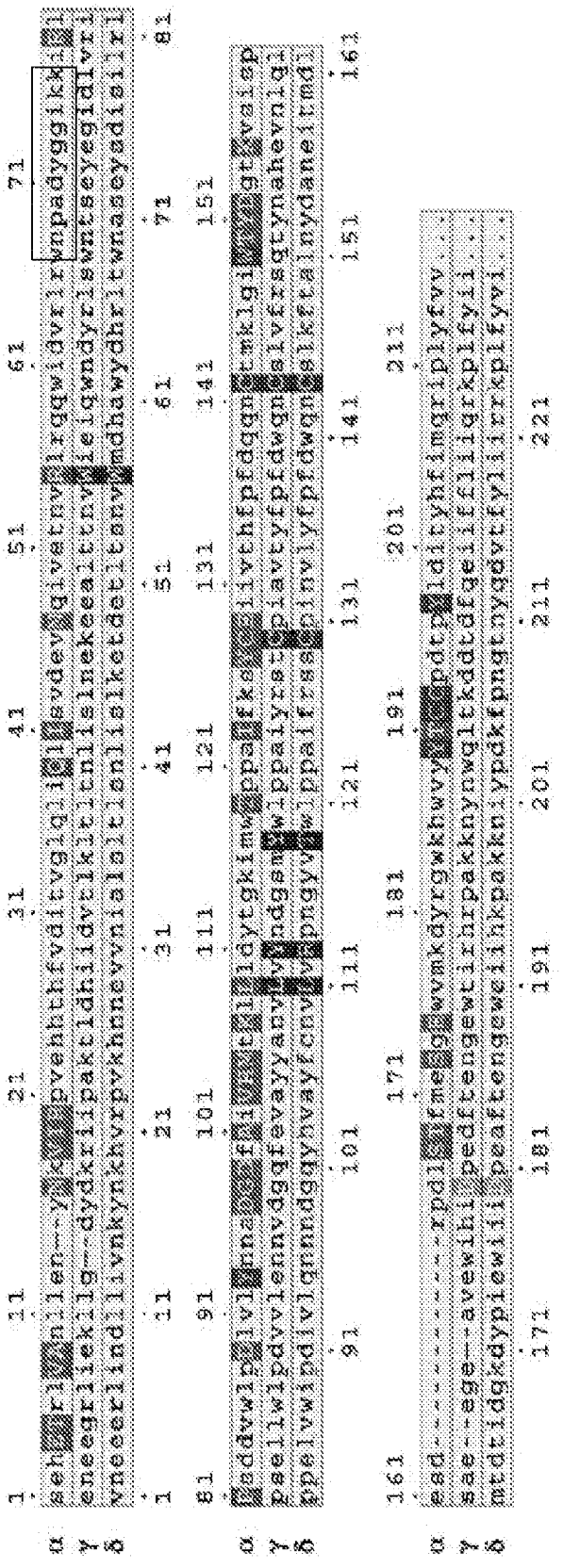
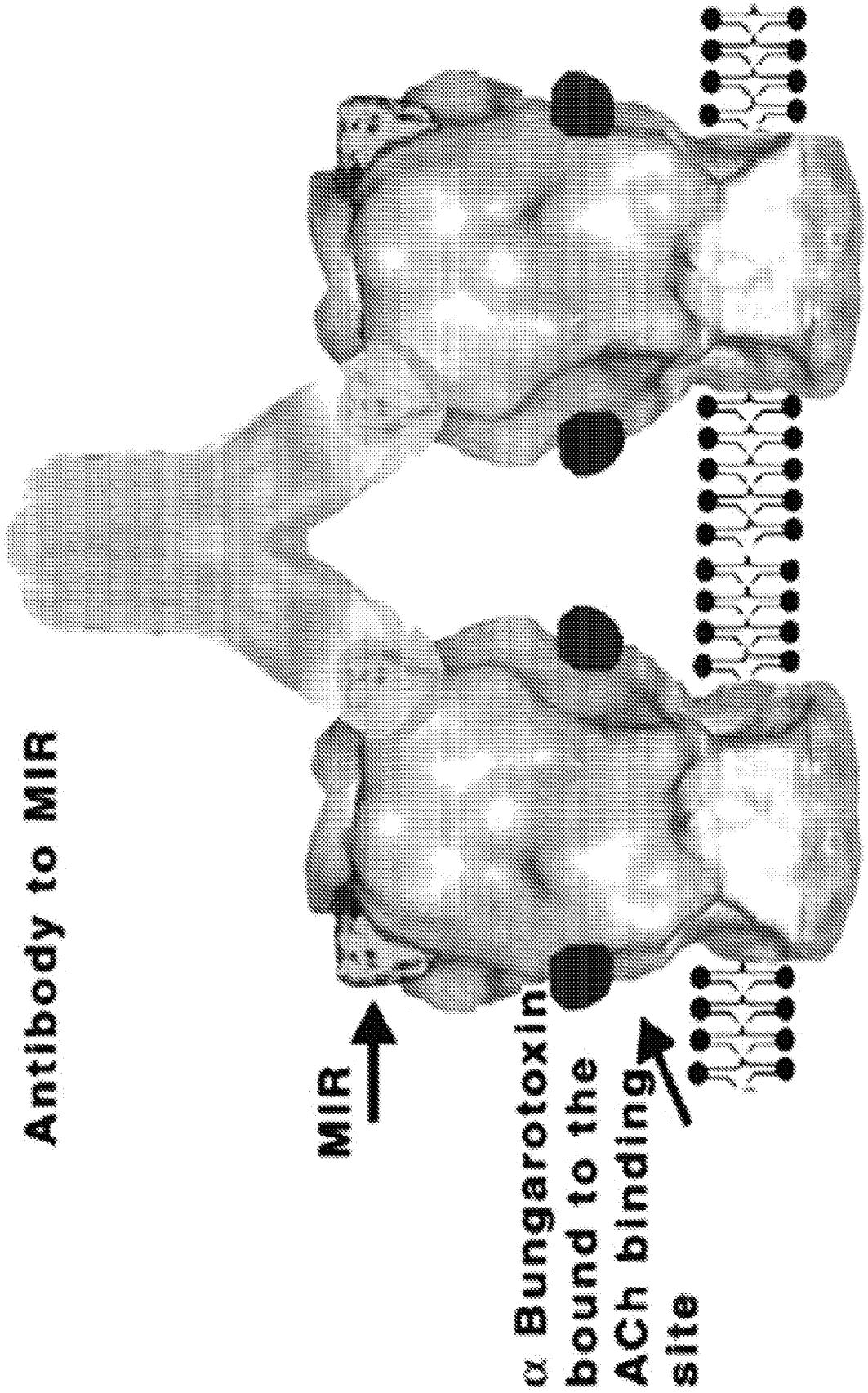


Figure 7


Antibody to MIR
MIR
α Bungarotoxin bound to the ACh binding site

INTERNATIONAL SEARCH REPORT

International application No.: PCT/US 11/38185

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/02; C12N 1/20; C12N 1/00 (2011.01)

USPC - 424/200.1 ; 435/252.1 , 435/252.8; 435/243

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 39/02; C12N 1/20; C12N 1/00 (201 1.01)

USPC - 424/200.1 ; 435/252.1 , 435/252.8; 435/243; 24/234.1; 435/471 ; 435/176, 435/177; 424/204.1

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

IPC(8) - A61K 39/02; C12N 1/20; C12N 1/00 (201 1.01) - see keyword below

USPC - 424/200.1 ; 435/252.1 , 435/252.8; 435/243; 24/234.1; 435/471 ; 435/176, 435/177; 424/204.1 - see keyword below

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

PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: autoantigen, alloantigen, microbial, microorganisms, bacteria, dead, inactivated, killed, oral tolerance, mucosal, mucous, oral, spray, nasal, intranasal, inhalation, transplant, autoimmune, graft, rejection, pharmaceutical carrier, auxiliary, excipient, treating, inhibiting, prevent, fungi, yeast, al

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2005/0272030 A 1 (Braun) 08 December 2005 (08.12.2005), Abstract, para [0002], [0043], [0044], [0048], [0055], [0121], [0145], and [0165]	2 --- 1, 3-4
Y	US 2009/0148389 A 1 (ROTTIERS et al.) 11 June 2009 (11.06.2009), Abstract, [0002], [0006], [0008], [0027], [0032], [0034], [0042], and [0062]	1, 3-4
Y	US 2003/0078208 A 1 (Wilkes) 24 April 2003 (24.04.2003), para [0019], [0058], [0076], and [0082]	3
A	SUN et al. Cholera toxin B subunit: An efficient transmucosal carrier-delivery system for induction of peripheral immunological tolerance. Proc Natl Acad Sci U S A. 1994, Vol. 91(23), p. 10795-9. Entire document especially Abstract	1-4
A	MOTA et al. Genetic transformation of novel isolates of chicken Lactobacillus bearing probiotic features for expression of heterologous proteins: a tool to develop live oral vaccines. BMC Biotechnol. 2006 , Vol. 6(2), PDF file pg 1-1 1. Entire document, especially Abstract; and pg 5, col 2	1-4
A	HUIBREGTSE et al. Induction of antigen-specific tolerance by oral administration of Lactococcus lactis delivered immunodominant DQ8-restricted gliadin peptide in sensitized nonobese diabetic Abo Dq8 transgenic mice. J Immunol. 2009, Vol. 183(4), p. 2390-6. Epub 2009 Jul 27. Entire document, especially Abstract	1-4

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 September 201 1 (01 .09.201 1)	09 SEP 2011

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/38185

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Hanninen, Prevention of Autoimmune Type 1 Diabetes via Mucosal Tolerance: Is Mucosal Autoantigen Administration As Safe and Effective As It Should? Scand. J. Immunol. 2000, Vol. 52, p. 217-225. Entire document, especially Abstract; and pg 223, col 1, Conclusions	1-4