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(71) Applicant: **COUR PHARMACEUTICALS DEVELOPMENT COMPANY INC.** [US/US]; 2215 Sanders Road, Suite 425, Northbrook, Illinois 60062 (US).

(72) Inventor: **GETTS, Daniel R.**; 2215 Sanders Road, Suite 425, Northbrook, Illinois 60062 (US).

(74) Agents: **NEVILLE, Katherine, L.** et al.; Marshall, Gerstein & Borun LLP, 233 S. Wacker Drive, 6300 Willis Tower, Chicago, Illinois 60606-6357 (US).

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(54) Title: TIMPS ENCAPSULATING JAPANESE CEDAR POLLEN EPITOPES

(57) Abstract: The present invention provides compositions comprising particles with a negative zeta potential that encapsulate one or more epitopes associated with Japanese cedar pollen. Methods of inducing immunological tolerance to Japanese cedar pollen by administering said particles are also provided.

TIMPS ENCAPSULATING JAPANESE CEDAR POLLEN EPITOPES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/293,261, filed February 9, 2016, the content of which is incorporated herein by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: COUR_014_02WO_SeqList_ST25.txt, date recorded: February 9, 2017, file size: 12 kb).

BACKGROUND OF THE INVENTION

[0003] Japanese cedar pollinosis is a common allergic disease in Japan caused by inhalation of the pollen of the Japanese cedar (*Cryptomeria japonica*). The prevalence of Japanese cedar pollinosis in Japan may be as high as 30% in individual, non-randomized populations in schools, factories, and local communities, and it is estimated that up to 20 million people may suffer from the disease. As such, the disease is a major public health problem in Japan in part due to the severity of symptoms, high prevalence, poor spontaneous recovery rate, and high healthcare expenses associated with controlling the disease.

[0004] Japanese cedar pollinosis is a serious type I allergic disease. Type I allergic diseases are mediated by aberrant Th2-polarized immune responses to otherwise innocuous environmental antigens (*e.g.*, Japanese cedar pollen) characterized by elevated systemic levels of allergen-specific immunoglobulin E (IgE), mast cell degranulation, and the release of histamine and other chemical mediators of allergy. The primary clinical approaches to the treatment of allergic diseases consist generally of antigen (Ag) avoidance (*e.g.*, allergen avoidance) and symptom control by targeting acute effector molecules with drugs such as antihistamines, leukotriene inhibitors, or broad-acting glucocorticoids. However, such treatments fail to address the development of the underlying aberrant Th2-biased immune response that drives allergic inflammation. Alternative approaches, such as specific

immunotherapy (SIT), wherein patients are exposed to gradually increasing doses of soluble Ag delivered mucosally or subcutaneously are also used clinically. SIT induces both regulatory and Th1 responses that inhibit established Th2 responses. As such, SIT can result in the deviation of the immune responses to environmental allergens away from a pathologic, Th2-biased response and towards a protective or regulatory Th1/T regulatory response. However, administration of soluble Ag to sensitized patients carries considerable risks of adverse reactions, thereby necessitating slow dose escalation or co-administration of the antigen with additional drugs that minimize anaphylaxis, such as omalizumab.

[0005] Therefore, improved methods to safely and effectively induce Ag-specific tolerance remain a sought after clinical tool for the treatment of allergic disease in pre-sensitized subjects. Nanoparticles encapsulating allergenic peptides have been previously demonstrated to reduce allergen-induced Th2 responses in *in vivo* models (*See* U.S. Publication No. 2015-0209293, incorporated herein by reference in its entirety). It is therefore possible to encapsulate pollens derived from a variety of environmental sources into nanoparticles for use in the treatment of allergic diseases, such as Japanese cedar pollinosis. Further, the Japanese cedar pollen antigen CRYJ1 has a high degree of similarity to the Jun a 1 antigen of mountain cedar, the Cha o 1 antigen of Japanese cypress, and the Cup a 1 antigen of *Cupressus arizonica*, suggesting that particular antigens from Japanese cedar pollen may mediate allergic antibody responses that are cross-reactive across multiple conifer pollens.

[0006] However, attempts to encapsulate Japanese cedar pollen into particles suitable for treatment of allergic diseases have been only partially successful. This is likely because Japanese cedar pollen is highly viscous, even at low concentrations. As such, SIT treatment methods for Japanese cedar pollinosis remain limited. Given the substantial prevalence of the disease, specifically in Japan and neighboring countries, as well as the likelihood of antibody cross-reactivity between conifer pollens there is a need in the art for a safe and effective means to induce tolerance to cedar pollen antigens (*e.g.*, CRYJ1 and CRYJ2) in subjects that suffer from Japanese cedar pollinosis, or in subjects at risk for developing Japanese cedar pollinosis.

SUMMARY OF THE INVENTION

[0007] In some embodiments, the present invention provides compositions (*e.g.*, for induction of antigen-specific tolerance) comprising a carrier particle (*e.g.*, PLG particle) that embeds or is attached to one or more epitopes from Japanese cedar pollen. In

certain embodiments, the carrier particle is a poly(lactide-*co*-glycolide) (PLG) particle with a negative zeta potential.

[0008] Certain embodiments of the present invention are directed to a composition comprising a biodegradable particle comprising one or more encapsulated antigenic epitopes from Japanese cedar pollen, wherein the biodegradable particle has a negative zeta potential. In particular embodiments, the biodegradable particle comprises poly(lactide-co-glycolide) (PLG). In some embodiments, the biodegradable particle comprises PLG with a copolymer ratio of about 50:50 of polylactic acid:polyglycolic acid. In particular embodiments, the surface of the biodegradable particle is carboxylated. In some embodiments, the carboxylation is achieved by using poly(ethylene-maleic anhydride) (PEMA), or poly(acrylic acid) (PAA).

[0009] In particular embodiments, the biodegradable particle has a zeta potential of about -100 mV to about 0 mV. In certain embodiments, the biodegradable particle has a zeta potential of about -50 mV to about -40 mV. In some embodiments, the biodegradable particle has a zeta potential of about -75 mV to about -50 mV. In certain embodiments the biodegradable particle has a zeta potential of about -50 mV.

[0010] In particular embodiments, the biodegradable particle has a diameter of between about 0.1 μm to about 10 μm. In some embodiments, the biodegradable particle has a diameter of between about 0.3 μm to about 5 μm. In certain embodiments, the biodegradable particle has a diameter of between about 0.5 μm to about 3 μm. In particular embodiments, the biodegradable particle has a diameter of between about 0.5 μm to about 1 μm. In some embodiments, the biodegradable particle has a diameter of about 0.2 μm to about 0.7 μm. In particular embodiments, the biodegradable particle has a diameter of about 0.5 μm.

[0011] Certain embodiments of the present invention are directed to a composition comprising a biodegradable particle with a negative zeta potential that comprises one or more encapsulated antigenic epitopes from Japanese cedar pollen. In some embodiments, the one or more encapsulated antigenic epitopes from Japanese cedar pollen comprises Cry j 1, Cry j 2, Cry j 3, Cry j 4, Cry j IFR, Cry j Chitinase, Cry j Asp, Cry j LTP, and/or Cry j CPA9. In particular embodiments, the one or more encapsulated antigenic epitopes from Japanese cedar pollen comprises CRYJ1 or a fragment or variant thereof. In some embodiments, CRYJ1 has an amino acid sequence of MDNPIDSSWRGDSNWAQNRMKLADSAVGFGSSTMGGKGGDLYTVTNSDDDPVNP APGTLRYGATRDRPLWIIFSGNMNIKLKMPMYIAGYKTFDGRGAQVYIGNGGPSVFI

KRVSNVIIHGLHLYGSSTSVLGNVLINESFGVEPVHPQDGDALTLRTATNIWIDHNSFS
NSSDGLVDVTLSSTGVTISNNLFFNHHKVMLLGHDDAYSDDKSMKVTVAFNQFGPN
SGQRMPRARYGLVHVANNNYDPWTIYAIGGSSNPTILSEGNSFTAPNESYKKQVTIRI
GSKTSSSSSNWVWQSTQDVFYNGAYFVSSGKYEGGNIYTKKEAFN (SEQ ID NO: 1).

[0012] In some embodiments, the fragment of CRYJ1 comprises at least 10, at least 20, at least 30, at least 40, or at least 50 consecutive amino acids with at least a 90% sequence identity to SEQ ID NO: 1. In certain embodiments, the variant of CRYJ1 has an amino acid sequence with at least a 70%, at least a 75%, at least an 80%, at least an 85%, at least a 90%, at least a 95%, at least a 98%, or at least a 99% sequence identity to SEQ ID NO: 1. In some embodiments, the fragment of CRYJ1 is selected from the group consisting of p16–30, p81–95, p106–120, p111–125, p211–225, and p301–315.

[0013] In particular embodiments, the one or more encapsulated antigenic epitopes from Japanese cedar pollen comprises CRYJ2 or a fragment or variant thereof. In some embodiments, CRYJ2 has an amino acid sequence of VENGNATPQLTKNAGVLTSSLSKRCRKVEHSRHDAINIFNVEKYGAVGDGKHDSTE
AFSTAWQAASKKPSAMLLVPGNKKFVVNNLFFNGPSQPHFTFKVDGIIAAYQNPAS
WKNNRIWLQFAKLTGFTLMGKGVIDGQGKQWWAGQSKWVNGREISNDRDRPTAIK
FDFSTGLIIQGLKLMNSPEFHLVFGNSEGVKIIGISITAPRDSPNTDGIDIFASKNFHLQK
NTIGTGDDSVAIGTGSSNIVIEDLISGPGHGISIGSLGRENSRAEVSYVHVNGAKFIDTQ
NGLRIKTWQGGSGMASHIIYENVEMINSENPILINQFYSTSASASQNQRSAVQIQDVT
YKNIRGTSATAAAIQLKSSDSMPSKDIKLSDISLKLTSGKIASSL (SEQ ID NO: 2).

[0014] In some embodiments, the fragment of CRYJ2 comprises at least 10, at least 20, at least 30, at least 40, or at least 50 consecutive amino acids with at least a 90% sequence identity to SEQ ID NO: 2. In certain embodiments, the variant of CRYJ2 has an amino acid sequence with at least a 70%, at least a 75%, at least an 80%, at least an 85%, at least a 90%, at least a 95%, at least a 98%, or at least a 99% sequence identity to SEQ ID NO: 2. In some embodiments, the fragment of CRYJ2 is selected from the group consisting of p66–80, p81–95, p141–155, p186–200, p236–250, p346–360, p351–365, and p336–350.

[0015] In particular embodiments, biodegradable particles described herein comprise two or more encapsulated antigenic epitopes from Japanese cedar pollen proteins. In some embodiments, the two or more encapsulated epitopes are contained in a fusion protein, wherein the two or more encapsulated epitopes in the fusion protein are separated by a cleavable linker. In certain embodiments, the amino acid sequence of the cleavable linker is cleavable by a protease located in the phagolysosome of a cell and/or a protease located in the

cytosol of the cell. In some embodiments, the amino acid sequence of the cleavable linker is cleavable by a protease located in the phagolysosome of a cell and a protease located in the cytosol of the cell. In particular embodiments, the cleavable linker a furin sensitive linker or cathepsin sensitive linker. In certain embodiments, the cleavable linker is a furin sensitive linker. In some embodiments, the cleavable linker is a cathepsin sensitive linker. In particular embodiments, the cathepsin sensitive linker is sensitive to cleavage by one or more of cathepsin A, cathepsin B, cathepsin C, cathepsin D, cathepsin E, cathepsin F, cathepsin G, cathepsin H, cathepsin K, cathepsin L, cathepsin O, cathepsin W, and/or cathepsin Z. In some embodiments, the amino acid sequence of the linker is Gly-Ala-Val-Val-Arg-Gly-Ala (SEQ ID NO: 3).

[0016] In particular embodiments, the one or more encapsulated antigenic epitopes from Japanese cedar pollen is covalently coupled to the biodegradable particle. In certain embodiments the one or more encapsulated antigenic epitopes from Japanese cedar pollen is covalently coupled to the biodegradable particle by a conjugate molecule. In some embodiments, the conjugate molecule comprises a carbodiimide compound. In particular embodiments, the carbodiimide compound comprises 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC).

[0017] Particular embodiments are directed to a pharmaceutical composition comprising a biodegradable particle described herein. In some embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier. In particular embodiments, the pharmaceutical composition further comprises pharmaceutically acceptable excipients. Certain embodiments are directed to a lyophilized composition comprising a biodegradable particles described herein.

[0018] Certain embodiments are directed to a method of inducing antigen-specific tolerance to Japanese cedar pollen in a subject comprising administering to the subject an effective amount of a pharmaceutical composition described herein. Particular embodiments are directed to a method for the treatment of a Japanese cedar pollen allergy in a subject in need thereof comprising administering a pharmaceutical composition described herein. Some embodiments are directed to a method for the prevention of a Japanese cedar pollen allergy in a subject in need thereof comprising administering the pharmaceutical composition described herein.

[0019] Particular embodiments are directed to a method of inducing antigen-specific tolerance to Japanese cedar pollen in a subject comprising reconstituting the lyophilized particles described herein to obtain a reconstituted pharmaceutical composition

and administering the reconstituted pharmaceutical composition to the subject. Certain embodiments are directed to a method for the treatment of a Japanese cedar pollen allergy in a subject in need thereof comprising reconstituting lyophilized particles described herein to obtain a reconstituted pharmaceutical composition and administering the reconstituted pharmaceutical composition to the subject. Some embodiments are directed to a method for the prevention of a Japanese cedar pollen allergy in a subject in need thereof comprising reconstituting the lyophilized particles described herein to obtain a reconstituted pharmaceutical composition and administering the reconstituted pharmaceutical composition to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. 1 shows a model of how administration of biodegradable particles with negative zeta potential encapsulating antigenic epitopes from Japanese cedar pollen provides an effective treatment of Japanese cedar pollen allergy.

[0021] FIG. 2 shows the viscosity of JCP extract (FIG. 2A), an exemplary illustration of a biodegradable particle with negative zeta potential that encapsulates antigenic epitopes of Japanese cedar pollen (TIMP-JCP) (FIG. 2B), and a schematic of the double-emulsion process (FIG. 2 C).

[0022] FIG. 3 shows SDS-PAGE analysis of JCP extract and recombinant JCP proteins.

[0023] FIG. 4 shows particle characteristics for TIMPs encapsulation JCP extract.

[0024] FIG. 5 shows a schematic of the acute inflammatory mouse model (FIG. 5A) and antibody responses on Day 21 after sensitization (FIG. 5B-5D).

[0025] FIG. 6 shows body temperature changes after JCP sensitization, TIMP treatment, and JCP challenge.

[0026] FIG. 7 shows scratching, sneezing, and coughing scores after JCP sensitization, TIMP treatment, and JCP challenge.

[0027] FIG. 8 shows cytokine production from splenocytes from JCP sensitized and challenged mice treated with TIMP-JCP or TIMP-OVA.

[0028] FIG. 9 shows antibody responses on Day 30 after sensitization with JCP.

[0029] FIG. 10 shows serum levels of histamine (FIG. 10A) and MCPT-1 (FIG. 10B).

[0030] FIG. 11 illustrates the effect of administration route on the resultant immune response.

DETAILED DESCRIPTION OF THE INVENTION

[0031] The present inventors have found that nanoparticles that embed an antigen can induce tolerance to autoimmune disease and decrease the immune response. These particles, therefore, may be useful in the treatment of any disease or condition characterized by an excessive inflammatory immune response, such as allergy to Japanese cedar pollen.

[0032] "Particle" as used herein refers to any non-tissue derived composition of matter, it may be a sphere or sphere-like entity, bead, or liposome. The term "particle", the term "immune modifying particle", the term "carrier particle", and the term "bead" may be used interchangeably depending on the context. Additionally, the term "particle" may be used to encompass beads and spheres.

[0033] "Negatively charged particle" as used herein refers to particles which have been modified to possess a net surface charge that is less than zero.

[0034] "Carboxylated particles" or "carboxylated beads" or "carboxylated spheres" includes any particle that has been modified to contain a carboxyl group on its surface. In some embodiments the addition of the carboxyl group enhances phagocyte/monocyte uptake of the particles from circulation, for instance through the interaction with scavenger receptors such as MARCO. Carboxylation of the particles can be achieved using any compound which adds or incorporates carboxyl groups, including, but not limited to, poly(ethylene-alt-maleic anhydride) (PEMA).

[0035] "Antigenic moiety" as used herein refers to any moiety, for example a peptide that is recognized by the host's immune system. Examples of antigenic moieties include, but are not limited to, autoantigens, enzymes, and/or bacterial or viral proteins, peptides, drugs or components. Without being bound by theory, while the carboxylated beads themselves may be recognized by the immune system, the carboxylated beads with nothing more attached thereto are not considered an "antigenic moiety" for the purposes of the invention.

[0036] "Naked beads" or "naked particles" or "naked spheres" as used herein refers to beads, particles or spheres that have not been carboxylated.

[0037] "Pro-inflammatory mediators" or "pro-inflammatory polypeptides" as used herein refers to polypeptides or fragments thereof which induce, maintain, or prolong

inflammation in a subject. Examples of pro-inflammatory mediators include, but are not limited to, cytokines and chemokines.

[0038] As used herein, the term "Inflammatory monocyte" refers to any myeloid cell expressing any combination of CD14/CD26 and CCR2.

[0039] As used herein, the term "inhibitory neutrophil" refers to neutrophils, and/or monocyte derived suppressor cells.

[0040] As used herein, the term "Th cell" or "helper T cell" refers to $CD4^+$ cells. $CD4^+$ T cells assist other white blood cells with immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. T cells become activated when they are presented with peptide antigens by MHC class II molecules, which are expressed on the surface of antigen-presenting cells (APCs).

[0041] As used herein, the term "Th1 cell" refers to a subset of Th cells which produce pro-inflammatory mediators. Th1 cells secrete cytokines to facilitate immune response and play a role in host defense against pathogens in part by mediating the recruitment of neutrophils and macrophages to infected tissues. Th1 cells secrete cytokines including IFN-γ, IL-2, IL-10, and TNF α/β to coordinate defense against intracellular pathogens such as viruses and some bacteria.

[0042] As used herein, the term "Th2 cell" refers to a subset of Th cells that mediate the activation and maintenance of the antibody-mediated immune response against extracellular parasites, bacteria, allergens, and toxins. Th2 cells mediate these functions by producing various cytokines such as IL-4, IL-5, IL-6, IL-9, IL-13, and IL-17E (IL-25) that are responsible for antibody production, eosinophil activation, and inhibition of several macrophage functions, thus providing phagocyte-independent protective responses.

[0043] As used herein, the term "Th17 cell" refers to a subset of Th cells. Th17 cells secrete cytokines to facilitate immune response and play a role in host defense against pathogens by mediating the recruitment of neutrophils and macrophages to infected tissues. TH17 cells secrete cytokines such as IL-17, IL-21, IL-22, IL-24, IL-26 and TNFα to coordinate defense against extracellular pathogens including fungi and bacteria.

[0044] "Coupled" as used herein refers to an antigen fixed to the outside of a particle or encapsulated within a particle. Thus, an antigen coupled to a particle includes both surface coupling as well as encapsulation within the particle.

[0045] The term "IMP" as used herein refers to immune-modifying particles which are not coupled to an antigen. The term "TIMP" as used herein refers to tolerizing

immune modifying particles which are coupled to an antigen. In some embodiments, the antigen is attached to the surface of the TIMP. In other embodiments, the antigen is encapsulated within the TIMP.

[0046] The particle may have any particle shape or conformation. However, in some embodiments it is preferred to use particles that are less likely to clump in vivo. Examples of particles within these embodiments are those that have a spherical shape.

[0047] Another aspect of the invention relates to a composition which comprises an immune modified particle having a negative zeta potential and free from antigenic moieties. In a further embodiment, the invention provides compositions comprising an immune modified particle with a negative zeta potential coupled to an antigen. In a further embodiment, the antigen is coupled to the outside of the particle. In a preferred embodiment, the antigen is encapsulated within the particle.

[0048] Yet another aspect of the invention relates to a process for the preparation an immune modified particle with a negative zeta potential and free from antigenic moieties. The process involves contacting an immune modified particle precursor with a buffer solution under conditions effective to form the immune modified particle with a negative zeta potential. In some embodiments of this invention, the immune modified particle precursor is formed via co-polymerization. The particle microstructure may depend on the method of co-polymerization.

[0049] In some embodiments, an antigenic peptide molecule is coupled to the carrier particle (*e.g.* immune modified particle) by a conjugate molecule and/or linker group. In some embodiments, coupling of the antigenic peptide and/or apoptotic signaling molecule to the carrier (*e.g.*, PLG particle) comprises one or more covalent and/or non-covalent interactions. In some embodiments, the antigenic peptide is attached to the surface of the carrier particle with a negative zeta potential. In some embodiments, the antigenic peptide is encapsulated within the carrier particle with a negative zeta potential.

[0050] In one embodiment, the buffer solution contacting the immune modified particle may have a basic pH. Suitable basic pH for the basic solution include 7.1, 7.5, 8.0, 8.5, 9.5, 10.0 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, and 13.5. The buffer solution may also be made of any suitable base and its conjugate. In some embodiments of the invention, the buffer solution may include, without limitation, sodium bicarbonate, potassium bicarbonate, lithium bicarbonate, potassium dihydrogen phosphate, sodium dihydrogen phosphate, or lithium dihydrogen phosphate and conjugates thereof.

[0051] In one embodiment of the invention, the immune modified particles contain co-polymers. These co-polymers may have varying molar ratio. Suitable co-polymer ratio of present immune modified particles may be 25:75, 30:70, 35:65, 40:60, 45:55, 50:50, 55:45, 60:40, 65:35, 70:30, 75:25, 80:20, 81:19, 82:18, 83:17, 84:16, 85:15, 86:14, 87:13, 88:12, 89:11, 90:10, 91;:9, 92:8, 93:7, 94:6, 95:5, 96:4, 97:3, 98:2, 99:1, or 100:0. In another embodiment, the co-polymer may be periodical, statistical, linear, branched (including star, brush, or comb co-polymers) co-polymers. In some embodiments, the co-polymers ratio may be, but not limited to, polystyrene:poly(vinyl carboxylate)/80:20, polystyrene: poly(vinyl carboxylate)/90:10, poly(vinyl carboxylate):polystyrene/80:20, poly(vinyl carboxylate):polystyrene/90:10, polylactic acid: polyglycolic acid/50:50, polylactic acid: polyglycolic acid/80:20, or polylactic acid: polyglycolic acid/90:10.

[0052] In one embodiment, the particles of the invention are made by adding a composition comprising the polymer (*e.g.* PLGA) to a solution of poly(ethylene-maleic anhydride) (PEMA). The concentration of PEMA in the solution can be between about 0.1% and about 10%. In one embodiment, the concentration of PEMA in the solution is between about 0.2% and about 5%. In another embodiment, the concentration of PEMA in the solution is between about 0.1% and 4%. In another embodiment, the concentration of PEMA in the solution is between about 0.1% and 2%. In another embodiment, the concentration of PEMA in the solution is between about 0.5% and 1%. In one embodiment, the percentage of PEMA in solution is 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6% 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5% or 10%. In one embodiment, the percentage of PEMA in the solution is about 0.5%. In another embodiment, the percentage of PEMA in the solution is about 1.0%. Other compounds that may be used include, but are not limited to, poly(ethylene-*alt*-maleic anhydride), poly(isobutylene-*co*-maleic acid), poly(methyl vinyl ether-*alt*-maleic acid), poly(methyl vinyl ether-*alt*-maleic acid monoethyl ester), poly(methyl vinyl ether-*alt*-maleic anhydride), poly(methyl vinyl ether-*alt*-maleic anhydride) cross-linked with 1,9-decadiene powder, poly(styrene-*alt*-maleic acid) sodium salt, poly(vinyl alcohol), poly(acrylic acid), and/or sodium deoxycholate..

[0053] In one embodiment, the particle is a liposome. In a further embodiment, the particle is a liposome composed of the following lipids at the following molar ratios – 30:30:40 phosphatidylcholine:phosphatidylglycerol:cholesterol. In yet a further embodiment, the particle is encapsulated within a liposome.

[0054] It is not necessary that each particle be uniform in size, although the particles must generally be of a size sufficient to be sequestered in the spleen or liver and

trigger phagocytosis or uptake through receptor or non-receptor mediated mechanism by an antigen presenting cell, including endothelial cell or other MPS cell. Preferably, the particles are microscopic or nanoscopic in size, in order to enhance solubility, avoid possible complications caused by aggregation *in vivo* and to facilitate pinocytosis. Particle size can be a factor for uptake from the interstitial space into areas of lymphocyte maturation. A particle having a diameter of from about 0.1 μm to about 10 μm is capable of triggering phagocytosis. Thus in one embodiment, the particle has a diameter within these limits. In another embodiment, the particle has an average diameter of about 0.3 μm to about 5 μm. In still another embodiment, the particle has an average diameter of about 0.5 μm to about 3 μm. In another embodiment, the particle has an average diameter of about 0.2 μm to about 2μm. In a further embodiment the particle has an average size of about 0.1 μm, or about 0.2 μm or about 0.3 μm or about 0.4 μm or about 0.5 μm or about 1.0 μm or about 1.5 μm or about 2.0 μm or about 2.5 μm or about 3.0 μm or about 3.5 μm or about 4.0 μm or about 4.5 μm or about 5.0 μm. In a particular embodiment the particle has an average size of about 0.5 μm. In some embodiments, the particle has an average diameter between about 0.5 μm and about 0.95 μm. For example, in such embodiments, the particle has an average diameter of about 0.5 μm, 0.55 μm, 0.6 μm, 0.65 μm, 0.7 μm, 0.75 μm, 0.8 μm, 0.85 μm, 0.9 μm, or about 0.95 μm. In particular embodiments, the particle has an average diameter of about 0.7 μm. In some embodiments, the overall weight of the particles is at least about 1000 kDa. In some embodiments, the overall weight of the particles is about 1000 kDa, 1100 kDa, 1200 kDa, 1300 kDa, 1400 kDa, 1500 kDa, 1600 kDa, 1700 kDa, 1800 kDa, 1900 kDa, 2000 kDa , 2500 kDa, 3000 kDa , 3500 kDa , 4000 kDa, 4500 kDa, 5000 kDa, or more.

[0055] In some embodiments, the overall weights of the particles are less than about 10,000 kDa, less than about 5,000 kDa, or less than about 1,000 kDa, 500 kDa, 400 kDa, 300 kDa, 200 kDa, 100 kDa, 50 kDa, 20 kDa, 10 kDa. The particles in a composition need not be of uniform diameter. By way of example, a pharmaceutical formulation may contain a plurality of particles, some of which have a diameter of about 0.5 μm, while others have a diameter of about 1.0 μm. By way of additional example, a pharmaceutical formulation may contain a plurality of particles, some of which have a diameter of about 0.7 μm, while others have a diameter of about 0.5 μm to about 0.95 μm. Any mixture of particle sizes within these given ranges will be useful.

[0056] The particles of the current invention can possess a particular zeta potential. In certain embodiments, the zeta potential is negative. In one embodiment, the zeta

potential is less than (*e.g.*, more negative than) about -100 mV. In one embodiment, the zeta potential is less than (*e.g.*, more negative than) about -50 mV. In certain embodiments, the particles possess a zeta potential between -100 mV and 0 mV. In a further embodiment, the particles possess a zeta potential between -75 mV and 0 mV. In a further embodiment, the particles possess a zeta potential between -60 mV and 0 mV. In a further embodiment, the particles possess a zeta potential between -50 mV and 0 mV. In still a further embodiment, the particles possess a zeta potential between -40 mV and 0 mV. In a further embodiment, the particles possess a zeta potential between -30 mV and 0 mV. In a further embodiment, the particles possess a zeta potential between -20 mV and +0 mV. In a further embodiment, the particles possess a zeta potential between -10 mV and -0 mV. In a further embodiment, the particles possess a zeta potential between -100mV and -50mV. In another particular embodiment, the particles possess a zeta potential between -75 mV and -50mV. In a particular embodiment, the particles possess a zeta potential between -50 mV and -40mV. In another particular embodiment, the particles possess a zeta potential of less than (*e.g.*, more negative than) about -40mV. In another particular embodiment, the particles possess a zeta potential of at least about -30mV. In another particular embodiment, the particles possess a zeta potential of less than (*e.g.*, more negative than) about -30mV.

[0057] The particles of the present invention can possess a ratio of antigen to polymer (*e.g.*, μg of antigen/mg of polymer). In some embodiments, the ratio of antigen to polymer is between about 1 μg/mg to at least about 5 μg/mg. For example, in some embodiments, the ratio of antigen to polymer is about 1 μg/mg, 1.5 μg/mg, 2 μg/mg, 2.5 μg/1 mg, 3.0 μg/mg, 3.5 μg/mg, 4 μg/mg, 4.5 μg/mg, or about 5 μg/mg. In some embodiments, the ratio of antigen to polymer is at least about 5 μg/mg. For example, in some embodiments, the ratio of antigen to polymer is at least about 5 μg/mg, 5.5 μg/mg, 6 μg/mg, 6.5 μg/mg, 7 μg/mg, 7.5 μg/mg, 8 μg/mg, 8.5 μg/mg, 9.0 μg/mg, 9.5 μg/mg, 10 μg/mg, 10.5 μg/mg, 11 μg/mg, 11.5 μg/mg, 12 μg/mg, 12.5 μg/mg, 13 μg/mg, 13.5 μg/mg, 14 μg/mg, 14.5 μg/mg, or about 15 μg/mg.

[0058] In some embodiments, the charge of a carrier (*e.g.*, positive, negative, neutral) is selected to impart application-specific benefits (*e.g.*, physiological compatibility, beneficial surface-peptide interactions, etc.). In some embodiments, a carrier has a net neutral or negative charge (*e.g.*, to reduce non-specific binding to cell surfaces which, in general, bear a net negative charge). In certain embodiments carriers are capable of being conjugated, either directly or indirectly, to an antigen to which tolerance is desired (also referred to herein as an antigen-specific peptide, antigenic peptide, autoantigen, inducing antigen or tolerizing

antigen). In some instances, a carrier has multiple binding sites (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10... 20... 50... 100, or more) in order to have multiple copies of an antigen-specific peptide, or multiple different peptides, exposed on the surface (*e.g.*, to increase the likelihood of a tolerance response). In some embodiments, a carrier displays a single type of antigenic peptide. In some embodiments, a carrier displays multiple different antigenic peptides on the surface. In some embodiments, a carrier surface displays functional groups for the covalent attachment of selected moieties (*e.g.*, antigenic peptides). In some embodiments, carrier surface functional groups provide sites for non-covalent interaction with selected moieties (*e.g.*, antigenic peptides). In some embodiments, a carrier has a surface to which conjugating moieties may be adsorbed without chemical bond formation.

[0059] The size and charge of the particles are critical for tolerance induction. While the particles will differ in size and charge based on the antigen encapsulated within them, in general, particles of the current invention are effective at inducing tolerance when they are between about 100 nanometers and about 1500 nanometers and have a charge of 0 to about -70 mV and are most effective at inducing tolerance when they are 400-800 nanometers and have a charge of between about -25mV and -70mV. Furthermore, due in part to the concentration of the particles and presence of sucrose and D-mannitol in the lyophilization process, the average particle size and charge of the particles can be slightly altered in the lyophilization process. As used herein, the term "post-synthesis size" and "post synthesis charge" refer to the size and charge of the particle prior to lyophilization. The term "post lyophilization size" and "post lyophilization charge" refer to the size and charge of the particle after lyophilization.

[0060] In some embodiments, the particle is non-metallic. In these embodiments the particle may be formed from a polymer. In a preferred embodiment, the particle is biodegradable in an individual. In this embodiment, the particles can be provided in an individual across multiple doses without there being an accumulation of particles in the individual. Examples of suitable particles include polystyrene particles, PLGA particles, PLURIONICS stabilized polypropylene sulfide particles, and diamond particles. Preferably the particle surface is composed of a material that minimizes non-specific or unwanted biological interactions. Interactions between the particle surface and the interstitium may be a factor that plays a role in lymphatic uptake. The particle surface may be coated with a material to prevent or decrease non-specific interactions. Steric stabilization by coating particles with hydrophilic layers such as poly(ethylene glycol) (PEG) and its copolymers such as PLURONICS® (including copolymers of poly(ethylene glycol)-block-poly(propylene

glycol)-block-poly(ethylene glycol)) may reduce the non-specific interactions with proteins of the interstitium as demonstrated by improved lymphatic uptake following subcutaneous injections. All of these facts point to the significance of the physical properties of the particles in terms of lymphatic uptake. Biodegradable polymers may be used to make all or some of the polymers and/or particles and/or layers. Biodegradable polymers may undergo degradation, for example, by a result of functional groups reacting with the water in the solution. The term "degradation" as used herein refers to becoming soluble, either by reduction of molecular weight or by conversion of hydrophobic groups to hydrophilic groups. Polymers with ester groups are generally subject to spontaneous hydrolysis, *e.g.*, polylactides and polyglycolides.

[0061] Particles of the present invention may also contain additional components. For example, carriers may have imaging agents incorporated or conjugated to the carrier. An example of a carrier nanosphere having an imaging agent that is currently commercially available is the Kodak X-sight nanospheres. Inorganic quantum-confined luminescent nanocrystals, known as quantum dots (QDs), have emerged as ideal donors in FRET applications: their high quantum yield and tunable size-dependent Stokes Shifts permit different sizes to emit from blue to infrared when excited at a single ultraviolet wavelength. (Bruchez, et al., Science, 1998, 281, 2013; Niemeyer, C. M Angew. Chem. Int. Ed. 2003, 42, 5796; Waggoner, A. Methods Enzymol. 1995, 246, 362; Brus, L. E. J. Chem. Phys. 1993, 79, 5566). Quantum dots, such as hybrid organic/inorganic quantum dots based on a class of polymers known as dendrimers, may be used in biological labelling, imaging, and optical biosensing systems. (Lemon, et al., J. Am. Chem. Soc. 2000, 122, 12886). Unlike the traditional synthesis of inorganic quantum dots, the synthesis of these hybrid quantum dot nanoparticles does not require high temperatures or highly toxic, unstable reagents. (Etienne, et al., Appl. Phys. Lett. 87, 181913, 2005).

[0062] Particles can be formed from a wide range of materials. The particle is preferably composed of a material suitable for biological use. For example, particles may be composed of glass, silica, polyesters of hydroxy carboxylic acids, polyanhydrides of dicarboxylic acids, or copolymers of hydroxy carboxylic acids and dicarboxylic acids. More generally, the carrier particles may be composed of polyesters of straight chain or branched, substituted or unsubstituted, saturated or unsaturated, linear or cross-linked, alkanyl, haloalkyl, thioalkyl, aminoalkyl, aryl, aralkyl, alkenyl, aralkenyl, heteroaryl, or alkoxy hydroxy acids, or polyanhydrides of straight chain or branched, substituted or unsubstituted, saturated or unsaturated, linear or cross-linked, alkanyl, haloalkyl, thioalkyl, aminoalkyl, aryl,

aralkyl, alkenyl, aralkenyl, heteroaryl, or alkoxy dicarboxylic acids. Additionally, carrier particles can be quantum dots, or composed of quantum dots, such as quantum dot polystyrene particles (Joumaa et al. (2006); Langmuir 22: 1810-6). Carrier particles including mixtures of ester and anhydride bonds (*e.g.*, copolymers of glycolic and sebacic acid) may also be employed. For example, carrier particles may comprise materials including polyglycolic acid polymers (PGA), polylactic acid polymers (PLA), polysebacic acid polymers (PSA), poly(lactic-co-glycolic) acid copolymers (PLGA or PLG; the terms are interchangeable), [rho]oly(lactic-co-sebacic) acid copolymers (PLSA), poly(glycolic-co-sebacic) acid copolymers (PGSA), polypropylene sulfide polymers, poly(caprolactone), chitosan, etc. Other biocompatible, biodegradable polymers useful in the present invention include polymers or copolymers of caprolactones, carbonates, amides, amino acids, orthoesters, acetals, cyanoacrylates and degradable urethanes, as well as copolymers of these with straight chain or branched, substituted or unsubstituted, alkanyl, haloalkyl, thioalkyl, aminoalkyl, alkenyl, or aromatic hydroxy- or di-carboxylic acids. In addition, the biologically important amino acids with reactive side chain groups, such as lysine, arginine, aspartic acid, glutamic acid, serine, threonine, tyrosine and cysteine, or their enantiomers, may be included in copolymers with any of the aforementioned materials to provide reactive groups for conjugating to antigen peptides and proteins or conjugating moieties. Biodegradable materials suitable for the present invention include diamond, PLA, PGA, polypropylene sulfide, and PLGA polymers. Biocompatible but non-biodegradable materials may also be used in the carrier particles of the invention. For example, non-biodegradable polymers of acrylates, ethylene-vinyl acetates, acyl substituted cellulose acetates, non-degradable urethanes, styrenes, vinyl chlorides, vinyl fluorides, vinyl imidazoles, chlorosulphonated olefins, ethylene oxide, vinyl alcohols, TEFLON® (DuPont, Wilmington, Del.), and nylons may be employed.

[0063] The particles of the instant invention can be manufactured by any means commonly known in the art. Exemplary methods of manufacturing particles include, but are not limited to, microemulsion polymerization, interfacial polymerization, precipitation polymerization, emulsion evaporation, emulsion diffusion, solvent displacement, and salting out (Astete and Sabliov, J. Biomater. Sci. Polymer Edn., 17:247-289(2006)). Manipulation of the manufacturing process for PLGA particles can control particle properties (*e.g.* size, size distribution, zeta potential, morphology, hydrophobicity/hydrophilicity, polypeptide entrapment, etc.). The size of the particle is influenced by a number of factors including, but not limited to, the concentration of PLGA, the solvent used in the manufacture of the particle,

the nature of the organic phase, the surfactants used in manufacturing, the viscosity of the continuous and discontinuous phase, the nature of the solvent used, the temperature of the water used, sonication, evaporation rate, additives, shear stress, sterilization, and the nature of any encapsulated antigen or polypeptide.

[0064] Particle size is affected by the polymer concentration; higher particles are formed from higher polymer concentrations. For example, an increase in PLGA concentration from 1% to 4% (w/v) can increase mean particle size from about 205 nm to about 290 nm when the solvent propylene carbonate is used. Alternatively, in ethyl acetate and 5% Pluronic F-127, an increase in PLGA concentration from 1% to 5% (w/v) increases the mean particle size from 120 nm to 230 nm.

[0065] The viscosity of the continuous and discontinuous phase is also an important parameter that affects the diffusion process, a key step in forming smaller particles. The size of the particles increases with an increase in viscosity of the dispersed phase, whereas the size of the particles decreases with a more viscous continuous phase. In general, the lower the phase ratio of organic to aqueous solvent, the smaller the particle size.

[0066] Homogenizer speed and agitation also affect particle size; in general, higher speeds and agitation cause a decrease in particle size, although there is a point where further increases in speed and agitation no longer decrease particle size. There is a favorable impact in the size reduction when the emulsion is homogenized with a high pressure homogenizer compared with just high stirring. For example, at a phase ratio of 20% in 5% PVA, the mean particle size with stirring is 288 nm and the mean particle size with homogenization (high pressure of 300 bars) is 231 nm.

[0067] An important size reduction of the particles can be achieved by varying the temperature of the water added to improve the diffusion of the solvent. The mean particle size decreases with an increase in water temperature.

[0068] The nature of the polypeptide encapsulated in the particle also affects particle size. In general, encapsulation of hydrophobic polypeptides leads to the formation of smaller particles compared with the encapsulation of more hydrophilic polypeptides. In the double emulsion process, the entrapment of more hydrophilic polypeptides is improved by using high molecular mass PLGA and a high molecular mass of the first surfactant which causes a higher inner phase viscosity. The interaction between the solvent, polymer, and polypeptide affects the efficiency of incorporating the polypeptide into the particle.

[0069] The PLGA molecular mass impacts the final mean particle size. In general, the higher the molecular mass, the higher the mean particle size. For example, as the

composition and molecular mass of PLGA varies (*e.g.* 12 to 48 kDa for 50 : 50 PLGA; 12 to 98 kDa for 75 : 25 PLGA) the mean particle size varies (about 102 nm -154 nm; about 132 nm to 152 nm respectively). Even when particles are the same molecular mass, their composition can affect average particle size; for example, particles with a 50 : 50 ratio generally form particles smaller than those with a 75 : 25 ratio. The end groups on the polymer also affects particle size. For example, particles prepared with ester end-groups form particles with an average size of 740nm (PI=0.394) compared with the mean size for the acid PLGA end-group is 240 nm (PI=0.225).

[0070] The solvent used can also affect particle size; solvents that reduce the surface tension of the solution also reduce particle size.

[0071] The organic solvent is removed by evaporation in a vacuum to avoid polymer and polypeptide damage and to promote final particle size reduction. Evaporation of the organic solvent under vacuum is more efficient in forming smaller particles. For example, evaporation in vacuum produces a mean particle size around 30% smaller than the mean particle size produced under a normal rate of evaporation.

[0072] The amplitude of the sonication wavelength also affects the particle characteristics. The amplitude of the wavelength should be over 20% with 600 to 800 s of sonication to form sable miniemulsions with no more droplet size changes. However, the main draw-back of sonication is the lack of monodispersity of the emulsion formed.

[0073] Organic phases that may be used in the production of the particles of the invention include, but are not limited to, ethyl acetate, methyl ethyl ketone, propylene carbonate, and benzyl alcohol. The continuous phases that may be used, include but are not limited to the surfactant poloxamer 188.

[0074] A variety of surfactants can be used in the manufacturing of the particles of the invention. The surfactant can be anionic, cationic, or nonionic. Surfactants in the poloxamer and poloaxamines family are commonly used in particle synthesis. Surfactants that may be used, include, but are not limited to PEG, Tween-80, gelatin, dextran, pluronic L-63, PVA, methylcellulose, lecithin and DMAB. Additionally, biodegradable and biocompatible surfactants including, but not limited to, vitamin E TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate). In certain embodiments, two surfactants are needed (*e.g.* in the double emulsion evaporation method). These two surfactants can include a hydrophobic surfactant for the first emulsion, and a hydrophobic surfactant for the second emulsion.

[0075] Solvents that may be used in the production of the particles of the invention include, but are not limited to, acetone, Tetrahydrofuran (THF), chloroform, and members of the chlorinate family, methyl chloride. The choice of organic solvents require two selection criteria: the polymer must be soluble in this solvent, and the solvent must be completely immiscible with the aqueous phase.

[0076] Salts that may be used in the production of the particles of the invention include, but are not limited to magnesium chloride hexahydrate, magnesium acetate tetrahydrate.

[0077] Common salting-out agents include, but are not limited to, electrolytes (*e.g.* sodium chloride, magnesium acetate, magnesium chloride), or non-electrolytes (*e.g.* sucrose).

[0078] The stability and size of the particles of the invention may be improved by the addition of compounds including, but not limited to, fatty acids or short chains of carbons. The addition of the longer carbon chain of lauric acid is associated with the improvement of particle characteristics. Furthermore, the addition of hydrophobic additives can improve the particle size, incorporation of the polypeptide into the particle, and release profile. Preparations of particles can be stabilized by lyophilization. The addition of a cryoprotectant such as trehalose can decrease aggregation of the particles upon lyophilization.

[0079] Suitable beads which are currently available commercially include polystyrene beads such as FluoSpheres (Molecular Probes, Eugene, Oreg.).

[0080] In some embodiments, the present invention provides systems comprising: (a) a delivery scaffold configured for the delivery of chemical and/or biological agents to a subject; and (b) antigen-coupled poly(lactide-*co*-glycolide) particles for induction of antigen-specific tolerance. In some embodiments, at least a portion of said delivery scaffold is microporous. In some embodiments, the antigen-coupled poly(lactide-*co*-glycolide) particles are encapsulated within said scaffold. In some embodiments, the chemical and/or biological agents are selected from the group consisting of: protein, peptide, small molecules, nucleic acids, cells, and particles. In some embodiments, chemical and/or biological agents comprise cell, and said cells comprise pancreatic islet cells.

[0081] Physical properties are also related to a nanoparticle's usefulness after uptake and retention in areas having immature lymphocytes. These include mechanical properties such as rigidity or rubberiness. Some embodiments are based on a rubbery core, *e.g.*, a poly(propylene sulfide) (PPS) core with an overlayer, *e.g.*, a hydrophilic overlayer, as in PEG, as in the PPS-PEG system recently developed and characterized for systemic (but not

targeted or immune) delivery. The rubbery core is in contrast to a substantially rigid core as in a polystyrene or metal nanoparticle system. The term rubbery refers to certain resilient materials besides natural or synthetic rubbers, with rubbery being a term familiar to those in the polymer arts. For example, cross-linked PPS can be used to form a hydrophobic rubbery core. PPS is a polymer that degrades under oxidative conditions to polysulphoxide and finally polysulphone, transitioning from a hydrophobic rubber to a hydrophilic, water-soluble polymer. Other sulphide polymers may be adapted for use, with the term sulphide polymer referring to a polymer with a sulphur in the backbone of the mer. Other rubbery polymers that may be used are polyesters with glass transition temperature under hydrated conditions that is less than about 37° C. A hydrophobic core can be advantageously used with a hydrophilic overlayer since the core and overlayer will tend not to mingle, so that the overlayer tends to sterically expand away from the core. A core refers to a particle that has a layer on it. A layer refers to a material covering at least a portion of the core. A layer may be adsorbed or covalently bound. A particle or core may be solid or hollow. Rubbery hydrophobic cores are advantageous over rigid hydrophobic cores, such as crystalline or glassy (as in the case of polystyrene) cores, in that higher loadings of hydrophobic drugs can be carried by the particles with the rubbery hydrophobic cores.

[0082] Another physical property is the surface's hydrophilicity. A hydrophilic material may have a solubility in water of at least 1 gram per liter when it is uncrosslinked. Steric stabilization of particles with hydrophilic polymers can improve uptake from the interstitium by reducing non-specific interactions; however, the particles' increased stealth nature can also reduce internalization by phagocytic cells in areas having immature lymphocytes. The challenge of balancing these competing features has been met, however, and this application documents the creation of nanoparticles for effective lymphatic delivery to DCs and other APCs in lymph nodes. Some embodiments include a hydrophilic component, *e.g.*, a layer of hydrophilic material. Examples of suitable hydrophilic materials are one or more of polyalkylene oxides, polyethylene oxides, polysaccharides, polyacrylic acids, and polyethers. The molecular weight of polymers in a layer can be adjusted to provide a useful degree of steric hindrance in vivo, *e.g.*, from about 1,000 to about 100,000 or even more; artisans will immediately appreciate that all the ranges and values within the explicitly stated ranges are contemplated, *e.g.*, between 10,000 and 50,000.

[0083] The nanoparticles may incorporate functional groups for further reaction. Functional groups for further reaction include electrophiles or nucleophiles; these are convenient for reacting with other molecules. Examples of nucleophiles are primary

amines, thiols, and hydroxyls. Examples of electrophiles are succinimidyl esters, aldehydes, isocyanates, and maleimides.

[0084] A great variety of means, well known in the art, may be used to conjugate antigenic peptides and proteins to carriers. These methods include any standard chemistries which do not destroy or severely limit the biological activity of the antigen peptides and proteins, and which allow for a sufficient number of antigen peptides and proteins to be conjugated to the carrier in an orientation which allows for interaction of the antigen peptide or protein with a cognate T cell receptor. Generally, methods are preferred which conjugate the C-terminal regions of an antigen peptide or protein, or the C-terminal regions of an antigen peptide or protein fusion protein, to the earner. The exact chemistries will, of course, depend upon the nature of the earner material, the presence or absence of C-terminal fusions to the antigen peptide or protein, and/or the presence or absence of conjugating moieties.

[0085] Functional groups can be located on the particle as needed for availability. One location can be as side groups or termini on the core polymer or polymers that are layers on a core or polymers otherwise tethered to the particle. For instance, examples are included herein that describe PEG stabilizing the nanoparticles that can be readily functionalized for specific cell targeting or protein and peptide drug delivery.

[0086] Conjugates such as ethylene carbodiimide (ECDI), hexamethylene diisocyanate, propyleneglycol di-glycidylether which contain 2 epoxy residues, and epichlorohydrin may be used for fixation of peptides or proteins to the carrier surface. Without being bound by theory, ECDI is suspected of carrying out two major functions for induction of tolerance: (a) it chemically couples the protein/peptides to the cell surface via catalysis of peptide bond formation between free amino and free carboxyl groups; and (b) it induces the carrier to mimic apoptotic cell death such that they are picked up by host antigen presenting cells (which may include endothelial cells) in the spleen and induce tolerance. It is this presentation to host T-cells in a non-immunogenic fashion that leads to direct induction of anergy in autoreactive cells. In addition, ECDI serves as a potent stimulus for the induction of specific regulatory T cells.

[0087] In one series of embodiments, the antigen peptides and proteins are bound to the carrier via a covalent chemical bond. For example, a reactive group or moiety near the C-terminus of the antigen (*e.g.*, the C-terminal carboxyl group, or a hydroxyl, thiol, or amine group from an amino acid side chain) may be conjugated directly to a reactive group or moiety on the surface of the carrier (*e.g.*, a hydroxyl or carboxyl group of a PLA or PGA

polymer, a terminal amine or carboxyl group of a dendrimer, or a hydroxyl, carboxyl or phosphate group of a phospholipid) by direct chemical reaction. Alternatively, there may be a conjugating moiety which covalently conjugates to both the antigen peptides and proteins and the carrier, thereby linking them together.

[0088] Reactive carboxyl groups on the surface of a carrier may be joined to free amines (*e.g.*, from Lys residues) on the antigen peptide or protein, by reacting them with, for example, 1 -ethyl-3-[3,9-dimethyl aminopropyl] carbodiimide hydrochloride (EDC) or N-hydroxysuccinimide ester (NHS). Similarly, the same chemistry may be used to conjugate free amines on the surface of a carrier with free carboxyls (*e.g.*, from the C-terminus, or from Asp or Glu residues) on the antigen peptide or protein. Alternatively, free amine groups on the surface of a carrier may be covalently bound to antigen peptides and proteins, or antigen peptide or protein fusion proteins, using sulfo-SIAB chemistry, essentially as described by Arano et al. (199I) Chem. 2:71-6.

[0089] In another embodiment, a non-covalent bond between a ligand bound to the antigen peptide or protein and an anti-ligand attached to the carrier may conjugate the antigen to the carrier. For example, a biotin ligase recognition sequence tag may be joined to the C-terminus of an antigen peptide or protein, and this tag may be biotinylated by biotin ligase. The biotin may then serve as a ligand to non-covalently conjugate the antigen peptide or protein to avidin or streptavidin which is adsorbed or otherwise bound to the surface of the carrier as an anti-ligand. Alternatively, if the antigen peptides and proteins are fused to an immunoglobulin domain bearing an Fc region, as described above, the Fc domain may act as a ligand, and protein A, either covalently or non-covalently bound to the surface of the carrier, may serve as the anti-ligand to non-covalently conjugate the antigen peptide or protein to the carrier. Other means are well known in the art which may be employed to non-covalently conjugate antigen peptides and proteins to carriers, including metal ion chelation techniques (*e.g.*, using a poly-His tag at the C-terminus of the antigen peptide or protein or antigen peptide or protein fusion proteins, and a Ni^{+}-coated carrier), and these methods may be substituted for those described here.

[0090] Conjugation of a nucleic acid moiety to a platform molecule can be effected in any number of ways, typically involving one or more crosslinking agents and functional groups on the nucleic acid moiety and platform molecule. Linking groups are added to platforms using standard synthetic chemistry techniques. Linking groups can be added to nucleic acid moieties using standard synthetic techniques. The practitioner has a number of choices for antigens used in the combinations of this invention. The inducing

antigen present in the combination contributes to the specificity of the tolerogenic response that is induced. It may or may not be the same as the target antigen, which is the antigen present or to be placed in the subject being treated which is a target for the unwanted immunological response, and for which tolerance is desired.

[0091] An inducing antigen of this invention may be a polypeptide, polynucleotide, carbohydrate, glycolipid, or other molecule isolated from a biological source, or it may be a chemically synthesized small molecule, polymer, or derivative of a biological material, providing it has the ability to induce tolerance according to this description when combined with the mucosal binding component.

[0092] In some embodiments, the present invention provides a carrier (*e.g.*, immune modifying particle) coupled to one or more peptides, polypeptides, and/or proteins. In some embodiments, a carrier (*e.g.*, PLG carrier), such as those described herein, are effective to induce antigen-specific tolerance and/or prevent the onset of an immune related disease (such as EAE in a mouse model) and/or diminish the severity of a pre-existing immune related disease. In some embodiments, the compositions and methods of the present invention can cause T cells to undertake early events associated with T-cell activation, but do not allow T-cells to acquire effector function. For example, administration of compositions of the present invention can result in T-cells having a quasi-activated phenotype, such as CD69 and/or CD44 upregulation, but do not display effector function, such as indicated by a lack of IFN-γ or IL-17 synthesis. In some embodiments, administration of compositions of the present invention results in T-cells having a quasi-activated phenotype without having conversion of naive antigen-specific T-cells to a regulatory phenotype, such as those having $CD25^{+/}Foxp3^{+}$ phenotypes.

[0093] In some embodiments, the surface of a carrier (*e.g.*, particle) comprises chemical moieties and/or functional groups that allow attachment (*e.g.*, covalently, non-covalently) of antigenic peptides and/or other functional elements to the carrier. In some embodiments, the number, orientation, spacing, etc. of chemical moieties and/or functional groups on the carrier (*e.g.*, particle) vary according to carrier chemistry, desired application, etc.

[0094] In some embodiments, a carrier comprises one or more biological or chemical agents adhered to, adsorbed on, encapsulated within, and/or contained throughout the carrier. In some embodiments, a chemical or biological agent is encapsulated in and/or contained throughout the particles. The present invention is not limited by the nature of the chemical or biological agents. Such agents include, but are not limited to, proteins, nucleic

acid molecules, small molecule drugs, lipids, carbohydrates, cells, cell components, and the like. In some embodiments, two or more (*e.g.*, 3, 4, 5, etc.) different chemical or biological agents are included on or within the carrier. In some embodiments, agents are configured for specific release rates. In some embodiments, multiple different agents are configured for different release rates. For example, a first agent may release over a period of hours while a second agent releases over a longer period of time (*e.g.*, days, weeks, months, etc.). In some embodiments, the carrier or a portion thereof is configured for slow-release of biological or chemical agents. In some embodiments, the slow release provides release of biologically active amounts of the agent over a period of at least 30 days (*e.g.*, 40 days, 50 days, 60 days, 70 days, 80 days, 90 days, 100 days, 180 days, etc.). In some embodiments, the carrier or a portion thereof is configured to be sufficiently porous to permit ingrowth of cells into the pores. The size of the pores may be selected for particular cell types of interest and/or for the amount of ingrowth desired. In some embodiments, the particles comprise the antigen of interest without other non-peptide active agents, such as drugs or immunomodulators. Furthermore, in some embodiments the particles of the invention do not contain immunostimulatory or immunosuppressive peptides in addition to the antigen of interest. Furthermore, in some embodiments, the particles do not contain other proteins or peptides (*e.g.* costimulatory molecules, MHC molecules, immunostimulatory peptides or immunosuppressive peptides) either on the surface or encapsulated within the particle.

[0095] Encapsulation of the antigen, biological, and/or chemical agents in the particle of the invention has been surprisingly found to induce immunological tolerance and has several advantages. First, the encapsulated particles have a slower cytokine response. Second, when using multiple antigens, biological, and/or chemical agents, encapsulation removes the competition between these various molecules that might occur if the agents were attached to the surface of the particle. Third, encapsulation allows more antigens, biological, and/or chemical agents to be incorporated with the particle. Fourth, encapsulation allows for easier use of complex protein antigens or organ homogenates (*e.g.* pancreas homogenate for type 1 diabetes or peanut extract in peanut allergy). Finally, encapsulation of antigens, biological, and/or chemical agents within the particle instead of conjugation to the surface of the particle maintains the net negative charge on the surface of the particle. The encapsulation of the antigen, biological, and/or chemical agents in the particles of the invention may be performed by any method known in the art. In one embodiment, polypeptide antigens are encapsulated in the particles by a double-emulsion process. In a further embodiment, the polypeptide antigens are water soluble.

[0096] In another embodiment, the polypeptide antigens are encapsulated in the particles by a single-emulsion process. In a further embodiment, the polypeptide antigens are more hydrophobic. Sometimes, the double emulsion process leads to the formation of large particles which may result in the leakage of the hydrophilic active component and low entrapment efficiencies. The coalescence and Ostwald ripening are two mechanisms that may destabilize the double-emulsion droplet, and the diffusion through the organic phase of the hydrophilic active component is the main mechanism responsible of low levels of entrapped active component. In some embodiments, it may be beneficial to reduce the nanoparticle size. One strategy to accomplish this is to apply a second strong shear rate. The leakage effect can be reduced by using a high polymer concentration and a high polymer molecular mass, accompanied by an increase in the viscosity of the inner water phase and in increase in the surfactant molecular mass.

[0097] In certain embodiments, the present invention provides carriers having therein (or thereon) cells or other biological or chemical agents. Where cells are employed, the carriers are not limited to a particular type of cells. In some embodiments, the carriers have thereon pancreatic islet cells. In some embodiments, the microporous carriers additionally have thereon ECM proteins and/or exendin-4. The carriers are not limited to a particular type. In some embodiments, a carrier has regions of varying porosity (*e.g.*, varying pore size, pore depth, and/or pore density). In some embodiments, carriers have thereon (or therein) pharmaceutical agents, DNA, RNA, extracellular matrix proteins, exendin-4, etc. In certain embodiments, the present invention provides methods for transplanting pancreatic islet cells with such carriers. In certain embodiments of this invention, the inducing antigen is a single isolated or recombinantly produced molecule. For treating conditions where the target antigen is disseminated to various locations in the host, it is generally necessary that the inducing antigen be identical to or immunologically related to the target antigen. Examples of such antigens are most polynucleotide antigens, and some carbohydrate antigens (such as blood group antigens).

[0098] Any suitable antigens may find use within the scope of the present invention. In some embodiments, the inducing antigen contributes to the specificity of the tolerogenic response that is induced. The inducing antigen may or may not be the same as the target antigen, which is the antigen present or to be placed in the subject being treated which is a target for the unwanted immunological response, and for which tolerance is desired.

[0099] In certain embodiments of this invention, the inducing antigen is not in the same form as found in pollen, *e.g.*, a polypeptide from Japanese cedar pollen, but is a

fragment or derivative thereof. Inducing antigens of this invention include peptides based on a molecule of the appropriate specificity but adapted by fragmentation, residue substitution, labeling, conjugation, and/or fusion with peptides having other functional properties. The adaptation may be performed for any desirable purposes, including but not limited to the elimination of any undesirable property, such as toxicity or immunogenicity; or to enhance any desirable property, such as mucosal binding, mucosal penetration, or stimulation of the tolerogenic arm of the immune response. Terms such as CRYJ1 or CRYJ2, as used herein, refer not only to the intact subunit, but also to allotypic and synthetic variants, fragments, fusion peptides, conjugates, and other derivatives that contain a region that is homologous (preferably 70% identical, more preferably 80% identical and even more preferably 90% identical at the amino acid level) to at least 10 and preferably 20 consecutive amino acids of the respective molecule for which it is an analog, wherein the homologous region of the derivative shares with the respective parent molecule an ability to induce tolerance to the target antigen.

[00100] It is recognized that tolerogenic regions of an inducing antigen are often different from immunodominant epitopes for the stimulation of an antibody response. Tolerogenic regions are generally regions that can be presented in particular cellular interactions involving T cells. Tolerogenic regions may be present and capable of inducing tolerance upon presentation of the intact antigen. Some antigens contain cryptic tolerogenic regions, in that the processing and presentation of the native antigen does not normally trigger tolerance. An elaboration of cryptic antigens and their identification is found in International Patent Publication WO 94/27634.

[00101] In certain embodiments of this invention, two, three, or a higher plurality of inducing antigens is used. It may be desirable to implement these embodiments when there are a plurality of target antigens, or to provide a plurality of bystanders for the target. It may also be desirable to provide a cocktail of antigens to cover several possible alternative targets. For example, a cocktail of CRYJ1 and CRYJ2 fragments, as well as other epitopes derived from Japanese cedar pollen, could be used to tolerize a subject. In another example, a mixture of allergens may serve as inducing antigen for the treatment of atopy.

[00102] Inducing antigens can be prepared by a number of techniques known in the art, depending on the nature of the molecule. Polynucleotide, polypeptide, and carbohydrate antigens can be isolated from cells of the species to be treated in which they are enriched. Short peptides are conveniently prepared by amino acid synthesis. Longer proteins of known sequence can be prepared by synthesizing an encoding sequence or PCR-

amplifying an encoding sequence from a natural source or vector, and then expressing the encoding sequence in a suitable bacterial or eukaryotic host cell.

[00103] In certain embodiments of this invention, the combination comprises a complex mixture of antigens obtained from a pollen grain, one or more of which plays the role of inducing antigen. The antigens may be in the form of whole cells, either intact or treated with a fixative such as formaldehyde, glutaraldehyde, or alcohol. The antigens may be in the form of a lysate, created by detergent solubilization or mechanical rupture of cells or tissue, followed by clarification. The antigens may also be obtained by subcellular fractionation, particularly an enrichment of plasma membrane by techniques such as differential centrifugation, optionally followed by detergent solubilization and dialysis. Other separation techniques are also suitable, such as affinity or ion exchange chromatography of solubilized membrane proteins.

[00104] Allergens are other antigens for which tolerance of the immune response thereto is also desirable. In one embodiment, the antigen is a polypeptide associated with Japanese cedar pollen. In some embodiments, the antigen is CRYJ1. In certain embodiments, the antigen is CRYJ2.

[00105] In one embodiment, the particles of the invention are coupled to antigens comprising one or more epitopes associated with allergies, autoimmune diseases and/or inflammatory diseases or disorders. The antigens may comprise one or more copies of an epitope. In one embodiment, the antigens comprise a single epitope associated with one disease or disorder. In a further embodiment, the antigens comprise more than one epitope associated with the same disease or disorder. In yet a further embodiment, the antigens comprise more than one epitope associated with different diseases or disorders. In a further embodiment, the antigens comprise one or more epitopes associated with one or more allergies.

[00106] Further non-limiting examples of epitopes associated allergies to Japanese cedar pollen Table 1.

Table 1 – Polypeptide antigens associated with Japanese cedar pollen

Polypeptide	Representative Epitopes
CRYJ1	MDNPIDSSWRGDSNWAQNRMKLADSAVGFGSSTMGGKGGDLYT VTNSDDDPVNPAPGTLRYGATRDRPLWIIFSGNMNIKLKMPMYIAG YKTFDGRGAQVYIGNGGPSVFIKRVSNVIIHGLHLYGSSTSVLGNVL INESFGVEPVHPQDGDALTLRTATNIWIDHNSFSNSSDGLVDVTLSS TGVTISNNLFFNHHKVMLLGHDDAYSDDKSMKVTVAFNQFGPNSG QRMPRARYGLVHVANNNYDPWTIYAIGGSSNPTILSEGNSFTAPNE

	SYKKQVTIRIGSKTSSSSSNWVWQSTQDVFYNGAYFVSSGKYEGGN IYTKKEAFN (SEQ ID NO: 1)
CRYJ2	VENGNATPQLTKNAGVLTSSLSKRCRKVEHSRHDAINIFNVEKYGA VGDGKHDSTEAFSTAWQAASKKPSAMLLVPGNKKFVVNNLFFNGP SQPHFTFKVDGIIAAYQNPASWKNNRIWLQFAKLTGFTLMGKGVID GQGKQWWAGQSKWVNGREISNDRDRPTAIKFDFSTGLIIQGLKLM NSPEFHLVFGNSEGVKIIGISITAPRDSPNTDGIDIFASKNFHLQKNTI GTGDDSVAIGTGSSNIVIEDLISGPGHGISIGSLGRENSRAEVSYVHV NGAKFIDTQNGLRIKTWQGGSGMASHIIYENVEMINSENPILINQFY STSASASQNQRSAVQIQDVTYKNIRGTSATAAAIQLKSSDSMPSKDI KLSDISLKLTSGKIASSL (SEQ ID NO: 2)

[00107] Any suitable antigens associated with an allergy may find use within the scope of the present invention. In some embodiments, particles described herein are encapsulated with one or more antigens or epitopes associated with an allergy. In particular embodiments, the antigens or epitopes are associated with dust, pet allergens, tree pollen, grass pollen, mold, vegetable allergens, fruit allergens, nut allergens, seed allergens, spice allergens, grain allergens, seafood allergens, meat allergens, nematode allergens, latex allergens, and/or venom allergens.

[00108] In some embodiments, the antigenic epitope from Japanese cedar pollen comprises Cry j 1, Cry j 2, Cry j 3, Cry j 4, Cry j IFR, Cry j Chitinase, Cry j Asp, Cry j LTP, and/or Cry j CPA9, or fragments or variants thereof. In particular embodiments, the antigenic epitope from Japanese cedar pollen comprises a CRYJ1 polypeptide or a fragment or a variant thereof. In some embodiments, the CRYJ1 polypeptide comprises an amino acid sequence of SEQ ID NO: 1. In particular embodiments, the antigenic epitope from Japanese cedar pollen is a fragment of CRYJ1. In some embodiments, the fragment of CRYJ1 comprises ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, at least twenty, at least twenty-five, at least thirty, at least thirty-five, at least forty, at least forty-five, or at least fifty consecutive amino acids with at least 90%, at least 95%, at least 99%, or 100% sequence identity to SEQ ID NO: 1. In certain embodiments, the antigenic epitope is a variant of CRYJ1. In particular embodiments, the variant comprises an amino acid sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater than 99% sequence identity to SEQ ID NO: 1.

[00109] In some embodiments, the antigenic epitope from Japanese cedar pollen is a fragment of CRYJ1 selected from the group consisting of p16–30, p81–95, p106–120, p111–125, p211–225, and p301–315, which are described in Sone et al, J. Immunol. vol. 161(1) 448-457 (1998); incorporated herein by reference in its entirety.

[00110] In some embodiments, the antigenic epitope from Japanese cedar pollen comprises a CRYJ2 polypeptide or a fragment or a variant thereof. In some embodiments, the CRYJ2 polypeptide comprises an amino acid sequence of SEQ ID NO: 2. In particular embodiments, the antigenic epitope from Japanese cedar pollen is a fragment of CRYJ2. In some embodiments, the fragment of CRYJ2 comprises ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, at least twenty, at least twenty-five, at least thirty, at least thirty-five, at least forty, at least forty-five, or at least fifty consecutive amino acids with at least 90%, at least 95%, at least 99%, or 100% sequence identity to SEQ ID NO: 2. In certain embodiments, the antigenic epitope is a variant of CRYJ2. In particular embodiments, the variant comprises an amino acid sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater than 99% sequence identity to SEQ ID NO: 2

[00111] In some embodiments, the antigenic epitope from Japanese cedar pollen is a fragment of CRYJ2 selected from the group consisting of p66–80, p81–95, p141–155, p186–200, p236–250, p346–360, p351–365, and p336–350, which are described in Sone et al (1998).

[00112] In certain embodiments, the particles described herein encapsulate two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, fifteen or more, or twenty or more antigenic epitopes from Japanese cedar pollen. In particular embodiments, the two or more antigenic epitopes contained in a fusion protein. In some embodiments, the antigenic epitopes contained in the fusion protein are connected by a cleavable linker. A cleavable linker, as used herein, refers to an amino acid sequence that contains a specific cleavage site.

[00113] In some embodiments, the cleavable linker is five, six, seven, eight, nine, ten, greater than ten, greater than fifteen, greater than twenty, greater than twenty-five amino acids in length. In some embodiments, the cleavable linker is cleaved by a protease at a specific site. In particular embodiments, the cleavable linker contains more than one specific site that is cleaved by a protease. In some embodiments, the cleavable linker contains more than one specific site, wherein the specific sites are cleaved by different proteases. In some embodiments, the cleavable linker contains more than one specific site, wherein the specific sites are cleaved by the same protease.

[00114] In some embodiments, the cleavable linker is a Furin sensitive linker and/or a cathepsin sensitive linker. In particular embodiments, the cleavable linker is cleaved at a specific site on the linker by one or more of furin, cathepsin A, cathepsin B, cathepsin C,

cathepsin D, cathepsin E, cathepsin F, cathepsin G, cathepsin H, cathepsin K, cathepsin L, cathepsin O, cathepsin W, and/or cathepsin Z. In some embodiments, the cleavable liner comprises the amino acid sequence of SEQ ID NO: 3.

[00115] In some embodiments, the fusion protein contains two or more epitopes from the same protein. In particular embodiments, the fusion protein contains two or more epitopes from different proteins. In certain embodiments, the fusion protein comprises two or more epitopes from CRYJ1. In particular embodiments, the fusion protein comprises two or more epitopes from CRYJ2. In some embodiments, the fusion protein comprises epitopes from CRYJ1 and CRYJ2.

[00116] Combinations of antigens and/or epitopes can be tested for their ability to promote tolerance by conducting experiments with isolated cells or in animal models.

[00117] In some embodiments, the tolerance inducing compositions of the present invention contain an apoptosis signaling molecule (*e.g.*, in addition to an antigenic peptide or other antigenic molecule). In some embodiments, the apoptosis signaling molecule is coupled and/or associated with the surface of the carrier. In some embodiments an apoptotic signaling molecules allows a carrier to be perceived as an apoptotic body by antigen presenting cells of the host, such as cells of the host reticuloendothelial system; this allows presentation of the associated peptide epitopes in a tolerance-inducing manner. Without being bound by theory, this is presumed to prevent the upregulation of molecules involved in immune cell stimulation, such as MHC class I/II, and costimulatory molecules. These apoptosis signaling molecules may also serve as phagocytic markers. For example, apoptosis signaling molecules suitable for the present invention have been described in US Pat App No. 20050113297, which is hereby incorporated by reference in its entirety. Molecules suitable for the present invention include molecules that target phagocytes, which include macrophages, dendritic cells, monocytes, granulocytes and neutrophils.

[00118] In some embodiments, molecules suitable as apoptotic signaling molecules act to enhance tolerance of the associated peptides. Additionally, a carrier bound to an apoptotic signaling molecule can be bound by Clq in apoptotic cell recognition (Paidassi et al., (2008) J. Immunol. 180:2329-2338; herein incorporated by reference in its entirety). For example, molecules that may be useful as apoptotic signaling molecules include phosphatidyl serine, annexin-1, annexin-5, milk fat globule-EGF-factor 8 (MFG-E8), or the family of thrombospondins (*e.g.*, thrombospondin-1 (TSP-1)). Various molecules suitable for use as apoptotic signaling molecules with the present invention are discussed, for example, in U.S. Patent Publication No. 2012/0076831; herein incorporated by reference in its entirety).

[00119] In some embodiments, the apoptotic signaling molecule may be conjugated to the antigen-specific peptide. In some instances, the apoptotic signaling molecule and antigen-specific peptide are conjugated by the creation of a fusion protein. For example a fusion protein may comprise at least one antigen-specific peptide (or a fragment or a variant thereof) coupled to at least one molecule of an apoptotic signaling molecule (or a fragment or a variant thereof). For the creation of fusion proteins, the terms "fusion protein," "fusion peptide," "fusion polypeptide," and "chimeric peptide" are used interchangeably. Suitable fragments of the antigen-specific peptide include any fragment of the full-length peptide that retains the function of generating the desired antigen-specific tolerance function of the present invention. The fusion protein may be created by various means understood in the art (*e.g.*, genetic fusion, chemical conjugation, etc.). The two proteins may be fused either directly or via an amino acid linker. The polypeptides forming the fusion protein are typically linked C-terminus to N-terminus, although they can also be linked C-terminus to C-terminus, N-terminus to N-terminus, or N-terminus to C-terminus. The polypeptides of the fusion protein can be in any order. A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., Gene 40:39-46 (1985); Murphy et al., Proc. Natl. Acad. Sci. USA 83:8258-8262 (1986); U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180; herein incorporated by reference in their entireties. The linker sequence may generally be from 1 to about 50 amino acids in length. In some embodiments, linker sequences are not required and/or utilized, for example, when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

[00120] A proxy for tolerogenic activity is the ability of an intact antigen or fragment to stimulate the production of an appropriate cytokine at the target site. The immunoregulatory cytokine released by T suppressor cells at the target site is thought to be TGF-β (Miller et al., Proc. Natl. Acad. Sci. USA 89:421, 1992). Other factors that may be produced during tolerance are the cytokines IL4 and IL-10, and the mediator PGE. In contrast, lymphocytes in tissues undergoing active immune destruction secrete cytokines such as IL-I, IL-2, IL-6, and IFN-γ. Hence, the efficacy of a candidate inducing antigen can be evaluated by measuring its ability to stimulate the appropriate type of cytokines.

[00121] With this in mind, a rapid screening test for tolerogenic epitopes of the inducing antigen, effective mucosal binding components, effective combinations, or effective

modes and schedules of mucosal administration can be conducted using syngeneic animals as donors for in vitro cell assays. Animals are treated at a mucosal surface with the test composition, and at some time are challenged with parenteral administration of the target antigen in complete Freund's adjuvant. Spleen cells are isolated, and cultured in vitro in the presence of the target antigen at a concentration of about 50 μg/mL. Target antigen can be substituted with candidate proteins or sub-fragments to map the location of tolerogenic epitopes. Cytokine secretion into the medium can be quantitated by standard immunoassay.

[00122] The ability of the cells to suppress the activity of other cells can be determined using cells isolated from an animal immunized with the target antigen, or by creating a cell line responsive to the target antigen (Ben-Nun et al., Eur. J. Immunol. 11:195, 1981, herein incorporated by reference in its entirety). In one variation of this experiment, the suppressor cell population is mildly irradiated (about 1000 to 1250 rads) to prevent proliferation, the suppressors are co-cultured with the responder cells, and then tritiated thymidine incorporation (or MTT) is used to quantitate the proliferative activity of the responders. In another variation, the suppressor cell population and the responder cell population are cultured in the upper and lower levels of a dual chamber transwell culture system (Costar, Cambridge Mass.), which permits the populations to coincubate within 1 mm of each other, separated by a polycarbonate membrane (WO 93/16724). In this approach, irradiation of the suppressor cell population is unnecessary, since the proliferative activity of the responders can be measured separately.

[00123] In embodiments of the invention where the target antigen is already present in the individual, there is no need to isolate the antigen or precombine it with the mucosal binding component. For example, the antigen may be expressed in the individual in a certain fashion as a result of a pathological condition (such as inflammatory bowel disease or Celiac disease) or through digestion of a food allergen. Testing is performed by giving the mucosal binding component in one or more doses or formulations, and determining its ability to promote tolerization against the antigen *in situ*.

[00124] The effectiveness of compositions and modes of administration for treatment of specific disease can also be elaborated in a corresponding animal disease model. The ability of the treatment to diminish or delay the symptomatology of the disease is monitored at the level of circulating biochemical and immunological hallmarks of the disease, immunohistology of the affected tissue, and gross clinical features as appropriate for the model being employed. Non-limiting examples of animal models that can be used for testing are included in the following section. The invention contemplates modulation of

tolerance by modulating TH1 response, TH2 response, TH17 response, or a combination of these responses. Modulating TH1 response encompasses changing expression of, *e.g.*, interferon-gamma. Modulating TH2 response encompasses changing expression of, *e.g.*, any combination of IL-4, IL-5, IL-10, and IL-13. Typically an increase (decrease) in TH2 response will comprise an increase (decrease) in expression of at least one of IL-4, IL-5, IL-10, or IL-13; more typically an increase (decrease) in TH2 response will comprise an increase in expression of at least two of IL-4, IL-5, IL-10, or EL-13, most typically an increase (decrease) in TH2 response will comprise an increase in at least three of IL-4, IL-5, IL-10, or IL-13, while ideally an increase (decrease) in TH2 response will comprise an increase (decrease) in expression of all of IL-4, IL-5, IL-10, and IL-13. Modulating TH 17 encompasses changing expression of, *e.g.*, TGF-beta, IL-6, IL-21 and IL23, and effects levels of IL-17, IL-21 and IL-22. In some embodiments, the present invention contemplates modulation of tolerance by promoting one type of immune response over another (*e.g.*, immune-switching or immune-deviation). In such embodiments, an established immune response of one phenotype is suppressed or reduced and an immune response of a different phenotype is increased or enhanced. For example, immune-switching from a Th2 to a Th1 response in the context of an allergic immune reaction promotes the generation of an IgG2a antibody response and production of IFNγ and/or IL-12, leading to a decrease in allergen-specific IgE response and a decrease in T cell polarization to Th2 cells. Other suitable methods for assessing the effectiveness of compositions and methods of the present invention are understood in the art, as are discussed, for example, in U.S. Patent Publication No. 2012/0076831 (herein incorporated by reference in its entirety).

[00125] Certain embodiments of this invention relate to priming of immune tolerance in an individual not previously tolerized by therapeutic intervention. These embodiments generally involve a plurality of administrations of a combination of antigen and mucosal binding component. Typically, at least three administrations, frequently at least four administrations, and sometimes at least six administrations are performed during priming in order to achieve a long-lasting result, although the subject may show manifestations of tolerance early in the course of treatment. Most often, each dose is given as a bolus administration, but sustained formulations capable of mucosal release are also suitable. Where multiple administrations are performed, the time between administrations is generally between 1 day and 3 weeks, and typically between about 3 days and 2 weeks. Generally, the same antigen and mucosal binding component are present at the same concentration, and the administration is given to the same mucosal surface, but variations of any of these variables

during a course of treatment may be accommodated. Other embodiments of this invention relate to boosting or extending the persistence of a previously established immune tolerance. These embodiments generally involve one administration or a short course of treatment at a time when the established tolerance is declining or at risk of declining. Boosting is generally performed 1 month to 1 year, and typically 2 to 6 months after priming or a previous boost. This invention also includes embodiments that involve regular maintenance of tolerance on a schedule of administrations that occur semiweekly, weekly, biweekly, or on any other regular schedule.

[00126] The particles of the current invention can be given in any dose effective to dampen the inflammatory immune response in a subject in need thereof or to treat a bacterial or viral infection in a subject in need thereof. In certain embodiments, about 10^2 to about 10^{20} particles are provided to the individual. In a further embodiment between about 10^3 to about 10^{15} particles are provided. In yet a further embodiment between about 10^6 to about 10^{12} particles are provided. In still a further embodiment between about 10^8 to about 10^{10} particles are provided. In a preferred embodiment the preferred dose is 0.1% solids/ml. Therefore, for 0.5 μm beads, a preferred dose is approximately 4 x 10^9 beads, for 0.05μm beads, a preferred dose is approximately 4 x 10^{12} beads, for 3μm beads, a preferred dose is 2 x 10^7 beads. However, any dose that is effective in treating the particular condition to be treated is encompassed by the current invention.

[00127] The invention is useful for treatment of immune related disorders such as autoimmune disease, transplant rejection, enzyme deficiencies and allergic reactions. Substitution of a synthetic, biocompatible particle system to induce immune tolerance could lead to ease of manufacturing, broad availability of therapeutic agents, increase uniformity between samples, increase the number of potential treatment sites and dramatically reduce the potential for allergic responses to a carrier cell.

[00128] As used herein, the term "immune response" includes T cell mediated and/or B cell mediated immune responses. Exemplary immune responses include T cell responses, *e.g.*, cytokine production and cellular cytotoxicity. In addition, the term immune response includes immune responses that are indirectly effected by T cell activation, *e.g.*, antibody production (humoral responses) and activation of cytokine responsive cells, *e.g.*, macrophages. Immune cells involved in the immune response include lymphocytes, such as B cells and T cells ($CD4^+$, $CD8^+$, Th1 and Th2 cells); antigen presenting cells (*e.g.*, professional antigen presenting cells such as dendritic cells, macrophages, B lymphocytes, Langerhans cells, and nonprofessional antigen presenting cells such as keratinocytes, endothelial cells,

astrocytes, fibroblasts, oligodendrocytes); natural killer cells; myeloid cells, such as macrophages, eosinophils, mast cells, basophils, and granulocytes. In some embodiments, the modified particles of the present invention are effective to reduce inflammatory cell trafficking to the site of inflammation.

[00129] As used herein, the term "anergy," "tolerance," or "antigen-specific tolerance" refers to insensitivity of T cells to T cell receptor-mediated stimulation. Such insensitivity is generally antigen- specific and persists after exposure to the antigenic peptide has ceased. For example, anergy in T cells is characterized by lack of cytokine production, *e.g.*, IL-2. T-cell anergy occurs when T cells are exposed to antigen and receive a first signal (a T cell receptor or CD-3 mediated signal) in the absence of a second signal (a costimulatory signal). Under these conditions, re-exposure of the cells to the same antigen (even if re-exposure occurs in the presence of a costimulatory molecule) results in failure to produce cytokines and subsequently failure to proliferate. Thus, a failure to produce cytokines prevents proliferation. Anergic T cells can, however, proliferate if cultured with cytokines (*e.g.*, IL-2). For example, T cell anergy can also be observed by the lack of IL-2 production by T lymphocytes as measured by ELISA or by a proliferation assay using an indicator cell line. Alternatively, a reporter gene construct can be used. For example, anergic T cells fail to initiate DL-2 gene transcription induced by a heterologous promoter under the control of the 5' IL-2 gene enhancer or by a multimer of the API sequence that can be found within the enhancer (Kang et al. 1992 Science. 257:1134).

[00130] As used herein, the term "immunological tolerance" refers to methods performed on a proportion of treated subjects in comparison with untreated subjects where: a) a decreased level of a specific immunological response (thought to be mediated at least in part by antigen-specific effector T lymphocytes, B lymphocytes, antibody, or their equivalents); b) a delay in the onset or progression of a specific immunological response; or c) a reduced risk of the onset or progression of a specific immunological response. "Specific" immunological tolerance occurs when immunological tolerance is preferentially invoked against certain antigens in comparison with others. “Non-Specific” immunological tolerance occurs when immunological tolerance is invoked indiscriminately against antigens which lead to an inflammatory immune response. “Quasi-Specific” immunological tolerance occurs when immunological tolerance is invoked semi-discriminately against antigens which lead to a pathogenic immune response but not to others which lead to a protective immune response.

[00131] Tolerance to autoantigens and autoimmune disease is achieved by a variety of mechanisms including negative selection of self-reactive T cells in the thymus and

mechanisms of peripheral tolerance for those autoreactive T cells that escape thymic deletion and are found in the periphery. Examples of mechanisms that provide peripheral T cell tolerance include "ignorance" of self-antigens, anergy or unresponsiveness to autoantigen, cytokine immune deviation, and activation-induced cell death of self- reactive T cells. In addition, regulatory T cells have been shown to be involved in mediating peripheral tolerance. See, for example, Walker et al. (2002) Nat. Rev. Immunol. 2: 11-19; Shevach et al. (2001) Immunol. Rev. 182:58-67. In some situations, peripheral tolerance to an autoantigen is lost (or broken) and an autoimmune response ensues. For example, in an animal model for EAE, activation of antigen presenting cells (APCs) through TLR innate immune receptors was shown to break self-tolerance and result in the induction of EAE (Waldner et al. (2004) J. Clin. Invest. 113:990-997).

[00132] Accordingly, in some embodiments, the invention provides methods for increasing antigen presentation while suppressing or reducing TLR7/8, TLR9, and/or TLR 7/8/9 dependent cell stimulation. As described herein, administration of particular modified particles results in antigen presentation by DCs or APCs while suppressing the TLR 7/8, TLR9, and/or TLR7/8/9 dependent cell responses associated with immunostimulatory polynucleotides. Such suppression may include decreased levels of one or more TLR-associated cytokines.

[00133] As discussed above this invention provides novel compounds that have biological properties useful for the treatment of Mac-1 and LFA-1 mediated disorders.

[00134] Accordingly, in another aspect of the present invention, pharmaceutical compositions are provided, which comprise the immune modifying particles and optionally comprise a pharmaceutically acceptable carrier. In certain embodiments, these compositions optionally further comprise one or more additional therapeutic agents. Alternatively, the modified particles of the current invention may be administered to a patient in need thereof in combination with the administration of one or more other therapeutic agents. For example, additional therapeutic agents for conjoint administration or inclusion in a pharmaceutical composition with a compound of this invention may be an approved anti-inflammatory agent, or it may be any one of a number of agents undergoing approval in the Food and Drug Administration that ultimately obtain approval for the treatment of any disorder characterized by an uncontrolled inflammatory immune response or a bacterial or viral infection. It will also be appreciated that certain of the modified particles of present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative thereof.

[00135] The pharmaceutical compositions of the present invention additionally comprise a pharmaceutically acceptable carrier, which, as used herein, 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, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) 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 compounds 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 sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatine; 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 as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogenfree 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.

[00136] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, 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.

[00137] The particles of the invention may be administered orally, nasally, intravenously, intramuscularly, ocularly, transdermally, intraperitoneally, or subcutaneously. In one embodiment, the particles of the invention are administered intravenously.

[00138] The effective amounts and method of administration of the present invention for modulation of an immune response can vary based on the individual, what condition is to be treated and other factors evident to one skilled in the art. Factors to be considered include route of administration and the number of doses to be administered. Such factors are known in the art and it is well within the skill of those in the art to make such determinations without undue experimentation. A suitable dosage range is one that provides the desired regulation of immune. Useful dosage ranges of the carrier, given in amounts of carrier delivered, may be, for example, from about any of the following: 0.5 to 10 mg/kg, 1 to 9 mg/kg, 2 to 8 mg/kg, 3 to 7 mg/kg, 4 to 6 mg/kg, 5 mg/kg, 1 to 10 mg/kg, 5 to 10 mg/kg. Alternatively, the dosage can be administered based on the number of particles. For example, useful dosages of the carrier, given in amounts of carrier delivered, may be, for example, about 10^6, 10^7, 10^8, 10^9, 10^{10}, or greater number of particles per dose. The absolute amount given to each patient depends on pharmacological properties such as bioavailability, clearance rate and route of administration. Details of pharmaceutically acceptable carriers, diluents and excipients and methods of preparing pharmaceutical compositions and formulations are provided in Remmingtons Pharmaceutical Sciences 18th Edition, 1990, Mack Publishing Co., Easton, Pa., USA, which is hereby incorporated by reference in its entirety.

[00139] The effective amount and method of administration of the particular carrier formulation can vary based on the individual patient, desired result and/or type of disorder, the stage of the disease and other factors evident to one skilled in the art. The route(s) of administration useful in a particular application are apparent to one of skill in the art. Routes of administration include but are not limited to topical, dermal, transdermal, transmucosal, epidermal, parenteral, gastrointestinal, and naso-pharyngeal and pulmonary, including transbronchial and transalveolar. A suitable dosage range is one that provides sufficient IRP-containing composition to attain a tissue concentration of about 1-50 μM as measured by blood levels. The absolute amount given to each patient depends on pharmacological properties such as bioavailability, clearance rate and route of administration.

[00140] The present invention provides carrier formulations suitable for topical application including, but not limited to, physiologically acceptable implants, ointments, creams, rinses and gels. Exemplary routes of dermal administration are those which are least invasive such as transdermal transmission, epidermal administration and subcutaneous injection.

[00141] Transdermal administration is accomplished by application of a cream, rinse, gel, etc. capable of allowing the carrier to penetrate the skin and enter the blood stream. Compositions suitable for transdermal administration include, but are not limited to, pharmaceutically acceptable suspensions, oils, creams and ointments applied directly to the skin or incorporated into a protective carrier such as a transdermal device (so-called "patch"). Examples of suitable creams, ointments etc. can be found, for instance, in the Physician's Desk Reference. Transdermal transmission may also be accomplished by iontophoresis, for example using commercially available patches which deliver their product continuously through unbroken skin for periods of several days or more. Use of this method allows for controlled transmission of pharmaceutical compositions in relatively great concentrations, permits infusion of combination drugs and allows for contemporaneous use of an absorption promoter.

[00142] Parenteral routes of administration include but are not limited to electrical (iontophoresis) or direct injection such as direct injection into a central venous line, intravenous, intramuscular, intraperitoneal, intradermal, or subcutaneous injection. Formulations of carrier suitable for parenteral administration are generally formulated in USP water or water for injection and may further comprise pH buffers, salts bulking agents, preservatives, and other pharmaceutically acceptable excipients. Immunoregulatory polynucleotide for parenteral injection may be formulated in pharmaceutically acceptable sterile isotonic solutions such as saline and phosphate buffered saline for injection.

[00143] Gastrointestinal routes of administration include, but are not limited to, ingestion and rectal routes and can include the use of, for example, pharmaceutically acceptable powders, pills or liquids for ingestion and suppositories for rectal administration.

[00144] Naso-pharyngeal and pulmonary administration include are accomplished by inhalation, and include delivery routes such as intranasal, transbronchial and transalveolar routes. The invention includes formulations of carrier suitable for administration by inhalation including, but not limited to, liquid suspensions for forming aerosols as well as powder forms for dry powder inhalation delivery systems. Devices

suitable for administration by inhalation of carrier formulations include, but are not limited to, atomizers, vaporizers, nebulizers, and dry powder inhalation delivery devices.

[00145] Injectable preparations, for example, 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.

[00146] The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00147] In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension or crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

[00148] In some embodiments, the synthetic, biodegradable particles of the present invention provide ease of manufacturing, broad availability of therapeutic agents, and increased treatment sites. In particular embodiments, surface-functionalized biodegradable poly(lactide-*co*-glycolide) particles with a high density of surface carboxylate groups, synthesized using the surfactant poly(ethylene-*alt*-maleic anhydride) provide a carrier that

offers numerous advantages over other carrier particles and/or surfaces. Experiments conducted during development of embodiments of the present invention demonstrated the conjugation of peptides (*e.g.*, $PLP_{139-151}$ peptide) to these particles. Such peptide-coupled particles have shown that they are effective for the prevention of disease development and the induction of immunological tolerance (*e.g.*, in the SJL/J $PLP_{139-151}$ /CFA-induced R-EAE murine model of multiple sclerosis). Peptide coupled carriers of the present invention provide numerous advantages over other tolerance induction structures. In some embodiments, the particles are biodegradable, and therefore will not persist for long times in the body. The time for complete degradation can be controlled. In some embodiments, particles are functionalized to facilitate internalization without cell activation (*e.g.*, phosphatidylserine loaded into PLG microspheres). In some embodiments, particles incorporate targeting ligands for a specific cell population. In some embodiments, anti-inflammatory cytokines such as IL-10 and TGF-β, are included on or within particles to limit activation of the cell type that is internalizing the particles and to facilitate the induction of tolerance via energy and/or deletion and the activation of regulatory T cells. The composition of the particles has been found to affect the length of time the particles persist in the body and tolerance requires rapid particle uptake and clearance/degradation. Since ratios of over 50:50 lactide:glycolide slow the degradation rate, the particles of the invention have a lactide:glycolide ratio of about 50:50 or below. In one embodiment the particles of the invention have about a 50:50 D,L-lactide:glycolide ratio.

[00149] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the modified particles are 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. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[00150] Solid compositions of a similar type 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. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type 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.

[00151] The modified particles can also be in micro-encapsulated form with one or more excipients as noted above. The 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 the active compound may be admixed with at least one inert diluent such as sucrose, lactose and starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the modified particles 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.

[00152] The present invention encompasses pharmaceutically acceptable topical formulations of the inventive modified particles. The term "pharmaceutically acceptable topical formulation", as used herein, means any formulation which is pharmaceutically acceptable for intradermal administration of modified microparticles of the invention by application of the formulation to the epidermis. In certain embodiments of the invention, the topical formulation comprises a carrier system. Pharmaceutically effective carriers include, but are not limited to, solvents (*e.g.*, alcohols, poly alcohols, water), creams, lotions, ointments, oils, plasters, liposomes, powders, emulsions, microemulsions, and buffered solutions (*e.g.*, hypotonic or buffered saline) or any other carrier known in the art for topically administering pharmaceuticals. A more complete listing of art-known carriers is

provided by reference texts that are standard in the art, for example, Remington's Pharmaceutical Sciences, 16th Edition, 1980 and 17th Edition, 1985, both published by Mack Publishing Company, Easton, Pa., the disclosures of which are incorporated herein by reference in their entireties. In certain other embodiments, the topical formulations of the invention may comprise excipients. Any pharmaceutically acceptable excipient known in the art may be used to prepare the inventive pharmaceutically acceptable topical formulations. Examples of excipients that can be included in the topical formulations of the invention include, but are not limited to, preservatives, antioxidants, moisturizers, emollients, buffering agents, solubilizing agents, other penetration agents, skin protectants, surfactants, and propellants, and/or additional therapeutic agents used in combination to the modified particles. Suitable preservatives include, but are not limited to, alcohols, quaternary amines, organic acids, parabens, and phenols. Suitable antioxidants include, but are not limited to, ascorbic acid and its esters, sodium bisulfite, butylated hydroxytoluene, butylated hydroxyanisole, tocopherols, and chelating agents like EDTA and citric acid. Suitable moisturizers include, but are not limited to, glycerine, sorbitol, polyethylene glycols, urea, and propylene glycol. Suitable buffering agents for use with the invention include, but are not limited to, citric, hydrochloric, and lactic acid buffers. Suitable solubilizing agents include, but are not limited to, quaternary ammonium chlorides, cyclodextrins, benzyl benzoate, lecithin, and polysorbates. Suitable skin protectants that can be used in the topical formulations of the invention include, but are not limited to, vitamin E oil, allatoin, dimethicone, glycerin, petrolatum, and zinc oxide.

[00153] In certain embodiments, the pharmaceutically acceptable topical formulations of the invention comprise at least the modified particles of the invention and a penetration enhancing agent. The choice of topical formulation will depend or several factors, including the condition to be treated, the physicochemical characteristics of the inventive compound and other excipients present, their stability in the formulation, available manufacturing equipment, and costs constraints. As used herein the term "penetration enhancing agent" means an agent capable of transporting a pharmacologically active compound through the stratum corneum and into the epidermis or dermis, preferably, with little or no systemic absorption. A wide variety of compounds have been evaluated as to their effectiveness in enhancing the rate of penetration of drugs through the skin. See, for example, Percutaneous Penetration Enhancers, Maibach H. I. and Smith H. E. (eds.), CRC Press, Inc., Boca Raton, Fla. (1995), which surveys the use and testing of various skin penetration enhancers, and Buyuktimkin et al., Chemical Means of Transdermal Drug Permeation

Enhancement in Transdermal and Topical Drug Delivery Systems, Gosh T. K., Pfister W. R., Yum S. I. (Eds.), Interpharm Press Inc., Buffalo Grove, Ill. (1997). In certain exemplary embodiments, penetration agents for use with the invention include, but are not limited to, triglycerides (*e.g.*, soybean oil), aloe compositions (*e.g.*, aloe-vera gel), ethyl alcohol, isopropyl alcohol, octolyphenylpolyethylene glycol, oleic acid, polyethylene glycol 400, propylene glycol, N-decylmethylsulfoxide, fatty acid esters (*e.g.*, isopropyl myristate, methyl laurate, glycerol monooleate, and propylene glycol monooleate) and N-methylpyrrolidone.

[00154] In certain embodiments, the compositions may be in the form of ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. In certain exemplary embodiments, formulations of the compositions according to the invention are creams, which may further contain saturated or unsaturated fatty acids such as stearic acid, palmitic acid, oleic acid, palmito-oleic acid, cetyl or oleyl alcohols, stearic acid being particularly preferred. Creams of the invention may also contain a non-ionic surfactant, for example, polyoxy-40-stearate. In certain embodiments, the active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, eardrops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms are made by dissolving or dispensing the compound in the proper medium. As discussed above, penetration enhancing agents can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

[00155] The modified particles can be administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the modified particles. A nonaqueous (*e.g.*, fluorocarbon propellant) suspension could be used.

[00156] Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronics®, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

[00157] It will also be appreciated that the modified particles and pharmaceutical compositions of the present invention can be formulated and employed in combination therapies, that is, the compounds and pharmaceutical compositions can be formulated with or administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an inventive compound may be administered concurrently with another anti-inflammatory agent), or they may achieve different effects (*e.g.*, control of any adverse effects).

[00158] In certain embodiments, the pharmaceutical compositions containing the modified particles of the present invention further comprise one or more additional therapeutically active ingredients (*e.g.*, anti-inflammatory and/or palliative). For purposes of the invention, the term "Palliative" refers to treatment that is focused on the relief of symptoms of a disease and/or side effects of a therapeutic regimen, but is not curative. For example, palliative treatment encompasses painkillers, antinausea medications and anti-sickness drugs.

[00159] The invention provides methods of regulating an immune response in an individual, preferably a mammal, more preferably a human, comprising administering to the individual the modified particles described herein. Methods of immunoregulation provided by the invention include those that suppress and/or inhibit an innate immune response or an adaptive immune response, including, but not limited to, an immune response stimulated by immunostimulatory polypeptides or viral or bacterial components.

[00160] The modified particles are administered in an amount sufficient to regulate an immune response. As described herein, regulation of an immune response may be humoral and/or cellular, and is measured using standard techniques in the art and as described herein.

[00161] In some embodiments, compositions described herein are administered along with (*e.g.*, concurrent with, prior to, or following) an implant (*e.g.*, device) and/or transplant (*e.g.*, tissue, cells, organ) to mediate, negate, regulate and/or reduce the immune response associated therewith.

[00162] In certain embodiments, the individual suffers from a disorder associated with unwanted immune activation, such as allergic disease or condition, allergy

and asthma. An individual having an allergic disease or asthma is an individual with a recognizable symptom of an existing allergic disease or asthma. Tolerance can be induced in such an individual, for example, by particles complexed inhaled substances (*e.g.* Japanese cedar pollen proteins) which elicit the allergic reaction.

[00163] In some embodiments, the invention relates to uses of compositions of this invention prior to the onset of an allergy, *e.g.* an allergy to Japanese cedar pollen. In particular embodiments, the invention relates to uses of the compositions of this invention to inhibit an ongoing or existing allergy. In some embodiments, the invention relates to ameliorating an allergy or an allergic response in a subject. By ameliorating it is meant to include treating, preventing, or suppressing the allergy or allergic response in the subject.

[00164] In some embodiments, compositions of the present invention (*e.g.*, PLG carrier coupled to antigenic molecule associated with Japanese cedar pollen allergy) find use with one or more scaffolds, matrices, and/or delivery systems (See, *e.g.*, U.S. Patent Publication No. 2009/0238879; U.S. Patent Nos. 7,846,466; 7,427,602; 7,029,697; 6,890,556; 6,797,738; and 6,281,256; herein incorporated by reference in their entireties). In some embodiments, particles (*e.g.*, antigen-coupled PLG particles) are associated with, adsorbed on, embedded within, conjugated to, etc. a scaffold, matrix, and/or delivery system (*e.g.*, for delivery of chemical/biological material, cells, tissue, and/or an organ to a subject). In some embodiments, a scaffold, matrix, and/or delivery system (*e.g.*, for delivery of chemical/biological material, cells, tissue, and/or an organ to a subject) comprises and/or is made from materials described herein (*e.g.*, PLG conjugated to one or more antigenic peptides).

[00165] In some embodiments, microporous scaffolds (*e.g.*, for transplanting biological material (*e.g.*, cells, tissue, etc.) into a subject) are provided. In some embodiments, microporous scaffolds are provided having thereon agents (*e.g.*, extracellular matrix proteins, exendin-4) and biological material (*e.g.*, pancreatic islet cells). In some embodiments, the scaffolds are used in the treatment of diseases (*e.g.*, type 1 diabetes), and related methods (*e.g.*, diagnostic methods, research methods, drug screening). In some embodiments, scaffolds are provided with the antigen-conjugated carriers described herein on and/or within the scaffold. In some embodiments, scaffolds are produced from antigen conjugated materials (*e.g.*, antigen conjugated PLG).

[00166] In some embodiments, a scaffold and/or delivery system comprises one or more layers and/or has one or more chemical and/or biological entities/agents (*e.g.*, proteins, peptide-conjugated particles, small molecules, cells, tissue, etc.), see, *e.g.*, U.S.

Patent Publication No. 2009/0238879; herein incorporated by reference in its entirety. In some embodiments, antigen-coupled particles are co-administered with a scaffold delivery system to elicit induction of immunological tolerance to the scaffold and the associated materials. In some embodiments, microporous scaffold is administered to a subject with particles described herein on or within the scaffold. In some embodiments, antigen-coupled particles coupled to a scaffold delivery system. In some embodiments, a scaffold delivery system comprises antigen-coupled particles.

[00167] Various modification, recombination, and variation of the described features and embodiments will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although specific embodiments have been described, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes and embodiments that are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims. For example, U.S. Application No. 62/221,504, PCT Application Nos. PCT/US2016/068423 and PCT/US2017/012173 (each of which is herein incorporated by reference in their entirety); U.S. Patent Publication Nos. 2012/0076831, 2002/0045672, 2005/0090008, 2006/0002978, 2009/0238879, 2012/0076831, 2015/0209293, 2015/0283218 (each of which is herein incorporated by reference in their entirety); and U.S. Patent Nos. 7,846,466; 7,427,602; 7,029,697; 6,890,556; 6,797,738; and 6,281,256 (each of which is herein incorporated by reference in their entirety) provide details, modifications, and variations that find use in various embodiments described herein.

[00168] All publications and patents mentioned in the present application and/or listed below are herein incorporated by reference in their entireties.

EXAMPLES

[00169] The following examples are provided to further illustrate the advantages and features of the invention, but are not intended to limit the scope of this disclosure.

Example 1: Encapsulation of recombinant Japanese cedar pollen proteins into TIMPs

[00170] As described above, the use of particles encapsulating Japanese cedar pollen (JCP) extract in the treatment of Japanese cedar pollinosis remains limited due to the challenges of encapsulating JCP, which remains highly viscous in solution even at low concentrations. Experiments were performed to prepare TIMP particles encapsulating JCP

(TIMP-JCP^P) using a double emulsion-solvent evaporation method (FIG. 2C). Briefly, 200 µL of 5 mg/mL JCP solution was added to 0.5 mL 20% w/v PLGA in DCM or 400 µL of 5 mg/mL JCP solution was added to 1.0 mL 20% w/v PLGA in DCM, depending on the batch of JCP extract being tested. Each solution was emulsified by sonication to generate a primary emulsion. The PEMA solution (10 mL 1% w/v aqueous PEMA) was then added to the emulsion and re-emulsified by sonication to generate a secondary emulsion. The emulsion was poured into 200 mL 0.5% w/v aqueous PEMA under stirring. The particles were purified, lyophilized, and stored for future use at -20 degrees. Antigen dose of particles was determined as previously described by dissolving particles in DMSO prior to CBQCA analysis. Nanoparticle size and zeta–potential in water was measured using dynamic light scattering on a Zetasizer Nano ZSP (Malvern Instruments, Westborough, MA). Lot-release criteria require TIMP-JCP^P diameter to be 700nm ± 250nm, charge of at least -30 mV and contain at least 5 µg antigen/mg of PLGA. Conditions for particle fabrication and results of JCP encapsulation are shown in FIG. 4.

[00171] Initial attempts to encapsulate total JCP extract into TIMPs were only partially successful, as the maximum antigen loading achieved was approximately 1 µg protein/mg TIMP or less (FIG. 4). This was potentially due to the high viscosity of JCP, even at low concentrations (FIG. 2A). Therefore, experiments were performed to determine the optimal conditions for encapsulating recombinant JCP proteins (CRY1 and CRY2, Lifeome) into TIMPs (TIMP-JCP^R, schematic shown in FIG. 2B).

[00172] TIMP-JCP^R particles were generated using low molecular weight PLGA (0.17 dL/g), Sugi basic protein (CRYJ1, 55,180 Da), and polygalacturonase (CRYJ2, 59,070 Da). Purity of the recombinant proteins was assessed by SDS-PAGE (FIG. 3C) and compared to previously determined results (Y. Mitobe *et al.*, *Regulatory toxicology and pharmacology:* RTP 2015 71). Conditions for TIMP-JCP^R particle fabrication are shown in Table 2.

Table 2: Conditions for TIMP-JCP^R particle fabrication

Particle	PLGA	Recombinant protein	Continuous phase 1% PEMA	Extraction phase 0.5% PEMA
TIMP-CRYJ103-56-1 (Theoretical 15 µg/mL)	50 mg (100 mg/mL; 0.5 mL DCM)	0.75 mg (4 mg/mL; 186 µL MilliQ)	2.5 mL	20 mL
TIMP-CRYJ103-56-2 (Theoretical 7.5	50 mg (100 mg/mL; 0.5 mL DCM)	0.375 mg (4 mg/mL; 94 µL MilliQ)	2.5 mL	20 mL

µg/mL)				
TIMP-CRYJ203-56-3 (Theoretical 15 µg/mL)	50 mg (100 mg/mL; 0.5 mL DCM)	0.75 mg (8 mg/mL; 94 µL MilliQ)	2.5 mL	20 mL
TIMP-CRYJ203-56-4 (Theoretical 7.5 µg/mL)	50 mg (100 mg/mL; 0.5 mL DCM)	0.375 mg (4 mg/mL; 47 µL MilliQ)	2.5 mL	20 mL

[00173] CRYJ1 or CRYJ2 was encapsulated using the double-emulsion protocol for particle formulation (schematic shown in FIG. 2C) and described above. Protein concentration and burst release for were determined by CBQCA for each sample. Results for these analyses are shown below in Table 3. Size and particle charge were determined by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS or ZSP and results are shown below in Table 4.

Table 3: Loading of CRYJ1 and CRYJ2 in TIMPs

Particle	Protein loading (µg protein /mg of TIMP)	Percent protein release upon reconstitution
TIMP-CRYJ103-56-1	13.1 ± 2.7	Not detected[a]
TIMP-CRYJ103-56-2	5.5 ± 4.2	Not detected[a]
TIMP-CRYJ203-56-3	10.2 ± 0.7	12.8
TIMP-CRYJ203-56-4	1.7 ± 0.5	0.7

[a] below the limit of detection

Table 4: Measurement of particle size and zeta potential

Particle	Z-average size (nm)	Zeta potential (mV)	PDI
TIMP-CRYJ103-56-1	846.7 ± 39.0	-45.2 ± 0.7	0.380
TIMP-CRYJ103-56-2	775.9 ± 23.6	-37.3 ± 0.2	0.380
TIMP-CRYJ203-56-3	981 ± 9.6	-37.1 ± 1.4	0.370
TIMP-CRYJ203-56-4	1084 ± 7.7	-39.1 ± 0.8	0.410

Example 2: Murine model of JCP allergy

[00174] Mouse models of the acute allergic response to inhaled allergens have been widely used opt elucidate the mechanisms underlying the immunologic and inflammatory responses in asthma, and for the identification and investigation of novel targets for controlling allergic inflammation.

[00175] The nature of the acute inflammatory model may be influenced by the choice of mouse strain, the allergen, and the sensitization and challenge protocol (FIG. 5A).

Briefly, Japanese cedar pollen extract (Cedar pollen extract-Cj; LSL-LG5280; Lo# 153101, Cosmo Bio USA) was re-suspended in MilliQ water at 1 mg/mL. The JCP solution was diluted 1:1 in Imject Alum (Thermo Scientific, Cat. # 77161), such that the final concentration of alum was 20 mg/mL. On day 0, Balb/c mice were injected intraperitoneally with 100 µL JCP+Alum solution (i.e. 50 µg JCP adsorbed to 2 mg/mL of alum, n=10). Control mice were injected with alum diluted in PBS (n=10). A second equivalent dose was administered to the mice on day 14. Mice were retro-orbitally bled to collect serum for antibody analysis on day 21 (FIG. 5B-5D). On day 22, a portion of the mice from each group were tolerized with a single 2.5 mg dose of either TIMP-OVA (n=5) or TIMP-JCP (n=4) administered intravenously via the tail vein. Additionally, mice are tolerized with TIMP-OVA or TIMP-JCP prior to sensitization with JCP.

[00176] On days 28 and 29, mice were intranasally challenged with 100 µg of JCP extract in 20 µL MilliQ water. Mice were monitored for scratching (FIG. 7) and body temperature changes (FIG. 6) for 1 hour after the challenge on Day 29. After 1 hour, blood was collected to assess serum levels of MCPT-1 (FIG. 10B) and histamine (FIG. 10A).

[00177] On day 30, mice were again bleed for analysis of antibodies in the serum (FIG. 9A- 9C). Mice were then sacrificed and the spleens from each mouse were collected and homogenized for *ex vivo* cytokine analysis and proliferation (FIG. 8). Spleens and were harvested, processed into red blood cell-free single cell suspensions and incubated with 1, 25 or 50 µg/mL JCP complete RPMI culture medium at 37°C for 48hr. 50 µL supernatant was removed for cytokine analysis and cells were then pulsed with 1 µCi/well [3H]TdR for the last 24 hours of culture. Proliferation was determined by [3H]TdR incorporation as detected by a Topcount Microplate Scintillation Counter (PerkinElmer, Waltham, MA).

[00178] These data indicate that treatment with JCP-TIMPs after JCP sensitization was sufficient reduce IFNγ, IL-17, and IL-5 production in *ex vivo* analyses. Similar results were observed for IL-4, IL-10 and IL-13 levels when splenocytes were stimulated with higher doses of JCP. Antibody levels remained unchanged after TIMP treatment.

Example 3: Murine model of JCP allergy and treatment with CRYJ1 and CRYJ2 TIMPs

Allergic airway inflammation model

[00179] Mice are immunized intraperitoneally (i.p.) with two doses of 10 μg CRYJ1 and CRYJ2, the major Japanese cedar pollen allergens in alum (3 mg) or alum and PBS alone. Mice are then challenged for 20 min with aerosolized CRYJ1 and CRYJ2 (10 mg/ml) for three consecutive days prior to tissue harvest.

Analysis of bronchoalveolar lavage eosinophils

[00180] Lungs are flushed with 1 mL of bronchoalveolar lavage fluid (BALF; 1 mM EDTA and 10% FBS in PBS). Total cell counts are determined and samples are cytospun onto slides and DiffQuik (Siemens, Newark, DE) stained for differential cell counts.

Airway histology

[00181] Lungs are collected and fixed in formalin and processed into paraffin. Parafin sections are stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS).

JCP-specific IgE

[00182] Serum is collected from mice upon sacrifice and CRYJ1/2-specific IgE is quantified by sandwich ELISA. Anti-mouse IgE (BD Biosciences) is used as the capture antibody and biotinylated CRYJ1/2 (prepared using EZ-Link Sulfo-NHS-LC-Biotin kit from Pierce [Rockford, IL]) is used as a secondary reagent. The quantity of CRYJ1/2-IgE is to be determined by a standard curve generated using purified mouse CRYJ1/2-IgE.

Cytokine quantification

[00183] BAL fluid and supernatants from recall response cultures (harvested at 48 h) are assayed for IL-4, IL-5, IL-13, IL-10, IL-17 and IFNγ by magnetic Milliplex MAP multiplex assay (Millipore, Billerica, MA).

Production and/or sourcing of Cedar pollen antigen

[00184] Purified cedar pollen extract is sourced from the Japanese Aerology Society. In addition, recombinant Japanese cedar pollen antigen is to be manufactured as previously described (Fujimura T, Int. Arch. Allergy Immunol. 2015;168(1):32-43). The amino acid sequences for the immunodominant epitopes in JCP are CRYJ1 (accession number AB081309; SEQ ID NO: 1) and CRYJ2 (accession number AB211810; SEQ ID NO: 2).

Production of research batch of TIMP-JCP

[00185] Recombinant JCP antigens are encapsulated into TIMP as described above. 200 mg of TIMP-CRYJ1 and/or CRYJ2 (TIMP-JCP^R) and 200 mg TIMP-OVA control will be used for efficacy testing in the mouse model of JCP allergy. Six groups of 5-10 mice will be treated with TIMP-OVA or TIMP-JCP^R given as either a one dose (Groups 1-3) or two dose (Groups 3-6) regimen. Animals are sensitized (either before or after TIMP treatment) to JCP antigen. On day 28 and 29 airway challenge will be performed and efficacy of treatment determined using the primary read outs and success measures described below. Note that group 7 is to be sensitized to OVA and is being used as positive allergy control, as these mice will be challenged with OVA instead of JCP.

Animal POC: TIMP-JCP treatment prior to sensitization

[00186] Six groups of 5-10 mice are treated as shown in Table 5 with TIMP-OVA or TIMP-JCP^R given as either a one dose (Groups 1-3) or two dose (Groups 4-6) regimen. Animals will then be sensitized to JCP antigen. ON day 28 and 29 airway challenge is performed and efficacy of treatment is determined using the primary read outs and success measures described below. Note that Group 7 is to be sensitized to OVA and is used as a positive allergy control, as these mice are challenged with OVA instead of JCP.

TABLE 5: Experimental Design: TIMP-JCP treatment prior to sensitization

Group	n	Sensitization	Treatment
Group 1	5-10	JCP	1 dose TIMP-JCP^P
Group 2	5-10	JCP	1 dose TIMP-JCP^R
Group 3	5-10	JCP	1 dose TIMP-OVA
Group 4	5-10	JCP	2 doses TIMP-JCP^P
Group 5	5-10	JCP	2 doses TIMP-JCP^R
Group 6	5-10	JCP	2 doses TIMP-OVA
Group 7	5-10	OVA	None
Group 8	5-10	None	None

Experiment is blinded and repeated 3 times.

Animal POC- TIMP-JCP treatment post sensitization

[00187] Six groups of mice are treated as shown in Table 6 below with TIMP-JCP^R or TIMP-OVA *after sensitization to JCP*. Two dose regimens are tested. This includes a one TIMP dose regimen (groups 1-3) and a two dose regimen (groups 4-6), separated by 7 days. On day 28 and 29 air challenge is performed and efficacy of treatment is determined using the primary read outs and success measures described below.

TABLE 6: Experimental Design: TIMP-JCP treatment post sensitization

Group	n	Sensitization	Treatment
Group 1	5-10	JCP	1 dose TIMP-JCP^P
Group 2	5-10	JCP	1 dose TIMP-JCP^R
Group 3	5-10	JCP	1 dose TIMP-OVA
Group 4	5-10	JCP	2 doses TIMP-JCP^P
Group 5	5-10	JCP	2 doses TIMP-JCP^R
Group 6	5-10	JCP	2 doses TIMP-OVA
Group 7	5-10	OVA	None

Experiment is blinded and repeated 3 times.

Primary Readouts and Success Measures in Animal Model

[00188] Primary readouts and success measures are measured in mice receiving TIMP-JCP^R treatment prior to sensitization. TIMP-JCP^R treated mice do not have a change in temperature compared to baseline temperature following air challenge. There is no change in blood eosinophil and mast cell numbers compared to baseline measures in TIMP-JCP^R treated mice. TIMP-JCP^R treated mice have significantly reduced JCP-specific IgE compared to TIMP-OVA treated mice sensitized to JCP. TIMP-JCP^R treated mice also have significantly reduced systemic IL-4, IL-5, and IL-13 compared to TIMP-OVA treated mice sensitized to JCP. TIMP-JCP^R compared to TIMP-OVA treated mice.

[00189] Primary readouts and success measures are measured in mice receiving TIMP-JCP^R treatment after sensitization. TIMP-JCP^R treated mice do not have a change in temperature compared to baseline temperature following air challenge. There is no change in blood eosinophil and mast cell numbers compared to baseline measures in TIMP-JCP^R treated mice. TIMP-JCP^R treated mice have significantly reduced JCP-specific IgE

compared to TIMP-OVA treated mice sensitized to JCP. TIMP-JCP^R treated mice also have significantly reduced JCP-specific systemic IL-4, IL-5, and IL-13 compared to TIMP-OVA treated mice sensitized to JCP. TIMP-JCP^R treated mice also have reduced specific antigen production compared to TIMP-OVA treated mice.

Example 4: Clinical Study for TIMP-JCP

[00190] A clinical study is performed to evaluate the efficacy and safety of TIMP-JCP in a single-center, non-blinded, placebo controlled study in Japanese Seasonal Allergy Rhinitis (SAR) patients exposed to quantitative high-density Japanese cedar pollen.

[00191] The primary objective of the clinical study is to analyse and evaluate changes in total nasal symptom score (TNSS) in Japanese SAR patients treated with oral TIMP-JCP during high-density exposure to Japanese cedar pollen.

[00192] The secondary objectives are to analyze and evaluate changes in Total Symptom Score (TSS), symptom scores, amount of nasal discharge, number of sneeze, and patient's impression in patents with allergic Rhinitis, and to evaluate the safety of TIMP-JCP administered in intravenous route in patients receiving high-density exposure to pure Japanese cedar pollen.

General Study Design.

[00193] The safety and efficacy of TIMP-JCP is determined in patients allergic to Japanese cedar pollen, using an environmental exposure unit. There are numerous environmental exposure units (EEU), however, this study works in collaboration with the Japan Health Supporting network unit in the Wakayama prefecture. Free from the many limitations of conventional approaches, the EEU enables a precise and controlled study to be designed and executed (A similar study design is described by Enomoto, *et al.*, 2009). The below diagram outlines the dosing and event schedules.

Subjects

[00194] There are 25 total subjects in the clinical study. Five subjects are treated with placebo. Twenty subjects are treated with TIMP-JCP.

Diagnosis and criteria for inclusion

[00195] **Inclusion criteria:**

• Japanese patients with SAR.

• Patients with a history of symptoms of Japanese cedar pollinosis for at least 2 years.

• A positive IgE (for Japanese cedar pollen antigen): Determined by Fluorescence-Enzyme Immunoassay (FEIA) or Chemiluminescent Enzyme Immunoassay (CLEIA) within 1.5 years prior to the day of the screening exposure test.

• Patients with TNSS of 8 or more and nasal congestion score of 2 (moderate) or more at least one assessment point of 90 to 150 minutes after the start of the screening exposure. (TNSS) and nasal congestion scores may be at any other assessment points if it meets the criteria) • Age ≥20 and ≤65 years (no gender preferences).

• Patients with signed informed consent.

[00196] Exclusion criteria:

• Patient with symptoms of Perennial allergic rhinitis.

• Patients with severe asthma, bronchiectasis, severe hepatic, renal, or cardiac dysfunction, hematological, endocrine disease, and other serious complications.

• Patients with nasal diseases (hypertrophic rhinitis, paranasal sinusitis, nasal polyps, deviation of the nasal septum, etc.) or eye diseases that could interfere with judgment of the efficacy of TIMP-JCP

• Patients with evidence of upper and/or lower respiratory tract inflammation (acute rhinitis, chronic rhinitis, congestive rhinitis, atrophic rhinitis, purulent nasal discharge, sinusitis in the presence of cold-like, etc.) on the day of treatment exposure.

• Patients who have taken any of the following medications that may affect the evaluation of TIMP-JCP (including anti-contestants and immune modifying agents).

[00197] Additional Exclusion Criteria:

[00198] Within 2 weeks prior to the day of first dose:

• Anti-allergic drugs, antihistamines (H1 and H2 blockers: oral administration, nose drops, eye drops, injection, and topical use), anticholinergic agents, vasoconstrictor nose drops, antihistamine-containing cold remedies, agents that can be expected to have an anti-allergic/ antihistaminic effect (including Chinese medicines and glycyrrhizin), and other agents that are indicated for allergic symptoms (sneezing, rhinorrhea, nasal congestion, and eye itching etc.).

• Steroids (oral, inhaled, nose drops, eye drops, or topical use), immunosuppressants (oral, topical use, or injected), azole fungicides, and histamine containing gamma-globulin preparations.

• Azole fungicides, macrolide antibiotics, and preparations containing aluminum hydroxide/magnesium hydroxide.

[00199] Within 4 weeks prior to the day of the screening exposure test:

• Depot steroid preparations.

[00200] Within 6 months prior to the day of the screening exposure test: Steroid injections.

• Steroid injections

[00201] Within 1 year prior to the day of the screening exposure test:

• Patients receiving maintenance therapy for specific hypo-sensitization or receiving nonspecific alternative therapy

• Patients who are participating in another study or who have previously participated in another study within the previous 6 months prior to the informed consent.

• Patients who are considered by the Investigator/sub-investigator to be unsuitable for enrolment in the study for any other criterion.

• Patient with a history of hypersensitivity to antihistamines or antihistaminic agent (fexofenadine HCI is included), and the pseudoephedrine hydrochloride.

• Patients who are participating in another study or who have previously participated in another study within the previous 6 months prior to the day of the screening exposure test.

• Women who are pregnant, may be pregnant, or currently breast-feeding.

[00202] **Safety and Stopping Criteria**

[00203] If any of the following parameters are met the study may be put on hold at the discretion of the data safety monitoring board:

a) If one or more unexpected drug related serious adverse events (SAE's) are reported to the data safety monitoring board.

b) Excessive and/or unexpected adverse events not clearly relatable to study drug.

c) Unexpected patient death(s)

Dosage, duration, and mode of administration:

[00204] **Drug:** TIMP-JCP, Poly(lactic-co-glycolic acid) tolerogenic immune modifying

[00205] **Dose and Form:** TIMP-JCP is provided as sterile lyophilized white powder in a vial. Each vial contains 500mg of TIMP-JCP. TIMP-JCP is given at a dose of [adjust per Phase 1 results –initial assumption is 10mg/kg].

[00206] **Duration of Treatment/Study:** Patient enrolment occurs following patient screening and informed consent. Dosing begins upon recruitment into the study. Dosing will be given at day 0 and once more at day 14. The first high-density Japanese cedar pollen exposure occurs at day 18-21 (patients are exposed to 8,000 grains/m^3 of JCP for 5 hours in environmental exposure unit). The second high-density Japanese cedar pollen exposure occurs at day 32-35 (patients are exposed to 8,000 grains/m3 of JCP for 5 hours in environmental exposure unit). Follow up visits occur at days 42, 49, and 56. The final follow-up visit occurs at day 63, which is 4 weeks after the second exposure.

[00207] **Mode of Administration:** Reconstituted TIMP-JCP is to be diluted in normal saline (NS; 0.9% Sodium Chloride, NaCl) by slow IV infusion over 30 minutes. TIMP-JCP may be discontinued at any time if the Investigator determines that alternative therapy should be administered

Criteria for evaluation

[00208] **Safety:**

• Incidence and severity of AE's, plus AE's of special interest (including standard reporting criteria for hypersensitivity and immune-mediated reactions).

• Vital signs (axillary temperature, blood pressure in a sitting position, and pulse rate in a sitting position)

• Standard laboratory evaluations

• 12-lead ECG Laboratory test (haematology, biochemistry, and urinalysis)

• Prick test for Japanese cedar pollen

• Parameters developed by antibody and histamine release test.

[00209] **Primary End Points:**

[00210] TNSS: Total score of sneezing, Rhinorrhea, Nasal Congestion and itchy nose. Each symptom is assessed by the patients in the following five categories; 1=none (no symptoms); 2=mild (symptoms present but easily tolerated); 3=moderate (awareness of symptoms; bothersome, but tolerable); 4=severe (definite awareness of symptoms; difficult to tolerate, but does not interfere with the activities of daily living), and 5=very severe (difficult to tolerate and interferes with the activities of daily living)

[00211] **Secondary efficacy endpoints:**

[00212] Immune monitoring:

• Antibody: IgG antibody, specific IgG antibody (anti-JCP, anti-CRYJ1, and anti-CRYJ2), specific IgG4 antibody (anti-JCP), IgE antibody, specific IgE antibody (anti-JCP)

• Cytokines (IFN-gamma, IL-4, IL-5, IL-10, IL-12, and IL-13)

• Immune cell phenotyping (Tregs, effector T cells and monocytes)

• Peripheral B and T cell responses to Japanese cedar pollen.

• Change in TSS, changes in each symptom score, nasal discharge amount, number of sneeze, and patient's impression.

• Nasal discharge amount: The weight of tissue papers after blowing patient's nose is measured every 1 hour. A difference from the weight before blowing the nose is calculated as nasal discharge amount.

• Number of sneezing: Patients recorded number of sneezing by themselves every 1 hour.

[00213] Pharmacokinetics (PK):

• Serum concentrations of TIMP-JCP measured daily in sparse sampling design during study drug administration.

[00214] **Conclusion:**

[00215] Subjects treated with TIMP-JCP show less occurrence and severity of allergic reactions to Japanese cedar pollen exposure. This is manifested by reduced TNSS in TIMP-JCP-treated subjects compared to placebo-treated subjects. JCP-treated subjects also have reduced levels specific IgG antibody (anti-JCP, anti-CRYJ1, and anti-CRYJ2), specific IgG4 antibody (anti-JCP), IgE antibody, specific IgE antibody (anti-JCP); reduced Peripheral B and T cell responses to Japanese cedar pollen; and lower TSS scores, symptom scores, nasal discharge amount, and number of sneezes compared to placebo group.

[00216] While specific embodiments of the invention have been described and illustrated, such embodiments should be considered illustrative of the invention only and not as limiting the invention as construed in accordance with the accompanying claims.

[00217] All patents, applications and other references cited herein are incorporated by reference in their entireties.

CLAIMS:

1. A composition comprising a biodegradable particle comprising one or more encapsulated antigenic epitopes from Japanese cedar pollen, wherein the biodegradable particle has a negative zeta potential.

2. The composition of claim 1, wherein the biodegradable particle comprises poly(lactide-co-glycolide) (PLG).

3. The composition of any of claims 1 or 2, wherein the biodegradable particle comprises PLG with a copolymer ratio of about 50:50 of polylactic acid:polyglycolic acid.

4. The composition of any of claims 1-3, wherein the surface of the biodegradable particle is carboxylated.

5. The composition of claim 4, wherein the carboxylation is achieved by using poly(ethylene-alt-maleic anhydride) (PEMA), poly(acrylic acid), or sodium deoxycholate.

6. The composition of any of claims 1-5, wherein the biodegradable particle has a zeta potential of about -100 mV to about 0 mV.

7. The composition of any of claims 1-6, wherein the biodegradable particle has a zeta potential of about -50 mV to about -40 mV.

8. The composition of any of claims 1-7, wherein the biodegradable particle has a zeta potential of about -75 mV to about -50 mV.

9. The composition of any of claims 1-8, wherein the biodegradable particle has a zeta potential of about -50 mV.

10. The compositions of any of claims 1-9, wherein the biodegradable particle has a zeta potential of about -30 mV.

11. The compositions of any of claims 1-10, wherein the biodegradable particle has a zeta potential of about -40 mV.

12. The composition of any of claims 1-11, wherein the biodegradable particle has a diameter of between about 0.1 μm to about 10 μm.

13. The composition of any of claims 1-12, wherein the biodegradable particle has a diameter of between about 0.3 μm to about 5 μm.

14. The composition of any of claims 1-13, wherein the biodegradable particle has a diameter of between about 0.5 μm to about 3 μm.

15. The composition of any of claims 1-14, wherein the biodegradable particle has a diameter of between about 0.5 μm to about 1 μm.

16. The composition of any of claims 1-15, wherein the biodegradable particle has a diameter of about 0.2 μm to about 0.7 μm.

17. The composition of claim 16, wherein the biodegradable particle has a diameter of about 0.7 μm.

18. The composition of claim 16, wherein the biodegradable particle has a diameter of about 0.5 μm.

19. The composition of any of claims 1-18, wherein the one or more encapsulated antigenic epitopes from Japanese cedar pollen comprises CRYJ1 or a fragment or variant thereof.

20. The composition of claim 19, wherein CRYJ1 has an amino acid sequence of SEQ ID NO: 1.

21. The composition of claim 20, wherein the fragment of CRYJ1 comprises at least 10, at least 20, at least 30, at least 40, or at least 50 consecutive amino acids with at least a 90% sequence identity to SEQ ID NO: 1.

22. The composition of claim 20, wherein the variant of CRYJ1 has an amino acid sequence with at least a 70%, at least a 75%, at least an 80%, at least an 85%, at least a 90%, at least a 95%, at least a 98%, or at least a 99% sequence identity to SEQ ID NO: 1.

23. The composition of claim 20, wherein the fragment of CRYJ1 is selected from the group consisting of p16–30, p81–95, p106–120, p111–125, p211–225, and p301–315.

24. The composition of any of claims 1-23, wherein the one or more encapsulated antigenic epitopes from Japanese cedar pollen comprises CRYJ2 or a fragment or variant thereof.

25. The composition of claim 24, wherein CRYJ2 has an amino acid sequence of SEQ ID NO: 2.

26. The composition of claim 25, wherein the fragment of CRYJ2 comprises at least 10, at least 20, at least 30, at least 40, or at least 50 consecutive amino acids with at least a 90% sequence identity to SEQ ID NO: 2.

27. The composition of claim 25, wherein the variant of CRYJ2 has an amino acid sequence with at least a 70%, at least a 75%, at least an 80%, at least an 85%, at least a 90%, at least a 95%, at least a 98%, or at least a 99% sequence identity to SEQ ID NO: 2.

28. The composition of claim 25, wherein the fragment of CRYJ2 is selected from the group consisting of p66–80, p81–95, p141–155, p186–200, p236–250, p346–360, p351–365, and p336–350.

29. The composition of any of claims 1-28, wherein the biodegradable particles comprise an antigen to polymer ratio between 1 μg/mg and 15 μg/mg.

30. The composition of claim 29, wherein the biodegradable particles comprise an antigen to polymer ratio between 5 μg/mg and 15 μg/mg.

31. The composition of any of claims 1-30, wherein the biodegradable particles comprise an antigen to polymer ratio of at least about 5 μg/mg.

32. The composition of any of claims 1-31, wherein the biodegradable particles comprise two or more encapsulated antigenic epitopes from Japanese cedar pollen proteins.

33. The composition of claim 32, wherein the two or more encapsulated epitopes are contained in a fusion protein, wherein the two or more encapsulated epitopes in the fusion protein are separated by a cleavable linker.

34. The composition of claim 33, wherein the amino acid sequence of the cleavable linker is cleavable by a protease located in the phagolysosome of a cell and/or a protease located in the cytosol of the cell.

35. The composition of claim 34, wherein the amino acid sequence of the cleavable linker is cleavable by a protease located in the phagolysosome of a cell and a protease located in the cytosol of the cell.

36. The composition of claims 34 or 35, wherein the cleavable linker a furin sensitive linker or cathepsin sensitive linker.

37. The composition of any of claims 34-36, wherein the cleavable linker is a furin sensitive linker.

38. The composition of any of claims 34-36, wherein the cleavable linker is a cathepsin sensitive linker.

39. The composition of claim 38, wherein the cathepsin sensitive linker is sensitive to cleavage by one or more of cathepsin A, cathepsin B, cathepsin C, cathepsin D, cathepsin E, cathepsin F, cathepsin G, cathepsin H, cathepsin K, cathepsin L, cathepsin O, cathepsin W, and/or cathepsin Z.

40. The composition of claim 34, wherein the amino acid sequence of the linker is Gly-Ala-Val-Val-Arg-Gly-Ala (SEQ ID NO: 3).

41. The composition of any of claims 1-40, wherein the one or more encapsulated antigenic epitopes from Japanese cedar pollen is covalently coupled to the biodegradable particle.

42. The composition of claim 41, wherein the one or more encapsulated antigenic epitopes from Japanese cedar pollen is covalently coupled to the biodegradable particle by a conjugate molecule.

43. The composition of claim 42, wherein the conjugate molecule comprises a carbodiimide compound.

44. The composition of claim 43, wherein the carbodiimide compound comprises 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC).

45. A composition comprising a biodegradable particle comprising one or more encapsulated antigenic epitopes from Japanese cedar pollen, wherein the biodegradable particle has a negative zeta potential of at least about -30 mV, a diameter of about 0.7 μm, and an antigen to polymer ratio of at least about 5 μg/mg.

46. A pharmaceutical composition comprising a biodegradable particle of any of claims 1-45.

47. The pharmaceutical composition of claim 46, further comprising a pharmaceutically acceptable carrier.

48. The pharmaceutical composition of claim 47, further comprising pharmaceutically acceptable excipients.

49. A lyophilized composition comprising the biodegradable particles of any of claims 1-45.

50. A method of inducing antigen-specific tolerance to Japanese cedar pollen in a subject comprising administering to the subject an effective amount of the pharmaceutical composition from any of claims 46-49.

51. A method for the treatment of a Japanese cedar pollen allergy in a subject in need thereof comprising administering the pharmaceutical composition of any of claims 46-49.

52. A method for the prevention of a Japanese cedar pollen allergy in a subject in need thereof comprising administering the pharmaceutical composition of any of claims 46-49.

53. A method of inducing antigen-specific tolerance to Japanese cedar pollen in a subject comprising reconstituting the lyophilized particles of claim 49 to obtain a reconstituted pharmaceutical composition and administering the reconstituted pharmaceutical composition to the subject.

54. A method for the treatment of a Japanese cedar pollen allergy in a subject in need thereof comprising reconstituting the lyophilized particles of claim 49 to obtain a reconstituted pharmaceutical composition and administering the reconstituted pharmaceutical composition to the subject.

55. A method for the prevention of a Japanese cedar pollen allergy in a subject in need thereof comprising reconstituting the lyophilized particles of claim 49 to obtain a reconstituted pharmaceutical composition and administering the reconstituted pharmaceutical composition to the subject.

FIG. 1

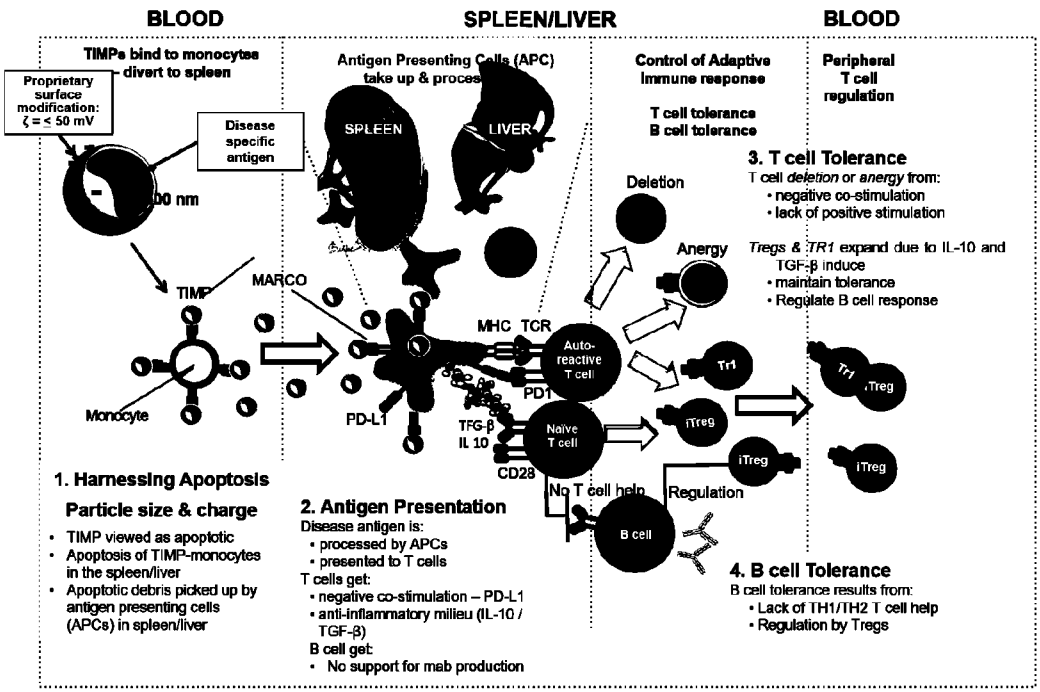

BLOOD
SPLEEN/LIVER
BLOOD
TIMPs bind to monocytes divert to spleen
Antigen Presenting Cells (APC) take up & proces
Control of Adaptive Immune response
T cell tolerance
B cell tolerance
Peripheral T cell regulation
Proprietary surface modification: ζ = < 50 mV
Disease specific antigen
SPLEEN
LIVER
nm
TIMP
MARCO
Monocyte
MHC
TCR
PD1
PD-L1
TFG-β
IL 10
CD28
Auto-reactive T cell
Naive T cell
Deletion
Anergy
Tr1
iTreg
No T cell help
Regulation
B cell
3. T cell Tolerance
T cell deletion or anergy from:
• negative co-stimulation
• lack of positive stimulation
Tregs & TR1 expand due to IL-10 and TGF-β induce
• maintain tolerance
• Regulate B cell response
1. Harnessing Apoptosis
Particle size & charge
• TIMP viewed as apoptotic
• Apoptosis of TIMP-monocytes in the spleen/liver
• Apoptotic debris picked up by antigen presenting cells (APCs) in spleen/liver
2. Antigen Presentation
Disease antigen is:
• processed by APCs
• presented to T cells
T cells get:
• negative co-stimulation – PD-L1
• anti-inflammatory milieu (IL-10 / TGF-β)
B cell get:
• No support for mab production
4. B cell Tolerance
B cell tolerance results from:
• Lack of TH1/TH2 T cell help
• Regulation by Tregs

FIG. 2

FIG. 2A

FIG. 2B

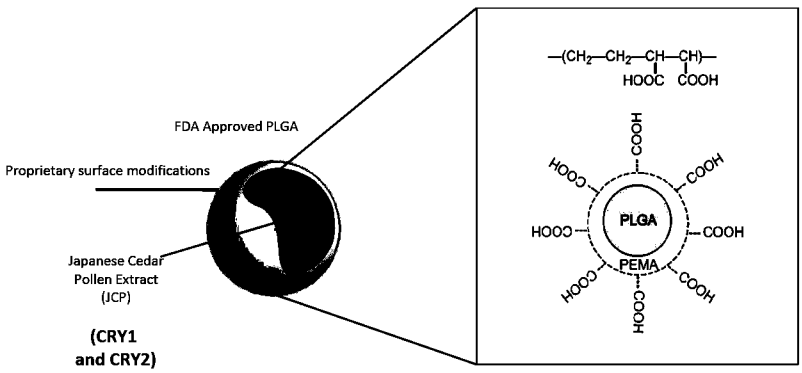


FIG. 2, Cont.

FIG. 2C

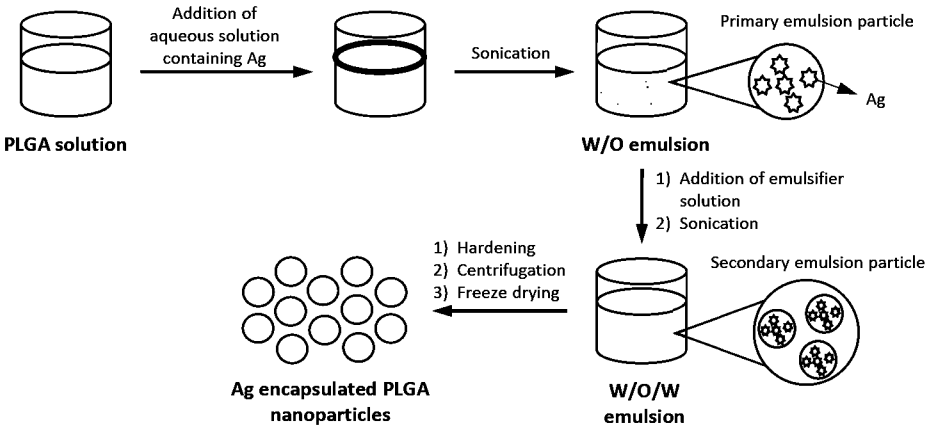


FIG. 3

FIG. 3A

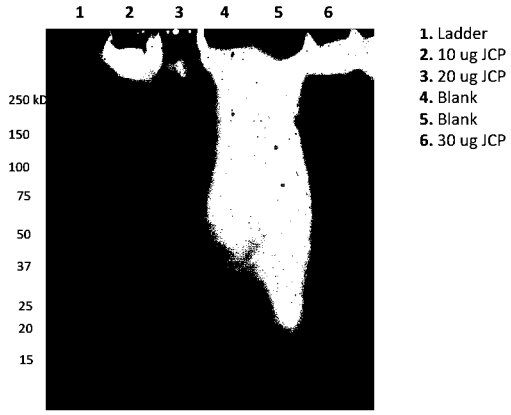
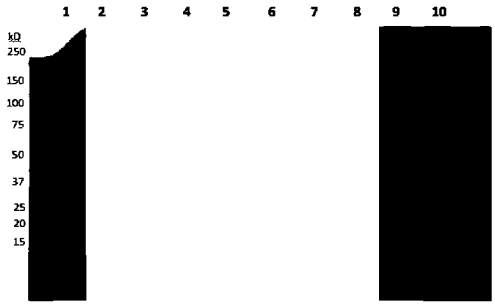


FIG. 3B



1. Molecular weight markers; 9. Reduced CryJ1; 10. Reduced CryJ2

FIG. 4

Batch	Concentration of JCP	Volume of JCP added	PLGA	Concentration of PLGA	Volume of PLGA added	Solvent	Comments
TIMP-JCP001	5 mg/mL	200 µL	LMW	200 mg/mL	500 µL	DCM	100% sonication amplitude
TIMP-JCP002	5 mg/mL	400 µL	LMW	100 mg/mL	1000 µL	DCM	100% sonication amplitude
TIMP-JCP003	5 mg/mL	200 µL	LMW	100 mg/mL	500 µL	DCM	50% sonication amplitude
TIMP-JCP004	5 mg/mL	200 µL	HMW	100 mg/mL	500 µL	DCM	50% sonication amplitude

Batch	Size (nm)	Zeta potential (mV)	PDI	Loading (µg/mg)	Encapsulation efficiency (%)
TIMP-JCP001	612.3 ± 7.1	-41.7 ± 1.5	0.247	0.34 ± 0.1	3.4 ± 0.6
TIMP-JCP002	442.7 ± 9.1	-39.0 ± 1.7	0.226	0.41 ± 0.02	2.1 ± 0.1
TIMP-JCP003	330.7 ± 2.1	-37.3 ± 0.9	0.263	0.28 ± 0.4	1.42 ± 1.8
TIMP-JCP004	993.3 ± 16.6	-33.7 ± 1.0	0.291	1.11 ± 0.2	5.6 ± 1.1

FIG. 5

FIG. 5A

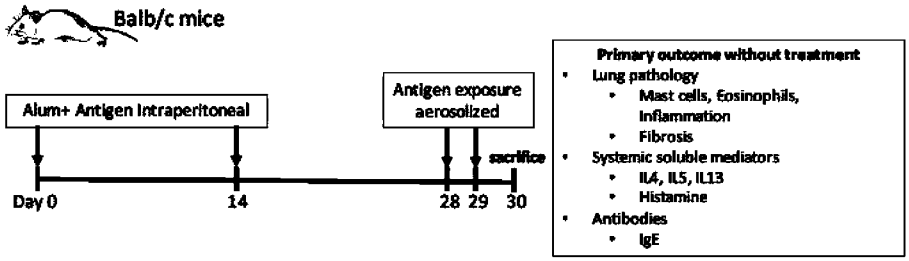


FIG. 5B

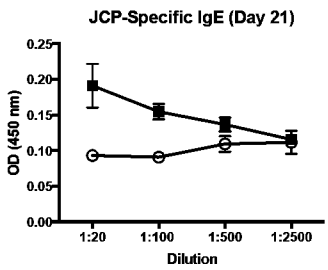


FIG. 5C

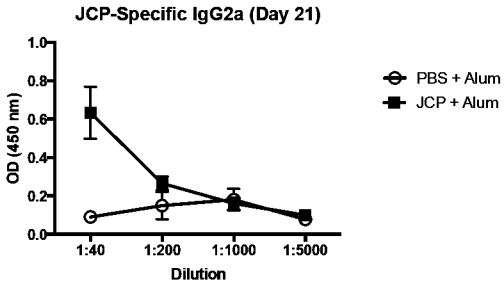


FIG. 5D

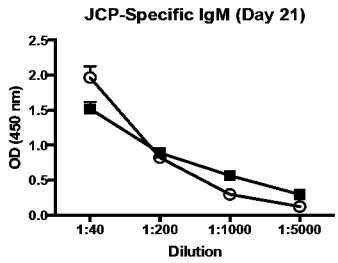


FIG. 6

Time	PBS+Alum (No particle Treatment)				
	Tail	Back	Right	Left	None
0	36	34.9	35.5	35.8	35.4
10	34.7	34.8	35.1	35.4	33.8
20	36.1	35.3	35.7	36	33.4
30	36.1	35	34.9	34.8	34.2
45	36	35.5	35.7	35.6	34.7
60	36.8	35.7	35.1	35.4	35.1
300	36.1	35.9	35.3	35.8	36

Time	TIMP-OVA Treated (Sensitized with JCP + Alum)				
	Tail	Back	Right	Left	None
0	36.2	37	36.6	36.4	37.1
10	34.4	35.6	34.4	36.5	36.2
20	36.4	36.6	34.2	36.6	36.7
30	36.5	36.4	34.9	35.7	36.5
45	35.4	36	34.6	36.5	36.5
60	36.5	35.9	36.6	35.7	36.6
300	35.6	35.1	35.7	36.4	36

Time	TIMP-JCP Treated (Sensitized with JCP + Alum)				
	Tail	Left	None	Back	Right
0	35.2	35.4	37		
10	34.1	34.3	32.5		
20	35.9	35.1	33.2		
30	36.6	35.8	34.5	N/A	N/A
45	36.1	35.3	36.2		
60	35.7	36	35		
300	35.7	35.5	35.9		

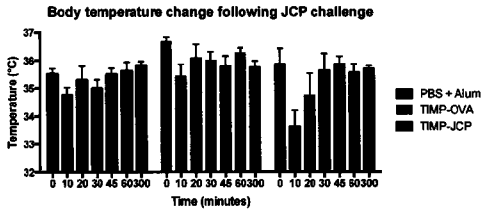


FIG. 7

Scratching Score						
Treatment	Mouse 1 T	Mouse 2 B	Mouse 3 R	Mouse 4 L	Mouse 5 N	Mean
PBS + Alum	4	5	11	16	4	8
TIMP-OVA	3	7	1	2	7	4
TIMP-JCP	11	NA	NA	16	1	9.33

Sneezing Score						
Treatment	Mouse 1 T	Mouse 2 B	Mouse 3 R	Mouse 4 L	Mouse 5 N	Mean
PBS + Alum	6	0	2	1	0	1.8
TIMP-OVA	2	0	0	1	1	0.8
TIMP-JCP	4	NA	NA	2	0	2

Coughing Score						
Treatment	Mouse 1 T	Mouse 2 B	Mouse 3 R	Mouse 4 L	Mouse 5 N	Mean
PBS + Alum	6	10	3	4	6	5.8
TIMP-OVA	14	15	24	12	4	13.8
TIMP-JCP	27	NA	NA	10	10	15.67

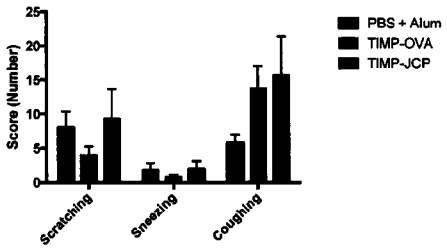


FIG. 8

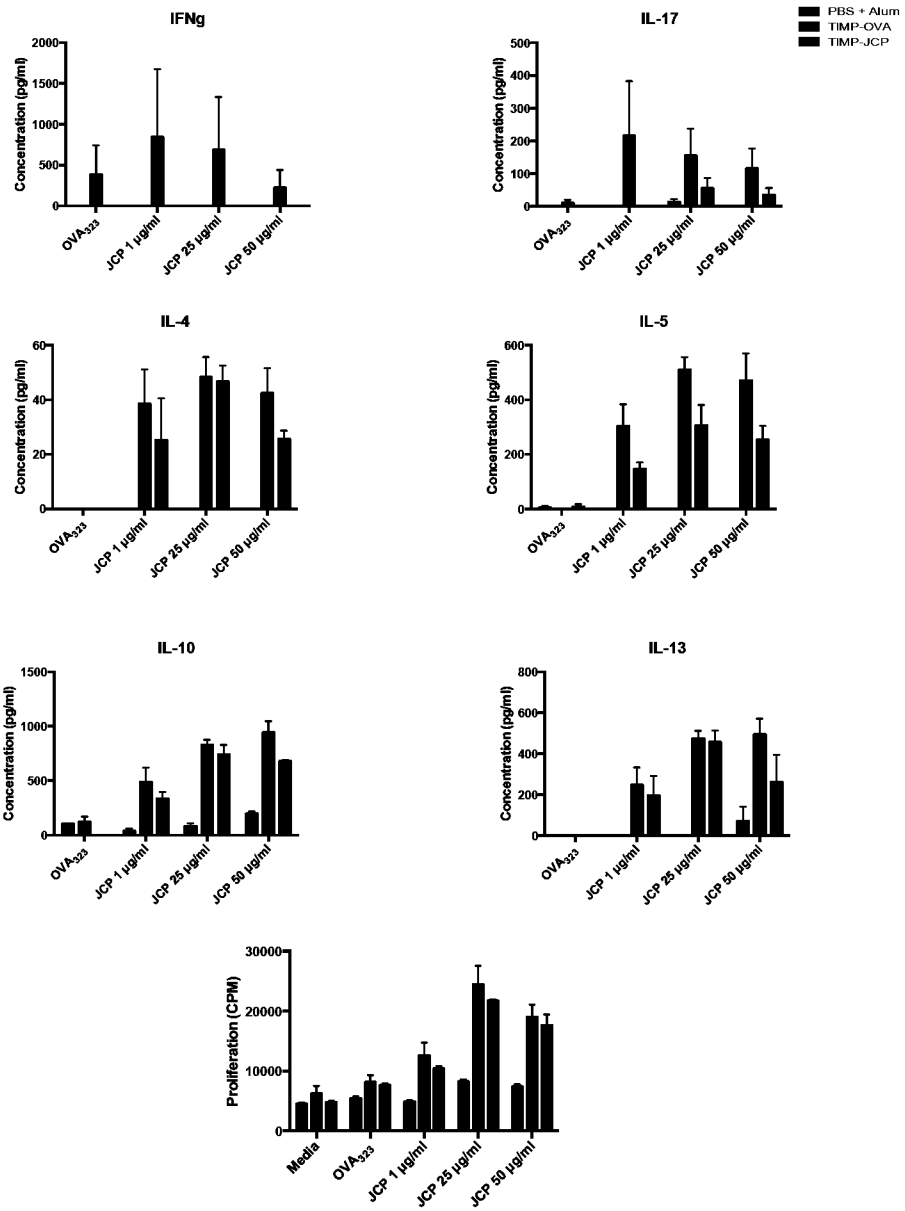

PBS + Alum
TIMP-OVA
TIMP-JCP
IFNg
IL-17
IL-4
IL-5
IL-10
IL-13
Proliferation
Concentration (pg/ml)
Proliferation (CPM)
OVA323
JCP 1 µg/ml
JCP 25 µg/ml
JCP 50 µg/ml
Media

FIG. 9

FIG. 9A

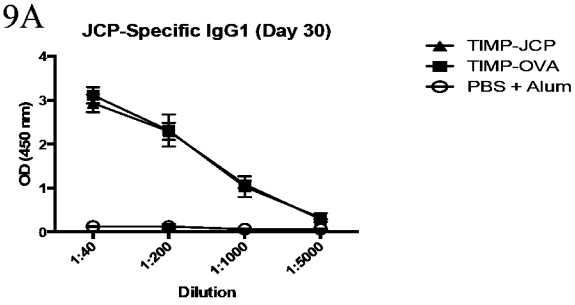


FIG. 9B

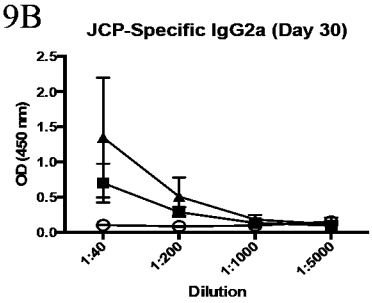


FIG. 9C

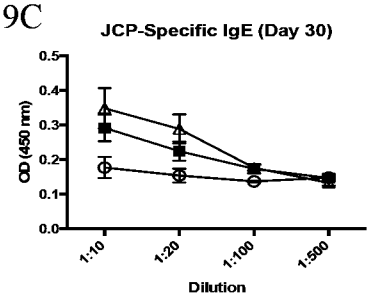


FIG. 10

FIG. 10A

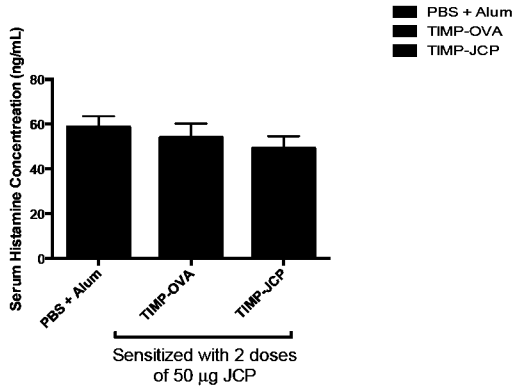


FIG. 10B

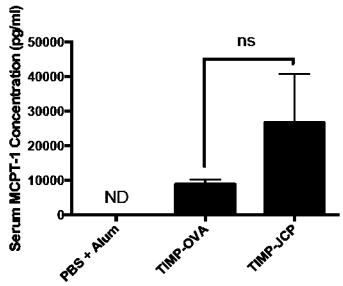
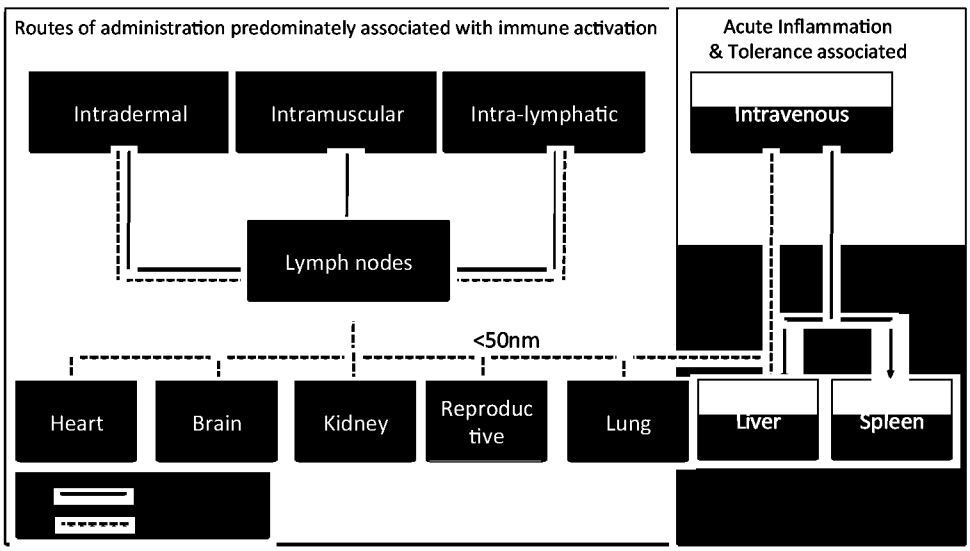


FIG. 11


Routes of administration predominately associated with immune activation
Intradermal
Intramuscular
Intra-lymphatic
Lymph nodes
<50nm
Heart
Brain
Kidney
Reproduc tive
Lung
Acute Inflammation & Tolerance associated
Intravenous
Liver
Spleen

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/17248

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/17248

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-44, 46-55
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/17248

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00, A61K 9/16 (2017.01)
CPC - A61K 39/0008, A61K 9/1647, A61K 39/00

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)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2015/0283218 A1 (NORTHWESTERN UNIVERSITY) 08 October 2015 (08.10.2015) abstract; para [0013]-[0021]; [0100].	1-3, 45
Y	US 2009/0156480 A1 (AKASHI et al.) 18 June 2009 (18.06.2009) para [0049]-[0050].	1-3, 45

☐ 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
29 March 2017	27 APR 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774