**Title:** ALLERGEN-SPECIFIC INDUCED TOLERGENIC DENDRITIC CELLS FOR ALLERGY THERAPY

**Abstract:** Disclosed are allergen-specific induced tolerogenic dendritic cells (iTDCs), as well as related compositions and methods.

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ALLERGEN-SPECIFIC INDUCED TOLEROGENTIC DENDRITIC CELLS FOR
ALLERGY THERAPY

RELATED APPLICATIONS
This application claims the benefit under 35 U.S.C. §119 of United States provisional application 61/531,103; United States provisional application 61/531,106; United States provisional application 61/531,109; United States provisional application 61/531,112; United States provisional application 61/531,115; United States provisional application 61/531,121; United States provisional application 61/531,124; United States provisional application 61/531,127; United States provisional application 61/531,131; United States provisional application 61/531,140; and United States provisional application 61/531,231; all filed September 6, 2011, the entire contents of each of which are incorporated herein by reference.

FIELD OF THE INVENTION
This invention relates to methods of administering allergen-specific induced tolerogenic dendritic cell (itDC) compositions to reduce an allergic response to an allergen in a subject, and related compositions. The methods and compositions allow for the shift to tolerogenic immune response development specific to allergens. The methods and compositions provided, therefore, can be used to generate a tolerogenic immune response in a subject that is experiencing or at risk of experiencing allergic responses against an allergen.

BACKGROUND OF THE INVENTION
Allergic responses in a subject are generally exaggerated and undesired but may be reduced through the use of immunosuppressant drugs. Conventional immunosuppressant drugs, however, are broad-acting. Additionally, in order to maintain immunosuppression, immunosuppressant drug therapy is generally a life-long proposition. Unfortunately, the use of broad-acting immunosuppressants are associated with a risk of severe side effects, such as tumors, infections, nephrotoxicity and metabolic disorders. Accordingly, new immunosuppressant therapies would be beneficial.

SUMMARY OF THE INVENTION
In one aspect, a method comprising administering to a subject allergen-specific induced tolerogenic dendritic cells (itDCs) in an amount effective to reduce an allergic
response to an allergen in the subject, wherein the allergen-specific itDCs present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of the allergen, and wherein the subject is experiencing or is at risk of experiencing the allergic response to the allergen is provided. In another aspect, a method comprising reducing an allergic response to an allergen in a subject by administering allergen-specific itDCs to the subject, wherein the allergen-specific itDCs present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of the allergen is provided. In another aspect, a method comprising administering to a subject a composition according to a protocol that was previously shown to reduce an allergic response to an allergen in one or more test subjects, wherein the composition comprises allergen-specific itDCs, and wherein the allergen-specific itDCs present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of the allergen is provided.

In one embodiment, the method further comprises providing or identifying the subject.

In another embodiment, the allergen induces or is expected to induce an undesired immune response in the subject.

In another embodiment, the allergen-specific itDCs are in or are administered in an amount effective to reduce an undesired immune response in the subject. In another embodiment, the undesired immune response is allergen-specific antibody production. In another embodiment, the undesired immune response is allergen-specific CD4+ T cell proliferation and/or activity.

In another embodiment, the allergen comprises an asthma antigen, a hay fever antigen, a hives antigen, an eczema antigen, a plant allergen, an insect sting allergen, an insect allergen, an animal allergen, a fungal allergen, a drug allergen, a pet allergen, a latex allergen, a mold allergen, a cosmetic allergen or a food allergen. In another embodiment, the food allergen comprises a milk allergen, an egg allergen, a nut allergen, a fish allergen, a shellfish allergen, a soy allergen, a legume allergen, a seed allergen or a wheat allergen. In another embodiment, the plant allergen is a ragweed allergen. In another embodiment, the allergen is associated with hay fever or allergic asthma.

In another embodiment, the method further comprises assessing the undesired immune response to the allergen in the subject prior to and/or after the administration of the allergen-specific itDCs. In another embodiment, the assessing is performed on a sample obtained from the subject.
In another embodiment, one or more maintenance doses of the allergen-specific itDCs are administered to the subject.

In another embodiment, the subject has or is at risk of having an allergy. In another embodiment, the allergy is allergic asthma, hay fever, hives, eczema, a plant allergy, an insect sting allergy, an insect allergy, an animal allergy, a fungal allergy, a drug allergy, a pet allergy, a latex allergy, a mold allergy, a cosmetic allergy or a food allergy. In another embodiment, the food allergy is a milk allergy, an egg allergy, a nut allergy, a fish allergy, a shellfish allergy, a soy allergy, a legume allergy, a seed allergy or a wheat allergy. In another embodiment, the plant allergy is a ragweed allergy. In another embodiment, the allergy is allergic asthma or hay fever.

In another embodiment, the administering is by parenteral, intraarterial, intranasal or intravenous administration or by injection to lymph nodes or anterior chamber of the eye or by local administration to an organ or tissue of interest. In another embodiment, the administering is by subcutaneous, intrathecal, intraventricular, intramuscular, intraperitoneal, intracoronary, intrapancreatic, intrahepatic or bronchial injection.

In another aspect, a method, comprising combining itDCs with MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen.

In one embodiment, the allergen induces or is expected to induce an undesired immune response in a subject. In another embodiment, the undesired immune response is allergen-specific antibody production. In another embodiment, the undesired immune response is allergen-specific CD4+ T cell proliferation and/or activity.

In another embodiment, the allergen comprises an asthma antigen, a hay fever antigen, a hives antigen, an eczema antigen, a plant allergen, an insect sting allergen, an insect allergen, an animal allergen, a fungal allergen, a drug allergen, a pet allergen, a latex allergen, a mold allergen, a cosmetic allergen or a food allergen. In another embodiment, the food allergen comprises a milk allergen, an egg allergen, a nut allergen, a fish allergen, a shellfish allergen, a soy allergen, a legume allergen, a seed allergen or a wheat allergen. In another embodiment, the plant allergen is a ragweed allergen. In another embodiment, the allergen is associated with hay fever or allergic asthma.

In another embodiment, the method further comprises collecting the itDCs after combining with the epitopes of the allergen. In another embodiment, the method further comprises making a dosage form comprising the allergen-specific itDCs. In another
embodiment, the method further comprises making the allergen-specific itDCs or the dosage form available to a subject for administration. In another embodiment, the allergen-specific itDCs are in an amount effective to reduce an undesired immune response in a subject. In another embodiment, the method further comprises assessing an undesired immune response to the allergen with the allergen-specific itDCs. In another embodiment, the assessing is performed in a subject. In another embodiment, the assessing is performed on a sample from the subject. In another embodiment, the undesired immune response is allergen-specific antibody production. In another embodiment, the undesired immune response is allergen-specific CD4+ T cell proliferation and/or activity.

In another aspect, a composition comprising allergen-specific itDCs wherein the allergen-specific itDCs present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen is provided.

In another embodiment, the allergen induces or is expected to induce an undesired immune response in a subject. In another embodiment, the undesired immune response is allergen-specific antibody production. In another embodiment, the undesired immune response is allergen-specific CD4+ T cell proliferation and/or activity.

In another embodiment, the allergen comprises an asthma antigen, a hay fever antigen, a hives antigen, an eczema antigen, a plant allergen, an insect sting allergen, an insect allergen, an animal allergen, a fungal allergen, a drug allergen, a pet allergen, a latex allergen, a mold allergen, a cosmetic allergen or a food allergen. In another embodiment, the food allergen comprises a milk allergen, an egg allergen, a nut allergen, a fish allergen, a shellfish allergen, a soy allergen, a legume allergen, a seed allergen or a wheat allergen. In another embodiment, the plant allergen is a ragweed allergen. In another embodiment, the allergen is associated with hay fever or allergic asthma.

In another embodiment, the allergen-specific itDCs are produced by any of the methods provided. In another embodiment, the allergen-specific itDCs are as defined in any of the compositions and methods provided. In another embodiment, the composition further comprises a pharmaceutically acceptable excipient.

In another aspect, a dosage form comprising any of the compositions provided is provided.

In another aspect, a process for producing a composition comprising allergen-specific induced tolerogenic dendritic cells (itDCs), the process comprising combining itDCs with MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell
epitopes of an allergen is provided. In one embodiment, said process comprises the steps as
defined in any of the methods provided.

In another aspect, a composition comprising allergen-specific induced tolerogenic
dendritic cells (itDCs) obtainable by any of the methods and processes provided is provided.

In another aspect, a composition comprising: (i) induced tolerogenic dendritic cells;
and (ii) MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no
B cell epitopes of an allergen is provided. In one embodiment, the allergen is any of the
allergens provided herein.

In another aspect, any of the compositions or dosage forms provided may be for use in
therapy or prophylaxis.

In another aspect, any of the compositions or dosage forms provided may be for use in
a method of therapy or prophylaxis of an allergy in a subject or in any of the methods
provided.

In another aspect, a use of any of the compositions or dosage forms provided for the
manufacture of a medicament for use in a method of therapy or prophylaxis of an allergy in a
subject or in any of the methods provided is provided.

In another aspect, a composition comprising MHC Class I-restricted and/or MHC
Class II-restricted epitopes but substantially no B cell epitopes of an allergen for use in a
method comprising:

(i) providing MHC Class I-restricted and/or MHC Class II-restricted epitopes but
substantially no B cell epitopes of the allergen;

(ii) providing allergen-specific induced tolerogenic dendritic cells (itDCs) by
loading DCs with the epitopes of step (i); and

(iii) administering the allergen-specific itDCs to a subject prior to, concomitantly
with or after exposure to the allergen is provided.

In another aspect, allergen-specific itDCs for use in a method of therapy or
prophylaxis of allergy in a subject, said method comprising:

(i) providing MHC Class I-restricted and/or MHC Class II-restricted epitopes but
substantially no B cell epitopes of an allergen;

(ii) providing allergen-specific itDCs by loading DCs with the epitopes of step (i); and

(iii) administering the allergen-specific itDCs to said subject prior to,
concomitantly with or after exposure to the allergen is provided.
In another aspect, allergen-specific itDCs for use in a method comprising:

(i) providing MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen;

(ii) providing allergen-specific itDCs by loading DCs with the epitopes of step (i);

(iii) administering the allergen-specific itDCs to a subject is provided.

In another embodiment, any of the compositions or allergen-specific itDCs provided are as defined in any of the methods or compositions provided or the allergen is any of the allergens provided.

In another aspect, a dosage form comprising any of the compositions or allergen-specific itDCs provided is provided.

In embodiments of any of the compositions provided herein, the composition may further comprise an agent that enhances the migratory behavior (e.g., to an organ or tissue of interest) of the itDCs, including the allergen-specific itDCs. In embodiments of any of the methods provided herein, the method may further comprise administering an agent that enhances the migratory behavior of the itDCs.

In embodiments of any of the compositions and methods provided herein, the itDCs are not XCR1+ and/or CD8CC+ itDCs. In other embodiments of any of the compositions and methods provided herein, the itDCs are not derived from XCR1+ and/or CD8CC+ DCs.

In an embodiment of any of the compositions and methods provided herein, the allergens are peptides. Such allergens, in some embodiments, comprise at least an epitope as described anywhere herein but may also comprise additional amino acids that flank one or both ends of the epitope. In embodiments, the allergens comprise a whole allergenic protein. These allergens may be combined with the itDCs or precursors thereof to ultimately form the allergen-specific itDCs.

In an embodiment of any of the compositions and methods provided herein, the allergens comprise multiple types of allergens. In some embodiments, the allergens comprise multiple types of peptides that comprise the same epitopic sequence or different epitopic sequences.

**BRIEF DESCRIPTION OF FIGURES**
**Fig. 1** demonstrates that antigen-specific itDCs, including antigen-specific itDCs loaded with antigen using synthetic nanocarriers, effectively reduce the production of antigen-specific antibodies.

**Fig. 2** demonstrates a reduction in the number of antigen-specific B cells with the itDCs, even with the administration of the strong immune stimulant, Cpg.

**DETAILED DESCRIPTION OF THE INVENTION**

Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified materials or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting of the use of alternative terminology to describe the present invention.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety for all purposes.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to "a cell" includes a mixture of two or more such cells or a plurality of such cells, reference to "a DNA molecule" includes a mixture of two or more such DNA molecules or a plurality of such DNA molecules, and the like.

As used herein, the term "comprise" or variations thereof such as "comprises" or "comprising" are to be read to indicate the inclusion of any recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, element, characteristics, properties, method/process steps or limitations) but not the exclusion of any other integer or group of integers. Thus, as used herein, the term "comprising" is inclusive and does not exclude additional, unrecited integers or method/process steps.

In embodiments of any of the compositions and methods provided herein, "comprising" may be replaced with "consisting essentially of" or "consisting of. The phrase "consisting essentially of" is used herein to require the specified integer(s) or steps as well as those which do not materially affect the character or function of the claimed invention. As used herein, the term "consisting" is used to indicate the presence of the recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of
integers (e.g. features, element, characteristics, properties, method/process steps or limitations) alone.

A. INTRODUCTION

As previously mentioned, current conventional immunosuppressants are broad acting and generally result in an overall systemic down regulation of the immune system. The compositions and methods provided herein can achieve immune suppression in a more targeted and directed manner, for example, through the presentation to specific immune cells of specific antigens. It is believed that the administration of allergen-specific itDCs that present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen can cause a reduction in the amount of undesired allergic responses and result in beneficial tolerogenic immune responses specific to the allergen. As shown herein in the Examples, itDCs presenting antigen successfully reduced the production of antigen-specific antibodies. The reduction of the production if IgG antibodies is reflective of the production of immunoglobulins in general and can be extended to IgE antibodies, which have particular relevance to allergy and allergic responses. This effect was demonstrated using itDCs loaded with antigen using synthetic nanocarriers as well as of a particular subset of itDCs, CD103. Antigen-specific itDCs also successfully reduced the proliferation of antigen-specific B cells. These results demonstrate the utility of the compositions and methods provided herein to promote tolerogenic immune responses in subjects who are experiencing or are at risk of experiencing allergic responses to allergens. Such subjects include those who have or are at risk of having an allergy.

The inventors have unexpectedly and surprisingly discovered that the problems and limitations noted above can be overcome by practicing the invention disclosed herein. In particular, the inventors have unexpectedly discovered that it is possible to produce allergen-specific itDCs by combining itDCs with MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen and that these allergen-specific itDCs can be expected to reduce undesired immune responses to the allergen. Allergens may be combined with the itDCs in the form of the full length allergen itself or a fragment or derivative thereof or in the form of one or more cells that express the allergen. The cells may be in their native cellular form or they may be processed into a form suitable for uptake by the itDCs before combining with the itDCs. In embodiments, the processing comprises obtaining a cell suspension, a cell lysate, a cell homogenate, cell exosomes, cell
debris, conditioned medium, or a partially purified protein preparation from the cells that
express the antigen. In other embodiments, the processing comprises obtaining proteins,
protein fragments, fusion proteins, peptides, peptide mimeotypes, altered peptides, fusion
peptides from materials obtained from the cells. In other embodiments, the allergen is
combined with the itDCs in the presence of an agent that enhances the uptake, processing or
presentation of epitopes. The allergen-loading provided by such methods allows for the
production of itDCs specific to the allergen, and, thus, can result in allergen-specific itDCs.
In some embodiments, the allergen-specific itDCs are generated by contacting naïve itDCs
with allergens as provided above and elsewhere herein (e.g., as the full length allergens,
polypeptides or peptides that comprise the desired epitopes, or the epitopes themselves).

Allergen-specific itDCs can be administered to a subject in order to ameliorate an
undesired allergic response. In one aspect, a method comprising administering to a subject
allergen-specific itDCs in an amount effective to reduce an allergic response to an allergen in
the subject, wherein the allergen-specific itDCs present MHC Class I-restricted and/or MHC
Class II-restricted epitopes but substantially no B cell epitopes of an allergen is provided.
The subject may be one that is experiencing or is at risk of experiencing the allergic response
to the allergen. In another aspect, a method comprising reducing an allergic response to an
allergen in a subject by administering allergen-specific itDCs to the subject, wherein the
allergen-specific itDCs present MHC Class I-restricted and/or MHC Class II-restricted
epitopes but substantially no B cell epitopes of an allergen, is provided. In yet another
aspect, a method comprising administering to a subject according to a protocol that was
previously shown to reduce an allergic response to an allergen in one or more test subjects,
where the composition comprises allergen-specific itDCs, wherein the allergen-specific itDCs
present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no
B cell epitopes of an allergen, is provided.

Compositions of the allergen-specific itDCs are also provided. Allergen-specific
itDCs may be produced according to the methods provided and may, for example, reduce an
allergic response to an allergen. In embodiments, the allergen-specific itDCs present one or
more MHC Class I-restricted epitopes. In some embodiments, the allergen-specific itDCs
present or further present MHC Class II-restricted epitopes. In other embodiments, the
allergen-specific itDCs present substantially no B cell epitopes of the allergen, such as when
the presence of such epitopes would exacerbate an undesired immune response. In
embodiments, the methods of producing allergen-specific itDCs comprise combining itDCs
with MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen.

In embodiments, the allergen-specific iTDCs provided may be administered as one or more maintenance doses, such as to a subject that is exposed to or will be exposed to an allergen. In embodiments, the compositions provided are administered such that the allergic response is reduced for a certain length of time. Examples of such lengths of time are provided elsewhere herein.

In yet another aspect, dosage forms of any of the compositions provided herein are provided. Such dosage forms can be administered to a subject in need thereof (e.g., in need of allergic response reduction). Such a subject may be one that has or is at risk of having an allergy.

The invention will now be described in more detail below.

B. DEFINITIONS

"Administering" or "administration" means providing a material to a subject in a manner that is pharmacologically useful.

"Allergens" are any substances that can cause an undesired (e.g., a Type 1 hypersensitive) immune response (i.e., an allergic response or reaction) in a subject. Allergens include, but are not limited to, plant allergens (e.g., pollen, ragweed allergen), insect allergens, insect sting allergens (e.g., bee sting allergens), animal allergens (e.g., pet allergens, such as animal dander or cat Fel d 1 antigen), latex allergens, mold allergens, fungal allergens, cosmetic allergens, drug allergens, food allergens, dust, insect venom, viruses, bacteria, etc. Food allergens include, but are not limited to milk allergens, egg allergens, nut allergens (e.g., peanut or tree nut allergens, etc. (e.g., walnuts, cashews, etc.)), fish allergens, shellfish allergens, soy allergens, legume allergens, seed allergens and wheat allergens. Insect sting allergens include allergens that are or are associated with bee stings, wasp stings, hornet stings, yellow jacket stings, etc. Insect allergens also include house dust mite allergens (e.g., Der PI antigen) and cockroach allergens. Drug allergens include allergens that are or are associated with antibiotics, NSAIDs, anaesthetics, etc. Pollen allergens include grass allergens, tree allergens, weed allergens, flower allergens, etc.

Subjects that develop or are at risk of developing an undesired immune response to any of the allergens provided herein may be treated with any of the compositions and methods provided herein. Subjects that may be treated with any of the compositions and methods provided also
include those who have or are at risk of having an allergy to any of the allergens provided. "Allergens associated with an allergy" are allergens that generate an undesired immune response that results in, or would be expected by a clinician to result in, alone or in combination with other allergens, an allergic response or reaction or a symptom of an allergic response or reaction in a subject. "Type(s) of allergens" means molecules that share the same, or substantially the same, antigenic characteristics in the context of an undesired immune response. In some embodiments, the allergens may be proteins, polypeptides, peptides, lipoproteins or are contained or expressed in cells.

It is intended that epitopes of an allergen may be presented by the itDCs as provided herein. The epitopes themselves may be combined with the DCs or proteins, polypeptides, peptides, etc. that comprise these epitopes may be combined with the DCs. Thus an allergen itself or a portion thereof that comprises the epitopes may be combined with the DCs in the methods and compositions provided herein. The epitopes in the compositions and methods provided herein can be presented for recognition by cells of the immune system such as by, for example, T cells. Such epitopes may normally be recognized by and trigger an immune response in a T cell via presentation by a major histocompatibility complex molecule (MHC), but in the compositions provided herein the presentation of such epitopes by the itDCs can result in tolerogenic immune responses. In some embodiments, substantially no B cell epitopes are presented, such as when the inclusion of the B cell epitopes would exacerbate an undesired immune response and thus, the allergens or portions thereof, in some embodiments, substantially comprise no B cell epitopes.

An allergen can be combined with the DCs in the same form as what a subject is exposed to that causes an undesired immune response but may also be a fragment or derivative thereof. When a fragment or derivative, however, a desired immune response to the form encountered by such a subject is the preferable result with the compositions and methods provided.

"Allergen-specific", when referring to an immune response, refers to any immune response that results from the presence of the allergen, or portion thereof, or that generates molecules that specifically recognize or bind the allergen. For example, where the immune response is allergen-specific antibody production, antibodies are produced that specifically bind the allergen. As another example, where the immune response is allergen-specific B cell or CD4+ T cell proliferation and/or activity, the proliferation and/or activity results from
recognition of the allergen, or portion thereof, alone or in complex with MHC molecules, B cell receptors, etc.

The term "allergen-specific itDCs" refers to itDCs that present antigens associated with an allergen and modulate immune responses specific to the allergen (e.g., induce tolerance to the allergen or reduce an undesired immune response to the allergen in a subject). Preferably, the allergen-specific itDCs present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of the allergen (e.g., that elicit an undesired immune response, such as anaphylaxis). In some embodiments, allergen-specific itDCs present only a single epitope, while in other embodiments, allergen-specific itDCs present a plurality of epitopes. In some embodiments, allergen-specific itDCs are generated by antigen-loading of itDCs, for example, naive itDCs that have not been exposed to an antigen. In some embodiments, allergen-specific itDCs are administered to a subject and induce a tolerogenic reaction to an allergen in the subject. Antigen-loading is achieved, in some embodiments, by combining itDCs with the allergen (provided in any of the forms provided herein).

An "allergy" also referred to herein as an "allergic condition," is any condition where there is an undesired (e.g., a Type 1 hypersensitive) immune response (i.e., allergic response or reaction) to a substance. Such substances are referred to herein as allergens. Allergies or allergic conditions include, but are not limited to, allergic asthma, hay fever, hives, eczema, plant allergies, bee sting allergies, pet allergies, latex allergies, mold allergies, cosmetic allergies, food allergies, allergic rhinitis or coryza, topic allergic reactions, anaphylaxis, atopic dermatitis, hypersensitivity reactions and other allergic conditions. The allergic reaction may be the result of an immune reaction to any allergen. In some embodiments, the allergy is a food allergy. Food allergies include, but are not limited to, milk allergies, egg allergies, nut allergies, fish allergies, shellfish allergies, soy allergies or wheat allergies.

"Amount effective" in the context of a composition or dosage form for administration to a subject refers to an amount of the composition or dosage form that produces one or more desired immune responses in the subject, for example, the generation of a tolerogenic immune response. Therefore, in some embodiments, an amount effective is any amount of a composition provided herein that produces one or more of these desired immune responses. This amount can be for in vitro or in vivo purposes. For in vivo purposes, the amount can be one that a clinician would believe may have a clinical benefit for a subject in need of antigen-
specific tolerization. Such subjects include those that have or are at risk of having an allergy.

Amounts effective can involve only reducing the level of an undesired immune response, although in some embodiments, it involves preventing an undesired immune response altogether. Amounts effective can also involve delaying the occurrence of an undesired immune response. An amount that is effective can also be an amount of a composition provided herein that produces a desired therapeutic endpoint or a desired therapeutic result. Amounts effective, preferably, result in a tolerogenic immune response in a subject to an antigen. The achievement of any of the foregoing can be monitored by routine methods.

In some embodiments of any of the compositions and methods provided, the amount effective is one in which the desired immune response persists in the subject for at least 1 week, at least 2 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 9 months, at least 1 year, at least 2 years, at least 5 years, or longer. In other embodiments of any of the compositions and methods provided, the amount effective is one which produces a measurable desired immune response, for example, a measurable decrease in an immune response (e.g., to a specific antigen), for at least 1 week, at least 2 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 9 months, at least 1 year, at least 2 years, at least 5 years, or longer.

Amounts effective will depend, of course, on the particular subject being treated; the severity of a condition, disease or disorder; the individual patient parameters including age, physical condition, size and weight; the duration of the treatment; the nature of concurrent therapy (if any); the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reason.

In some embodiments, doses of the iTDCs in the compositions of the invention can range from a single cell to about $10^{12}$ cells. In some embodiments, the number of iTDCs administered to a subject can range from about 1 cell/kg body weight to about $10^8$ cells/kg.
In some embodiments, the number of itDCs administered is the smallest number that produces a desired immune response in the subject. In some embodiments, the dose is the largest number of itDCs that can be administered without generating an undesired effect in the subject, for example, an undesired side effect. Useful doses include, in some embodiments, cell populations of greater than $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$, $10^8$, $10^9$ or $10^{10}$ itDCs per dose. Other examples of useful doses include from about $1 \times 10^4$ to about $1 \times 10^6$, about $1 \times 10^6$ to about $1 \times 10^8$ or about $1 \times 10^8$ to about $1 \times 10^{10}$ itDCs per dose.

"Antigen" means a B cell antigen or T cell antigen. "Type(s) of antigens" means molecules that share the same, or substantially the same, antigenic characteristics. In some embodiments, antigens may be proteins, polypeptides, peptides, lipoproteins, glycolipids, polynucleotides, polysaccharides or are contained or expressed in cells. In some embodiments, such as when the antigens are not well defined or characterized, the antigens may be contained within a cell or tissue preparation, cell debris, cell exosomes, conditioned media, etc. and are provided as such. An antigen can be combined with the DCs in the same form as what a subject is exposed to that causes an undesired immune response but may also be a fragment or derivative thereof. When a fragment or derivative, however, a desired immune response to the form encountered by such a subject is the preferable result with the compositions and methods provided.

"Antigen-specific" refers to any immune response that results from the presence of the antigen, or portion thereof, or that generates molecules that specifically recognize or bind the antigen. For example, where the immune response is antigen-specific antibody production, antibodies are produced that specifically bind the antigen. As another example, where the immune response is antigen-specific B cell or CD4+ T cell proliferation and/or activity, the proliferation and/or activity results from recognition of the antigen, or portion thereof, alone or in complex with MHC molecules, by B cells, etc.

"Assessing an immune response" refers to any measurement or determination of the level, presence or absence, reduction, increase in, etc. of an immune response in vitro or in vivo. Such measurements or determinations may be performed on one or more samples obtained from a subject. Such assessing can be performed with any of the methods provided herein or otherwise known in the art.

An "at risk" subject is one in which a health practitioner believes has a chance of having a disease, disorder or condition as provided herein or is one a health practitioner believes has a chance of experiencing an undesired immune response as provided herein.
"B cell antigen" means any antigen that is or recognized by and triggers an immune response in a B cell (e.g., an antigen that is specifically recognized by a B cell or a receptor thereon). In some embodiments, an antigen that is a T cell antigen is also a B cell antigen. In other embodiments, the T cell antigen is not also a B cell antigen. B cell antigens include, but are not limited to proteins, peptides, etc.

"Cells processed into a form suitable for uptake by the itDCs" refers to cells that were treated or processed to a form suitable for antigen-loading of itDCs, such as naïve itDCs. In embodiments, the processing comprises obtaining a cell suspension, a cell lysate, a cell homogenate, cell exosomes, cell debris, conditioned medium, or a partially purified protein preparation. In other embodiments, the processing comprises obtaining proteins, protein fragments, fusion proteins, peptides, peptide mimeotypes, altered peptides, fusion peptides from the cells. In some embodiments, the processing includes an enrichment of cells from a cell population that displays a relevant antigen. In some embodiments, the enrichment results in a cell population that is at least 80%, at least 90%, at least 95%, at least 98%, at least 99% or 100% homogeneous in regard to an antigen of interest (i.e., the aforementioned percentages refer to the percent of cells in a population that express an antigen of interest). In some embodiments, the processing includes a purification of the cells, for example, from a mixed population of cells, or from a culture medium. In some embodiments, the processing comprises lysis of the cells to generate a crude cell lysate comprising antigen of interest. In some embodiments, the purification comprises fusing the cells to naïve itDCs, for example, by methods of electric pulse or chemical-induced cell fusion that are known to those of skill in the art. Additional methods of processing cells into a form suitable for uptake by itDCs are known to those of skill in the art and the invention is not limited in this respect.

The term "combining" refers to actively contacting one material, such as a population of cells with another material, such as another population of cells, or processed forms thereof, thus creating a mix or combination of materials, cell populations and/or processed forms. The term includes, in some embodiments, a combination under conditions that do not result in cell fusion. In other embodiments, the term includes contacting under conditions under which at least some of the cells of one population fuse with some of the cells of another population. Preferably, the combining of itDCs, or precursors thereof, with antigens of interest (provided in any of the forms provided herein) comprises contacting the itDCs, or precursors thereof, ex vivo.
"Concomitantly" means administering two or more substances to a subject in a manner that is correlated in time, preferably sufficiently correlated in time so as to provide a modulation in an immune response. In embodiments, concomitant administration may occur through administration of two or more substances in the same dosage form. In other embodiments, concomitant administration may encompass administration of two or more substances in different dosage forms, but within a specified period of time, preferably within 1 month, more preferably within 1 week, still more preferably within 1 day, and even more preferably within 1 hour.

"Dendritic cells," also referred to herein as "DCs," are antigen-presenting immune cells that process antigenic material and present it to other cells of the immune system, most notably to T cells. Immature DCs function to capture and process antigens. When DCs endocytose antigens, they process the antigens into smaller fragments, generally peptides, that are displayed on the DC surface, where they are presented to, for example, antigen-specific T cells through MHC molecules. After uptake of antigens, DCs migrate to the lymph nodes. Immature dendritic cells are characterized by high endocytic and micropinocytotic function. During maturation, DCs can be prompted by various signals, including signaling through Toll-like receptors (TLR), to express co-stimulatory signals that induce cognate effector T cells (Teff) to become activated and to proliferate, thereby initiating a T-cell mediated immune response to the antigen. Alternatively, DCs can present antigen to antigen-specific T cells without providing co-stimulatory signals (or while providing co-inhibitory signals), such that Teff are not properly activated. Such presentation can cause, for example, death or anergy of T cells recognizing the antigen, or can induce the generation and/or expansion of regulatory T cells (Treg). The term "dendritic cells" includes differentiated dendritic cells, immature, and mature dendritic cells. These cells can be characterized by expression of certain cell surface markers (e.g., CD1 lc, MHC class II, and at least low levels of CD80 and CD86), CD1 lb, CD304 (BDCA4)). In some embodiments, DCs express CD8, CD103, CD1d, etc. Other DCs can be identified by the absence of lineage markers such as CD3, CD14, CD19, CD56, etc. In addition, dendritic cells can be characterized functionally by their capacity to stimulate alloresponses and mixed lymphocyte reactions (MLR).

"Derived" means prepared from a material or information related to a material but is not "obtained" from the material. Such materials may be substantially modified or processed forms of materials taken directly from a biological material. Such materials also include materials produced from information related to a biological material.
"Differentiated" cells are cells that have acquired a functional cell type and cannot or do not differentiate into another cell type. Examples of differentiated cells include, but are not limited to, β-cells, Tregs, Teffs, muscle cells, neurons, glial cells, and hepatocytes. Cells that are "pluripotent" are cells that have the potential to develop, or differentiate, into all fetal or adult cell types, but typically lack the potential to develop into placental cells. Non-limiting examples of pluripotent cells include embryonic stem cells and induced pluripotent stem (iPS) cells.

"Dosage form" means a pharmacologically and/or immunologically active material in a medium, carrier, vehicle, or device suitable for administration to a subject.

"Epitope", also known as an antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by, for example, antibodies, B cells, or T cells. As used herein, "MHC Class I-restricted epitopes" are epitopes that are presented to immune cells by MHC class I molecules found on nucleated cells. "MHC Class II-restricted epitopes" are epitopes that are presented to immune cells by MHC class II molecules found on antigen presenting cells (APCs), for example, on professional antigen-presenting immune cells, such as on macrophages, B cells, and dendritic cells; or on non-hematopoietic cells, such as hepatocytes. "B cell epitopes" are molecular structures that are recognized by antibodies or B cells.

contents of each of which are incorporated herein by reference for disclosure of methods and algorithms for the identification of epitopes.

Other examples of epitopes that can be combined with or presented by the itDCs provided herein include any of the allergen-associated MHC Class II-restricted and B cell epitopes as provided as SEQ ID NOs: 1-516. Without wishing to being bound by any particular theory, MHC Class II-restricted epitopes include those set forth in SEQ ID NOs: 1-338 and B cell epitopes include those set forth in SEQ ID NOs: 339-516.

"Generating" means causing an action, such as an immune response (e.g., a tolerogenic immune response) to occur, either directly oneself or indirectly, such as, but not limited to, an unrelated third party that takes an action through reliance on one's words or deeds.

"Humoral immune response" means any immune response that results in the production or stimulation of B cells and/or the production of antibodies. Methods for assessing whether a humoral response is induced are known to those of ordinary skill in the art and include assessing antibody response by measuring antibody titers and/or assessing the number and/or activity of CD4+ T and/or B cells. Any humoral immune response against an antigen as provided herein, such as where tolerance against the antigen would be beneficial to a subject, can be undesired. An antigen associated with such humoral immune responses means an antigen that when administered to a subject can result in one or more of the undesired humoral immune responses (e.g., results in undesired antibody production against the antigen or undesired CD4+ T cell or B cell proliferation or activity specific to the antigen). The production of antibodies is referred to herein as an "antibody response". "Antibody titer" means a measurable level of antibodies. In some embodiments, the antibodies are antibodies of a certain isotype, such as IgG, IgE or a subclass thereof.

Methods for measuring antibody titers are known in the art and are described elsewhere herein. Methods for measuring CD4+ T or B cell proliferation or activity are also known in the art or described elsewhere herein.

"Identifying" is any action or set of actions that allows a clinician to recognize a subject as one who may benefit from the methods and compositions provided herein. Preferably, the identified subject is one who is in need of a tolerogenic immune response as provided herein. The action or set of actions may be either directly oneself or indirectly, such as, but not limited to, an unrelated third party that takes an action through reliance on one's words or deeds.
"Induced tolerogenic DCs" refers to dendritic cells capable of suppressing immune responses or generating tolerogenic immune responses, such as antigen-specific T cell-mediated immune responses, e.g., by reducing effector T cell responses to specific antigens, by effecting an increase in the number of antigen-specific regulatory T cells, etc. Induced tolerogenic DCs can be characterized by antigen specific tolerogenic immune response induction ex vivo and/or in vivo. Such induction refers to an induction of tolerogenic immune responses to one or more antigens of interest presented by the induced tolerogenic dendritic cells. In embodiments, induced tolerogenic dendritic cells have a tolerogenic phenotype that is characterized by at least one, if not all, of the following properties i) capable of converting naive T cells to Foxp3+ T regulatory cells ex vivo and/or in vivo (e.g., inducing expression of FoxP3 in the naive T cells); ii) capable of deleting effector T cells ex vivo and/or in vivo; iii) retain their tolerogenic phenotype upon stimulation with at least one TLR agonist ex vivo (and, in some embodiments, increase expression of costimulatory molecules in response to such stimulus); and/or iv) do not transiently increase their oxygen consumption rate upon stimulation with at least one TLR agonist ex vivo.

Starting populations of cells comprising dendritic cells and/or dendritic cell precursors may be "induced" by treatment, for example, ex vivo to become tolerogenic. In some embodiments, starting populations of dendritic cells or dendritic cell precursors are differentiated into dendritic cells prior to, as part of, or after induction, for example using methods known in the art that employ cytokines and/or maturation factors. In some embodiments, induced dendritic cells comprise fully differentiated dendritic cells. In some embodiments, induced dendritic cells comprise both immature and mature dendritic cells. In some embodiments, induced dendritic cells are enriched for mature dendritic cells.

"Load" refers to the amount of antigen combined with the dendritic cells and taken up and/or presented, preferably on their surface. Dendritic cells can be loaded with antigen according to methods described herein. In some embodiments, it is desirable to assess the level of antigen-loading achieved. For example, in some embodiments, it is desirable, to confirm that loading is sufficient to achieve a tolerogenic immune response in a subject. In some embodiments, the tolerogenic immune response is a certain level of antigen-specific CD4+ T cell, CD8+ T cell or B cell proliferation and/or activity. In other embodiments, the tolerogenic immune response is a certain level of antigen-specific antibody production. In other embodiments, the tolerogenic immune response is a certainly level of regulatory cell production and/or activity. In yet other embodiments, the tolerogenic immune response is a
certain level of regulatory (e.g., anti-inflammatory) cytokine production. Antigen-loading of dendritic cells can be assessed, for example, by assessing whether a population of iTDCs is able to induce a tolerogenic response in vitro, for example, when contacted with non-adherent peripheral blood mononuclear cells (PBMCs). In some embodiments, the iTDCs are contacted with a regulatory T cell (Treg) precursor population, or a population of cells comprising such a precursor, under conditions and for a time sufficient to induce activation and/or proliferation of the Treg cells. In some embodiments, the presence and/or the number or frequency of the Treg cells is measured after a time sufficient for induction and/or proliferation, for example, with an ELISPOT assay, which allows for single-cell detection.

Alternatively, the presence or the number of Treg cells can be determined indirectly, for example, by measuring a molecule secreted by the Treg cells, or a cytokine specific for activation of Treg cells. In some embodiments, the presence of Treg cells in the cell population contacted with the iTDCs indicates that antigen-loading is sufficient. In some embodiments, the number of Treg cells measured is compared to a control or reference number, for example, the number of antigen-specific Treg cells present or expected to be present in a sample not contacted with the iTDCs or contacted with naïve DCs. In some embodiments, if the number of Treg cells in the cell population contacted with the iTDCs is statistically significantly higher than the control or reference number, the antigen-loading of the iTDCs is indicated to be sufficient. In embodiments, the load is a function of the amount of Treg cells generated as compared to one or more reference or control numbers. In other embodiment, the load is a function of the amount of antigen combined with the iTDCs in addition to in addition to the activity observed and/or one or more reference or control numbers.

"Maintenance dose" refers to a dose that is administered to a subject, after an initial dose has resulted in an immunosuppressive (e.g., tolerogenic) response in a subject, to sustain a desired immunosuppressive (e.g., tolerogenic) response. A maintenance dose, for example, can be one that maintains the tolerogenic effect achieved after the initial dose, prevents an undesired immune response in the subject, or prevents the subject becoming a subject at risk of experiencing an undesired immune response, including an undesired level of an immune response. In some embodiments, the maintenance dose is one that is sufficient to sustain an appropriate level of a desired immune response.

"MHC" refers to major histocompatibility complex, a large genomic region or gene family found in most vertebrates that encodes MHC molecules that display fragments or
epitopes of processed proteins on the cell surface. The presentation of MHC:peptide on cell surfaces allows for surveillance by immune cells, usually a T cell. There are two general classes of MHC molecules: Class I and Class II. Generally, Class I MHC molecules are found on nucleated cells and present peptides to cytotoxic T cells. Class II MHC molecules are found on certain immune cells, chiefly macrophages, B cells and dendritic cells, collectively known as professional APCs. The best-known genes in the MHC region are the subset that encodes antigen-presenting proteins on the cell surface. In humans, these genes are referred to as human leukocyte antigen (HLA) genes.

"Obtained" means taken directly from a material and used with substantially no modification and/or processing.

"Pharmaceutically acceptable excipient" means a pharmacologically inactive material used together with the itDCs, including antigen-specific itDCs, to formulate the inventive compositions. Pharmaceutically acceptable excipients comprise a variety of materials known in the art, including but not limited to saccharides (such as glucose, lactose, and the like), preservatives such as antimicrobial agents, reconstitution aids, colorants, saline (such as phosphate buffered saline), and buffers.

"Protocol " refers to any dosing regimen of one or more substances to a subject. A dosing regimen may include the amount, frequency and/or mode of administration. In some embodiments, such a protocol may be used to administer one or more compositions of the invention to one or more test subjects. Immune responses in these test subject can then be assessed to determine whether or not the protocol was effective in reducing an undesired immune response or generating a desired immune response (e.g., the promotion of a tolerogenic effect). Any other therapeutic and/or prophylactic effect may also be assessed instead of or in addition to the aforementioned immune responses. Whether or not a protocol had a desired effect can be determined using any of the methods provided herein or otherwise known in the art. For example, a population of cells may be obtained from a subject to which a composition provided herein has been administered according to a specific protocol in order to determine whether or not specific immune cells, cytokines, antibodies, etc. were reduced, generated, activated, etc. Useful methods for detecting the presence and/or number of immune cells include, but are not limited to, flow cytometric methods (e.g., FACS) and immunohistochemistry methods. Antibodies and other binding agents for specific staining of immune cell markers, are commercially available. Such kits typically include staining
reagents for multiple antigens that allow for FACS-based detection, separation and/or quantitation of a desired cell population from a heterogeneous population of cells.

"Providing a subject" is any action or set of actions that causes a clinician to come in contact with a subject and administer a composition provided herein thereto or to perform a method provided herein thereupon. Preferably, the subject is one who is in need of a tolerogenic immune response as provided herein. The action or set of actions may be either directly oneself or indirectly, such as, but not limited to, an unrelated third party that takes an action through reliance on one's words or deeds.

"Subject" means animals, including warm blooded mammals such as humans and primates; avians; domestic household or farm animals such as cats, dogs, sheep, goats, cattle, horses and pigs; laboratory animals such as mice, rats and guinea pigs; fish; reptiles; zoo and wild animals; and the like.

"Substantially no B cell epitopes" refers to the absence of B cell epitopes in an amount (by itself, within the context of the antigen, in conjunction with a carrier or in conjunction with an inventive composition) that stimulates substantial activation of a B cell response. In embodiments, a composition with substantially no B cell epitopes does not contain a measurable amount of B cell epitopes of an antigen. In other embodiments, such a composition may comprise a measurable amount of B cell epitopes of an antigen but said amount is not effective to generate a measurable B cell immune response (by itself, within the context of the antigen, in conjunction with a carrier or in conjunction with an inventive composition), such as antigen-specific antibody production or antigen-specific B cell proliferation and/or activity, or is not effective to generate a significant measurable B cell immune response (by itself, within the context of the antigen, in conjunction with a carrier or in conjunction with an inventive composition). In some embodiments, a significant measurable B cell immune response is one that produces or would be expected to produce an adverse clinical result in a subject. In other embodiments, a significant measurable B cell immune response is one that is greater than the level of the same type of immune response (e.g., antigen-specific antibody production or antigen-specific B cell proliferation and/or activity) produced by a control antigen (e.g., one known not to comprise B cell epitopes of the antigen or to stimulate B cell immune responses). In some embodiments, a significant measurable B cell immune response, such as a measurement of antibody titers (e.g., by ELISA) is 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold or more greater than the same type of response produced by a control (e.g., control
antigen). In other embodiments, a composition with substantially no B cell epitopes is one that produces little to no antigen-specific antibody titers (by itself, within the context of the antigen, in conjunction with a carrier or in conjunction with an inventive composition). Such compositions include those that produce an antibody titer (as an EC50 value) of less than 500, 400, 300, 200, 100, 50, 40, 30, 20 or 10. In other embodiments, a significant measurable B cell immune response, is a measurement of the number or proliferation of B cells that is 10%, 25%, 50%, 100%, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold or more greater than the same type of response produced by a control. Other methods for measuring B cell responses are known to those of ordinary skill in the art.

In embodiments, to ensure that a composition comprises substantially no B cell epitopes, antigens are selected such that they do not comprise B cell epitopes for loading onto the itDCs, or precursors thereof, as provided herein. In other embodiments, to ensure that a composition comprises substantially no B cell epitopes of an antigen, the itDCs, or precursors thereof, are produced and tested for B cell immune responses (e.g., antigen-specific antibody production, B cell proliferation and/or activity). Compositions that exhibit the desired properties may then be selected.

"T cell antigen" means a CD4+ T-cell antigen or CD8+ cell antigen. "CD4+ T-cell antigen" means any antigen that is recognized by and triggers an immune response in a CD4+ T-cell e.g., an antigen that is specifically recognized by a T-cell receptor on a CD4+T cell via presentation of the antigen or portion thereof bound to a Class II major histocompatibility complex molecule (MHC). "CD8+ T cell antigen" means any antigen that is recognized by and triggers an immune response in a CD8+ T-cell e.g., an antigen that is specifically recognized by a T-cell receptor on a CD8+T cell via presentation of the antigen or portion thereof bound to a Class I major histocompatibility complex molecule (MHC). In some embodiments, an antigen that is a T cell antigen is also a B cell antigen. In other embodiments, the T cell antigen is not also a B cell antigen. T cell antigens generally are proteins or peptides.

"Tolerogenic immune response" means any immune response that can lead to immune suppression specific to an antigen or a cell, tissue, organ, etc. that expresses such an antigen. Such immune responses include any reduction, delay or inhibition in an undesired immune response specific to the antigen or cell, tissue, organ, etc. that expresses such antigen. Such immune responses also include any stimulation, production, induction, promotion or recruitment in a desired immune response specific to the antigen or cell, tissue,
organ, etc. that expresses such antigen. Tolerogenic immune responses, therefore, include the absence of or reduction in an undesired immune response to an antigen that can be mediated by antigen reactive cells as well as the presence or promotion of suppressive cells. Tolerogenic immune responses as provided herein include immunological tolerance. To "generate a tolerogenic immune response" refers to the generation of any of the foregoing immune responses specific to an antigen or cell, tissue, organ, etc. that expresses such antigen. The tolerogenic immune response can be the result of MHC Class I-restricted presentation and/or MHC Class II-restricted presentation and/or B cell presentation and/or presentation by CD1d, etc.

Tolerogenic immune responses include any reduction, delay or inhibition in CD4+ T cell, CD8+ T cell or B cell proliferation and/or activity. Tolerogenic immune responses also include a reduction in antigen-specific antibody production. Tolerogenic immune responses can also include any response that leads to the stimulation, induction, production or recruitment of regulatory cells, such as CD4+ Treg cells, CD8+ Treg cells, Breg cells, etc. In some embodiments, the tolerogenic immune response, is one that results in the conversion to a regulatory phenotype characterized by the production, induction, stimulation or recruitment of regulatory cells.

Tolerogenic immune responses also include any response that leads to the stimulation, production or recruitment of CD4+ Treg cells and/or CD8+ Treg cells. CD4+ Treg cells can express the transcription factor FoxP3 and inhibit inflammatory responses and auto-immune inflammatory diseases (Human regulatory T cells in autoimmune diseases. Cvetanovich GL, Hafler DA. Curr Opin Immunol. 2010 Dec;22(6):753-60. Regulatory T cells and autoimmunity. Vila J, Isaacs JD, Anderson AE. Curr Opin Hematol. 2009 Jul;16(4):274-9). Such cells also suppress T-cell help to B-cells and induce tolerance to both self and foreign antigens (Therapeutic approaches to allergy and autoimmunity based on FoxP3+ regulatory T-cell activation and expansion. Miyara M, Wing K, Sakaguchi S. J Allergy Clin Immunol. 2009 Apr;123(4):749-55). CD4+ Treg cells recognize antigen when presented by Class II proteins on APCs. CD8+ Treg cells, which recognize antigen presented by Class I (and Qa-1), can also suppress T-cell help to B-cells and result in activation of antigen-specific suppression inducing tolerance to both self and foreign antigens. Disruption of the interaction of Qa-1 with CD8+ Treg cells has been shown to dysregulate immune responses and results in the development of auto-antibody formation and an auto-immune lethal systemic-lupus-erythematosus (Kim et al., Nature. 2010 Sep 16, 467 (7313): 328-32). CD8+
Treg cells have also been shown to inhibit models of autoimmune inflammatory diseases including rheumatoid arthritis and colitis (CD4+CD25+ regulatory T cells in autoimmune arthritis. Oh S, Rankin AL, Caton AJ. Immunol Rev. 2010 Jan;233(1):97-111. Regulatory T cells in inflammatory bowel disease. Boden EK, Snapper SB. Curr Opin Gastroenterol. 2008 Nov;24(6):733-41. In some embodiments, the compositions provided can effectively result in both types of responses (CD4+ Treg and CD8+ Treg). In other embodiments, FoxP3 can be induced in other immune cells, such as macrophages, iNKT cells, etc., the compositions provided herein can result in one or more of these responses as well.

Tolerogenic immune responses also include, but are not limited to, the induction of regulatory cytokines, such as Treg cytokines; induction of inhibitory cytokines; the inhibition of inflammatory cytokines (e.g., IL-4, IL-1β, IL-5, TNF-cc, IL-6, GM-CSF, IFN-γ, IL-2, IL-9, IL-12, IL-17, IL-18, IL-21, IL-22, IL-23, M-CSF, C reactive protein, acute phase protein, chemokines (e.g., MCP-1, RANTES, MIP-lcc, MIP-1β, MIG, ITAC or IP-10), the production of anti-inflammatory cytokines (e.g., IL-4, IL-13, IL-10, etc.), chemokines (e.g., CCL-2, CXCL8), proteases (e.g., MMP-3, MMP-9), leukotrienes (e.g., CysLT-1, CysLT-2), prostaglandins (e.g., PGE2) or histamines; the inhibition of polarization to a Thl7, Thl or Th2 immune response; the inhibition of effector cell-specific cytokines: Thl7 (e.g., IL-17, IL-25), Thl (IFN-γ), Th2 (e.g., IL-4, IL-13); the inhibition of Thl-, Th2- or Thl7-specific transcription factors; the inhibition of proliferation of effector T cells; the induction of apoptosis of effector T cells; the induction of tolerogenic dendritic cell-specific genes; the induction of FoxP3 expression; the inhibition of IgE induction or IgE-mediated immune responses; the inhibition of antibody responses (e.g., antigen-specific antibody production); the inhibition of T helper cell response; the production of TGF-β and/or IL-10; the inhibition of effector function of autoantibodies (e.g., inhibition in the depletion of cells, cell or tissue damage or complement activation); etc. In some embodiments, the tolerogenic immune response includes the production of anti-inflammatory cytokines (e.g., IL-4 and/or IL-10). In some embodiments, the tolerogenic immune response is the reduction of antigen-specific antibodies and/or CD4+ T helper cells and/or B cells. Assessing CD4+ T helper cell or B cell stimulation may include analyzing CD4+ T helper cell or B cell number, phenotype, activation and/or cytokine production.

Any of the foregoing may be measured in vivo in one or more animal models or may be measured in vitro. One of ordinary skill in the art is familiar with such in vivo or in vitro
measurements. Undesired immune responses or tolerogenic immune responses can be monitored using, for example, methods of assessing immune cell number and/or function, tetramer analysis, ELISPOT, flow cytometry-based analysis of cytokine expression, cytokine secretion, cytokine expression profiling, gene expression profiling, protein expression profiling, analysis of cell surface markers, PCR-based detection of immune cell receptor gene usage (see T. Clay et al., "Assays for Monitoring Cellular Immune Response to Active Immunotherapy of Cancer" Clinical Cancer Research 7:1127-1135 (2001)), etc. Undesired immune responses or tolerogenic immune responses may also be monitored using, for example, methods of assessing protein levels in plasma or serum, T cell or B cell proliferation and functional assays, etc. In some embodiments, tolerogenic immune responses can be monitored by assessing the induction of FoxP3. In addition, specific methods are described in more detail in the Examples.

Preferably, tolerogenic immune responses lead to the inhibition of the development, progression or pathology of the diseases, disorders or conditions described herein. Whether or not the inventive compositions can lead to the inhibition of the development, progression or pathology of the diseases, disorders or conditions described herein can be measured with animal models of such diseases, disorders or conditions. In some embodiments, the reduction of an undesired immune response or generation of a tolerogenic immune response may be assessed by determining clinical endpoints, clinical efficacy, clinical symptoms, disease biomarkers and/or clinical scores. Undesired immune responses or tolerogenic immune responses can also be assessed with diagnostic tests to assess the presence or absence of a disease, disorder or condition as provided herein. Undesired immune responses can further be assessed by methods of measuring proteins levels and/or function in a subject. In embodiments, methods for monitoring or assessing undesired allergic responses include assessing an allergic response in a subject by skin reactivity and/or allergen-specific antibody production.

In some embodiments, monitoring or assessing the generation of an undesired immune response or a tolerogenic immune response in a subject can be prior to the administration of a composition of allergen-specific iTDCs provided herein and/or prior to exposure to an allergen. In other embodiments, assessing the generation of an undesired immune response or tolerogenic immune response can be after administration of a composition of allergen-specific iTDCs provided herein and/or after exposure to an allergen. In some embodiments, the assessment is done after administration of the composition of
allergen-specific iTDCs, but prior to exposure to an allergen. In other embodiments, the assessment is done after exposure to an allergen, but prior to administration of the composition. In still other embodiments, the assessment is performed prior to both the administration of the allergen-specific iTDCs and the exposure to an allergen, while in yet other embodiments the assessment is performed after administration of both the allergen-specific iTDCs and the exposure to an allergen. In further embodiments, the assessment is performed both prior to and after the administration of the allergen-specific iTDCs and/or the exposure to an allergen. In still other embodiments, the assessment is performed more than once on the subject to determine that a desirable immune state is maintained in the subject, such as a subject that has or is at risk of having an allergy.

An antibody response can be assessed by determining one or more antibody titers. "Antibody titer" means a measurable level of antibody production. Methods for measuring antibody titers are known in the art and include Enzyme-linked Immunosorbent Assay (ELISA). In embodiments, the antibody response can be quantitated, for example, as the number of antibodies, concentration of antibodies or titer. The values can be absolute or they can be relative. Assays for quantifying an antibody response include antibody capture assays, enzyme-linked immunosorbent assays (ELISAs), inhibition liquid phase absorption assays (ILPAAs), rocket Immunoelectrophoresis (RIE) assays and line Immunoelectrophoresis (LIE) assays. When an antibody response is compared to another antibody response the same type of quantitative value (e.g., titer) and method of measurement (e.g., ELISA) is preferably used to make the comparison.

An ELISA method for measuring an antibody titer, for example, a typical sandwich ELISA, may consist of the following steps (i) preparing an ELISA-plate coating material such that the antibody target of interest is coupled to a substrate polymer or other suitable material (ii) preparing the coating material in an aqueous solution (such as PBS) and delivering the coating material solution to the wells of a multiwell plate for overnight deposition of the coating onto the multiwell plate (iii) thoroughly washing the multiwell plate with wash buffer (such as 0.05% Tween-20 in PBS) to remove excess coating material (iv) blocking the plate for nonspecific binding by applying a diluent solution (such as 10% fetal bovine serum in PBS), (v) washing the blocking/diluent solution from the plate with wash buffer (vi) diluting the serum sample(s) containing antibodies and appropriate standards (positive controls) with diluent as required to obtain a concentration that suitably saturates the ELISA response (vii) serially diluting the plasma samples on the multiwell plate such to
cover a range of concentrations suitable for generating an ELISA response curve (viii) incubating the plate to provide for antibody-target binding (ix) washing the plate with wash buffer to remove antibodies not bound to antigen (x) adding an appropriate concentration of a secondary detection antibody in same diluent such as a biotin-coupled detection antibody capable of binding the primary antibody (xi) incubating the plate with the applied detection antibody, followed by washing with wash buffer (xii) adding an enzyme such as streptavidin-HRP (horse radish peroxidase) that will bind to biotin found on biotinylated antibodies and incubating (xiii) washing the multiwell plate (xiv) adding substrate(s) (such as TMB solution) to the plate (xv) applying a stop solution (such as 2N sulfuric acid) when color development is complete (xvi) reading optical density of the plate wells at a specific wavelength for the substrate (450 nm with subtraction of readings at 570 nm) (xvi) applying a suitable multiparameter curve fit to the data and defining half-maximal effective concentration (EC50) as the concentration on the curve at which half the maximum OD value for the plate standards is achieved.

"Undesired immune response" refers to any undesired immune response that results from exposure to an antigen, promotes or exacerbates a disease, disorder or condition provided herein (or a symptom thereof), or is symptomatic of a disease, disorder or condition provided herein, etc. Such immune responses generally have a negative impact on a subject's health or is symptomatic of a negative impact on a subject's health.

C. INVENTIVE COMPOSITIONS

Provided herein are methods and compositions and dosage forms related to allergen-specific induced tolerogenic dendritic cells useful for reducing allergic responses and promoting the generation of tolerogenic immune responses to allergens. Preferably, such allergen-specific iTDCs are produced by the methods provided herein through the combining of iTDCs, or precursors thereof, with antigens that comprise MHC Class I-restricted and/or MHC Class II-restricted epitopes of an allergen but substantially no B cell epitopes of the allergen. Such iTDCs are useful for the suppression, inhibition, prevention, or delay of the onset of an undesired allergic response in a subject, as described in more detail elsewhere herein. Such subjects include those that have or are at risk of having an allergy.

Some embodiments of this invention provide the aforementioned allergen-specific iTDCs. These iTDCs are capable of suppressing an immune response to an antigen presented by it by, for example, increasing the number of antigen-specific Treg cells and/or decreasing
the number of antigen-specific effector T cells. Treg cells are described elsewhere herein, while effector cells can be characterized by certain markers of activation, e.g., cytokine production. In some embodiments, effector T cells are CD4+ and/or reducing CD4+ T cell help.

The induced tolerogenic dendritic cells for use in the compositions and methods provided have a tolerogenic phenotype that is characterized by, for example, at least one of the following properties i) capable of converting naïve T cells to Foxp3+ T regulatory cells ex vivo and in vivo; ii) capable of deleting effector T cells ex vivo and in vivo; iii) retain their tolerogenic phenotype upon stimulation with at least one TLR agonist ex vivo (and in some embodiments, increase expression of costimulatory molecules with the same stimulus); and/or iv) do not transiently increase their oxygen consumption rate upon stimulation with at least one TLR agonist ex vivo. In some embodiments, the itDCs have at least 2 of the above properties. In some embodiments, the itDCs have at least 3 of the above properties. In yet some embodiments, the itDCs have all 4 of the above properties. Induced tolerogenic DCs that convert naïve T cells to Foxp3+ T regulatory cells are itDCs that induce expression of the transcription factor Foxp3 in naïve T cells, e.g., in the absence of cell division, such that naïve T cells that did not previously express Foxp3 are induced to express Foxp3 and become T reg cells. In addition to expression of Foxp3, T regulatory cells (Treg cells) express CD25 and are capable of sustained suppression of effector T cell responses.

It is known in the art that stimulation of Toll-like receptors (TLR) on the surface of DCs promotes DC activation, allowing DCs to induce proliferation of effector T cells. However, the itDCs described herein for use in the compositions and methods provided maintain their tolerogenic phenotype (are tolerogenically locked) even after being contacted with a maturation stimulus ex vivo, e.g., after stimulation with at least one TLR agonist. The presence of the tolerogenic phenotype of the cells can be demonstrated functionally, e.g., by confirming that cells treated with a maturation stimulus retain their functional tolerogenic phenotype as described herein. In some embodiments, induced tolerogenic dendritic cells treated with a maturation stimulus increase expression of costimulatory molecules (as compared to the level of expression of costimulatory molecules prior to stimulation), but retain their tolerogenic phenotype. Exemplary costimulatory molecules include one or more of CD80, CD86, and ICOS ligand. In some embodiments, induced tolerogenic dendritic cells treated with a maturation stimulus increase their expression of class II molecules and/or migratory capacities (as compared to the level of expression of class II molecules prior to
stimulation), but retain their tolerogenic phenotype. Tolerogenically locked itDCs may be produced by a tolerogenic locking protocol in which dendritic cells or dendritic cell precursors are treated in an ex vivo environment with a tolerogenic locking agent which renders them capable of, for example, at least one of: i) converting naïve T cells to Foxp3+ T regulatory cells ex vivo and ii) deleting effector T cells ex vivo. Further methods of producing tolerogenically locked itDCs are described in more detail below.

In embodiments, the antigens that are presented by the allergen-specific itDCs provided are any of the allergens provided or portions or derivatives thereof. In embodiments, the antigens are combined with the itDCs, or precursors thereof, in the presence of an agent that enhances the uptake, processing or presentation of antigens. Preferably, the loading of an antigen on the itDCs of the compositions and methods provided will lead to a tolerogenic immune response against the antigen and/or the cells in, by or on which the antigen is expressed. The antigens include any associated with an allergy or allergens that stimulate or are expected to stimulate an allergic response in a subject.

In some embodiments, the composition of the invention are formulated as a dosage form. Appropriate carriers or vehicles for administration (e.g., for pharmaceutical administration) of cells are compatible with cell viability and are known in the art. Such carriers may optionally include buffering agents or supplements that promote cell viability. In some embodiments, cells to be administered are formulated with one or more additional agents, e.g., survival enhancing factors or pharmaceutical agents. In some embodiments, cells are formulated with a liquid carrier which is compatible with survival of the cells.

Compositions according to the invention, therefore, may further comprise pharmaceutically acceptable excipients. The compositions may be made using conventional pharmaceutical manufacturing and compounding techniques to arrive at useful dosage forms. Techniques suitable for use in practicing the present invention may be found in Handbook of Industrial Mixing: Science and Practice, Edited by Edward L. Paul, Victor A. Atiemo-Obeng, and Suzanne M. Kresta, 2004 John Wiley & Sons, Inc.; and Pharmaceutics: The Science of Dosage Form Design, 2nd Ed. Edited by M. E. Auten, 2001, Churchill Livingstone. In an embodiment, the compositions are suspended in sterile saline solution for injection together with a preservative.

Typical inventive compositions may comprise inorganic or organic buffers (e.g., sodium or potassium salts of phosphate, carbonate, acetate, or citrate) and pH adjustment agents (e.g., hydrochloric acid, sodium or potassium hydroxide, salts of citrate or acetate,
amino acids and their salts) antioxidants (e.g., ascorbic acid, alpha-tocopherol), surfactants (e.g., polysorbate 20, polysorbate 80, polyoxyethylene9-10 nonyl phenol, sodium desoxycholate), solution and/or cryo/lyo stabilizers (e.g., sucrose, lactose, mannitol, trehalose), osmotic adjustment agents (e.g., salts or sugars), antibacterial agents (e.g., benzoic acid, phenol, gentamicin), antifoaming agents (e.g., polydimethylsiloxane), preservatives (e.g., thimerosal, 2-phenoxyethanol, EDTA), polymeric stabilizers and viscosity-adjustment agents (e.g., polyvinylpyrrolidone, poloxamer 488, carboxymethylcellulose) and co-solvents (e.g., glycerol, polyethylene glycol, ethanol).

In some embodiments, a cell, antigen, etc., may be isolated. Isolated refers to the element being separated from its native environment and present in sufficient quantities to permit its identification or use. This means, for example, the element may be (i) selectively produced by expression cloning or (ii) purified as by chromatography or electrophoresis. Isolated elements may be, but need not be, substantially pure. Because an isolated element may be admixed with a pharmaceutically acceptable excipient in a pharmaceutical preparation, the element may comprise only a small percentage by weight of the preparation. The element is nonetheless isolated in that it has been separated from the substances with which it may be associated in living systems, i.e., isolated from other lipids or proteins. Any of the elements provided herein may be isolated. Any of the antigens provided herein can be included in the compositions in isolated form.

D. METHODS OF MAKING AND USING THE INVENTIVE COMPOSITIONS

Some aspects of this invention provide methods of generating allergen-specific itDCs and related compositions, and some aspects provide methods of using the allergen-specific itDCs provided herein. The allergen-specific itDCs may be produced from itDCs generated by the methods provided herein that are combined with antigen associated with an allergen to produce allergen-specific itDCs. The allergen-specific itDCs may also be produced from itDCs generated according to the methods provided in PCT Publication, WO2011/09833.

In one embodiment, a protocol for producing itDCs for use in the methods provided employs one or more respirostatic agents for treatment of dendritic cells or dendritic cell precursors ex vivo to produce induced tolerogenic DCs capable of antigen specific tolerance induction by, for example, i) converting naïve T cells into FoxpP3+ CD4+ regulatory T cells, and/or ii) deleting effector T cells. In another embodiment, a protocol employs at least one agent which tolerogenically locks dendritic cells or dendritic cell precursors ex vivo to
produce induced tolerogenic DCs capable of antigen specific tolerance induction by, for example, i) converting naive T cells into FoxpP3+ CD4+ regulatory T cells, and/or ii) deleting effector T cells.

In some embodiments, itDCs are generated by treating a starting population of cells comprising dendritic cell precursors and/or dendritic cells with a tolerogenic stimulus. To obtain starting cell populations which comprise dendritic cell precursors and/or dendritic cells, samples of cells, tissues, or organs comprising dendritic cell precursors or dendritic cells are isolated from a subject, e.g., a human subject, using methods known in the art.

In some embodiments, a starting population which comprises dendritic cells and/or dendritic cell precursors is derived from splenic tissue. In some embodiments, a starting cell population which comprises dendritic cells and/or dendritic cell precursors is derived from thymic tissue. In some embodiments, a starting cell population which comprises dendritic cells and/or dendritic cell precursors is derived from bone marrow. In some embodiments, a starting cell population which comprises dendritic cells and/or dendritic cell precursors is derived from peripheral blood, e.g., from whole blood or from a sub-population obtained from blood, for example, via leukopheresis.

In some embodiments, a starting population of cells comprises dendritic cell precursors. In some embodiments, a population of cells comprising dendritic cell precursors can be harvested from the peripheral blood using standard mononuclear cell leukopheresis, a technique that is well known in the art. Dendritic cell precursors can then be collected, e.g., using sequential buoyant density centrifugation steps. For example, the leukopheresis product can be layered over a buoyant density solution (specific gravity = 1.077 g/mL) and centrifuged at 1,000 g for 20 minutes to deplete erythrocytes and granulocytes. The interface cells are collected, washed, layered over a second buoyant density solution (specific gravity = 1.065 g/mL), and centrifuged at 805 g for 30 minutes to deplete platelets and low-density monocytes and lymphocytes. The resulting cell pellet is enriched for dendritic cell precursors. Alternatively, a kit, such as EasySep Human Myeloid DC Enrichment Kit, designed to isolate dendritic cells from fresh blood or ammonium chloride-lysed leukopheresis by negative selection may also be used.

In some embodiments, a starting population of cells comprising dendritic cells can be obtained using methods known in the art. Such a population may comprise myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), and/or dendritic cells generated in culture from monocytes (e.g., MO-DC, MDDC). In some embodiments, dendritic cells and/or
dendritic cell precursors can also be derived from a mixed cell population containing such cells (e.g., from the circulation or from a tissue or organ). In certain embodiments, the mixed cell population containing DC and/or dendritic cell precursors is enriched such that DC and/or dendritic cell precursors make up greater than 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, 99.5%, 99.9% or more) of the cell population. In some embodiments, the dendritic cells described herein are purified by separation from some or all non-dendritic cells in a cell population. In exemplary embodiments, cells can be purified such that a starting population comprising dendritic cells and/or dendritic cell precursors contains at least 50% or more dendritic cells and/or dendritic cell precursors, e.g., a purity of 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, 99.5%, 99.9% or more.

In some embodiments, dendritic cells can be isolated using the techniques described in Current Protocols in Immunology, Wiley Interscience, November 19, 2009, or in Woo et al., Transplantation, 58:484 (1994), the entire contents of which are incorporated herein by reference. Those skilled in the art are able to implement modifications to the foregoing methods of isolating cells comprising dendritic cells and/or dendritic cell precursors without the exercise of undue experimentation. In some embodiments, dendritic cells can be purified using fluorescence-activated cell sorting for antigens present on their surface, e.g., CD11c in the case of certain dendritic cells. In some embodiments, DCs present in a starting population of cells express CD11c. In some embodiments, DCs and/or dendritic cell precursors present in a starting population of cells express class II molecules. A starting population of cells may be monitored for expression of various cell surface markers (e.g., including CD11c) using techniques known in the art.

In some embodiments, a population of cells comprising dendritic cells and/or dendritic cell precursors can be obtained from pluripotential cells present in blood as PBMCs. Although most easily obtainable from blood, the pluripotential cells may also be obtained from any tissue in which they reside, including bone marrow and spleen tissue. These pluripotential cells typically express CD14, CD32, CD68 and CD115 monocyte markers with little or no expression of CD83, p55 or accessory molecules such as CD40 and CD86.

In some embodiments, dendritic cell precursors can be differentiated into dendritic cells using methods known in the art prior to, during, or after treatment with at least one agent in a protocol to prepare induced tolerogenic dendritic cells. For example, when cultured in the presence of cytokines such as a combination of GM-CSF and IL-4 or IL-13, the pluripotential cells give rise to the immature dendritic cells. In some embodiments, FLT3
Ligand can be used for this purpose. For example, in some embodiments, a starting population of cells comprising dendritic cells and/or dendritic cell precursors can be cultured ex vivo in the presence of one or more agents which promote differentiation of DCs. In some embodiments, one or more of GMCSF or IL-4 is used to promote the development of DCs ex vivo, e.g., by culture for 1-15 days, 2-10 days, 3-9 days, 4-8 days, or 5-6 days or such other time to obtain sufficient differentiation. In some embodiments, induced dendritic cells are fully differentiated (either prior to, during, or after induction to produce induced tolerogenic dendritic cells).

In some embodiments, a starting population of cells comprising DCs and/or DC precursors can be obtained from PBMCs. Methods of obtaining PBMCs from blood, using methods such as differential sedimentation through an appropriate medium, e.g. Ficoll-Hypaque [Pharmacia Biotech, Uppsala, Sweden], are well known and suitable for use in this invention. In a preferred embodiment of the invention, the pluripotential cells are obtained by depleting populations of PBMCs of platelets, and T and B lymphocytes. Various methods may be used to accomplish the depletion of the non-pluripotential cells. According to one method, immunomagnetic beads labeled with antibodies specific for cells to be removed, e.g., T and/or B lymphocytes, either directly or indirectly may be used to remove the T and B cells from the PBMC population. T cells may also be depleted from the PBMC population by rosetting with neuramimidase treated red blood cells as described by O'Dherty (1993), which is incorporated herein by reference. In some embodiments, to produce 3 million mature dendritic cells, approximately 40 ml of blood can be processed. In some embodiments, 4 to 8 x 10^7 pluripotential PBMC give rise to approximately 3 million mature dendritic cells.

Cultures of immature dendritic cells may be obtained by culturing the pluripotent cells in the presence of cytokines which promote their differentiation for a time sufficient to achieve the desired level of differentiation, e.g., from 1-10 days, from 2-9 days, from 3-8 days, or from 4-7 days. As an example, a combination of GM-CSF and IL-4 at a concentration of each at between about 200 to about 2000 U/ml, between about 500 and 1000 U/ml, or about 800 U/ml (GM-CSF) and 1000 U/ml (IL-4) produces significant quantities of the immature dendritic cells. A combination of GM-CSF (10-200 ng/ml) and IL-4 (5-50 ng/ml) can also be used. It may also be desirable to vary the concentration of cytokines at different stages of the culture such that freshly cultured cells are cultured in the presence of higher concentrations of IL-4 (1000 U/ml) than established cultures (500 U/ml IL-4 after 2 days in culture). Other cytokines such as IL-13 may be found to substitute for IL-4. In some
embodiments, FLT3 ligand can be used for this purpose. Other protocols for this purpose are known in the art.

Methods for obtaining these immature dendritic cells from adherent blood mononuclear fractions are described in Romani et al. (1994); and Sallustio and Lanzavecchia, 1994) both of which are incorporated herein by reference. Briefly, lymphocyte depleted PBMCs are plated in tissue culture plates at a density of about 1 million cells/cm2 in complete culture medium containing cytokines such as GM-CSF and IL-4 at concentrations of each at between about 800 to 1000 U/ml and IL-4 is present at about 1000 U/ml.

In some embodiments, the source of immature dendritic cells is a culture of proliferating dendritic cell precursors prepared according to a method described in Steinman et al. International application PCT/US93/03141, which is incorporated herein by reference. Since the dendritic cells prepared from the CD34+ proliferating precursors mature to dendritic cells expressing mature characteristics it is likely that they also pass through a development stage where they are pluripotent.

In some embodiments, a starting population of cells comprising dendritic cells can be enriched for the presence of mature dendritic cells by contacting the immature dendritic cells with a dendritic cell maturation factor. As referred to herein, the dendritic cell maturation factor may actually be one or more specific substances which act alone or with another agent to cause the maturation of the immature dendritic cells, for example, with one or more of an adjuvant, a TLR agonist, a CD40 agonist, an inflammasome activator, an inflammatory cytokine, or combinations thereof.

The tolerogenic stimuli includes substances which, alone or in combination, induce a dendritic cell or a dendritic cell precursor to become tolerogenic, e.g., by inducing the dendritic cell to become capable of increasing the proportion of antigen specific Treg cells to antigen specific Teff cells in a cell population. More specifically, induced tolerogenic dendritic cells are produced by one or more agents which induce a tolerogenic phenotype in the DCs characterized by, for example, at least one of the following properties i) induced tolerogenic DCs are capable of converting naive T cells to Foxp3+ T regulatory cells ex vivo and in vivo; ii) induced tolerogenic DCs are capable of deleting effector T cells ex vivo and in vivo; iii) induced tolerogenic DCs retain their tolerogenic phenotype upon stimulation with at least one TLR agonist ex vivo (while in some embodiments, they increase expression of costimulatory molecules); and/or iv) induced tolerogenic DCs do not transiently increase their oxygen consumption rate upon stimulation with at least one TLR agonist ex vivo.
Exemplary tolerogenic stimuli include those agents which do not increase mitochondrial activation (e.g., as measured by oxygen consumption) or which disrupt electron transport in cells. Other exemplary tolerogenic stimuli include those agents which tolerogenically lock induced DCs into a tolerogenic phenotype. Exemplary tolerogenic stimuli include agents include inhibitors of mammalian Target of Rapamycin (mTOR), agonists of TGFP pathway signaling, statins, purinergic receptor pathway antagonists, and agents which inhibit mitochondrial electron transport, either alone or in combination. In some embodiments, a tolerogenic stimulus does not consist of rapamycin alone. In some embodiments, a tolerogenic stimulus does not consist of an mTOR inhibitor alone.

In some embodiments, after treatment with one or more tolerogenic stimuli (such as those set forth below, known in the art, or identified using the methods described herein) the cells may be removed from the agents, e.g., by centrifugation and/or by washing prior to further manipulation.

Exemplary agents that can constitute a tolerogenic stimulus include, but are not limited to mTOR inhibitors, TGFP pathway agonists, statins, purinergic receptor pathway agonists, and certain agents disrupting electron transport. It should be appreciated that additional tolerogenic stimuli, for example, additional agents that can constitute a tolerogenic stimulus, are known to those of skill in the art, and that the invention is not limited in this respect.

For example, in some embodiments, the invention provides methods of producing a population of cells comprising induced tolerogenic DCs, wherein the method comprises contacting a starting population of cells comprising dendritic cells or dendritic cell precursors ex vivo with a tolerogenic stimulus. In some embodiments, the tolerogenic stimulus comprises at least one agent that promotes the induction of tolerogenic dendritic cells, or that results in the emergence of iTDCs in the cell population. In some embodiments, the at least one agent is selected from the group consisting of: i) an mTOR inhibitor and a TGFP agonist; ii) a statin; iii) an mTOR inhibitor and a statin; iv) an mTOR inhibitor, a TGFP agonist, and a statin; v) a purinergic receptor antagonist; vi) a purinergic receptor antagonist and a statin; vii) a purinergic receptor antagonist and an mTOR inhibitor; viii) a purinergic receptor antagonist, an mTOR inhibitor and a TGFP agonist; ix) a purinergic receptor antagonist, an mTOR inhibitor, a TGFP agonist and a statin; x) an agent which disrupts mitochondrial electron transport in the DCs; xi) an agent which disrupts mitochondrial electron transport in the DCs and an mTOR inhibitor; xii) an agent which disrupts
mitochondrial electron transport in the DCs and a statin; xiii) an agent which disrupts mitochondrial electron transport in the DCs, an mTOR inhibitor, and a TGFP agonist; and xiv) an agent which disrupts mitochondrial electron transport in the DCs, an mTOR inhibitor, a TGFP agonist, and a statin.

In some embodiments, the at least one agent is selected from the group consisting of: i) an mTOR inhibitor and a TGFP agonist; ii) a statin; iii) an mTOR inhibitor, a TGFP agonist, and a statin; iv) a purinergic receptor antagonist; and v) an agent which disrupts mitochondrial electron transport in the DCs.

In some embodiments, the at least one agent is a respirostatic agent or an agent that promotes respirostatic tolerance.

In some embodiments, the at least one agent comprises an mTOR inhibitor and a TGFP agonist. In some embodiments, the mTOR inhibitor comprises rapamycin or a derivative or analog thereof. In some embodiments, the TGFP agonist is selected from the group consisting of TGFpi, TGFP2, TGFP3, and mixtures thereof. In some embodiments, the at least one agent comprises a purinergic receptor antagonist. In some embodiments, the purinergic receptor antagonist binds to a purinergic receptor selected from the group consisting of P1, P2X, P2X7, and P2Y. In some embodiments, the purinergic receptor antagonist is oxidized ATP.

In some embodiments, the starting population of cells comprising dendritic cells or dendritic cell precursors is contacted with the at least one agent for a period of time sufficient for the induction of tolerogenic dendritic cells, or the emergence of such cells in the population. In some embodiments, the starting population of cells is contacted with the at least one agent for less than 10h. In some embodiments, the starting population of cells is contacted with the at least one agent for about 30 min, about 1h, about 2h, about 3h, about 4h, about 5h, about 6h, about 7h, about 8h, or about 9h. In some embodiments, the starting population of cells is contacted with the at least one agent for about 1-3 h, for example, for 2 h. In some embodiments, the starting population of cells is contacted with a composition comprising at least one agent selected from the group consisting of: a purinergic receptor antagonist, an mTOR inhibitor, a TGFP receptor antagonist, a statin, an agent which disrupts mitochondrial electron transport in the DCs for less than 10 h.

Some exemplary agents that constitute a tolerogenic stimulus are described in more detail below:

1. mTOR Inhibitors
In some exemplary embodiments, a tolerogenic stimulus for use in the instant invention comprises or consists of an mTOR inhibitor. mTOR inhibitors suitable for practicing the invention include inhibitors or antagonists of mTOR or mTOR-induced signaling. mTOR inhibitors include rapamycin and analogs, portions, or derivatives thereof, e.g., Temsirolimus (CCI-779), everolimus (RAD001) and deforolimus (AP23573).

Additional rapamycin derivatives include 42- and/or 31-esters and ethers of rapamycin, which are disclosed in the following patents, all hereby incorporated by reference in their entirety: alkyl esters (U.S. Pat. No. 4,316,885); aminoalkyl esters (U.S. Pat. No. 4,650,803); fluorinated esters (U.S. Pat. No. 5,100,883); amide esters (U.S. Pat. No. 5,118,677); carbamate esters (U.S. Pat. No. 5,118,678); silyl ethers (U.S. Pat. No. 5,120,842); aminoesters (U.S. Pat. No. 5,130,307); acetal (U.S. Pat. No. 5,141,413); aminodiesters (U.S. Pat. No. 5,162,333); sulfonate and sulfate esters (U.S. Pat. No. 5,177,203); esters (U.S. Pat. No. 5,221,670); alkoxyesters (U.S. Pat. No. 5,233,036); O-aryl, -alkyl, -alkenyl, and -alkynyl ethers (U.S. Pat. No. 5,258,389); carbonate esters (U.S. Pat. No. 5,260,300); arylcarbonyl and alkoxy carbonyl carbamates (U.S. Pat. No. 5,262,423); carbamates (U.S. Pat. No. 5,302,584); hydroxyesters (U.S. Pat. No. 5,362,718); hindered esters (U.S. Pat. No. 5,385,908); heterocyclic esters (U.S. Pat. No. 5,385,909); gem-disubstituted esters (U.S. Pat. No. 5,385,910); amino alkanolic esters (U.S. Pat. No. 5,389,639); phosphorylcarbamate esters (U.S. Pat. No. 5,391,730); carbamate esters (U.S. Pat. No. 5,411,967); carbamate esters (U.S. Pat. No. 5,434,260); amidino carbamate esters (U.S. Pat. No. 5,463,048); carbamate esters (U.S. Pat. No. 5,480,988); carbamate esters (U.S. Pat. No. 5,480,989); carbamate esters (U.S. Pat. No. 5,489,680); hindered N-oxide esters (U.S. Pat. No. 5,491,231); biotin esters (U.S. Pat. No. 5,504,091); O-alkyl ethers (U.S. Pat. No. 5,665,772); and PEG esters of rapamycin (U.S. Pat. No. 5,780,462). The preparation of these esters and ethers are disclosed in the patents listed above. 27-esters and ethers of rapamycin are disclosed in U.S. Pat. No. 5,256,790, which is hereby incorporated by reference in its entirety. Oximes, hydrazones, and hydroxylamines of rapamycin are disclosed in U.S. Pat. Nos. 5,373,014, 5,378,836, 5,023,264, and 5,563,145, which are hereby incorporated by reference in their entirety. The preparation of these oximes, hydrazones, and hydroxylamines are disclosed in the foregoing patents. The preparation of 42-oxorapamycin is disclosed in U.S. Pat. No. 5,023,263, which is hereby incorporated by reference in its entirety.

Other mTOR inhibitors include PI-103, XL765, Torin1, PP242, PP30, NVP-BEZ235, and OST027. Additional mTOR inhibitors include LY294002 and wortmannin. Other

In some embodiments, an mTOR inhibitor (e.g., rapamycin or a variant or derivative thereof) is used in combination with one or more statins. In some embodiments, an mTOR inhibitor (e.g., rapamycin or a variant or derivative thereof) is used in combination with a TGFβ pathway agonist.

2. TGFβ Pathway Agonists

In some exemplary embodiments, a tolerogenic stimulus for use in the instant invention comprises or consists of one or more TGFp agonists. TGFp agonists suitable for practicing the invention include substances that stimulate or potentiate responses induced by TGFβ signaling. In some embodiments, a TGFp pathway agonist is acts by modulating TGFβ receptor-mediated signaling. In some embodiments, a TGFp pathway agonist is a TGFp mimetic, e.g., a small molecule having TGF-like activity (e.g., biaryl hydroxamates, A-16 1906 as described in Glaser et al. 2002. Molecular Cancer Therapeutics 1:759-768, or other histone deacetylase inhibitors (such as spiruchostatins A and B or diheteropeptin).

In exemplary embodiments, a TGFp receptor agonist useful for practicing the invention is TGFp, including TGFp1, TGFp2, TGFp3, variants thereof, and mixtures thereof. Additional TGFp agonists are described in Patent Publication No. US2009014394A1, the entire contents of which are incorporated herein by reference.

In particular embodiments, the foregoing TGFp agonists are used in the presence of an mTOR inhibitor for producing induced tolerogenic DC.

3. Statins

Statins are HMG-CoA reductase inhibitors, a class of drug used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Exemplary statins include atorvastatin (Lipitor and Torvast), fluvastatin (Lescol), lovastatin (Mevacor, Altocor, Altoprev), pitavastatin (Livalo, Pitava), pravastatin (Pravachol, Selektine, Lipostat), rosuvastatin (Crestor), simvastatin (Zocor, Lipex). In some embodiments, at least one statin is used alone for producing induced tolerogenic dendritic cells. In some embodiments, at least one statin is used in combination with an mTOR inhibitor.

4. Purinergic Receptor Pathway Antagonists
In some exemplary embodiments, a tolerogenic stimulus for use in the instant invention comprises or consists of one or more purinergic agonists. Purinergic receptor pathway antagonists suitable for practicing the invention include inhibitors or antagonists of purinergic receptor activity or purinergic receptor signaling. Particular purinergic receptor antagonists include compounds that inhibit the activity of or signaling through the purinergic receptors PI, P2X, P2X7, and/or P2Y. These receptors bind extracellular adenosine triphosphate (ATP). In some embodiments, a purinergic receptor antagonist useful for practicing the invention is oxidized ATP (oATP).


5. Agents Which Disrupt Electron Transport
In some embodiments, an agent which disrupts electron transport can be used to induce tolerogenicity in dendritic cells. Such agents include, e.g., rotenone, antimycinA, and oligomycin.

6. Combinations of Agents

In some exemplary embodiments, the tolerogenic stimulus comprises or consists of a combination of agents, e.g., a cocktail of agents, for example, more than one of the agents set forth above. Exemplary tolerogenic stimuli include at least one respirostatic or tolerogenic locking agent which can be used to produce induced tolerogenic dendritic cells. In some embodiments, the at least one agent comprises an mTOR inhibitor and a TGFβ agonist. In some embodiments, the at least one agent comprises a statin. In some embodiments, the at least one agent comprises an mTOR inhibitor and a statin. In some embodiments, the at least one agent comprises an mTOR inhibitor, a TGFβ agonist, and a statin. In some embodiments, the at least one agent comprises a purinergic receptor antagonist. In some embodiments, the at least one agent comprises a purinergic receptor antagonist and a statin. In some embodiments, the at least one agent comprises a purinergic receptor antagonist and an mTOR inhibitor. In some embodiments, the at least one agent comprises a purinergic receptor antagonist, an mTOR inhibitor and a TGFβ agonist. In some embodiments, the at least one agent comprises a purinergic receptor antagonist, an mTOR inhibitor, a TGFβ agonist and a statin. In some embodiments, the at least one agent comprises an agent which disrupts mitochondrial electron transport in the DCs. In some embodiments, the at least one agent comprises an agent which disrupts mitochondrial electron transport in the DCs and an mTOR inhibitor. In some embodiments, the at least one agent comprises an agent which disrupts mitochondrial electron transport in the DCs and a statin. In some embodiments, the at least one agent comprises an agent which disrupts mitochondrial electron transport in the DCs, an mTOR inhibitor, and a TGFβ agonist. In some embodiments, the at least one agent comprises an agent which disrupts mitochondrial electron transport in the DCs, an mTOR inhibitor, a TGFP agonist, and a statin.

In some exemplary embodiments, the tolerogenic stimulus comprises or consists of a combination of agents selected from the group consisting of: i) an mTOR inhibitor (e.g., rapamycin or a variant or derivative thereof); a TGFβ agonist (e.g., TGFP); ii) a statin; an mTOR inhibitor (e.g., rapamycin or a variant or derivative thereof), a TGFP agonist (e.g.,
TGFB, and a statin; iv) a purinergic receptor antagonist (e.g., oATP); and v) an agent which
 disrupts mitochondrial electron transport in the DCs (e.g., rotenone).

7. Concentrations of Tolerogenic Stimuli

Exemplary concentrations of tolerogenic stimuli for producing induced tolerogenic
 cells can be readily determined by a person of skill in the art by titration of the stimulus on a
 starting population of cells in culture and testing the phenotype of the induced cells ex vivo.
 In some embodiments, a concentration of agent is chosen which has the desired effect on
 oxygen consumption rate (e.g., no change in the rate or a reduction in the rate) in dendritic
 cells. In some embodiments, a concentration of agent is chosen which has the desired effect
 on the induction of Treg cells. In exemplary embodiments, tolerogenic stimuli are used at a
 concentrations of 1 pM to 10 mM, for example, 1, 10, 25, 50, 100, 200, 300, 400, 500, 600,
 700, 800, 900 or 1000 pM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or
 1000 nM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 µM, or
 about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 mM, and ranges
 therein. In some embodiments, tolerogenic stimuli are used at concentrations of 1 pg/mL and
 10 mg/mL, for example, 1 pg/mL, 10 pg/mL, 100 pg/mL, 200 pg/mL, 300 pg/mL, 400
 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL, 1 ng/mL, 10 ng/mL, 100
 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL,
 900 ng/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500
 µg/mL, 600 µg/mL, 700 µg/mL, 800 µg/mL, 900 µg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4
 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 10 mg/mL, and ranges
 therein.

In some embodiments, an mTOR inhibitor (e.g., rapamycin or a derivative or variant
 thereof) is used as a tolerogenic stimulus at a concentration of 1 pM to 10 mM, for example,
 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 pM, about 1, 10, 25, 50,
 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 nM, about 1, 10, 25, 50, 100, 200, 300,
 400, 500, 600, 700, 800, 900 or 1000 µM, or about 1, 10, 25, 50, 100, 200, 300, 400, 500,
 600, 700, 800, 900 or 1000 mM, and ranges therein. In exemplary embodiments, an mTOR
 inhibitor e.g., rapamycin is used at a concentration of 1 µM or 10 nM. In some embodiments,
 an mTOR inhibitor (e.g., rapamycin or a derivative or variant thereof) is used at a
 concentration of 1 pg/mL and 10 mg/mL, for example, 1 pg/mL, 10 pg/mL, 100 pg/mL, 200
 pg/mL, 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL,
 1 ng/mL, 10 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL,
700 ng/mL, 800 ng/mL, 900 ng/mL, 1 µg/mL, 5 µg/ml, 10 µg/mL, 100 µg/mL, 200 µg/mL, 
300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL, 800 µg/mL, 900 µg/mL, 1 
mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, 
or 10 mg/mL, and ranges therein.

In some embodiments, one or more statins are used as a tolerogenic stimulus at a 
concentration of 1 pg/mL and 10 mg/mL, for example, 1 pg/mL, 10 pg/mL, 100 pg/mL, 200 
pg/mL, 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL, 
1 ng/mL, 10 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 
700 ng/mL, 800 ng/mL, 900 ng/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL, 200 µg/mL, 300 
µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL, 800 µg/mL, 900 µg/mL, 1 mg/mL, 
2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 10 
mg/mL, and ranges therein. In some embodiments, a statin is used at a concentration of 1 pM 
to 10 mM, for example, 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 
Pm, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 nM, about 1, 10, 
25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 mM, and ranges therein. In some exemplary 
embodiments, a statin is used at a concentration of about 10, 30, 50, 75, 100, or 300 µM.

In some embodiments, a TGFP agonist is used as a tolerogenic stimulus at a 
concentration of 1 pg/mL and 10 mg/mL, for example, 1 pg/mL, 10 pg/mL, 100 pg/mL, 200 
pg/mL, 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL, 
1 ng/mL, 10 ng/mL, 20 ng/mL, 30 ng/mL, 50 ng/mL, 75 ng/mL, 100 ng/mL, 200 ng/mL, 300 
ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, 1 µg/mL, 
10 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 
µg/mL, 800 µg/mL, 900 µg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 
mg/mL, 7 mg/mL, 8 mg/mL and ranges therein. In some 
embodiments, a TGFP agonist is used at a concentration of 1 pM to 10 mM, for example, 1, 
10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 pM, about 1, 10, 25, 50, 100, 
200, 300, 400, 500, 600, 700, 800, 900 or 1000 nM, about 1, 10, 25, 50, 100, 
200, 300, 400, 500, 600, 700, 800, 900 or 1000 mM, and ranges therein. In some exemplary 
embodiments, TGFP is used as a tolerogenic stimulus at a concentration of 20 ng/mL.

In some embodiments, a purinergic receptor antagonist (e.g., oATP) is used as a 
tolerogenic stimulus at a concentration of 1 pg/mL and 10 mg/mL, for example, 1 pg/mL, 10
pg/mL, 100 pg/mL, 200 pg/mL, 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL, 800 µg/mL, 900 µg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 10 mg/mL, and ranges therein. In some embodiments, a purinergic receptor antagonist is used at a concentration of 1 pM to 10 mM, for example, 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 pM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 nM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 mM, and ranges therein. In exemplary embodiments, oATP is used as a tolerogenic stimulus at a concentration of 100 uM-1 mM.

In some embodiments, an agent which disrupts mitochondrial electron transport is used as a tolerogenic stimulus at a concentration of 1 pg/mL and 10 mg/mL, for example, 1 pg/mL, 10 pg/mL, 100 pg/mL, 200 pg/mL, 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL, 800 µg/mL, 900 µg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 10 mg/mL, and ranges therein. In some embodiments, an agent which disrupts mitochondrial electron transport is used at a concentration of 1 pM to 10 mM, for example, 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 pM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 nM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 µM, or about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 mM, and ranges therein. In some embodiments, when combinations of agents are used, the concentration of each may be reduced.

8. Timing of Exposure

In general, exposure of a starting population of cells comprising dendritic cells and/or dendritic cell precursors to at least one tolerogenic stimulus is of a time sufficient to create induced tolerogenic dendritic cells, e.g., as demonstrated by a tolerogenic phenotype. In some embodiments, cells, for example, a starting population of cells comprising dendritic
cells and/or dendritic cell precursors, are contacted with at least one tolerogenic stimulus for at least one hour. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least two hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least three hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least four hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least five hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least six hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least seven hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least eight hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least nine hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least eleven hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least twelve hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least thirteen hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least fourteen hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least fifteen hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least sixteen hours.

In some embodiments, cells, for example, a starting population of cells comprising dendritic cells and/or dendritic cell precursors, are contacted with at least one tolerogenic stimulus for from one to seventy two hours, e.g., from two to forty eight hours, from three to twenty four hours, from four to sixteen hours, from five to twelve hours, from four to ten hours, from five to eight hours.

In some embodiments, cells, for example, a starting population of cells comprising dendritic cells and/or dendritic cell precursors, are contacted with at least one tolerogenic stimulus for at least one hour and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least two hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least three hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least four hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least five hours and less than ten hours. In some embodiments, cells are contacted with at least one
tolerogenic stimulus for at least six hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least seven hours and less than ten hours. Some such embodiments, which employ shorter incubation times than previously taught or suggested in the art are described in some, but not all of the appended Examples. In some embodiments, such shorter incubation times are employed for treatment of starting populations of cells comprising or enriched for fully differentiated dendritic cells (e.g., populations of cells which have been treated to differentiate dendritic cell precursors). In some embodiments, such shorter incubation times are employed for treatment of starting populations of cells comprising dendritic cell precursors (e.g., populations of cells which have not been treated to differentiate dendritic cell precursors). In some embodiments, shorter incubation time improves yields of viable cells and can be used for treatment of cells with mTOR inhibitors (e.g., rapamycin and variants or derivatives thereof) alone. In addition, these short incubation times can be used to produce tolerogenic dendritic cells using e.g., respirostatic or tolerogenic locking agents.

In some embodiments, mitochondrial respiration of cells can be tested to ensure that treatment with an inducing agent, for example, an agent that constitutes a tolerogenic stimulus, results in an appropriate response. For example, in some embodiments, $O_2$ consumption (the oxygen consumption rate; OCR) by cells can be measured. For example, induced tolerogenic dendritic cells can be tested to ensure that $O_2$ consumption decreases or does not increase. OCR can be measured, e.g., using an analyzer such as the Seahorse XF24 flux analyzer of Clark electrode. In some embodiments, a different assay can also be used to confirm the effect of an agent on mitochondrial function. For example, in some embodiments, mRNA levels of the expression of one or more of PGC-1α, PGC-1β, PRC, or other molecules involved in mitochondrial function, such as estrogen-related receptor α, NRF-1, NRF-2, Sp1, YY1, CREB and MEF-2/E-box factors can be measured. For example, induced tolerogenic dendritic cells exposed to a tolerogenic stimulus can be tested to ensure that levels of PGC-1α mRNA do not increase or decrease. Other methods of testing mitochondrial function which are known in the art can also be used for this purpose.

For example, alternative readouts of DC metabolism can be measured. For example, glucose uptake (e.g., using derivatized glucose) can be measured, as can the presence of reactive oxygen species (e.g., using DCF-DA). In some embodiments, lactic acid production (which is elevated with increased glycolysis and/or decreased mitochondrial activity) can be measured. In some embodiments, the extracellular acidification rate (ECAR) can be
measured and is reflective of lactic acid production by glycolysis or pyruvate overload. The Seahorse SF24 flux analyzer can be used for this purpose. In yet some embodiments, cellular ATP/ADP ratios may be measured (e.g., using commercially available kits or as in Nagel et al. 2010. Methods Mol. Biol. 645:123-31). Increased levels of ATP and decreased levels of ADP have been recognized in proliferating cells and are a measure of activation.

In some embodiments, whether the induced tolerogenic dendritic cells have, for example, at least one of the following properties can be tested ex vivo using methods known in the art and/or described herein i) the ability to convert naïve T cells to Foxp3+ T regulatory cells ex vivo; ii) the ability to delete effector T cells ex vivo; iii) the ability to express costimulatory molecules but retain their tolerogenic phenotype upon stimulation with at least one TLR agonist ex vivo; and/or iv) the ability to remain respirostatic upon stimulation with at least one TLR agonist ex vivo.

To make the antigen-specific iTDCs, the iTDCs are contacted, or "loaded," with the antigen of interest. Alternatively, precursors, such as dendritic cells before they are induced to have the tolerogenic phenotype as provided herein, can be loaded with the antigen of interest. These dendritic cells may then be further manipulated to form iTDCs. iTDCs of the invention may express an antigen of interest intrinsically (e.g., the antigen may be an intrinsic antigen such as a germline gene product such as a self protein, polypeptide, or peptide), in which case they will not need to be further modified.

In some embodiments, dendritic cells which do not already express the antigen of interest such that it can be recognized by immune cells are made to express the antigen of interest or are contacted with the antigen of interest, e.g., by being bathed or cultured with the antigen, such that the dendritic cells will display the antigen on their surface for presentation (e.g., after processing or by directly binding to MHC).

In some embodiments, iTDCs can be directly contacted with e.g., bathed in or pulsed with) antigen. In other embodiments, the cells may express the antigen or may be engineered to express an antigen by transfecting the cells with an expression vector directing the expression of the antigen of interest such that the antigen is expressed and then displayed on the surface of the DCs. The antigen of interest may be provided in the form as elsewhere described herein, e.g., by contacting the iTDCs with an antigen or a cell that expresses the antigen. Accordingly, in some embodiments, prior to, during, and/or following treatment with a tolerogenic stimulus, the cells are exposed to antigen. In some embodiments, before the cells have been induced with a tolerogenic stimulus, the cells are exposed to antigen. In
some embodiments, after the cells have been induced with a tolerogenic stimulus, the cells are exposed to antigen. The antigen may be provided as a population of cells, processed forms thereof, a crude preparation comprising many proteins, polypeptides, and/or peptides (e.g., a lysate or extract) or may comprise one or more purified proteins, polypeptides, or peptides. Such proteins, polypeptides, or peptides can be naturally occurring, chemically synthesized, or expressed recombinantly.

For example, in some embodiments, cells are contacted with an antigen which is heterogeneous, e.g., which comprises more than one protein, polypeptide, or peptide. In some embodiments, such a protein antigen is a cell lysate, extract or other complex mixture of proteins. In some embodiments, an antigen with which cells are contacted comprises or consists of a protein which comprises a number of different immunogenic peptides. In some embodiments, the cells are contacted with the intact antigen and the antigen is processed by the cells. In some embodiments, the cells are contacted with purified components of the antigen, e.g., a mixture of immunogenic peptides, which may be further processed or may bind directly to MHC molecules on the cells.

In some embodiments, the cells are cultured in the presence of antigen for an appropriate amount of time (e.g., for 4 hours or overnight) under certain conditions (e.g., at 37°C). In other embodiments, the cells are sonicated with antigen or the antigen is sonicated in buffer before loading.

In some embodiments, the antigen is targeted to surface receptors on DCs, e.g., by making antigen-antibody complexes (Fanger 1996), Ag-Ig fusion proteins (You et al. 2001) or heat shock protein-peptide constructs (Suzue K 1997, Arnold-Schild 1999, Todryk 1999). In some embodiments, non-specific targeting methods such as cationic liposome association with Ag (Ignatius 2000), apoptotic bodies from tumor cells (Rubartelli 1997, Albert 1998a, Albert 1998b), or cationic fusogenic peptides (Laus 2000) can be used.

In some embodiments, the antigen comprises or consists of a polypeptide that can be endocytosed, processed, and presented by dendritic cells. In some embodiments, the antigen comprises or consists of a short peptide that can be presented by dendritic cells without the need for processing. Short peptide antigens can bind to MHC class II molecules on the surface of dendritic cells. In some embodiments, peptide antigens can displace antigens previously bound to MHC molecules on the surface of dendritic cells. Thus, the antigen may be processed by the dendritic cells and presented or may be loaded onto MHC molecules on the surface of dendritic cells without processing. Those peptide(s) that can be presented by
the dendritic cell may appear on the surface in the context of MHC molecules for presentation to T cells. This can be demonstrated functionally (e.g., by measuring T cell responses to the cell) or by detecting antigen-MHC complexes using methods known in the art. This can also be demonstrated functionally by assessing the generation of one or more tolerogenic immune response by the antigen-specific itDCs (e.g., ability to activate antigen-specific T or B cells). Other methods are described elsewhere herein.

In some embodiments, cells are contacted with an antigen comprising more than one protein or more than one polypeptide or more than one peptide and the antigen is not purified to remove irrelevant or unwanted proteins, polypeptides, or peptides and the cells present those antigens which are processed and displayed. In some embodiments, the antigen used to contact dendritic cells comprises or consists of a single short peptide or polypeptide or mixture of peptides or polypeptides that are substantially pure, e.g., isolated from contaminating peptides or polypeptides. Likewise, the antigen can be a single polypeptide or peptide that is substantially pure and isolated from contaminating polypeptides or peptides.

Such short peptides and polypeptides can be obtained by suitable methods known in the art. For example, short peptides or polypeptides can be recombinantly expressed, purified from a complex protein antigen, or produced synthetically.

Alternatively, the antigen used to contact cells comprises or consists of a mixture of more than one short peptide or polypeptide, e.g., a mixture of two, three, four, five, six, seven, eight, nine, ten, twenty, thirty, forty, fifty, one hundred or more short peptides or polypeptides. The antigen used to contact cells can also comprise or consist of a more complex mixture of polypeptides. Use of a mixture of short peptides or polypeptides allows for the preparation of an induced dendritic cell population that is capable of, for example, modulating an antigen-specific T-cell mediated immune response to a number of distinct peptides or polypeptides. This is desirable when, for example, the immune response to be inhibited is an immune response against a complex antigen or particular cell types. In some embodiments, the antigen comprises a cell extract or cell lysate. In some embodiments, the antigen comprises a tissue extract or tissue lysate.

Other methods of loading antigen onto dendritic cells will be apparent to one of ordinary skill in the art (See, e.g., Dieckman et al. Int. Immunol. (May 2005) 17(5):621-635).

In some embodiments, the antigen is associated with allergic responses. In such embodiments, the antigen with which the dendritic cells are contacted with can comprise one or more allergens (e.g., one or more polypeptides or peptides derived therefrom). In some
embodiments, the antigen is a complex antigen, such as: a food protein (e.g., one or more proteins peptides or polypeptides derived from food, such as eggs, milk, wheat, soy, nuts, seeds, fish, shellfish, or gluten), pollen, mold, dust mites, or particular cell types or cells modified by exposure to a drug or chemical.

In some embodiments, the antigen comprises animal matter, such as one or more of animal dander, hair, urine or excrement. In some embodiments, the antigen comprises insect matter.

In some embodiments, the antigen comprises or consists of one or more peptides or polypeptides derived from food. In still some embodiments, the antigen comprises one or more peptides or polypeptides derived pollen. In some embodiments, the antigen comprises one or more peptides or polypeptides derived dust mites. In some embodiments, the antigen comprises one or more peptides or polypeptides derived from gluten. In some embodiments, the antigen comprises one or more peptides or polypeptides derived from myelin.

Other antigens that can be used with the methods of the invention can be envisioned by a person of skill in the art. For example, allergic disorders may have been associated with particular proteins, although specific peptide antigens important in such immune responses may not yet be known. Since proteins or mixtures of proteins can be used as antigen in the methods of the instant invention, one of skill in the art could readily determine what antigen or antigen mixture to use for loading dendritic cells to modulate immune responses to that particular antigen.

A wide range of antigen quantities can be used to contacting with the iTDCs. For example, in some embodiments, cells are contacted with antigen at concentrations ranging between 1 pg/mL and 10 mg/mL. In exemplary embodiments, cells are contacted with antigen at 1 pg/mL, 10 pg/mL, 100 pg/mL, 200 pg/mL, 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, 1 µg/mL, 10 µg/mL, 30 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL, 800 µg/mL, 900 µg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 10 mg/mL, and ranges therein. In some embodiments, cells are contacted with 100 µg/mL of antigen. In some embodiments, cells are contacted with antigen at a concentration of 1 pM to 10 mM, for example, 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 pM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 nM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600,
700, 800, 900 or 1000 µM, or about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 mM, and ranges therein.

In some embodiments, cells can be cocultured with antigen for a time sufficient to allow display of the antigen on the surface of the cells, e.g., 1-72 hours under appropriate conditions (e.g., 37°C in 5% CO2 atmosphere). For example, in some embodiments, cells are cocultured with antigen for about 1-72 hours, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 20, 24, 30, 35, 40, 45, 48, 50, 55, 60, 70, or 72 hours or such other time period which allows for processing and presentation or loading of antigen onto dendritic cells. Preferably, in some embodiments, the time sufficient is at least 2 hours. In other embodiments, the time sufficient is overnight. In yet other embodiment, the time sufficient is between 2 and 24 or between 2 and 12 hours. Such contacting can take place prior to induction of DCs or after induction and prior to further manipulation.

In some embodiments, the itDCs can be contacted with one or more maturation stimuli prior to administration to a subject. Treatment with a maturation stimulus can enhance the antigen presentation capacity of dendritic cells without blocking their tolerogenicity in the case of induced tolerogenic dendritic cells. Such maturation stimuli can include, but are not limited to, an adjuvant, a TLR agonist, a CD40 agonist, an inflammasome activator, or an inflammatory cytokine, and combinations thereof. Treatment of cells with maturation stimuli can be performed before, during, or following induction and/or contacting with antigen.

In some embodiments, the allergen-specific itDCs are administered to a subject by an appropriate route. The administering of the allergen-specific itDCs may be by parenteral, intraarterial, intranasal or intravenous administration or by injection to lymph nodes or anterior chamber of the eye or by local administration to an organ or tissue of interest. The administering may also be by subcutaneous, intrathecal, intraventricular, intramuscular, intraperitoneal, intracoronary, intrapancreatic, intrahepatic or bronchial injection. Administration can be rapid or can occur over a period of time.

Other agents may be administered by a variety of routes of administration, including but not limited to intraperitoneal, subcutaneous, intramuscular, intradermal, oral, intranasal, transmucosal, intramucosal, intravenous, sublingual, rectal, ophthalmic, pulmonary, transdermal, transcutaneous or by a combination of these routes. Routes of administration also include administration by inhalation or pulmonary aerosol. Techniques for preparing aerosol delivery systems are well known to those of skill in the art (see, for example, Sciarra...

The compositions of the inventions can be administered in effective amounts, such as the effective amounts described elsewhere herein. Doses contain varying amounts of populations of allergen-specific itDCs according to the invention. The amount of the cells or other agents present in the inventive dosage forms can be varied according to the nature of the antigens, the therapeutic benefit to be accomplished, and other such parameters. In some embodiments, dose ranging studies can be conducted to establish optimal therapeutic amount of the population of cells and/or the other agents to be present in the dosage form. In some embodiments, allergen-specific itDCs and/or the other agents are present in the dosage form in an amount effective to generate a tolerogenic immune response upon administration to a subject. It may be possible to determine amounts of the cells and/or other agents effective to generate a tolerogenic immune response using conventional dose ranging studies and techniques in subjects. Inventive dosage forms may be administered at a variety of frequencies. In a preferred embodiment, at least one administration of the dosage form is sufficient to generate a pharmacologically relevant response. In more preferred embodiments, at least two administrations, at least three administrations, or at least four administrations, of the dosage form are utilized to ensure a pharmacologically relevant response.

The quantity of allergen-specific itDCs to be administered to a subject can be determined by one of ordinary skill in the art. In some embodiments, amounts of cells can range from about 10^3 to about 10^{10} cells per dose. In exemplary embodiments, induced dendritic cells are administered in a quantity of about 10^5, 10^6, 10^7, 10^8, 10^9, or 10^{10} cells per dose. In other exemplary embodiments, intermediate quantities of cells are employed, e.g., 5 x 10^5, 5 x 10^6, 5 x 10^7, 5 x 10^8, 5 x 10^9, or 5 x 10^{10} cells. In some embodiments, subjects receive a single dose. In some embodiments, subjects receive multiple doses. Multiple doses may be administered at the same time, or they may be spaced at intervals over a number of days. For example, after receiving a first dose, a subject may receive subsequent doses of allergen-specific itDCs at intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21, 28, 30, 45, 60, or more days. As will be apparent to one of skill in the art, the quantity of cells and the appropriate times for administration may vary from subject to subject depending on factors including the duration and severity of disease, disorder or condition. To determine the
appropriate dosage and time for administration, skilled artisans may employ conventional clinical and laboratory means for monitoring the outcome of administration, e.g., on progression of a disorder in the subject or on humoral immune responses, Treg cell, Breg cell, B cell and/or T cell effector number and/or function. Such means include known biochemical and immunological tests for monitoring and assessing, for example, cytokine production, antibody production, inflammation, T-effector cell activity, allergic response, etc.

In some embodiments, a maintenance dose is administered to a subject after an initial administration has resulted in a tolerogenic response in the subject, for example to maintain the tolerogenic effect achieved after the initial dose, to prevent an undesired immune reaction in the subject, or to prevent the subject becoming a subject at risk of experiencing an undesired immune response or an undesired level of an immune response. In some embodiments, the maintenance dose is the same dose as the initial dose the subject received. In some embodiments, the maintenance dose is a lower dose than the initial dose. For example, in some embodiments, the maintenance dose is about \( \frac{3}{4} \), about \( \frac{2}{3} \), about \( \frac{1}{2} \), about \( V_3 \), about \( V_4 \), about \( V_8 \), about \( \frac{1}{10} \), about \( \frac{1}{20} \), about \( \frac{1}{25} \), about \( \frac{1}{50} \), about \( \frac{1}{100} \), about \( \frac{1}{1,000} \), about \( V_{10,000} \), about \( V_{100,000} \), or about \( \frac{1}{1,000,000} \) (weight/weight) of the initial dose.

Prophylactic administration of induced dendritic cells can be initiated prior to the onset of disease, disorder or condition or therapeutic administration can be initiated after a disorder, disorder or condition is established.

In some embodiments, administration of allergen-specific itDCs is undertaken e.g., prior to exposure to an allergen. In exemplary embodiments, induced tolerogenic dendritic cells are administered at one or more times including, but not limited to, 30, 25, 20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 days prior to exposure to an allergen. In addition or alternatively, allergen-specific itDCs can be administered to a subject concomitantly with or following exposure to an allergen. In exemplary embodiments, allergen-specific itDCs are administered at one or more times including, but not limited to, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, etc. days following exposure to an allergen.

In some embodiments, the use of allergen-specific itDCs will allow for administration of lower doses than that of immunosuppressants of the current standard of care, thereby reducing side effects.

It is to be understood that the cell populations, for example, compositions, and dosage forms of the invention can be made in any suitable manner, and the invention is in no way limited to compositions that can be produced using the methods described herein. Selection
of an appropriate method may require attention to the properties of the particular cell populations, compositions, and dosage forms, for example, with regard to their intended use.

For example, in some embodiments, inventive compositions are manufactured under sterile conditions or are generated using sterilized reagents. This can ensure that resulting composition are sterile or non-infectious, thus improving safety when compared to non-sterile compositions. This provides a valuable safety measure, especially when a subject receiving a cell population, composition, or dosage form provided herein has a defective or suppressed immune system, is suffering from infection, and/or is susceptible to infection.

The compositions and methods described herein can be used to induce or enhance a tolerogenic immune response and/or to suppress, modulate, direct or redirect an immune response for the purpose of immune suppression. The compositions and methods described herein can be used in the diagnosis, prophylaxis and/or treatment of diseases, disorders or conditions in which immune suppression or tolerance would confer a treatment benefit. Such diseases, disorders or conditions include allergies. The allergy may be to any allergen such as those provided and described elsewhere herein.

EXAMPLES

Example 1: Isolation of a Starting Population of Cells (Prophetic)

Starting populations are obtained from the bone marrow, the peripheral blood, or the spleen of a donor subject. In case of solid tissue being harvested or obtained from a subject, the tissue is digested or mechanically disrupted in order to obtain a cell suspension, for example, a single-cell suspension. In case of bone marrow or peripheral blood, the cells are separated from the non-cellular components and undesired cells, e.g., erythrocytes, B-lymphocytes and granulocytes are depleted. Bone marrow and peripheral blood cell populations are depleted of erythrocytes by hypotonic lysis. Erythroid precursors, B lymphocytes, T-lymphocytes, and granulocytes are removed by immunomagnetic bead depletion.

The obtained cell populations are enriched for dendritic cells and/or dendritic cell precursors by cell sorting for CD1 lc. For cell sorting, FACS or MACS are used in combination with a CD1 lc-antibody or CD1 lc immunomagnetic beads, respectively. Enriched populations of dendritic cells or dendritic cell precursors are more than 90% pure. Dendritic cell populations and dendritic precursor cell populations are cultured in a suitable
culture medium until further processing, e.g., in RPMI-1640 with 10% fetal calf serum, tglutamine, non-essential amino acids, sodium pyruvate, penicillin-streptomycin, HEPES, 2-mercaptoethanol, 1000 U/mL recombinant human granulocyte-macrophage colony-stimulating factor, and 1000 U/mL recombinant human IL-4 at 37°C.

Example 2: Induction of itDCs (Prophetic)

Starting populations of dendritic cells or dendritic precursor cells are contacted with a tolerogenic stimulus, here, with the mTOR inhibitor rapamycin and TGFP at 1000x each for 1h. An appropriate volume of a concentrated stock solution (e.g., 1000x) of each agent is added to the supernatant of the culture of the starting population to achieve the desired end concentration of the agent in the tissue culture medium. After the contacting time period has elapsed, cells are washed three times with PBS and transferred to culture medium not containing the tolerogenic stimulus. Respirostatic characteristics of the tolerogenic induction is monitored by assessing oxygen consumption of the cell populations.

For DC precursors after seven days in culture, tolerogenic characteristics of the DCs is assessed by contacting a population of naive T cells with some of the DCs generated and measuring induction of FoxP3 in the naive T cells, wherein cell populations containing cells that induce FoxP3 contain itDCs.

Example 3: Antigen-loading of itDCs (Prophetic)

Cultures of itDCs are contacted with an antigen of interest, for example, by contacting the itDCs with Der P1 antigen preparation. The itDCs are contacted with the antigen for 24 h at 37°C, and subsequently washed three times in PBS. Antigen-loaded itDCs are then cultured, or used according to methods described herein.

Example 4: Evaluating Tolerogenic Immune Response by T-cell Phenotypic Analysis (Prophetic)

A composition of the invention is injected subcutaneously into female Lewis rats. A control group of rats receives 0.1-0.2 ml of PBS. Nine to ten days after the injection, spleen and lymph nodes are harvested from the rats and single cell suspensions obtained by macerating tissues through a 40 μm nylon cell strainer. Samples are stained in PBS (1% FCS) with the appropriate dilution of relevant monoclonal antibodies. Propidium iodide staining cells are excluded from analysis. Samples are acquired on an LSR2 flow cytometer
(BD Biosciences, USA) and analyzed using FACS Diva software. The expression of markers CD25\textsuperscript{high}, CD27\textsuperscript{high}, CD86\textsuperscript{high}, CD1d\textsuperscript{high}, IL-10\textsuperscript{high}, TGF-\(\beta\textsuperscript{high}\), CD4 and FoxP3 is analyzed on the cells. The presence of CD4+CD25\textsuperscript{high}FoxP3+ cells suggests an induction of CD4+ Treg cells.

Example 5: Evaluating Tolerogenic Immune Response to Antigen In Vivo (Prophetic)

Balb/c mice are immunized with Der PI antigen in incomplete Freund’s adjuvant to induce antigen-specific T-cell proliferation (e.g., CD4+ T-cell proliferation), the level of which is assessed. Subsequently, a composition of the invention is administered in a dose-dependent manner. The same mice are then again exposed to the antigen, and the level of T-cell proliferation is again assessed. Changes in the T-cell population are then monitored with a reduction in T-cell proliferation upon subsequent challenge with the antigen indicating a tolerogenic immune response.

Example 6: Administration to a Subject to Suppress an Undesired Immune Response (Prophetic)

Antigen-specific itDCs are formulated into a dosage form suitable for administration (e.g., an injectable cell suspension) and an effective amount of the dosage form is administered to a subject having an undesired immune response.

Example 7: Administration to a Subject to Suppress an Undesired Immune Response to an Allergen (Prophetic)

Allergen-specific itDCs are generated according to methods described herein. Briefly, itDCs are generated by contacting itDCs with Der PI antigen. Allergen-specific itDCs are then formulated into an injectable cell suspension of about 10\textsuperscript{6} cells/ml in sterile, injectable saline. An effective amount of this injectable suspension, about 1ml, is administered to a subject having an allergic reaction to house dust mites. A decrease in the level of allergic reaction, or a complete suppression of the allergic reaction is expected in the subject after about one to four weeks after administration of the itDCs. This decrease is expected to result in an amelioration or complete regression of at least one clinically manifested symptom of an allergic reaction to house dust mites, for example, asthma, airway hypersensitivity, skin reactions, sneezing, nausea, rash, fatigue, headache, fever, dizziness, or chills. For one year after administration of the initial dose of itDCs, the subject receives a bi-
monthly maintenance dose of $10^6$ allergen-specific itDCs generated by contacting itDCs with Der PI antigen (a total of 6 maintenance doses). At the end of this treatment schedule, the subject is expected to show no or only a tolerable immune reaction to house dust mites.

**Example 8: Administration to a Subject to Suppress an Undesired Immune Response to House Dust Mites (Prophetic)**

Allergen-specific itDCs are generated according to methods described herein. Briefly, itDCs are generated by contacting itDCs with Der PI antigen or a portion thereof. Allergen-specific itDCs are then formulated into an injectable cell suspension of about $10^6$ cells/ml in sterile, injectable saline. An effective amount of this injectable suspension, about 1ml, is administered subcutaneously to a subject exhibiting an undesired immune response, such as an excessive Der PI-specific antibody production or CD4+ T cell proliferation and/or activity. A decrease in these undesired immune responses against the antigen is expected in the subject after about one to four weeks after administration of the allergen-specific itDCs. This decrease is expected to result in an amelioration or complete regression of Der PI-specific antibody production or CD4+ T cell proliferation and/or activity. Methods of assessing the level of Der PI-specific antibody production or CD4+ T cell proliferation and/or activity are provided elsewhere herein or are otherwise known to those of ordinary skill in the art.

**Example 9: Induced Tolerogenic itDCs Suppress Undesired Immune Responses to Antigen**

**In vitro Treatment of DCs to Yield Induced Tolerogenic DCs (itDCs)**

DCs were incubated for 2 hours under tissue culture conditions (37°C, 5%CO2) in Complete Media (CM, RPMI1640+10%Fetal Bovine Serum+Penicillin Streptomycin+L-Glutamate) with Rapamycin, (100nM) TGFp (20ng/ml) and Ova (luM). Cells were then washed 3 times in MACS Running Buffer (RB, 2%FBS+2mM EDTA in PBS) and counted. Cells were placed at 1-10x10^6/200ul in PBS and injected i.v. into experimental recipients.

**Nanocarrier (NP)**

Ovalbumin protein was purchased from Worthington Biochemical Corporation (730 Vassar Avenue, Lakewood, NJ 08701; Product Code 3048). PLGA with a lactide:glycolide
ratio of 3:1 and an inherent viscosity of 0.75 dL/g was purchased from SurModics Pharmaceuticals (756 Tom Martin Drive, Birmingham, AL 35211; Product Code 7525 DLG 7A). Polyvinyl alcohol (85-89% hydrolyzed) was purchased from EMD Chemicals (Product Number 1.41350.1001). PLA-PEG block co-polymer with a PEG block of approximately 5,000 Da and PLA block of approximately 20,000 Da was synthesized. Sodium cholate hydrate was purchased from Sigma-Aldrich Corp. (3050 Spruce Street, St. Louis, MO 63103; Product Code C6445).

Solutions were prepared as follows:

Solution 1: Ovalbumin @ 50 mg/mL in phosphate buffered saline solution. The solution was prepared by dissolving ovalbumin in phosphate buffered saline solution at room temperature. Solution 2: PLGA @ 100 mg/mL in methylene chloride. The solution was prepared by dissolving PLGA in pure methylene chloride. Solution 3: PLA-PEG @ 100 mg/mL in methylene chloride. The solution was prepared by dissolving PLA-PEG in pure methylene chloride. Solution 4: Polyvinyl alcohol @ 50 mg/mL and sodium cholate hydrate @ 10 mg/mL in 100 mM pH 8 phosphate buffer.

A primary water-in-oil emulsion was prepared first. W1/O1 was prepared by combining solution 1 (0.2 mL), solution 2 (0.75 mL), and solution 3 (0.25 mL) in a small pressure tube and sonicating at 50% amplitude for 40 seconds using a Branson Digital Sonifier 250. A secondary emulsion (W1/O1/W2) was then prepared by combining solution 4 (3.0 mL) with the primary W1/O1 emulsion, vortexing for 10 s, and sonicating at 30% amplitude for 60 seconds using the Branson Digital Sonifier 250.

The W1/O1/W2 emulsion was added to a beaker containing 70 mM pH 8 phosphate buffer solution (30 mL) and stirred at room temperature for 2 hours to allow the methylene chloride to evaporate and for the nanocarriers to form. A portion of the nanocarriers were washed by transferring the nanocarrier suspension to a centrifuge tube and centrifuging at 75,600xg and 4 °C for 35 min, removing the supernatant, and re-suspending the pellet in phosphate buffered saline. The washing procedure was repeated, and the pellet was re-suspended in phosphate buffered saline for a final nanocarrier dispersion of about 10 mg/mL.

Nanocarrier size was determined by dynamic light scattering. The amount of protein in the nanocarrier was determined by an o- phthalaldehyde fluorometric assay. The total dry-nanocarrier mass per mL of suspension was determined by a gravimetric method.

<table>
<thead>
<tr>
<th>Nanocarrier</th>
<th>Effective Diameter</th>
<th>Protein Content</th>
</tr>
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Immunization and Treatment

Group #1 of animals remained unimmunized as a control. All other groups were immunized (200µl of OVA (100µg in 40µM CpG)) using active immunization with OVA protein and CpG subcutaneously in the subscapular region. Group #2 were immunized but not treated to help appreciate the strength of the immune response induced. Groups #3-10 were treated (200µl DC i.v.) with different itDC products. The challenge route of administration was 20µl i.m. of OVA (10µg) or PBS. Five animals per group.

Treatments were carried out concomitantly with immunizations starting on day 0 as follows for the denoted groups. DCs used to treat groups 2-10 were incubated with 10µg OVA +/- 100ng/ml Rapamycin and 20ng/ml TGFp per animal.

1) Phosphate buffered saline (PBS), intravenously (i.v.),
2) Phosphate buffered saline (PBS), i.v.,
3) Dendritic cells (DCs) incubated with OVA in vitro, i.v.,
4) DCs incubated with OVA, Rapamycin (Rapa) and Tumor Growth Factor beta (TGFp) in vitro, i.v.,
5) DCs incubated with nanoparticles containing OVA (NPOVA) in vitro, i.v.,
6) DCs incubated with NPOVA, Rapamycin and TGFp in vitro, i.v.,
7) CD8 alpha positive (CD8a) DCs incubated with OVA in vitro, i.v.,
8) CD8a DCs incubated with OVA, Rapamycin (Rapa) and Tumor Growth Factor beta (TGFp) in vitro, i.v.,
9) CD103 positive (CD103) DCs incubated with OVA in-vitro, i.v.,
10) CD103 DCs incubated with OVA, Rapamycin (Rapa) and Tumor Growth Factor beta (TGFp) in vitro, i.v.

For each treatment day syngeneic donor mice were inoculated 10 days earlier with Fms-like tyrosine kinase 3 (FLT-3) ligand expressing melanoma cells s.s. (performed on days -10, 4, 18 in donor C57BL/6 age-matched mice). Flt3 ligand is a growth factor for DCs and allows for greater total number of DCs to be present in the spleen. This increased the number
of DCs more than 10-fold and allowed for more cells to be available for in vitro treatment and in vivo administration.

**Cell Sorting**

On treatment days the spleens from the FLT-3 melanoma inoculated animals were harvested and digested via liberase TM (Roche). The resulting slurry was filtered by 70µM nylon mesh and a series of magnetic activating cell sorting (MACS) separations was performed. First the cells were incubated with magnetic bead conjugated antibodies (Abs) specific for CD45R, DX5 and CD3. These cells were then run through a Miltenyi Biotec Automacs PRO automatic cell separator. The unlabeled cell fraction was then split into 3 groups. The first was incubated with bead conjugated Abs specific for CD1 1c the second was incubated with bead conjugated Abs specific for CD8a and the third was first incubated with biotin conjugated Abs specific for CD103 and then Abs conjugated to both streptavidin and beads. These cell separations were again performed on the AutoMacs PRO to yield enriched populations of CD1 lc+, CD8a+ and CD103+ DCs.

**Measurement of IgG**

The level of IgG antibodies were measured. This level is indicative of immunoglobulins in general, including IgEs, which are of particular relevance in allergy. Blocker Casein in PBS (Thermo Fisher, Catalog #37528) was used as diluent. 0.05% Tween-20 in PBS was used as wash buffer, prepared by adding 10 ml of Tween-20 ((Sigma, Catalog #P9416-100mL) to 2 liters of a 10X PBS stock (PBS: OmniPur® 10X PBS Liquid Concentrate, 4L, EMD Chemicals, Catalog #6505) and 18 Liters of deionized water.

OVA protein at a stock concentration of 5 mg/ml was used as a coating material. A 1:1000 dilution to 5 µg/ml was used as a working concentration. Each well of the assay plates was coated with 100 µl diluted OVA per well, plates were sealed with sealing film (VWR catalog #60941-120), and incubated overnight at 4°C. Costar9017 96-well Flat bottom plates were used as assay plates, Costar9017.

Low-binding polypropylene 96-well plate or tubes were used as set-up plates, in which samples were prepared before being transferred to the assay plate. The setup plates did not contain any antigen and, therefore, serum antibodies did not bind to the plate during the setup of the samples. Setup plates were used for sample preparation to minimize binding that might occur during preparation or pipetting of samples if an antigen-coated plate was used to
prepare the samples. Before preparing samples in the setup plate, wells were covered with diluent to block any non-specific binding and the plate was sealed and incubated at 4°C overnight.

Assay plates were washed three times with wash buffer, and wash buffer was completely aspirated out of the wells after the last wash. After washing, 300 µl diluent were added to each well of assay plate(s) to block non-specific binding and plates were incubated at least 2 hours at room temperature. Serum samples were prepared in the setup plate at appropriate starting dilutions. Starting dilutions were sometimes also prepared in 1.5 ml tubes using diluent. Appropriate starting dilutions were determined based on previous data, where available. Where no previous data was available, the lowest starting dilution was 1:40. Once diluted, 200 µl of the starting dilution of the serum sample was transferred from to the appropriate well of the setup plate.

An exemplary setup plate layout is described as follows: Columns 2 and 11 contained anti-Ovabumin monoclonal IgG2b isotype (AbCam, ab17291) standard, diluted to 1 µg/mL (1:4000 dilution). Columns 3-10 contained serum samples (at appropriate dilutions). Columns 1 and 12 were not used for samples or standards to avoid any bias of measurements due to edge effect. Instead, columns 1 and 12 contained 200 µl diluent. Normal mouse serum diluted 1:40 was used as a negative control. Anti-mouse IgG2a diluted 1:500 from 0.5mg/mL stock (BD Bioscience) was used as an isotype control.

Once all samples were prepared in the setup plate, the plate was sealed and stored at 4°C until blocking of the assay plates was complete. Assay plates were washed three times with wash buffer, and wash buffer was completely aspirated after the last wash. After washing, 100 µl of diluent was added to all wells in rows B-H of the assay plates. A 12-channel pipet was used to transfer samples from the setup plate to the assay plate. Samples were mixed prior to transfer by pipetting 150 µl of diluted serum up and down 3 times. After mixing, 150 µl of each sample was transferred from the setup plate and added to row A of the respective assay plate.

Once the starting dilutions of each sample were transferred from the setup plate to row A of the assay plate, serial dilutions were pipetted on the assay plate as follows: 50 µl of each serum sample was removed from row A using 12-channel pipet and mixed with the 100 µl of diluent previously added to each well of row B. This step was repeated down the entire plate. After pipetting the dilution of the final row, 50 µl of fluid was removed from the wells in the final row and discarded, resulting in a final volume of 100 µl in every well of the assay
plate. Once sample dilutions were prepared in the assay plates, the plates were incubated at room temperature for at least 2 hours.

After the incubation, plates were washed three times with wash buffer. Detection antibody (Goat anti-mouse anti-IgG, HRP conjugated, AbCam ab98717) was diluted 1:1500 (0.33 µg/mL) in diluent and 100 µL of the diluted antibody was added to each well. Plates were incubated for 1 hour at room temperature and then washed three times with wash buffer, with each washing step including a soak time of at least 30 seconds.

After washing, detection substrate was added to the wells. Equal parts of substrate A and substrate B (BD Biosciences TMB Substrate Reagent Set, catalog #555214) were combined immediately before addition to the assay plates, and 100 µL of the mixed substrate solution were added to each well and incubated for 10 minutes in the dark. The reaction was stopped by adding 50 µL of stop solution (2N H2SO4) to each well after the 10 minute period. The optical density (OD) of the wells was assessed immediately after adding the stop solution on a plate reader at 450 nm with subtraction at 570 nm. Data analysis was performed using Molecular Device’s software SoftMax Pro v5.4. In some cases, a four-parameter logistic curve-fit graph was prepared with the dilution on the x-axis (log scale) and the OD value on the y-axis (linear scale), and the half maximum value (EC50) for each sample was determined. The plate template at the top of the layout was adjusted to reflect the dilution of each sample (1 per column).

Results

Fig. 1 demonstrates that antigen-specific ITDCs, including antigen-specific ITDCs loaded with antigen using synthetic nanocarriers, effectively reduce the production of antigen-specific antibodies.

Example 10: Induced Tolerogenic ITDCs Suppress Undesired Immune Responses to Antigen

Materials and Methods

In vitro Treatment to Yield ITDCs

DCs were incubated for 2 hours under tissue culture conditions (37°C, 5% CO2) in Complete Media (CM, RPMI1640+10% Fetal Bovine Serum+Penicillin Streptomycin+L-
Glutamate) with Rapamycin, (100μM) TGFp (2ng/ml) and OVA<sup>323-339</sup> (1μM). Cells were then washed 3 times in MACS Running Buffer (RB, 2%FBS+2mM EDTA in PBS) filtered over 70μM nylon mesh and counted. Cells were equilibrated between treatment groups so that each animal received the same total number of DCs. Final cell prep was in 200ul PBS and injected i.v.

**Immunization**

For each treatment day syngeneic donor mice were inoculated 10 days earlier with Fms-like tyrosine kinase 3 (FLT-3) ligand expressing melanoma cells suscapularly. Flt3 ligand is a growth factor for DCs and allows for greater total number of DCs to be present in the spleen. This increased the number of DCs more than 10-fold and allowed for more cells to be available for in vitro treatment and in vivo administration.

On treatment days the spleens from the FLT-3 melanoma inoculated animals were harvested and digested via liberase. The resulting slurry was filtered by 70μM nylon mesh and a magnetic activating cell sorting (MACS) separation was performed. The cells were incubated with magnetic bead conjugated antibodies (Abs) specific for CD1 lc. These cells were then run through a Miltenyi Biotec Automacs PRO automatic cell separator. The labeled cells were then counted and prepped for treatment.

Animals received active immunization with OVA and GpG subcutaneously. All animals received immunization every 2 weeks at the same time they received the treatment. Each of these groups was split into subgroups to test the capacity of different treatments to modify the Ig titers induced. A control subgroup did not receive tolerogenic treatment. A subgroup received itDCs carrying OVA<sub>323-339</sub> peptide.

Immunization was administered via the following routes (values are per animal): 20 μl/limb of OVA+CpG (12^g OVA+10 μg CpG), both hind limbs s.c. Tolerogenic treatments were administered via the following route (values are per animal): 200 μl itDCs were provided at 100μg/ml of OVA<sub>323-339</sub> content.

**Measurement of IgG**

The level of IgG antibodies were measured. This level is indicative of immunoglobulins in general, including IgEs, which are of particular relevance in allergy. Blocker Casein in PBS (Thermo Fisher, Catalog #37528) was used as diluent. 0.05% Tween-20 in PBS was used as wash buffer, prepared by adding 10 ml of Tween-20 ((Sigma, Catalog
#P9416-100mL) to 2 liters of a 10x PBS stock (PBS: OmniPur® 10X PBS Liquid Concentrate, 4L, EMD Chemicals, Catalog #6505) and 18 Liters of deionized water.

OVA protein at a stock concentration of 5 mg/ml was used as a coating material. A 1:1000 dilution to 5 µg/ml was used as a working concentration. Each well of the assay plates was coated with 100 µl diluted OVA per well, plates were sealed with sealing film (VWR catalog #60941-120), and incubated overnight at 4°C. Costar9017 96-well Flat bottom plates were used as assay plates, Costar9017.

Low-binding polypropylene 96-well plate or tubes were used as set-up plates, in which samples were prepared before being transferred to the assay plate. The setup plates did not contain any antigen and, therefore, serum antibodies did not bind to the plate during the setup of the samples. Setup plates were used for sample preparation to minimize binding that might occur during preparation or pipetting of samples if an antigen-coated plate was used to prepare the samples. Before preparing samples in the setup plate, wells were covered with diluent to block any non-specific binding and the plate was sealed and incubated at 4°C overnight.

Assay plates were washed three times with wash buffer, and wash buffer was completely aspirated out of the wells after the last wash. After washing, 300 µl diluent were added to each well of assay plate(s) to block non-specific binding and plates were incubated at least 2 hours at room temperature. Serum samples were prepared in the setup plate at appropriate starting dilutions. Starting dilutions were sometimes also prepared in 1.5 ml tubes using diluent. Appropriate starting dilutions were determined based on previous data, where available. Where no previous data was available, the lowest starting dilution was 1:40. Once diluted, 200 µl of the starting dilution of the serum sample was transferred from to the appropriate well of the setup plate.

An exemplary setup plate layout is described as follows: Columns 2 and 11 contained anti-Ovabumin monoclonal IgG2b isotype (AbCam, ab17291) standard, diluted to 1 µg/mL (1:4000 dilution). Columns 3-10 contained serum samples (at appropriate dilutions). Columns 1 and 12 were not used for samples or standards to avoid any bias of measurements due to edge effect. Instead, columns 1 and 12 contained 200 µl diluent. Normal mouse serum diluted 1:40 was used as a negative control. Anti-mouse IgG2a diluted 1:500 from 0.5mg/mL stock (BD Bioscience) was used as an isotype control.

Once all samples were prepared in the setup plate, the plate was sealed and stored at 4°C until blocking of the assay plates was complete. Assay plates were washed three times
with wash buffer, and wash buffer was completely aspirated after the last wash. After washing, 100 µL of diluent was added to all wells in rows B-H of the assay plates. A 12-channel pipet was used to transfer samples from the setup plate to the assay plate. Samples were mixed prior to transfer by pipetting 150 µL of diluted serum up and down 3 times. After mixing, 150µL of each sample was transferred from the setup plate and added to row A of the respective assay plate.

Once the starting dilutions of each sample were transferred from the setup plate to row A of the assay plate, serial dilutions were pipetted on the assay plate as follows: 50 µL of each serum sample was removed from row A using 12-channel pipet and mixed with the 100 µL of diluent previously added to each well of row B. This step was repeated down the entire plate. After pipetting the dilution of the final row, 50 µL of fluid was removed from the wells in the final row and discarded, resulting in a final volume of 100 µL in every well of the assay plate. Once sample dilutions were prepared in the assay plates, the plates were incubated at room temperature for at least 2 hours.

After the incubation, plates were washed three times with wash buffer. Detection antibody (Goat anti-mouse anti-IgG, HRP conjugated, AbCam ab98717) was diluted 1:1500 (0.33 µg/mL) in diluent and 100 µL of the diluted antibody was added to each well. Plates were incubated for 1 hour at room temperature and then washed three times with wash buffer, with each washing step including a soak time of at least 30 seconds.

After washing, detection substrate was added to the wells. Equal parts of substrate A and substrate B (BD Biosciences TMB Substrate Reagent Set, catalog #555214) were combined immediately before addition to the assay plates, and 100 µL of the mixed substrate solution were added to each well and incubated for 10 minutes in the dark. The reaction was stopped by adding 50 µL of stop solution (2N H2S04) to each well after the 10 minute period. The optical density (OD) of the wells was assessed immediately after adding the stop solution on a plate reader at 450 nm with subtraction at 570 nm. Data analysis was performed using Molecular Device's software SoftMax Pro v5.4. In some cases, a four-parameter logistic curve-fit graph was prepared with the dilution on the x-axis (log scale) and the OD value on the y-axis (linear scale), and the half maximum value (EC50) for each sample was determined. The plate template at the top of the layout was adjusted to reflect the dilution of each sample (1 per column).

**Determination of %OVA+ Dividing B Cells**
Ovalbumin-!- B-cell division was assessed by flow cytometry. Splenocytes from experimental animals were stained with Cell Tracker Orange (CTO), a thiol-reactive fluorescent probe suitable for long-term cell labeling, and cultured in complete media at 37C, 5% CO₂ with Ovalbumin protein or peptide for 3 days. On day 3 the cells were washed, blocked with anti-CD 16/32 antibody and then stained with conjugated antibodies specific to B220 and CD19. Alexa 647 conjugated ovalbumin protein was also incubated with the cells to label Ovalbumin specific BCRs. Those splenocytes that were CD19+ B220+ OVA-Alexa647+ were assessed for proliferation by comparing the differential CTO staining. Those that were CTO low were labeled as proliferating Ovalbumin-i- B-cells and were compared to the CTO high Ovalbumin-i- B-cells to quantify the percentages.

Results

Fig. 2 demonstrates a reduction in the number of antigen-specific B cells with the itDCs, and even with the administration of the strong immune stimulant, CpG. These results demonstrate the reduction in undesired immune responses, such as those relevant to allergy and allergic responses, with itDCs presenting an MHC Class II-restricted epitope.
What is claimed is:

CLAIMS

1. A method comprising:
   administering to a subject allergen-specific induced tolerogenic dendritic cells (iTDCs) in an amount effective to reduce an allergic response to an allergen in the subject, wherein the allergen-specific iTDCs present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of the allergen, and wherein the subject is experiencing or is at risk of experiencing the allergic response to the allergen.

2. A method comprising:
   reducing an allergic response to an allergen in a subject by administering allergen-specific iTDCs to the subject, wherein the allergen-specific iTDCs present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of the allergen.

3. A method comprising:
   administering to a subject a composition according to a protocol that was previously shown to reduce an allergic response to an allergen in one or more test subjects;
   wherein the composition comprises allergen-specific iTDCs, and wherein the allergen-specific iTDCs present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of the allergen.

4. The method of any of claims 1-3, wherein the method further comprises providing or identifying the subject.

5. The method of any of claims A-Al, wherein the allergen induces or is expected to induce an undesired immune response in the subject.

6. The method of claim 5, wherein the undesired immune response is allergen-specific antibody production.
7. The method of claim 5, wherein the undesired immune response is allergen-specific CD4+ T cell proliferation and/or activity.

8. The method of any of claims 1-7, wherein the allergen comprises an asthma antigen, a hay fever antigen, a hives antigen, an eczema antigen, a plant allergen, an insect sting allergen, an insect allergen, an animal allergen, a fungal allergen, a drug allergen, a pet allergen, a latex allergen, a mold allergen, a cosmetic allergen or a food allergen.

9. The method of claim 8, wherein the food allergen comprises a milk allergen, an egg allergen, a nut allergen, a fish allergen, a shellfish allergen, a soy allergen, a legume allergen, a seed allergen or a wheat allergen.

10. The method of claim 8, wherein the plant allergen is a ragweed allergen.

11. The method of any of claims 1-8, wherein the allergen is associated with hay fever or allergic asthma.

12. The method of any of claims 1-11, wherein the method further comprises assessing an undesired immune response to the allergen in the subject prior to and/or after the administration of the allergen-specific itDCs.

13. The method of claim 12, wherein the undesired immune response is allergen-specific antibody production.

14. The method of claim 13, wherein the undesired immune response is allergen-specific CD4+ T cell proliferation and/or activity.

15. The method of any of claims 1-14, wherein one or more maintenance doses of the allergen-specific itDCs are administered to the subject.

16. The method of any of claims 1-15, wherein the subject has or is at risk of having an allergy.
17. The method of claim 16, wherein the allergy is allergic asthma, hay fever, hives, eczema, a plant allergy, an insect sting allergy, an insect allergy, an animal allergy, a fungal allergy, a drug allergy, a pet allergy, a latex allergy, a mold allergy, a cosmetic allergy or a food allergy.

18. The method of claim 17, wherein the food allergy is a milk allergy, an egg allergy, a nut allergy, a fish allergy, a shellfish allergy, a soy allergy, a legume allergy, a seed allergy or a wheat allergy.

19. The method of claim 17, wherein the plant allergy is a ragweed allergy.

20. The method of claim 17, wherein the allergy is allergic asthma or hay fever.

21. The method of any of claims 1-20, wherein the administering is by parenteral, intraarterial, intranasal or intravenous administration or by injection to lymph nodes or anterior chamber of the eye or by local administration to an organ or tissue of interest.

22. The method of any of claims 1-20, wherein the administering is by subcutaneous, intrathecal, intraventricular, intramuscular, intraperitoneal, intracoronary, intrapancreatic, intrahepatic or bronchial injection.

23. A method, comprising:
   combining itDCs with MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen.

24. The method of claim 23, wherein the allergen induces or is expected to induce an undesired immune response in a subject.

25. The method of claim 23 or 24, wherein the allergen comprises an asthma antigen, a hay fever antigen, a hives antigen, an eczema antigen, a plant allergen, an insect sting allergen, an insect allergen, an animal allergen, a fungal allergen, a drug allergen, a pet allergen, a latex allergen, a mold allergen, a cosmetic allergen or a food allergen.
26. The method of claim 25, wherein the food allergen comprises a milk allergen, an egg allergen, a nut allergen, a fish allergen, a shellfish allergen, a soy allergen, a legume allergen, a seed allergen or a wheat allergen.

27. The method of claim 25, wherein the plant allergen is a ragweed allergen.

28. The method of claim 25, wherein the allergen is associated with hay fever or allergic asthma.

29. The method of any of claims 23-28, wherein the method further comprises collecting the itDCs after combining with the epitopes of the allergen.

30. The method of any of claims 23-29, wherein the method further comprises making a dosage form comprising the allergen-specific itDCs.

31. The method of any of claims 23-30, wherein the method further comprises making the allergen-specific itDCs or the dosage form available to a subject for administration.

32. The method of any of claims 23-31, wherein the method further comprises assessing an undesired immune response to the allergen with the allergen-specific itDCs.

33. The method of claim 32, wherein the undesired immune response is allergen-specific antibody production.

34. The method of claim 32, wherein the undesired immune response is allergen-specific CD4+ T cell proliferation and/or activity.

35. A composition comprising allergen-specific itDCs wherein the allergen-specific itDCs present MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen.
36. The composition of claim 35, wherein the allergen induces or is expected to induce an undesired immune response in a subject.

37. The composition of claim 36, wherein the undesired immune response is allergen-specific antibody production.

38. The composition of claim 36, wherein the undesired immune response is allergen-specific CD4+ T cell proliferation and/or activity.

39. The composition of any of claims 35-38, wherein the allergen comprises an asthma antigen, a hay fever antigen, a hives antigen, an eczema antigen, a plant allergen, an insect sting allergen, an insect allergen, an animal allergen, a fungal allergen, a drug allergen, a pet allergen, a latex allergen, a mold allergen, a cosmetic allergen or a food allergen.

40. The composition of claim 39, wherein the food allergen comprises a milk allergen, an egg allergen, a nut allergen, a fish allergen, a shellfish allergen, a soy allergen, a legume allergen, a seed allergen or a wheat allergen.

41. The composition of claim 39, wherein the plant allergen is a ragweed allergen.

42. The composition of claim 39, wherein the allergen is associated with hay fever or allergic asthma.

43. The composition of any of claims 35-42, wherein the allergen-specific itDCs are produced by the method of any of claims 23-34.

44. The composition of any of claims 35-43, wherein the allergen-specific itDCs are as defined in any of claims 1-22.

45. The composition of any of claims 35-44, wherein the composition further comprises a pharmaceutically acceptable excipient.
46. A dosage form comprising the composition of any of claims 35-45.

47. A process for producing a composition comprising allergen-specific induced tolerogenic dendritic cells (itDCs), the process comprising combining itDCs with MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen.

48. The process of claim 47, wherein said process comprises the steps as defined in any one of claims 23-34.

49. A composition comprising allergen-specific induced tolerogenic dendritic cells (itDCs) obtainable by the process of claim 47 or 48.

50. A composition comprising: (i) induced tolerogenic dendritic cells; and (ii) MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen.

51. The composition of claim 50, wherein the allergen is as defined in any one of claims 39-42.

52. A composition of any one of claims 35-45 and 49-51 or dosage form of claim 46 for use in therapy or prophylaxis.

53. A composition of any one of claims 35-45 and 49-51 or dosage form of claim 46 for use in a method of therapy or prophylaxis of an allergy in a subject or in a method as defined in any one of claims 1-22.

54. Use of the composition of any one of claims 35-45 and 49-51 or dosage form of claim 46 for the manufacture of a medicament for use in a method of therapy or prophylaxis of an allergy in a subject or in a method as defined in any one of claims 1-22.

55. A composition comprising MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen for use in a method comprising:
(i) providing MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of the allergen;
(ii) providing allergen-specific induced tolerogenic dendritic cells (iTDCs) by loading DCs with the epitopes of step (i); and
(iii) administering the allergen-specific iTDCs to a subject prior to, concomitantly with or after exposure to the allergen.

56. Allergen-specific iTDCs for use in a method of therapy or prophylaxis of allergy in a subject, said method comprising:
(i) providing MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen;
(ii) providing allergen-specific iTDCs by loading DCs with the epitopes of step (i); and
(iii) administering the allergen-specific iTDCs to said subject prior to, concomitantly with or after exposure to the allergen.

57. Allergen-specific iTDCs for use in a method comprising:
(i) providing MHC Class I-restricted and/or MHC Class II-restricted epitopes but substantially no B cell epitopes of an allergen;
(ii) providing allergen-specific iTDCs by loading DCs with the epitopes of step (i); and
(iii) administering the allergen-specific iTDCs to a subject.

58. The composition of claim 55 or the allergen-specific iTDCs of claim 56 or 57 wherein the allergen-specific iTDCs are as defined in any of claims 1-22 or the allergen is as defined in any of claims 39-42.

59. A dosage form comprising the composition of any of claims 35-45, 49-53, 55 and 58 or the allergen-specific iTDCs of any of claims 56-58.
Fig. 1
Fig. 2

- % of OVA+ dividing B-cells
- PBS, ctrlDC, itDC
A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395(2006.01)i, A61K 39/38(2006.01)i, A61P 11/06(2006.01)i, A61P 37/00(2006.01)i

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)

A61K 39/395; A61K 39/00; A61P 37/02

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: induced tolerogenic dendritic cells, MHC Class I-restricted, MHC Class II-restricted, B cell epitopes, allergen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>RYAN T. FISCHER et al. 'Rapamycin-conditioned, alloantigen-pulsed myeloid dendritic cells present donor MHC class II-restricted, MHC Class I-restricted, B cell epitopes, allergen</td>
<td>23-28, 35-42, 47,50 ,51,55-57</td>
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</table>

Date of the actual completion of the international search

25 OCTOBER 2012 (25.10.2012)

Date of mailing of the international search report

25 OCTOBER 2012 (25.10.2012)

Name and mailing address of the ISA/KR

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Facsimile No. 82-42-472-7140

Authorized officer

Choi Sung Hee

Telephone No. 82-42-481-8740

Form PCT/ISA/210 (second sheet) (July 2009)
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.: 1-4, 8-22
   because they relate to subject matter not required to be searched by this Authority, namely:
   Claims 1-4 and 8-22 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☒ Claims Nos.: (See extra sheet.)
   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:

   (See extra sheet.)

3. ☒ Claims Nos.: 8, 11, 12, 15, 16, 21, 22, 29-32, 43-46, 48, 49, 52-54, 59
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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 an additional fee, this Authority did not invite payment of any additional fee.

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.
Continuation of Box No. II (2nd reason)

Claims Nos.: 5-22, 33, 34, 44-46, 52-54, 58, 59

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:

Claim 5 is unclear, since the claims which it refers to are not defined clearly, as shown in the following expression, "any of claims A-A1" (PCT Article 6).

Claims 6-22, 44-46, 52-54, 58, and 59 are not clear, since they either directly or indirectly refer to claim 5 which is not drafted in accordance with PCT Article 6.

Claims 9, 10, 13, 14, 17-20, 33, and 34 are unclear, since they either directly or indirectly refer to one of claims which are not drafted in accordance with PCT Rule 6.4(a).
<table>
<thead>
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<th>Patent document cited in search report</th>
<th>Publication date</th>
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