Title: ANTIGEN-SPECIFIC INDUCED TOLEROGENIC DENDRITIC CELLS TO REDUCE CYTOTOXIC T LYMPHOCYTE RESPONSES

Abstract: Disclosed are antigen-specific induced tolerogenic dendritic cells (iDCs) for reducing CD8+ T cell immune responses, as well as related compositions and methods.
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ANTIGEN-SPECIFIC INDUCED TOLEROGENIC DENDRITIC CELLS TO REDUCE CYTOTOXIC T LYMPHOCYTE RESPONSES

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 antigen-specific induced tolerogenic dendritic cell (itDC) compositions to reduce CD8+ T cell immune responses, such as antigen-specific CD8+ T cell proliferation and/or activity, and related compositions. The methods and compositions allow for the shift to tolerogenic immune response development specific to antigens. 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 undesired immune responses against the antigen.

BACKGROUND OF THE INVENTION

CD8+ T cells are involved in immune responses to antigens. Reducing their number and/or function can ameliorate undesired immune responses against antigens. Doing so, however, with conventional immunosuppressant drugs, which are broad-acting, may not be desirable. 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 antigen-specific induced tolerogenic dendritic cells (itDCs) in an amount effective to reduce an undesired CD8+ T cell immune response in the subject, wherein the subject is experiencing or is at risk of experiencing the undesired CD8+ T cell immune response against the antigen is provided. In another aspect, a method comprising reducing an undesired CD8+ T cell immune response in a subject by administering antigen-specific itDCs to the subject, wherein the antigen-specific itDCs present MHC Class I-restricted epitopes of an antigen is provided. In another aspect, a method comprising administering antigen-specific itDCs to a subject according to a protocol that was previously shown to reduce an undesired CD8+ T cell immune response to an antigen in one or more test subjects is provided.

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

In another embodiment, the antigen-specific itDCs present MHC Class I-restricted epitopes of the antigen. In another embodiment, the antigen-specific itDCs also present MHC Class II-restricted and/or B cell epitopes of the antigen. In another embodiment, the antigen-specific itDCs present substantially no B cell epitopes of the antigen. In another embodiment, the antigen-specific itDCs present substantially no MHC Class II-restricted epitopes of the antigen.

In another embodiment, the method further comprises assessing the undesired CD8+ T cell immune response in the subject prior to and/or after the administration of the antigen-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 antigen-specific itDCs are administered to the subject.

In another embodiment, the antigen-specific itDCs are in or are administered in an amount effective to reduce the proliferation and/or activity of antigen-specific CD8+ T cells.

In another embodiment, the antigen comprises an autoantigen, allergen or therapeutic protein, or is associated with an inflammatory disease, an autoimmune disease, organ or tissue rejection or graft versus host disease.
In another embodiment, the subject has or is at risk of having an autoimmune disease, an inflammatory disease, an allergy, organ or tissue rejection or graft versus host disease. In another embodiment, the subject has undergone or will undergo transplantation. In another embodiment, the subject has received, is receiving or will receive a therapeutic protein.

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, or precursors thereof, with MHC Class I-restricted epitopes of an antigen. In another embodiment, the itDCs, or precursors thereof, are also combined with MHC Class II-restricted epitopes and/or B cell epitopes of the antigen. In another embodiment, the itDCs, or precursors thereof, are combined with substantially no MHC Class II-restricted epitopes of the antigen. In another embodiment, the itDCs, or precursors thereof, are combined with substantially no B cell epitopes of the antigen.

In another embodiment, the method further comprises collecting the antigen-specific itDCs.

In another embodiment, the antigen comprises an autoantigen, allergen, therapeutic protein or is associated with an inflammatory disease, an autoimmune disease, organ or tissue rejection or graft versus host disease.

In another embodiment, the method further comprises making a dosage form comprising the antigen-specific itDCs. In another embodiment, the method further comprises making the antigen-specific itDCs or the dosage form available to a subject for administration.

In another embodiment, the method further comprises assessing the reduction of an undesired CD8+ T cell immune response with the antigen-specific itDCs. In another embodiment, wherein the assessing comprises determining the proliferation and/or activity of antigen-specific CD8+ T cells.

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

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

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

In another aspect, a process for producing a composition comprising antigen-specific itDCs, the process comprising combining itDCs, or precursors thereof, with MHC Class I-restricted epitopes of an antigen is provided. In another embodiment, the itDCs, or precursors thereof, are also combined with MHC Class II-restricted and/or B cell epitopes of the antigen. In another embodiment, the itDCs, or precursors thereof, are combined with substantially no MHC Class II-restricted epitopes of the antigen. In another embodiment, the itDCs, or precursors thereof, are combined with substantially no B cell epitopes of the antigen.

In another embodiment, the process comprises any of the steps of any of the methods provided herein.

In another aspect, a composition comprising antigen-specific itDCs obtainable by any of the methods or processes provided herein is provided.

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

In another aspect, any of the compositions or dosage form provided may be for use in therapy or prophylaxis.
In another aspect, any of the compositions or dosage form provided may be for use in a method of reducing an undesired CD8+ T cell immune response in a subject, in a method of therapy or prophylaxis of autoimmune disease, an inflammatory disease, an allergy, organ or tissue rejection or graft versus host disease or in any of the methods provided.

In another aspect, a use of any of the compositions or dosage forms for the manufacture of a medicament for use in a method of reducing an undesired CD8+ T cell immune response in a subject, in a method of therapy or prophylaxis of autoimmune disease, an inflammatory disease, an allergy, organ or tissue rejection or graft versus host disease or in any of the methods provided herein is provided.

In another embodiment, a composition comprising MHC Class I-restricted epitopes of an antigen for use in a method comprising:

(i) providing said MHC Class I-restricted epitopes;

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

(iii) administering the antigen-specific itDCs to a subject prior to, concomitantly with or after exposure to or administration of said MHC Class I-restricted epitopes of the antigen.

In another embodiment, the composition further comprises MHC Class II-restricted epitopes and/or B cell epitopes of the antigen, and the MHC Class II-restricted epitopes and/or B cell epitopes of the antigen are also provided in step (i). In another embodiment, the composition comprises substantially no MHC Class II-restricted epitopes of the antigen. In another embodiment, the composition comprises substantially no B cell epitopes of the antigen.

In another aspect, antigen-specific itDCs for use in a method of reducing an undesired CD8+ T cell immune response in a subject, said method comprising:

(i) providing MHC Class I-restricted epitopes of an antigen;

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

(iii) administering the antigen-specific itDCs to said subject prior to, concomitantly with or after exposure to or administration of a composition comprising MHC Class I-restricted epitopes of the antigen.

In another embodiment, MHC Class II-restricted epitopes and/or B cell epitopes of the antigen are also provided in step (i). In another embodiment, substantially no MHC Class II-
restricted epitopes of the antigen are provided. In another embodiment, substantially no B cell epitopes of the antigen are provided.

In another aspect, antigen-specific itDCs for use in a method comprising:

(i) providing MHC Class I-restricted epitopes of an antigen;

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

(iii) administering the antigen-specific itDCs to a subject.

In another embodiment, MHC Class II-restricted epitopes and/or B cell epitopes of the antigen are also provided in step (i). In another embodiment, substantially no MHC Class II-restricted epitopes of the antigen are provided. In another embodiment, substantially no B cell epitopes of the antigen are provided.

In another aspect, a dosage form comprising any of the compositions or antigen-specific itDCs provided herein 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 antigen-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 circulating itDCs. In other embodiments, the circulating itDCs are CD103+, CD1 lb+, XCR1+ or plasmacytoid itDCs and/or are not CD8CC+. In other embodiments, the circulating itDCs are CD103+ 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 embodiments of any of the compositions and methods provided the subject is a human.

In an embodiment of any of the compositions and methods provided herein, the antigens are peptides. Such antigens, 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 antigens comprise a whole antigenic protein. These antigens may be combined with the itDCs, or precursors thereof, to ultimately form the antigen-specific itDCs.
In an embodiment of any of the compositions and methods provided herein, the antigen comprise multiple types of antigens. In some embodiments, the antigens 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 effectively reduce the specific killing of cells expressing antigen.

**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

CD8+ T cells can be cytotoxic, recognizing "foreign" proteins on cells expressing the proteins and killing them. In some instances, however, the cytotoxicity is undesired. Therefore, ways to tolerize this mechanism by downregulating CD8+ T cell proliferation and/or activity and/or converting CD8+ T cells into regulatory CD8+ T cells would be beneficial. It is believed that the administration of itDCs that present MHC Class I-restricted epitopes can cause a reduction in the amount undesired CD8+ T cell immune responses and result in beneficial tolerogenic immune responses. As shown in the Examples, itDCs that present epitopes of ovalbumin protein successfully reduced the percentage of specific killing of cells expressing ovalbumin. Of particular interest for the compositions and methods provided are itDCs that are circulating itDCs as defined herein. Such itDCs, for example, CD103+ itDCs resulted in the aforementioned beneficial effect in the Examples.

Thus, 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 antigen-specific itDCs by combining itDCs with antigens that comprise MHC Class I-restricted epitopes. Such antigens can also comprise MHC Class II-restricted and/or B cell epitopes. Without being bound by any particular theory, such antigen-specific itDCs can result in the inhibition of the killing of cells that express antigen. This inhibition may be the result of the deletion or inhibition in the stimulation or activation of CD8+ T cells such that the CD8+ T cells are neutralized, do not proliferate and/or do not produce cytokines that result in CD4+ T cell help and/or the production of B cell immune responses, etc. The inhibition, as mentioned above, may also be the result of the conversion of CD8+ T cells to have a regulatory phenotype. The compositions provided herein, therefore, can be used for a number of treatment endpoints.
In embodiments, the antigens may be combined with the itDCs in the form of the antigen itself or a fragment or derivative thereof or in the form of one or more cells that express the antigen. The antigen, therefore, may be in the form of live cells 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 antigen is combined with the itDCs in the presence of an agent that enhances the uptake, processing or presentation of antigens. The antigen-loading provided by such methods allows for the production of itDCs specific to the antigen that can result in antigen-specific itDCs. In some embodiments, the antigen-specific itDCs are generated by contacting naïve itDCs with antigens as provided above and elsewhere herein.

Antigen-specific itDCs can be administered to a subject in order to ameliorate an undesired immune response. In one aspect, a method comprising administering to a subject antigen-specific itDCs, such as antigen-specific circulating itDCs, in an amount effective to reduce the generation of undesired CD8+ T cell immune responses against an antigen in the subject, wherein the subject is experiencing or is at risk of experiencing the undesired immune response against the antigen, is provided. In another aspect, a method comprising reducing the generation of an undesired CD8+ T cell immune response in a subject by administering antigen-specific itDCs, such as antigen-specific circulating itDCs, to the subject is provided. In yet another aspect, a method comprising administering to a subject according to a protocol that was previously shown to reduce the generation of CD8+ T cell immune responses against an antigen in one or more test subjects, where the composition comprises antigen-specific itDCs, such as antigen-specific circulating itDCs, is provided. The methods provided, in some embodiments, may further comprise administering a transplantable graft or therapeutic protein.

Compositions of the antigen-specific itDCs, such as antigen-specific circulating itDCs, are also provided. Antigen-specific itDCs may be produced according to the methods provided and may, for example, reduce an undesired CD8+ T cell immune response to an antigen. In
embodiments, the antigen-specific itDCs present one or more MHC Class I-restricted epitopes. In some embodiments, the antigen-specific itDCs also present MHC Class II-restricted epitopes and/or B cells epitopes. In other embodiments, the antigen-specific itDCs present substantially no MHC Class II-restricted epitopes and/or substantially no B cell epitopes. In embodiments, such compositions may also include a therapeutic protein or a transplantable graft. In other embodiments, the therapeutic protein or transplantable graft may be administered to a subject prior to, concomitantly with or after the administration of the antigen-specific itDCs, such as antigen-specific circulating itDCs. In embodiments, the antigen-specific itDCs provided may be administered as one or more maintenance doses, such as to a subject that has been receiving, is receiving or will receive a therapeutic protein or transplantable graft or that is exposed to or will be exposed to an allergen. In embodiments, the compositions provided are administered such that the generation of an undesired CD8+ T cell immune 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, such as a subject in need of antigen-specific tolerogenic immune responses via a downregulation of CD8+ T cells. Such a subject may be one that has or is at risk of having an inflammatory disease, an autoimmune disease, an allergy, organ or tissue rejection or graft versus host disease. Such a subject may also be one that has undergone or will undergo transplantation. Such a subject may also be one that has experienced, is experiencing or is expected to experience an undesired immune response to a therapeutic protein.

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.

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 "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, a reduction in the generation of an undesired immune response, such as undesired CD8+ T cell immune responses. 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 inflammatory disease, an autoimmune disease, an allergy, organ or tissue rejection or graft versus host disease. Such subjects also include those that have undergone or will undergo transplantation. Such subjects further include those that have experienced, are experiencing or are expected to experience an undesired immune response against a therapeutic protein.

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, on or by 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 CD8+ T cell proliferation and/or activity, the proliferation and/or activity results from recognition of the antigen, or portion thereof, generally in complex with MHC molecules.

"Antigens associated" with a disease, disorder or condition provided herein are antigens that can generate an undesired immune response against, as a result of, or in conjunction with, the disease, disorder or condition; the cause of the disease, disorder or condition (or a symptom or effect thereof); and/or can generate an undesired immune response that is a symptom, result or effect of the disease, disorder or condition. Preferably, in some embodiment use of an antigen associated with a disease, disorder or condition, etc. on the itDCs in the compositions and methods provided herein will lead to a tolerogenic immune response against the antigen and/or the cells in, by or on which the antigen is expressed. In one embodiment, the antigen associated with a disease, disorder or condition, etc. described herein can when presented by the described itDCs lead to a tolerogenic immune response that is specific to the disease, disorder or condition, etc. The antigens can be in the same form as expressed in a subject with the disease, disorder or condition but may also be a fragment or derivative thereof. When a fragment or derivative, however, a desired immune response to the form expressed in such a subject is the preferable result with the compositions and methods provided.
In one embodiment, the antigen is an antigen associated with an inflammatory disease, autoimmune disease, organ or tissue rejection or graft versus host disease. Such antigens include autoantigens, such as myelin basic protein, collagen (e.g., collagen type I), human cartilage gp 39, chromogranin A, gpl30-RAPS, proteolipid protein, fibrillarin, nuclear proteins, nucleolar proteins (e.g., small nucleolar protein), thyroid stimulating factor receptor, histones, glycoprotein gp 70, ribosomal proteins, pyruvate dehydrogenase dehydrolipoamide acetyltransferase, hair follicle antigens, human tropomyosin isoform 5, mitochondrial proteins, pancreatic β-cell proteins, myelin oligodendrocyte glycoprotein, insulin, glutamic acid decarboxylase (GAD), gluten and fragments or derivatives thereof. Other autoantigens are provided in Table 1 below.

Antigens also include those associated with organ or tissue rejection. Examples of such antigens include, but are not limited to, antigens from allogeneic cells, e.g., antigens from an allogeneic cell extract, and antigens from other cells, such as endothelial cell antigens.

Antigens also include those associated with an allergy. Such antigens may include allergens, which are described elsewhere herein.

Antigens also include those associated with a transplantable graft. Such antigens are associated with a transplantable graft, or an undesired immune response in a recipient of a transplantable graft that is generated as a result of the introduction of the transplantable graft in the recipient, that can be presented for recognition by cells of the immune system and that can generate an undesired immune response. Transplant antigens include those associated with organ or tissue rejection or graft versus host disease. Transplant antigens may be obtained or derived from cells of a biological material or from information related to a transplantable graft. Transplant antigens generally include proteins, polypeptides, peptides, lipoproteins, glycolipids, polynucleotides or are contained or expressed in cells. Information related to a transplantable graft is any information about a transplantable graft that can be used to obtain or derive transplant antigens. Such information includes information about antigens that would be expected to be present in or on cells of a transplantable graft such as, for example, sequence information, types or classes of antigens and/or their MHC Class I, MHC Class II or B cell presentation restrictions. Such information may also include information about the type of transplantable graft (e.g., autograft, allograft, xenograft), the molecular and cellular composition of the graft, the bodily location from which the graft is derived or to which the graft to be
transplanted (e.g., whole or partial organ, skin, bone, nerves, tendon, neurons, blood vessels, fat, cornea, etc.).

Antigens also include antigens associated with a therapeutic protein that can be presented for recognition by cells of the immune system and that can generate an undesired immune response against the therapeutic protein. Therapeutic protein antigens generally include proteins, polypeptides, peptides, lipoproteins, or are contained or expressed in, by or on cells.

Antigens, can be antigens that are fully defined or characterized. However, in some embodiments, an antigen is not fully defined or characterized. Antigens, therefore, also include those that are contained within a cell or tissue preparation, cell debris, cell exosome or conditioned media and can be delivered in such form in some embodiments.

"Antigen-specific itDCs" refers to itDCs that present antigens and modulate immune responses specific to the antigens. Such antigens may comprise MHC Class I-restricted and/or MHC Class II-restricted and/or B cell epitopes. In some embodiments, antigen-specific itDCs are generated by antigen-loading of itDCs, for example, naïve itDCs that have not been exposed to an antigen. In some embodiments, antigen-specific itDCs are administered to a subject and induce a tolerogenic reaction to the antigen in the subject. Antigen-loading is achieved, in some embodiments, by combining itDCs with the antigen (provided in any of the forms provided herein).

"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.

An "autoimmune disease" is any disease where the immune system mounts an undesired immune response against self (e.g., one or more autoantigens). In some embodiments, an autoimmune disease comprises an aberrant destruction of cells of the body as part of the self-targeted immune response. In some embodiments, the destruction of self manifests in the
malfunction of an organ, for example, the colon or pancreas. Examples of autoimmune diseases are described elsewhere herein. Additional autoimmune diseases will be known to those of skill in the art and the invention is not limited in this respect.

"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.

"Circulating itDCs" refers to itDCs that are capable of circulating in the peripheral blood or migrating to one or more organs or tissues in a subject, or that are produced from dendritic cells that are capable of circulating in the peripheral blood or migrating to one or more organs or tissues in a subject. Examples of types of circulating itDCs include itDCs that are CD103+,
CD1 lb+, XCR1+ or plasmacytoid itDCs. Another example of a type of circulating itDCs are those that are not CD8CC+. Still another example of such itDCs are migratory itDCs. In other embodiments, the circulating itDCs are CD103+ itDCs. Methods and reagents for enrichment of DCs for particular subsets are known in the art. Non-limiting examples of such methods are sorting by fluorescence-activated cell sorting (FACS) and magnetic cell sorting (MACS). Both technologies involve binding agents, for example, antibodies, that bind to a surface marker characteristic for a population of DCs of interest, for example, CD103, CD1 lb, XCR1, etc. and a separation step in which cells that bind to the binding agent are separated from cells that do not bind the binding agent. Populations of DC subsets can be isolated from various sources known to those of skill in the art, including, but not limited to, blood, e.g., peripheral blood or cord blood; lymphatic fluid; lymph nodes; bone marrow; thymus, liver or spleen.

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, CD1dl, 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. In some embodiments, the epitope itself is an antigen.


Other examples of epitopes that can be combined with or presented by the iDCs provided herein include any of the MHC Class I-restricted, MHC Class II-restricted and B cell epitopes as provided as SEQ ID NOs: 1-943. Without wishing to being bound by any particular
theory, MHC Class I-restricted epitopes include those set forth in SEQ ID NOs: 1-186, MHC Class II-restricted epitopes include those set forth in SEQ ID NOs: 187-537, and B cell epitopes include those set forth in SEQ ID NOs: 538-943. These epitopes include MHC Class I-restricted autoantigens, MHC Class II-restricted epitopes of allergens and B cell epitopes of autoantigens and allergens.

"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.

"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 naïve T cells to Foxp3+ T regulatory cells ex vivo and/or in vivo (e.g., inducing expression of FoxP3 in the naïve 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.

"Inflammatory disease" means any disease, disorder or condition in which undesired inflammation occurs.

"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 certain 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 antigen-specific CD8+ T cell number and/or activity.

"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%, 30%
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 that 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.

"Substantially no MHC Class II-restricted epitopes" refers to the absence of MHC Class II-restricted 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 CD4+ T cell immune response specific to the antigen. In embodiments, a composition with substantially no MHC Class II-restricted epitopes does not contain a measurable amount of MHC Class II-restricted epitopes of an antigen. In other embodiments, such a composition may comprise a measurable amount of MHC Class II-restricted epitopes of an antigen but said amount is not effective to generate a measurable CD4+ T cell immune response (by itself, within the context of the antigen, in conjunction with a carrier or in conjunction with an inventive composition) or is not effective to generate a significant measurable CD4+ T 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 CD4+ T 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 CD4+ T cell immune response is one that is greater than the level of the same type of immune response produced by a control antigen (e.g., one known not to comprise MHC Class II-restricted epitopes of the antigen or to stimulate CD4+ T cell immune responses). In embodiments, the compositions do not comprise MHC Class II-restricted epitopes (by itself, within the context of the antigen, in conjunction with a carrier or in conjunction with an
inventive composition) that generate antigen-specific CD4+ T cell immune responses or an undesired level thereof.

In embodiments, to ensure that a composition comprises substantially no MHC Class II-restricted antigens are selected such that they do not comprise MHC Class II-restricted epitopes for loading onto the itDCs, or precursors thereof, as provided herein. In other embodiments, to ensure that a composition comprises substantially no MHC Class II-restricted epitopes of an antigen, the itDCs, or precursors thereof, are produced and tested for CD4+ T cell immune responses (e.g., antigen-specific CD4+ T 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.

A "therapeutic protein" refers to any protein or protein-based therapy that may be administered to a subject and have a therapeutic effect. Such therapies include protein replacement and protein supplementation therapies. Such therapies also include the administration of exogenous or foreign protein, antibody therapies, and cell or cell-based therapies. Therapeutic proteins include enzymes, enzyme cofactors, hormones, blood clotting factors, cytokines, growth factors, monoclonal antibodies and polyclonal antibodies. Examples of other therapeutic proteins are provided elsewhere herein. Therapeutic proteins may be produced in, on or by cells and may be obtained from such cells or combined and/or administered in the form of such cells. In embodiments, the therapeutic protein is produced in, on or by mammalian cells, insect cells, yeast cells, bacteria cells, plant cells, transgenic animal cells, transgenic plant cells, etc. The therapeutic protein may be recombinantly produced in such
cells. The therapeutic protein may be produced in, on or by a virally transformed cell. The therapeutic protein may also be produced in, on or by autologous cells that have been transfected, transduced or otherwise manipulated to express it. Alternatively, the therapeutic protein may be combined with the iTDCs and/or administered as a nucleic acid or by introducing a nucleic acid into a virus, VLP, liposome, etc. and combining and/or administering such forms. Alternatively, the therapeutic protein may be obtained from such forms and combined and/or administered as the therapeutic protein itself. Subjects, therefore, include any subject that has received, is receiving or will receive any of the foregoing. Such subject includes subjects that have received, is receiving or will receive gene therapy, autologous cells that have been transfected, transduced or otherwise manipulated to express a therapeutic protein, polypeptide or peptide; or cells that express a therapeutic protein, polypeptide or peptide.

"Therapeutic protein antigen" means an antigen that is associated with a therapeutic protein that can be, or a portion of which, can be presented for recognition by cells of the immune system and can generate an undesired immune response (e.g., the production of therapeutic protein-specific antibodies) against the therapeutic protein. Therapeutic protein antigens generally include proteins, polypeptides, peptides, lipoproteins, or are contained or expressed in, on or by cells.

"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-1b, 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-1cc, 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, Th1 or Th2 immune response; the inhibition of effector cell-specific cytokines: Th17 (e.g., IL-17, IL-25), Th1 (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.

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 therapeutic 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 an itDC composition provided herein and/or prior to administration of a transplantable graft or therapeutic protein or 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 provided herein and/or and after administration of a transplantable graft or therapeutic protein or exposure to an allergen. In some embodiments, the assessment is done after administration of the composition, but prior to administration of a transplantable graft or therapeutic protein or exposure to an allergen. In other embodiments, the assessment is done after administration of a transplantable graft or therapeutic protein or 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 itDCs and administration of a transplantable graft or therapeutic protein or exposure to an allergen, while in yet other embodiments the assessment is performed after both the administration of itDCs and the administration of a
transplantable graft or therapeutic protein or exposure to an allergen. In further embodiments, the assessment is performed both prior to and after the administration of the iTDCs and/or administration of a transplantable graft or therapeutic protein or 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 inflammatory disease, an autoimmune disease, an allergy, organ or tissue rejections or graft versus host disease. Other subjects include those that have undergone or will undergo transplantation as well as those that have received, are receiving or will receive a therapeutic protein against which they have experienced, are experiencing or are expected to experience an undesired immune response.

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.

A "transplantable graft" refers to a biological material, such as cells, tissues and organs (in whole or in part) that can be administered to a subject. Transplantable grafts may be autografts, allografts, or xenografts of, for example, a biological material such as an organ, tissue, skin, bone, nerves, tendon, neurons, blood vessels, fat, cornea, pluripotent cells, differentiated cells (obtained or derived in vivo or in vitro), etc. In some embodiments, a transplantable graft is formed, for example, from cartilage, bone, extracellular matrix, or collagen matrices. Transplantable grafts may also be single cells, suspensions of cells and cells in tissues and organs that can be transplanted. Transplantable cells typically have a therapeutic function, for example, a function that is lacking or diminished in a recipient subject. Some non-limiting examples of transplantable cells are β-cells, hepatocytes, hematopoietic stem cells, neuronal stem cells, neurons, glial cells, or myelinating cells. Transplantable cells can be cells that are unmodified, for example, cells obtained from a donor subject and usable in transplantation without any genetic or epigenetic modifications. In other embodiments, transplantable cells can be modified cells, for example, cells obtained from a subject having a
genetic defect, in which the genetic defect has been corrected, or cells that are derived from reprogrammed cells, for example, differentiated cells derived from cells obtained from a subject.

"Transplantation" refers to the process of transferring (moving) a transplantable graft into a recipient subject (e.g., from a donor subject, from an in vitro source (e.g., differentiated autologous or heterologous native or induced pluripotent cells)) and/or from one bodily location to another bodily location in the same subject.

"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 antigen-specific induced tolerogenic dendritic cells useful for promoting the generation of tolerogenic immune responses by, for example, reducing antigen-specific CD8+ T cell immune responses. Preferably, in some embodiments, such itDCs are produced by the methods provided herein through the combining of itDCs, or precursors thereof, with antigens (in any of the forms provided). Such itDCs are useful for the suppression, inhibition, prevention, or delay of the onset of an undesired immune response in a subject, as described in more detail elsewhere herein. Such subjects include those that have or are at risk of having an inflammatory disease, an autoimmune disease, an allergy, organ or tissue rejection or graft versus host disease. Such subjects also include those that have been, are being or will be administered a therapeutic protein against which the subject has experienced or is expected to experience an undesired immune response. Such subjects also include those that have undergone or will undergo transplantation.

Some embodiments of this invention provide the aforementioned antigen-specific itDCs. These itDCs are capable of suppressing an immune response to an antigen presented by it by, for example, reducing CD8+ T cell immune responses, such as antigen-specific CD8+ T cell proliferation and/or activity.
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) 5
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 antigen-specific itDCs 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
of the antigens provided herein. Such antigens include antigens associated with an inflammatory
disease, autoimmune disease, allergy, organ or tissue rejection, graft versus host disease, a
transplantable graft and a therapeutic protein or portion thereof.

Therapeutic proteins include, but are not limited to, infusible therapeutic proteins,
enzymes, enzyme cofactors, hormones, blood clotting factors, cytokines and interferons, growth
factors, monoclonal antibodies, and polyclonal antibodies (e.g., that are administered to a subject
as a replacement therapy), and proteins associated with Pompe's disease (e.g., alglucosidase alfa,
rhGAA (e.g., Myozyme and Lumizyme (Genzyme)). Therapeutic proteins also include proteins
involved in the blood coagulation cascade. Therapeutic proteins include, but are not limited to,
Factor VIII, Factor VII, Factor IX, Factor V, von Willebrand Factor, von Heldebrant Factor,
tissue plasminogen activator, insulin, growth hormone, erythropoietin alfa, VEGF,
thrombopoietin, lysozyme, antithrombin and the like. Therapeutic proteins also include
adipokines, such as leptin and adiponectin. Other examples of therapeutic proteins are as
described below and elsewhere herein. Also included are fragments or derivatives of any of the
therapeutic proteins provided as the epitope, or protein, polypeptide or peptide that comprises the
epitope.

Examples of therapeutic proteins used in enzyme replacement therapy of subjects having
a lysosomal storage disorder include, but are not limited to, imiglucerase for the treatment of
Gaucher's disease (e.g., CEREZYME™), a-galactosidase A (a-gal A) for the treatment of Fabry
disease (e.g., agalsidase beta, FABRYZYME™), acid a-glucosidase (GAA) for the treatment of
Pompe disease (e.g., alglucosidase alfa, LUMIZYME™, MYOZYME™), arylsulfatase B for the treatment of Mucopolysaccharidoses (e.g., laronidase, ALDURAZYME™, idursulfase, ELAPRASETM, arylsulfatase B, NAGLAZYME™).

Examples of enzymes include oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases.

Examples of hormones include Melatonin (N-acetyl-5-methoxytryptamine), Serotonin, Thyroxine (or tetraiodothyronine) (a thyroid hormone), Triiodothyronine (a thyroid hormone), Epinephrine (or adrenaline), Norepinephrine (or noradrenaline), Dopamine (or prolactin inhibiting hormone), Antimullerian hormone (or Mullerian inhibiting factor or hormone), Adiponectin, Adrenocorticotropic hormone (or corticotropin), Angiotensinogen and angiotensin, Antidiuretic hormone (or vasopressin, arginine vasopressin), Atrial-natriuretic peptide (or atriopeptin), Calcitonin, Cholecystokinin, Corticotropin-releasing hormone, Erythropoietin, Follicle-stimulating hormone, Gastrin, Ghrelin, Glucagon, Glucagon-like peptide (GLP-1), GIP, Gonadotropin-releasing hormone, Growth hormone-releasing hormone, Human chorionic gonadotropin, Human placental lactogen, Growth hormone, Inhibin, Insulin, Insulin-like growth factor (or somatomedin), Leptin, Luteinizing hormone, Melanocyte stimulating hormone, Orexin, Oxytocin, Parathyroid hormone, Prolactin, Relaxin, Secretin, Somatostatin, Thrombopoietin, Thyroid-stimulating hormone (or thyrotropin), Thyrotropin-releasing hormone, Cortisol, Aldosterone, Testosterone, Dehydroepiandrosterone, Androstenedione, Dihydrotestosterone, Estradiol, Estrone, Estriol, Progesterone, Calcitriol (1,25-dihydroxyvitamin D3), Calcidiol (25-hydroxyvitamin D3), Prostaglandins, Leukotrienes, Prostacyclin, Thromboxane, Prolactin releasing hormone, Lipotropin, Brain natriuretic peptide, Neuropeptide Y, Histamine, Endothelin, Pancreatic polypeptide, Renin, and Enkephalin.

Examples of blood and blood coagulation factors include Factor I (fibrinogen), Factor II (prothrombin), tissue factor, Factor V (proaccelerin, labile factor), Factor VII (stable factor, proconvertin), Factor VIII (antihemophilic globulin), Factor IX (Christmas factor or plasma thromboplastin component), Factor X (Stuart-Prower factor), Factor Xa, Factor XI, Factor XII (Hageman factor), Factor XIII (fibrin-stabilizing factor), von Willebrand factor, prekallikrein (Fletcher factor), high-molecular weight kininogen (HMWK) (Fitzgerald factor), fibrinectin, fibrin, thrombin, antithrombin III, heparin cofactor II, protein C, protein S, protein Z, protein Z-
related protease inhibitor (ZPI), plasminogen, alpha 2-antiplasmin, tissue plasminogen activator (tPA), urokinase, plasminogen activator inhibitor-1 (PAI1), plasminogen activator inhibitor-2 (PAI2), cancer procoagulant, and epoetin alfa (Epogen, Procrit).

Examples of cytokines include lymphokines, interleukins, and chemokines, type 1 cytokines, such as IFN-γ, TGF-β, and type 2 cytokines, such as IL-4, IL-10, and IL-13.

Examples of growth factors include Adrenomedullin (AM), Angiopoietin (Ang), Autocrine motility factor, Bone morphogenetic proteins (BMPs), Brain-derived neurotrophic factor (BDNF), Epidermal growth factor (EGF), Erythropoietin (EPO), Fibroblast growth factor (FGF), Glial cell line-derived neurotrophic factor (GDNF), Granulocyte colony-stimulating factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), Growth differentiation factor-9 (GDF9), Hepatocyte growth factor (HGF), Hepatoma-derived growth factor (HDGF), Insulin-like growth factor (IGF), Migration-stimulating factor, Myostatin (GDF-8), Nerve growth factor (NGF) and other neurotrophins, Platelet-derived growth factor (PDGF), Thrombopoietin (TPO), Transforming growth factor alpha (TGF-α), Transforming growth factor beta (TGF-β), Tumour necrosis factor-alpha (TNF-α), Vascular endothelial growth factor (VEGF), Wnt Signaling Pathway, placental growth factor (P1GF), [(Foetal Bovine Somatotrophin)] (FBS), IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, and IL-7.


Examples of infusion therapy or injectable therapeutic proteins include, for example,

Tocilizumab (Roche/Actemra®), alpha-1 antitrypsin (Kamada/AAT), Hematide® (Affymax and Takeda, synthetic peptide), albinterferon alfa-2b (Novartis/Zalbin™), Rhucin® (Pharming Group, C1 inhibitor replacement therapy), tesamorelin (Theratechnologies/Egrifta, synthetic growth hormone-releasing factor), ocrelizumab (Genentech, Roche and Biogen), belimumab (GlaxoSmithKline/Benlysta®), pegloticase (Savient Pharmaceuticals/Krystexxa™), taliglucerase alfa (Protalix/Uplyso), agalsidase alfa (Shire/Replagal®), velaglucerase alfa (Shire).
Additional therapeutic proteins useful in accordance to aspects of this invention will be apparent to those of skill in the art, and the invention is not limited in this respect.

In some embodiments, the antigen-specific itDCs are combined with a transplantable graft or therapeutic protein, and such compositions are provided herein. In other embodiments, the antigen-specific itDCs are administered prior to, concomitantly with or after the administration of a transplantable graft, therapeutic protein, etc.

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 antigen-specific itDCs and related compositions, and some aspects provide methods of using the itDCs provided herein. The antigen-specific itDCs may be produced from itDCs generated by the methods provided herein that are combined with an antigen to produce antigen-specific itDCs. The antigen-specific itDCs may also be produced from itDCs generated according to the methods provided in PCT Publication, WO2011/109833.

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 naive 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., CD1 Ic in the case
of certain dendritic cells. In some embodiments, DCs present in a starting population of cells
express CDI Ic. 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 CD1Ic) 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 CD15 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 neuraminidase 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 pluripotential 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 Sallusto 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 naïve 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 mTOR inhibitor; 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 TGFp1, 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); aminesters (U.S. Pat. No. 5,130,307); acetals (U.S. Pat.
No. 5,51,413); 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 alkoxyaralkyl 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 alkanoic 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 carboxamides (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, Torinl, PP242, PP30, NVP-BEZ235, and
OST027. Additional mTOR inhibitors include LY294002 and wortmannin. Other inhibitors of
mTOR are described in U.S. Patent Nos. 7,504,397 and 7,659,274, and in Patent Publication
Nos. US20090304692A1; US20090099174A1, US20060199803A1, WO2008148074A3, the
entire contents of which are incorporated herein by reference.

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 TGF\(\beta\)
pathway agonist.

2. TGF\(\beta\) Pathway Agonists

In some exemplary embodiments, a tolerogenic stimulus for use in the instant invention
comprises or consists of one or more TGF\(\beta\) agonists. TGF\(\beta\) agonists suitable for practicing the
invention include substances that stimulate or potentiate responses induced by TGF\(\beta\) signaling.
In some embodiments, a TGF\(\beta\) pathway agonist is acts by modulating TGF\(\beta\) receptor-mediated
signaling. In some embodiments, a TGF\(\beta\) pathway agonist is a TGF\(\beta\) mimetic, e.g., a small
molecule having TGFP-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 TGF\(\beta\) receptor agonist useful for practicing the invention
is TGF\(\beta\), including TGF\(\beta\)1, TGF\(\beta\)2, TGF\(\beta\)3, variants thereof, and mixtures thereof. Additional
TGFβ agonists are described in Patent Publication No. US20090143394A1, the entire contents of which are incorporated herein by reference.

In particular embodiments, the foregoing TGFβ 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 P1, 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).

In some embodiments, purinergic receptor antagonists useful for practicing the invention include one or more of the compounds described in the following U.S. Patents, the entire contents of which are incorporated herein by reference: US7235549, US7214677, US7553972, US7241776, US7186742, US7176202, US6974812, US7071223, and US7407956. In some embodiments, purinergic receptor antagonists useful for practicing the invention include one or more of the compounds described in the following patent publications, the entire contents of which are incorporated herein by reference: WO2010018280A1, WO2008142194A1, WO2009074519A1, WO2008138876A1, WO2008 119825A3, WO2008119825A2,

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.

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, and a TGFβ 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., TGFβ); ii) a statin; an mTOR inhibitor (e.g., rapamycin or a variant or derivative thereof), a TGFβ agonist (e.g., TGFβ), 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 μM, or 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, 9 mg/mL, 10 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 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. In 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., αATP) 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 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, 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-la, PGC-lb, PRC, or other molecules involved in mitochondrial function, such as estrogen-related receptor a, NRF-1, NRF-2, Spl, 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-la 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.
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. For example, in some embodiments, where tolerance to an alloantigen is desired, itDCs which intrinsically express the alloantigen to which tolerance is desired, will not need to be manipulated to express an antigen of interest.

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
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). Such methods include assessing the level and/or function of therapeutic protein in a subject. 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

In some embodiments, the antigen is associated with allergic responses. In such
embodiments, the antigen which with 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 gluten. In some embodiments, the antigen
comprises one or more peptides or polypeptides derived myelin.

In exemplary embodiments, the antigen (or one of the antigens) with which the dendritic
cells are contacted in the foregoing methods is an antigen that is targeted by the immune system
of a subject with the disease, e.g., targeted by effector T cells, and such targeting contributes to
disease progression. Some exemplary antigens of this kind are described herein. Additional
antigens of this kind are well known to those of skill in the art, and the invention is not limited in
this respect. For example, in some embodiments, the antigen is associated with celiac disease
(CD). In such embodiments, the antigen with which the dendritic cells are contacted can be
derived from wheat, rye, or barley. In exemplary embodiments, the antigen can comprise gluten
or gliadin, or portions or mixtures thereof, for example, amino acids spanning from about amino acid 57 to amino acid 73 of A-gliadin.

In some embodiments, the antigen is associated with type I diabetes. In such embodiments, the antigen with which the dendritic cells are contacted can be one or more peptides or polypeptides derived from islet cells of the pancreas, e.g., can be a cell or tissue lysate or extract; a mixture of proteins or polypeptides or peptides; or one or more purified proteins, polypeptides or peptides.

In some embodiments, the antigen is associated with multiple sclerosis. In such embodiments, the antigen with which the dendritic cells are contacted can be one or more peptides or polypeptides derived from neural cell or tissue. For example, the antigen can be derived from axons, dendrites, neuronal cell bodies, oligodendrocytes, glia cells, microglia or Schwann cells. In particular embodiments, the antigen is myelin, or a component thereof, e.g., myelin basic protein.

In some embodiments, the antigen is associated with primary biliary cirrhosis. In such embodiments, the antigen with which the dendritic cells are contacted can be one or more peptides or polypeptides derived from bile duct cells, e.g., as a cell or tissue lysate or extract.

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, many autoimmune disorders 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 iDCs. 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, of the eye or by local administration to an organ or tissue of interest. The administering may also be performed prior to presentation intravenous or by injection. The cytokine, antigen, and/or adjuvant may be expressed, contacted, manipulated, or presented to the antigen-presenting cells. It is also possible to present antigen onto dendritic cells in the absence of an adjuvant, such as a TLR 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 antigen-specific itDCs and/or therapeutic protein, transplantable graft, etc. are administered to a subject by an appropriate route. The administering of the antigen-specific itDCs and/or transplantable graft and/or therapeutic protein, when expressed in a cell and administered as such, 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.

When not administered in cellular form, 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 and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp. 1694-1712; incorporated by reference). Other agents can likewise be administered by such routes.

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 antigen-specific iTDCs and/or varying amounts of therapeutic proteins or transplantable grafts 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, antigen-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 antigen-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 $10^5$ 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., $5 \times 10^5$, $5 \times 10^6$, $5 \times 10^7$, $5 \times 10^8$, $5 \times 10^9$, or $5 \times 10^{10}$ 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 antigen-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, etc. Such means include known biochemical and immunological tests for monitoring and assessing, for example, cytokine production, antibody production, inflammation, T-effector cell activity, organ or tissue rejection, allergic response, therapeutic protein level and/or function, 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 $\frac{1}{4}$, about $\frac{1}{3}$, about $\frac{1}{2}$, about $\frac{1}{3}$, about $\frac{1}{4}$, about $\frac{1}{8}$, about $\frac{1}{10}$, about $V_{20}$, about $V_{25}$, about $V_{50}$, about $V_{100}$, about $V_{1000}$, about $V_{10000}$, about $V_{100000}$, 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 antigen-specific itDCs is undertaken e.g., prior to administration of a therapeutic protein or transplantable graft or 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 administration of a therapeutic protein or transplantable graft or exposure to an allergen. In addition or alternatively, antigen-specific itDCs can be administered to an subject concomitantly with or following administration of a therapeutic protein or transplantable graft or exposure to an allergen. In exemplary embodiments, antigen-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 administration of a therapeutic protein or transplantable graft or exposure to an allergen.

In some embodiments, the use of antigen-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 inflammatory diseases, autoimmune diseases, allergies, organ or tissue rejection and graft versus host disease. The compositions and methods described herein can also be used in subjects who have undergone or will undergo transplantation. The compositions and methods described herein can also be used in subjects who have received, are receiving or will receive a therapeutic protein against which they have generated or are expected to generate an undesired immune response.

Autoimmune diseases include, but are not limited to, rheumatoid arthritis, multiple sclerosis, immune-mediated or Type I diabetes mellitus, inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis), systemic lupus erythematosus, psoriasis, scleroderma, autoimmune thyroid disease, alopecia areata, Grave's disease, Guillain-Barre syndrome, celiac disease, Sjogren's syndrome, rheumatic fever, gastritis, autoimmune atrophic gastritis, autoimmune hepatitis, insulinitis, oophoritis, orchitis, uveitis, phacogenic uveitis, myasthenia gravis, primary myxoedema, pernicious anemia, autoimmune haemolytic anemia, Addison's disease, scleroderma, Goodpasture's syndrome, nephritis, for example, glomerulonephritis, psoriasis, pemphigus vulgaris, pemphigoid, sympathetic ophthalmia, idiopathic thrombocytopoenic purpura, idiopathic feucopenia, Wegener's granulomatosis and poly/dermatomyositis.

Some additional exemplary autoimmune diseases, associated autoantigens, and autoantibodies, which are contemplated for use in the invention, are described in Table 1 below:

<table>
<thead>
<tr>
<th>Autoantibody Type</th>
<th>Autoantibody</th>
<th>Autoantigen</th>
<th>Autoimmune disease or disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antinuclear antibodies</td>
<td>Anti-SSA/Ro autoantibodies</td>
<td>ribonucleoproteins</td>
<td>Systemic lupus erythematosus, neonatal heart block, primary Sjogren's syndrome</td>
</tr>
<tr>
<td></td>
<td>Anti-La/SS-B autoantibodies</td>
<td>ribonucleoproteins</td>
<td>Primary Sjogren's syndrome</td>
</tr>
<tr>
<td></td>
<td>Anti-centromere antibodies</td>
<td>centromere</td>
<td>CREST syndrome</td>
</tr>
<tr>
<td></td>
<td>Anti-neuronal nuclear antibody-2</td>
<td>R[disambiguation needed]</td>
<td>Opsoclonus</td>
</tr>
<tr>
<td></td>
<td>Anti-dsDNA</td>
<td>double-stranded DNA</td>
<td>SLE</td>
</tr>
<tr>
<td>Antibody</td>
<td>Associated Protein(s)</td>
<td>Disease(s)</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Anti-Jol histidine-tRNA ligase</td>
<td>Inflammatory myopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Smith snRNP core proteins SLE</td>
<td>Systemic sclerosis (anti-Scl-70 antibodies)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-topoisomerase antibodies Type I</td>
<td>SLE and Drug-induced LE[2]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-histone antibodies histones</td>
<td>SLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-p62 nucleoprotein 62 antibodies</td>
<td>Primary biliary cirrhosis [3][4][5]</td>
<td></td>
<td></td>
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<tr>
<td>Anti-splOO antibodies SplOO nuclear antigen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-transglutaminase antibodies</td>
<td>Coeliac disease</td>
<td></td>
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<tr>
<td>Anti-tTG</td>
<td>Dermatitis herpetiformis</td>
<td></td>
<td></td>
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<tr>
<td>Anti-eTG</td>
<td>Miller-Fisher Syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-ganglioside antibodies ganglioside GQ1B</td>
<td>Acute motor axonal neuropathy (AMAN)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-ganglioside antibodies ganglioside GD3</td>
<td>Multifocal motor neuropathy with conduction block (MMN)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-actin antibodies actin</td>
<td>Coeliac disease anti-actin antibodies correlated with the level of intestinal damage [6][7]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver kidney microsomal type 1 antibody</td>
<td>Autoimmune hepatitis, [8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupus anticoagulant Anti-thrombin antibodies thrombin</td>
<td>Systemic lupus erythematosus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-neutrophil cytoplasmic antibody phospholipid</td>
<td>Antiphospholipid syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-ANCA proteins in neutrophil cytoplasm</td>
<td>Wegener's granulomatosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-ANCA neutrophil perinuclear</td>
<td>Microscopic polyangiitis, Churg-Strauss syndrome, systemic vasculitides (non-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoantibody</td>
<td>Target</td>
<td>Disease</td>
<td></td>
</tr>
<tr>
<td>------------------------------------</td>
<td>---------------------------------------------</td>
<td>----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid factor IgG</td>
<td>specific</td>
<td>Rheumatoid arthritis</td>
<td></td>
</tr>
<tr>
<td>Anti-smooth muscle antibody</td>
<td>smooth muscle</td>
<td>Chronic autoimmune hepatitis</td>
<td></td>
</tr>
<tr>
<td>Anti-mitochondrial antibody</td>
<td>mitochondria</td>
<td>Primary biliary cirrhosis [9]</td>
<td></td>
</tr>
<tr>
<td>Anti-SRP</td>
<td>signal recognition particle</td>
<td>Polymyositis [10]</td>
<td></td>
</tr>
<tr>
<td>exosome complex</td>
<td></td>
<td>Scleromyositis</td>
<td></td>
</tr>
<tr>
<td>nicotinic acetylcholine receptor</td>
<td></td>
<td>Myasthenia gravis</td>
<td></td>
</tr>
<tr>
<td>muscle-specific kinase (MUSK)</td>
<td></td>
<td>Myasthenia gravis</td>
<td></td>
</tr>
<tr>
<td>Anti-VGCC</td>
<td>voltage-gated calcium channel (P/Q-type)</td>
<td>Lambert-Eaton myasthenic syndrome</td>
<td></td>
</tr>
<tr>
<td>thyroid peroxidase (microsomal)</td>
<td></td>
<td>Hashimoto's thyroiditis</td>
<td></td>
</tr>
<tr>
<td>TSH receptor</td>
<td></td>
<td>Graves' disease</td>
<td></td>
</tr>
<tr>
<td>Hu</td>
<td></td>
<td>Paraneoplastic cerebellar syndrome</td>
<td></td>
</tr>
<tr>
<td>Yo (cerebellar Purkinje Cells)</td>
<td></td>
<td>Paraneoplastic cerebellar syndrome</td>
<td></td>
</tr>
<tr>
<td>amphipysin</td>
<td></td>
<td>Stiff person syndrome, paraneoplastic cerebellar syndrome</td>
<td></td>
</tr>
<tr>
<td>Anti-VGKC</td>
<td>voltage-gated potassium channel (VGKC)</td>
<td>Limbic encephalitis, Isaac's Syndrome (autoimmune neuromyotonia)</td>
<td></td>
</tr>
<tr>
<td>basal ganglia neurons</td>
<td></td>
<td>Sydenham's chorea, paediatric autoimmune neuropsychiatric disease associated with Streptococcus (PANDAS)</td>
<td></td>
</tr>
<tr>
<td>N-methyl-D-aspartate receptor (NMDA)</td>
<td></td>
<td>Encephalitis</td>
<td></td>
</tr>
<tr>
<td>glutamic acid</td>
<td></td>
<td>Diabetes mellitus type 1, stiff person</td>
<td></td>
</tr>
</tbody>
</table>
Inflammatory diseases include, but are not limited to, Alzheimer's, Ankylosing spondylitis, arthritis, asthma, atherosclerosis, Behcet's disease, chronic inflammatory demyelinating polyradiculoneuropathy, Crohn's disease, colitis, cystic fibrosis, dermatitis, diverticulitis, hepatitis, irritable bowel syndrome (IBS), lupus erythematous, muscular dystrophy, nephritis, Parkinson's, shingles and ulcerative colitis. Inflammatory diseases also include, for example, cardiovascular disease, chronic obstructive pulmonary disease (COPD), bronchiectasis, chronic cholecystitis, tuberculosis, Hashimoto's thyroiditis, sepsis, sarcoidosis, silicosis and other pneumoconioses, and an implanted foreign body in a wound, but are not so limited. As used herein, the term "sepsis" refers to a well-recognized clinical syndrome associated with a host's systemic inflammatory response to microbial invasion. The term "sepsis" as used herein refers to a condition that is typically signaled by fever or hypothermia, tachycardia, and tachypnea, and in severe instances can progress to hypotension, organ dysfunction, and even death.

In some embodiments, the inflammatory disease is non-autoimmune inflammatory bowel disease, post-surgical adhesions, coronary artery disease, hepatic fibrosis, acute respiratory distress syndrome, acute inflammatory pancreatitis, endoscopic retrograde cholangiopancreatography-induced pancreatitis, burns, atherogenesis of coronary, cerebral and peripheral arteries, appendicitis, cholecystitis, diverticulitis, visceral fibrotic disorders, wound healing, skin scarring disorders (keloids, hidradenitis suppurativa), granulomatous disorders (sarcoidosis, primary biliary cirrhosis), asthma, pyoderma gandrenosum, Sweet's syndrome, Behcet's disease, primary sclerosing cholangitis or an abscess. In some preferred embodiment the inflammatory disease is inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis).

In other embodiments, the inflammatory disease is an autoimmune disease. The autoimmune disease in some embodiments is rheumatoid arthritis, rheumatic fever, ulcerative colitis, Crohn's disease, autoimmune inflammatory bowel disease, insulin-dependent diabetes mellitus, diabetes mellitus, juvenile diabetes, spontaneous autoimmune diabetes, gastritis,
autoimmune atrophic gastritis, autoimmune hepatitis, thyroiditis, Hashimoto's thyroiditis, insulitis, oophoritis, orchitis, uveitis, phacogenic uveitis, multiple sclerosis, myasthenia gravis, primary myxoedema, thyrotoxicosis, pernicious anemia, autoimmune haemolytic anemia, Addison's disease, Anklosing spondylitis, sarcoidosis, scleroderma, Goodpasture's syndrome, Guillain-Barre syndrome, Graves' disease, glomerulonephritis, psoriasis, pemphigus vulgaris, pemphigoid, excema, bulous pemphigous, sympathetic ophalmia, idiopathic thrombocylopenic purpura, idiopathic feucopenia, Sjogren's syndrome, systemic sclerosis, Wegener's granulomatosis, poly/dermatomyositis, primary biliary cirrhosis, primary sclerosing cholangitis, lupus or systemic lupus erythematosus.

Graft versus host disease (GVHD) is a complication that can occur after a pluripotent cell (e.g., stem cell) or bone marrow transplant in which the newly transplanted material results in an attack on the transplant recipient's body. In some instances, GVHD takes place after a blood transfusion. Graft-versus-host-disease can be divided into acute and chronic forms. The acute or fulminant form of the disease (aGVHD) is normally observed within the first 100 days post-transplant, and is a major challenge to transplants owing to associated morbidity and mortality. The chronic form of graft-versus-host-disease (cGVHD) normally occurs after 100 days. The appearance of moderate to severe cases of cGVHD adversely influences long-term survival.

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, 1-glutamine, 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 10ng/ml 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 \( \text{O}_2 \) 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 naïve T cells with some of the DCs generated and measuring induction of FoxP3 in the naïve 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 autoantigen of interest 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<sup>high</sup>, CD27<sup>high</sup>, CD86<sup>high</sup>, CId<sup>high</sup>, IL-10<sup>high</sup>, TGF-β<sup>high</sup>, CD4 and FoxP3 is analyzed on the cells. The presence of CD8+CD25<sup>high</sup>FoxP3+ cells suggests an induction of CD8+ Treg cells.

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

Balb/c mice are immunized with an autoantigen in incomplete Freund's adjuvant to induce antigen-specific T-cell proliferation (e.g., CD8+ T-cell), 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 autoantigen, 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: In Vivo Reduction of an Undesired Immune Response (Prophetic)

Autoantigen-specific itDCs are generated according to methods described herein. Briefly, itDCs are generated by contacting itDCs with an autoantigen or portion thereof, and
autoantigen-specific itDCs. Autoantigen-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 to a subject having autoimmune disease exhibiting an undesired immune response, such as an undesired CD8+ T cell response against the autoantigen. A decrease in the undesired immune response against the autoantigen 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 the autoimmune disease. For one year after administration of the initial dose of itDCs, the subject receives a bi-monthly maintenance dose of $10^6$ autoantigen-specific itDCs (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 the autoantigen.

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

Insulin-specific itDCs are generated according to methods described herein. Briefly, itDCs are generated by contacting itDCs with insulin or portion thereof, and insulin-specific itDCs are subsequently collected. Insulin-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 insulin-specific CD8+ T cell proliferation and/or activity (e.g., killing of insulin expressing islet cells). A decrease in these undesired immune responses is expected in the subject after about one to four weeks after administration of the insulin-specific itDCs. This decrease is expected to result in an amelioration or complete regression of insulin-specific CD8+ T cell proliferation and/or activity. Methods of assessing the level of CD8+ 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 (1μM). Cells were then washed 3 times in MACS Running Buffer (RB, 2%FBS+2mM EDTA in PBS) and counted. Cells were placed at 1-10x10⁶/200ul in PBS and injected i.v. into experimental recipients.

**Immunization and Treatment**

C57B1/6 (B6) mice 6 weeks of age or older were immunized subscapularly (s.s.) with PBS on days 0, 14, 28 (Group 1) or Ovalbumin protein (OVA) and CpG 1826 oligonucleotides (CpG) (Groups 2-4) (25μg OVA+20μg CpG/animal). Group #1 of animals remained unimmunized as a control. Group #2 were immunized but not treated to help appreciate the strength of the immune response induced. Groups #3 and 4 were treated (200μ1DC i.v.) with different iTDC products. 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 3 and 4 were incubated with 10μg OVA +/- 100ng/ml Rapa and 20ng/ml TGFp per animal.

1) No immunization: Phosphate buffered saline (PBS), intravenously (i.v.),
2) OVA+CpG immunized, not treated,
3) OVA + CpG immunized, CD103 positive (CD103+) DCs incubated with OVA in-vitro, i.v.,
4) OVA + CpG immunized, 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 Harvesting

On day 39 spleens from syngeneic donor mice were harvested. The spleen cells were mashed into a single-cell suspension and split before being labeled with either 0.5uM or 5uM carboxyfluorescein succinimidyl esters (CFSE), an intracellular dye that tracks cells in vivo. The population labeled with 0.5uM CFSE was then further incubated with SIINFEKL (SEQ ID NO: 415) peptide, a Major Histocompatibility Complex (MHC) class I restricted peptide from OVA. These two differentially labeled cell populations were filtered and admixed at a 1:1 ratio before being injected i.v. into every mouse within the experiment.

On day 40 all mice were sacked and spleens were harvested. They were stored in PBS before being mashed into a single cell suspension using a syringe plunger and 70uM sieve. An RBC lysis was then performed using ammonium chloride solution and after washing a portion of the remaining splenocytes was analyzed by flow cytometry.

Cell Sorting

On treatment days the spleens from the FLT-3 melanoma inoculated animals were harvested and digested via liberase. The resulting slurry was filtered by 70uM 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 CD11c and the second 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 CD11c+ and CD103+ DCs.

Measurement of Specific Cell Killing

During manual processing of spleens a red blood cell lysis was performed. 3mls of red blood cell lysis buffer (RBC lyse) was added to a 50ml polypropylene centrifuge tube then a 70μm sieve was seated on top of the uncapped tube. 1ml of RBC lyse was pipetted over the sieve and a 5 minute timer was started just before a spleen was placed on it and mashed through it.
using the plunger of a 3ml syringe. Once the spleen was completely pulverized and no trace of redness was left in the remaining tissue 1ml of RBC lyse was pipetted over the sieve to wash out the remaining cells. After 5 minutes had elapsed 5mls of complete media (RPMI 1640 + 10% bovine serum v/v + L-glutamate + Penicillin Streptomycin, CM) was added to the tube. Tubes were then moved onto ice until they were spun and the cells pelleted and resuspended in FACS buffer. 7AAD was used to discriminate death cells. These preparations were then analyzed by flow cytometry and the content of transferred CFSElow and CFSEhigh cells was determined as compared to the immunized control.

Results

Fig. 1 demonstrates that antigen-specific itDCs, in particular the widely distributed circulating CD103+ subset, effectively reduced the percentage of specific killing of cells expressing antigen.
What is claimed is:

CLAIMS

1. A method comprising:
   administering to a subject antigen-specific induced tolerogenic dendritic cells (itDCs) in an amount effective to reduce an undesired CD8+ T cell immune response in the subject, wherein the subject is experiencing or is at risk of experiencing the undesired CD8+ T cell immune response against the antigen, and wherein the itDCs are circulating itDCs.

2. A method comprising:
   reducing an undesired CD8+ T cell immune response in a subject by administering antigen-specific itDCs to the subject, wherein the antigen-specific itDCs present MHC Class I-restricted epitopes of an antigen, and wherein the itDCs are circulating itDCs.

3. A method comprising:
   administering antigen-specific itDCs to a subject according to a protocol that was previously shown to reduce an undesired CD8+ T cell immune response to an antigen in one or more test subjects, and wherein the itDCs are circulating itDCs.

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 1, 3 and 4, wherein the antigen-specific itDCs present MHC Class I-restricted epitopes of the antigen.

6. The method of any of claims 1-5, wherein the antigen-specific itDCs also present MHC Class II-restricted and/or B cell epitopes of the antigen.

7. The method of any of claims 1-6, wherein the antigen-specific itDCs present substantially no B cell epitopes of the antigen.
8. The method of any of claims 1-7, wherein the antigen-specific itDCs present substantially no MHC Class II-restricted epitopes of the antigen.

9. The method of any of claims 1-8, wherein the method further comprises assessing the undesired CD8+ T cell immune response in the subject prior to and/or after the administration of the antigen-specific itDCs.

10. The method of any of claims 1-9, wherein one or more maintenance doses of the antigen-specific itDCs are administered to the subject.

11. The method of any of claims 1-10, wherein the antigen-specific itDCs are in or are administered in an amount effective to reduce the proliferation and/or activity of antigen-specific CD8+ T cells.

12. The method of any of claims 1-11, wherein the antigen comprises an autoantigen, allergen or therapeutic protein, or is associated with an inflammatory disease, an autoimmune disease, organ or tissue rejection or graft versus host disease.

13. The method of any of claims 1-12, wherein the subject has or is at risk of having an autoimmune disease, an inflammatory disease, an allergy, organ or tissue rejection or graft versus host disease.

14. The method of any of claims 1-12, wherein the subject has undergone or will undergo transplantation.

15. The method of any of claims 1-12, wherein the subject has received, is receiving or will receive a therapeutic protein.

16. The method of any of claims 1-15, 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.
17. The method of any of claims 1-15, wherein the administering is by subcutaneous, intrathecal, intraventricular, intramuscular, intraperitoneal, intracoronary, intrapancreatic, intrahepatic or bronchial injection.

18. A method, comprising:
   combining itDCs MHC Class I-restricted epitopes of an antigen, wherein the itDCs are circulating itDCs.

19. The method of claim 18, wherein the itDCs are also combined with MHC Class II-restricted epitopes and/or B cell epitopes of the antigen.

20. The method of claim 18 or 19, wherein the itDCs are combined with substantially no MHC Class U-restricted epitopes of the antigen.

21. The method of any of claims 18-20, wherein the itDCs are combined with substantially no B cell epitopes of the antigen.

22. The method of any of claims 18-21, wherein the method further comprises collecting the antigen-specific itDCs.

23. The method of any of claims 18-22, wherein the antigen comprises an autoantigen, allergen, therapeutic protein or is associated with an inflammatory disease, an autoimmune disease, organ or tissue rejection or graft versus host disease.

24. The method of any of claims 18-23, wherein the method further comprises making a dosage form comprising the antigen-specific itDCs.

25. The method of any of claims 18-24, wherein the method further comprises making the antigen-specific itDCs or the dosage form available to a subject for administration.

26. The method of any of claims 18-25, wherein the method further comprises assessing the reduction of an undesired CD8+ T cell immune response with the antigen-specific itDCs.
27. The method of any of claims 18-26, wherein the assessing comprises determining the proliferation and/or activity of antigen-specific CD8+ T cells.

28. A composition comprising antigen-specific itDCs, wherein the antigen-specific itDCs present MHC Class I-restricted epitopes of an antigen, and wherein the itDCs are circulating itDCs.

29. The composition of claim 28, wherein the antigen-specific itDCs also present MHC II-restricted epitopes and/or B cells of the antigen.

30. The composition of claim 28 or 29, wherein the antigen-specific itDCs present substantially no MHC Class II-restricted epitopes of the antigen.

31. The composition of any of claims 28-30, wherein the antigen-specific itDCs present substantially no B cell epitopes of the antigen.

32. The composition of any of claims 28-31, wherein the antigen-specific itDCs are produced by the method of any of claims 18-27.

33. The composition of any of claims 28-32, wherein the antigen-specific itDCs are as defined in any of claims 1-17.

34. The composition of any of claims 28-33, wherein the composition further comprises a pharmaceutically acceptable excipient.

35. A dosage form comprising the composition of any of claims 28-34.

36. A process for producing a composition comprising antigen-specific itDCs, the process comprising combining itDCs with MHC Class I-restricted epitopes of an antigen, wherein the itDCs are circulating itDCs.

37. The process of claim 36, wherein the itDCs are also combined with MHC Class II-restricted and/or B cell epitopes of the antigen.
38. The process of claim 36 or 37, wherein the itDCs are combined with substantially no MHC Class II-restricted epitopes of the antigen.

39. The process of any of claims 36-38, wherein the itDCs are combined with substantially no B cell epitopes of the antigen.

40. The process of any of claims 36-39, wherein said process comprises the steps as defined in any one of claims 18-27.

41. A composition comprising antigen-specific itDCs obtainable by the process of any one of claims 36-41.

42. A composition comprising: (i) induced tolerogenic dendritic cells; and (ii) MHC Class I-restricted epitopes of an antigen, wherein the itDCs are circulating itDCs.

43. The composition of claim 42, wherein the composition further comprises MHC Class II-restricted epitopes and/or B cell epitopes of the antigen.

44. The composition of claim 42 or 43, wherein the composition comprises substantially no MHC Class II-restricted epitopes of the antigen.

45. The composition of any of claims 42-44, wherein the composition comprises substantially no B cell epitopes of the antigen.

46. The composition of any of claims 42-45, wherein the antigen is as defined in claim 23.

47. A composition of any one of claims 28-34 and 41-46 or dosage form of claim 35 for use in therapy or prophylaxis.

48. A composition of any one of claims 28-34 and 41-46 or dosage form of claim 35 for use in a method of reducing an undesired CD8+ T cell immune response in a subject, in a method of therapy or prophylaxis of autoimmune disease, an inflammatory disease, an allergy, organ
or tissue rejection or graft versus host disease or in a method as defined in any one of claims 1-17.

49. Use of the composition of any one of claims 28-34 and 41-46 or dosage form of claim 35 for the manufacture of a medicament for use in a method of reducing an undesired CD8+ T cell immune response in a subject, in a method of therapy or prophylaxis of autoimmune disease, an inflammatory disease, an allergy, organ or tissue rejection or graft versus host disease or in a method as defined in any one of claims 1-17.

50. A composition comprising MHC Class I-restricted epitopes of an antigen for use in a method comprising:
   (i) providing said MHC Class I-restricted epitopes;
   (ii) providing antigen-specific itDCs by loading DCs with the epitopes of step (i); and
   (iii) administering the antigen-specific itDCs to a subject prior to, concomitantly with or after exposure to or administration of said MHC Class I-restricted epitopes of the antigen,
wherein the itDCs are circulating itDCs.

51. The composition of claim 50, wherein the composition further comprises MHC Class II-restricted epitopes and/or B cell epitopes of the antigen, and wherein the MHC Class II-restricted epitopes and/or B cell epitopes of the antigen are also provided.

52. The composition of claim 50 or 51, wherein the composition comprises substantially no MHC Class II-restricted epitopes of the antigen.

53. The composition of any of claims 50-52, wherein the composition comprises substantially no B cell epitopes of the antigen.

54. Antigen-specific itDCs for use in a method of reducing an undesired CD8+ T cell immune response in a subject, said method comprising:
   (i) providing MHC Class I-restricted epitopes of an antigen;
(ii) providing antigen-specific itDCs by loading DCs with the epitopes of step (i);
and

(iii) administering the antigen-specific itDCs to said subject prior to, concomitantly
with or after exposure to or administration of a composition comprising MHC
Class I-restricted epitopes of the antigen,

wherein the itDCs are circulating itDCs.

55. The antigen-specific itDCs of claim 54, wherein MHC Class II-restricted epitopes and/or
B cell epitopes of the antigen are also provided.

56. The antigen-specific itDCs of claim 54 or 55, wherein substantially no MHC Class II-
restricted epitopes of the antigen are provided.

57. The antigen-specific itDCs of any of claims 54-56, wherein substantially no B cell
epitopes of the antigen are provided.

58. Antigen-specific itDCs for use in a method comprising:

(i) providing MHC Class I-restricted epitopes of an antigen;

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

and

(iii) administering the antigen-specific itDCs to a subject,

wherein the itDCs are circulating itDCs.

59. The antigen-specific itDCs of claim 58, wherein MHC Class II-restricted epitopes and/or
B cell epitopes of the antigen are also provided.

60. The antigen-specific itDCs of claim 58 or 59, wherein substantially no MHC Class II-
restricted epitopes of the antigen are provided.

61. The antigen-specific itDCs of any of claims 58-60, wherein substantially no B cell
epitopes of the antigen are provided.
62. A dosage form comprising the compositions of any of claims 41-48 and 50-53 or the antigen-specific itDCs of any of claims 54-61.

63. The method of any of claims 1-27, the composition of any of claims 28-34, 41-48 and 50-53, the dosage form of claim 35 or 62, the process of any of claims 36-40, the use of claim 49 or the antigen-specific itDCs of any of claims 54-61, wherein the circulating itDCs are CD103+, CD1 lb+, XCR 1+ or plasmacytoid itDCs and/or are not CD8a+.

64. The method of any of claims 1-27, the composition of any of claims 28-34, 41-48 and 50-53, the dosage form of claim 35 or 62, the process of any of claims 36-40, the use of claim 49 or the antigen-specific itDCs of any of claims 54-61, wherein the circulating itDCs are CD103+.
Fig. 1
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/38(2006.01)i, A61K 48/00(2006.01)i, A61K 35/12(2006.01)1, A61K 39/395(2006.01)1, A61P 35/00(2006.01)i, A61P 29/00(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 39/38

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: antigen-specific, induced tolerogenic dendritic cells, MHC Class I-restricted, CDI lb, CD103, CD8+ T cells, immune response

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>
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</table>

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

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
30 OCTOBER 2012 (30.10.2012)

Date of mailing of the international search report
30 OCTOBER 2012 (30.10.2012)

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
189 Cheongsa-ro, So-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
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)
### Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. **Claims Nos.: 1-17, 64, 65**
   - Because they relate to subject matter not required to be searched by this Authority, namely:
     - Claims 1-17, 64, and 65 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.: 42**
   - 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 42 is not clear because it is worded in reference to claim 42, itself (PCT Article 6).

3. **Claims Nos.: 5-17, 21-27, 32-36, 40-42, 46-50, 54, 58, 62-65**
   - Because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. **As all 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.
<table>
<thead>
<tr>
<th>Category</th>
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<th>Relevant to claim No.</th>
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<td>Patent family member(s)</td>
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Form PCT/ISA/210 (patent family annex) (July 2009)