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Title: INDUCED TOLEROGENGIC DENDRITIC CELLS TO REDUCE SYSTEMIC INFLAMMATORY CYTOKINES

Abstract:
INDUCED TOLEROGENIC DENDRITIC CELLS TO REDUCE SYSTEMIC
INFLAMMATORY CYTOKINES

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 induced tolerogenic dendritic cell (itDC) compositions to reduce the generation of systemic inflammatory cytokines in a subject. Such subjects include those that are experiencing or are at risk of experiencing chronic systemic or chronic local inflammatory cytokine production at pathological levels. Such subjects include those that are experiencing or are at risk of experiencing chronic systemic or chronic local inflammation.

BACKGROUND OF THE INVENTION

Conventional strategies for generating immunosuppression associated with an undesired immune response are based on broad-acting immunosuppressive drugs. 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 induced tolerogenic dendritic cells (itDCs) in an amount effective to reduce the generation of systemic inflammatory cytokines in the subject, wherein the subject is experiencing or is at risk of...
experiencing chronic systemic inflammatory cytokine production at pathological levels is provided. In another aspect, a method comprising reducing the generation of chronically elevated systemic inflammatory cytokines in a subject by administering itDCs to the subject is provided. In yet another aspect, a method comprising administering itDCs to a subject according to a protocol that was previously shown to reduce the generation of systemic inflammatory cytokines in one or more test subjects, wherein the subject is experiencing or is at risk of experiencing chronic systemic inflammatory cytokine production at pathological levels is provided.

In one embodiment, the subject is experiencing or is at risk of experiencing chronic systemic or chronic local inflammation. In another embodiment, the method further comprises providing or identifying the subject.

In still another embodiment, the amount effective is effective to reduce the generation of IFN-γ, TNF-cc, IL-2, IL-5, IL-6, IL-8, IL-9, IL-12, IL-13, IL-17, IL-18, IL-21, IL-22, IL-23, IL-1β, GM-CSF, M-CSF, C reactive protein, acute phase proteins, MCP-1, RANTES, MIP-1cc, MIP-1β, MIG, ITAC and/or IP-10 in the subject. In yet another embodiment, the reduced generation of systemic inflammatory cytokines is chronic reduced generation of systemic inflammatory cytokines. In a further embodiment, the generation of systemic inflammatory cytokines is reduced in the subject for at least 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months or 1 year.

In yet another embodiment, the itDCs have been combined with an antigen associated with the chronic systemic inflammatory cytokine production before administering the itDCs to the subject. In still another embodiment, the antigen is a protein, polypeptide, lipoprotein, glycolipid, polynucleotide or is contained or expressed in, by or on a cell. In one embodiment, the antigen is an allergen, or is associated with an inflammatory disease, an autoimmune disease, inflammation associated with insulin resistance, organ or tissue rejection or graft versus host disease. In another embodiment, the antigen is an autoantigen. In yet another embodiment, the autoantigen comprises myelin basic protein, collagen, human cartilage gp 39, gpl30-RAPS, proteolipid protein, fibrillarin, a nuclear protein, a nucleolar protein, thyroid stimulating factor receptor, a histone, glycoprotein gp 70, a ribosomal protein, pyruvate dehydrogenase dehydrolipoamide acetyltransferase, a hair follicle antigen, human tropomyosin isoform 5, a mitochondrial protein, a pancreatic β-cell protein, a pancreatic islet cell protein, myelin oligodendrocyte glycoprotein, insulin, gluten or GAD. In
a further embodiment, the itDCs have not been combined with an antigen associated with the chronic systemic inflammatory cytokine production before administering the itDCs to the subject.

In yet a further embodiment, the method further comprises assessing the generation of systemic inflammatory cytokines in the subject prior to and/or after the administration of the itDCs. In still a further embodiment, one or more maintenance doses of the itDCs are administered to the subject. In one embodiment, the generation of an undesired antigen-specific immune response is also reduced in the subject.

In another embodiment, the method further comprises administering a transplantable graft. In yet another embodiment, the subject has or is at risk of having an inflammatory disease, an autoimmune disease (e.g., type I diabetes), an allergy or graft versus host disease. In still another embodiment, the subject has undergone or will undergo transplantation.

In a further embodiment, the administering of the itDCs or transplantable graft 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 yet a further embodiment, the administering is by subcutaneous, intrathecal, intraventricular, intramuscular, intraperitoneal, intracoronary, intrapancreatic, intrahepatic or bronchial injection. In still a further embodiment, any other agent is administered by intravenous, intramucosal, oral, subcutaneous, pulmonary, intranasal, intradermal or intramuscular administration.

In another aspect, a composition comprising induced tolerogenic dendritic cells (itDCs) for use in a method of reducing the generation of systemic inflammatory cytokines in a subject; reducing the generation of chronically elevated systemic inflammatory cytokines in a subject; treatment or prophylaxis of chronic systemic inflammatory cytokine production at pathological levels; treatment or prophylaxis of chronic local inflammatory cytokine production at pathological levels; treatment or prophylaxis of chronic local inflammation; treatment or prophylaxis of chronic systemic inflammation; treatment or prophylaxis of an inflammatory disease, an autoimmune disease, organ or tissue rejection or graft versus host disease; treatment of prophylaxis in a subject according to a protocol that was previously shown to reduce the generation of systemic inflammatory cytokines in one or more test subjects; or treatment or prophylaxis as defined in any of the methods provided herein is provided.
In yet another aspect, a use of a composition comprising induced tolerogenic dendritic cells (itDCs) for the manufacture of a medicament for use in any of the methods provided herein or any of the treatment and/or prophylaxis is provided. In one embodiment, in any of the compositions or uses provided herein the method comprises chronic reduction of systemic inflammatory cytokines. In another embodiment, the generation of systemic inflammatory cytokines is reduced for at least 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months or 1 year.

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. In embodiments of any of the methods provided herein, the method may further comprise administering an agent that enhances the migratory behavior of the itDCs.

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

In an embodiment of any of the compositions and methods provided herein, the antigens are peptides. Such antigens, in some embodiments, comprise at least an epitopic sequence but may also comprise additional amino acids that flank one or both ends of the epitopic sequence. In embodiments, the antigens consist of or consist essentially of an epitopic sequence.

In an embodiment of any of the compositions and methods provided herein, the antigens 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. In some embodiments, the multiple types of antigens are loaded onto the itDCs by combining itDCs, or precursors thereof, with a cell preparation (e.g., a cell extract, such as a islet cell extract).

**BRIEF DESCRIPTION OF THE FIGURE**

Fig. 1 shows the reduction of inflammatory cytokine/chemokine levels in NOD animals treated with itDCs loaded with insulin or antigens from an islet cell extract.

**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 context 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

It has been found that the administration of itDCs can reduce the generation of systemic inflammatory cytokines and result in beneficial immunosuppressive (e.g., tolerogenic) immune responses. This can be beneficial in subjects suffering from or are at risk of suffering from chronic systemic or chronic local cytokine production at pathological
levels. As shown in the Examples, itDCs that were loaded with insulin or antigens from an islet cell extract were found to reduce the level of IL-6, IL-12p70 and MCP-1 in NOD mice. A reduction in these potent inflammatory cytokines/chemokines demonstrates that itDCs as provided herein can lead to beneficial tolerogenic effects in subjects experiencing inflammation, such as chronic systemic inflammation.

A reduction in inflammatory cytokines can be the result of direct or indirect immune suppression. For example, it is believed that itDCs with immunosuppressive characteristics, or such itDCs loaded with antigen, can result in the reduction of pro-inflammatory cytokines by immune cells that interact with the itDCs (e.g., recognize antigen presented by the itDCs). The production of anti-inflammatory cytokines by immune cells that interact with the itDCs can also then lead to a reduction in pro-inflammatory cytokines. As another example, such itDCs can result in immune suppression through the presentation of antigen to immune cells that produce cytokines that affect other immune cells that play a role in inflammation, such as macrophages or the inflammasome. This invention is useful, therefore, to treat subjects who have or are at risk of having undesired inflammatory cytokine production, such as subjects who have or are at risk of having an inflammatory disease, autoimmune disease, graft versus host disease, organ or tissue rejection, an allergy, etc. Such subjects also include those who suffer from obesity. This invention is also useful for promoting tolerogenic immune responses in subjects who have undergone or will undergo transplantation.

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 reduce the generation of systemic inflammatory cytokines in a subject suffering from or are at risk of suffering from chronic systemic or chronic local cytokine production at pathological levels. The itDCs may or may not be antigen-specific itDCs produced by combining itDCs with an antigen that is associated with the chronic systemic or chronic local inflammatory cytokine production. In some embodiments, where tolerization to a specific antigen is desired, the itDCs are antigen-specific. In some embodiments, the antigen comprises MHC Class I-restricted and/or MHC Class II-restricted and/or B cell epitopes of the antigen. In other embodiments, the antigen comprises MHC Class I-restricted and/or MHC Class II-restricted but substantially no B cell epitopes of the antigen. In some embodiments, the itDCs are combined with antigen 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, and, thus, 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.

The itDCs can be administered to a subject in order to ameliorate systemic inflammatory cytokine production. In one aspect, a method comprising administering to a subject itDCs in an amount effective to reduce the generation of systemic inflammatory cytokines in the subject, wherein the subject is experiencing or is at risk of experiencing chronic systemic or chronic local inflammatory cytokine production at pathological levels is provided. In another aspect, a method comprising reducing the generation of chronically elevated systemic inflammatory cytokine production in a subject by administering 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 systemic inflammatory cytokine production in one or more test subjects, where the composition comprises itDCs is provided. The methods provided, in some embodiments, may further comprise administering an antigen. In embodiments, the antigen may be administered to a subject prior to, concomitantly with or after the administration of the itDCs. In other embodiments, the antigen is administered as antigen loaded on the itDCs. In embodiments, the 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 transplantable graft or that is exposed to or will be exposed to an allergen. In embodiments, the administration generates the desired immune response (e.g., the reduction of systemic inflammatory cytokines) for a certain length of time. Examples of such lengths of time are provided elsewhere herein.

In yet another aspect, compositions and dosage forms of any of the itDC compositions provided herein are provided. Such dosage forms can be administered to a subject in need.
thereof (e.g., in need of systemic inflammatory cytokine reduction), such as a subject that is experiencing or is at risk of experiencing chronic systemic or chronic local inflammatory cytokine production at pathological levels. Such a subject includes those that are experiencing or are at risk of experiencing chronic systemic or chronic local inflammation.

Such a subject may be one that has or is at risk of having an inflammatory disease, an autoimmune disease or graft versus host disease. Such a subject may also be one who suffers from obesity. Such a subject may also be one that has undergone or will undergo transplantation.

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, reduction in the production of systemic inflammatory cytokines. 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 further include those that have undergone or will undergo transplantation. Such subjects also include those suffering from obesity.

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, in some embodiments, preferably, result in a tolerogenic immune response in a subject to an antigen. The achievement of any of the foregoing can be monitored by routine methods.

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

Amounts effective will depend, of course, on the particular subject being treated; the severity of a condition, disease or disorder; the individual patient parameters including age, physical condition, size and weight; the duration of the treatment; the nature of concurrent therapy (if any); the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reason.
In some embodiments, doses of the itDCs in the compositions of the invention can range from a single cell to about \(10^{12}\) cells. In some embodiments, the number of itDCs administered to a subject can range from about 1 cell/kg body weight to about \(10^8\) cells/kg. In some embodiments, the number of itDCs administered is the smallest number that produces a desired immune response in the subject. In some embodiments, the dose is the largest number of itDCs that can be administered without generating an undesired effect in the subject, for example, an undesired side effect. Useful doses include, in some embodiments, cell populations of greater than \(10^2\), \(10^3\), \(10^4\), \(10^5\), \(10^6\), \(10^7\), \(10^8\), \(10^9\) or \(10^{10}\) itDCs per dose. Other examples of useful doses include from about \(1 \times 10^4\) to about \(1 \times 10^6\), about \(1 \times 10^6\) to about \(1 \times 10^8\) or about \(1 \times 10^8\) to about \(1 \times 10^{10}\) itDCs per dose.

"Antigen" means a B cell antigen or T cell antigen. "Type(s) of antigens" means molecules that share the same, or substantially the same, antigenic characteristics. In some embodiments, antigens may be proteins, polypeptides, peptides, lipoproteins, glycolipids, polynucleotides, polysaccharides or are contained or expressed in cells. In some embodiments, such as when the antigens are not well defined or characterized, the antigens are contained within a cell or tissue preparation, cell debris, cell exosomes, conditioned media, etc. and may be 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.

"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 embodiments, the use of an antigen associated with a disease, disorder or condition, etc. 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. The antigens can be in the same form as
expressed in a subject with the disease, disorder or condition, etc. 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 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 dehydrodripoamide
acyltransferase, 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 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
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 transplant that can be used to obtain or derive
transplantable graft 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 is to be transplanted (e.g., whole or partial organ, skin, bone, nerves, tendon, neurons, blood vessels, fat, cornea, etc.).

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 associated with inflammatory cytokine production" means any antigen that is recognized by the immune system and such recognition results, directly or indirectly, in the production of inflammatory cytokines.

"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. Such antigens may also be CD1d-restricted antigens. 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).
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.

The term "chronic" refers to a long-lasting effect or event and/or an effect or event that a clinician would classify as being chronic as compared to acute. In the context of inflammation, a chronic inflammatory response, in some embodiments, is a response that persists for at least about 1 week, at least about 2 weeks, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least
about 6 months, at least about 9 months, or at least about 1 year and/or is one that recurs in
the subject. Accordingly, chronically elevated systemic inflammatory cytokines are
cytokines, in some embodiments, the level of which are elevated in a subject, for example, as
compared to a control or reference level (e.g., a level found or expected in a healthy subject
or an average level of age- or sex-matched subjects), and such level persists for at least about
1 week, at least about 2 weeks, at least about 1 month, at least about 2 months, at least about
3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least
about 9 months, or at least about 1 year and/or recurs in the subject.

The term "combining" refers to actively contacting one material, such as 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 may express CD8, CD103, CD1Id, 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 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.

A number of epitopes are known to those of skill in the art, and exemplary epitopes


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"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 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. Tolerogenic dendritic cells have a tolerogenic phenotype that is characterized by, for example, 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 inflammatory cytokine production and/or 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 anti-inflammatory or pro-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 immune cells and the level of inflammatory cytokine is assessed. In other 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 embodiments, the load is a function of the amount of antigen combined with the iTDCs in addition to the activity observed and/or one or more reference or control numbers.

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

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

A subject suffering from "obesity" is one whom a clinician believe is overweight and would benefit from the compositions and methods provided herein. In some embodiments, the subject has a body mass index >30. In other embodiment, the subject is one who is morbidly obese (i.e., body mass index >40).

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

"Pathological levels of inflammatory cytokines" refers to inflammatory cytokine levels that are associated with a disease, disorder or condition, for example, an inflammatory disease or inflammation. In some embodiments, a pathological level of an inflammatory cytokine is a level that is above the level observed or expected in a healthy, age- or sex-matched subject and/or is a level that is observed or expected in a diseased, age- or sex-matched subject. In other embodiments, a pathological level is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 500-fold, or at least 1000-fold the level observed or expected in a healthy, age- or sex-matched subject. Subjects with pathological levels of inflammatory cytokines as provided herein include those that are experiencing or at risk of experiencing chronic systemic or chronic local inflammatory cytokines or chronic systemic or chronic local inflammation. In embodiments where the subject is experiencing or at risk of experiencing chronic local inflammatory cytokines or chronic local inflammation the chronic local inflammatory cytokine production or chronic local inflammation is reflected in the presence of systemic inflammatory cytokine production in the peripheral circulation. Such subjects may suffer from inflammatory or autoimmune diseases such as rheumatoid arthritis or irritable bowel disease. Other examples of such subjects or the diseases, disorders or conditions will be apparent to those of ordinary skill in the art and examples of which are provided elsewhere herein.

Examples of inflammatory cytokines are provided elsewhere herein and include IFN-γ, IL-2, IL-5, IL-9, IL-12, IL-13, IL-17, IL-18, IL-21, IL-22, IL-23, GM-CSF, M-CSF, C reactive protein, acute phase proteins, MCP-1, RANTES, MIP1cc, MIP-1β, MIG, ITAC, IP-10, etc., each of which may be produced or found systemically. The level of inflammatory cytokines can be assessed by various methods, including, but not limited to immunoassays.
and cell-based assays or as otherwise described herein or known in the art. In a clinical
context, a reduction in systemic inflammatory cytokines in a subject experiencing a disease,
disorder or condition, such as inflammatory disease and/or chronic systemic or chronic local
inflammation, can be assessed by monitoring the clinically manifested symptoms associated
therewith in the subject, wherein an amelioration of a symptom associated with the disease
correlates to a reduction in the level of inflammatory cytokines in the subject.

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

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

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

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

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

"T cell antigen" means a CD4+ T-cell antigen, CD8+ cell antigen or a CD1d-restricted 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). "CD1d-restricted antigen" means an antigen that comprise one or more epitopes that bind to, complex to or are presented by CD1d molecules. Generally, CD1d-restricted T cell antigens are lipids presented to invariant NKT cells. CD1d-restricted T cell antigens may comprise one or more lipids, or glycolipids, including but not limited to: galactosyldiacylglycerols (from Borrelia burgdorferi), lypophosphoglycan (from Leishmania donovani), endogenous or exogenous β-glucosylceramide, and phosphatidylinositol tetramannoside (PIM4) (from Mycobacterium leprae). For additional lipids and/or glycolipids useful as a CD1d-restricted antigens, see V. Cerundolo et al., "Harnessing invariant NKT cells in vaccination strategies." Nature Rev Immun, 9:28-38 (2009). 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, but may be other molecules such as lipids and glycolipids.
"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 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(l):97-lll. 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-a, IL-6, GM-CSF, IFN-γ, IL-2, IL-9, IL-12, IL-17, IL-18, IL-21, IL-22, IL-23, M-CSF, C reactive protein, acute phase protein, chemokines (e.g., MCP-1, RANTES, MIP-lcc, MIP-1β, MIG, ITAC or IP-10), the production of anti-inflammatory cytokines (e.g., IL-4, IL-13, IL-10, etc.), chemokines (e.g., CCL-2, CXCL8), proteases (e.g., MMP-3, MMP-9), leukotrienes (e.g., CysLT-1, CysLT-2), prostaglandins (e.g., PGE2) or histamines; the inhibition of polarization to a Thl7, Thl or Th2 immune response; the inhibition of effector cell-specific cytokines: Thl7 (e.g., IL-17, IL-25), Thl (IFN-γ), Th2 (e.g., IL-4, IL-13); the inhibition of Thl-, Th2- or Thl7-specific transcription factors; the inhibition of proliferation of effector T cells; the induction of apoptosis of effector T cells; the induction of tolerogenic dendritic cell-specific genes; the induction of FoxP3 expression; the inhibition of IgE induction or IgE-mediated immune responses; the inhibition of antibody responses (e.g., antigen-specific antibody production); the inhibition of T helper cell response; the production of TGF-β and/or IL-10; the inhibition of effector function of autoantibodies (e.g., inhibition in the depletion of cells, cell or tissue damage or complement activation); etc. In some embodiments, the tolerogenic immune response includes the production of anti-inflammatory cytokines (e.g., IL-4 and/or IL-10). In
some embodiments, the tolerogenic immune response is the reduction of antigen-specific antibodies and/or CD4+ T helper cells and/or B cells. Assessing CD4+ T helper cell or B cell stimulation may include analyzing CD4+ T helper cell or B cell number, phenotype, activation and/or cytokine production.

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

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

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 induced tolerogenic dendritic cells useful for reducing the generation of systemic inflammatory cytokines. Preferably, 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 chronic systemic or chronic local inflammatory cytokine production at pathological levels. Such subjects include those with an inflammatory disease, an autoimmune disease, an allergy or graft versus host disease. Such subjects also include those that have undergone or will undergo transplantation. Such subject also include those that suffer from obesity. Some embodiments of this invention provide the aforementioned iTDCs. These iTDCs are capable of reducing systemic inflammatory cytokine production in the aforementioned subjects.
The induced tolerogenic dendritic cells for use in the compositions and methods provided have a tolerogenic phenotype that is characterized by, for example, at least one of the following properties: i) capable of converting naïve T cells to Foxp3+ T regulatory cells ex vivo and in vivo; ii) capable of deleting effector T cells ex vivo and in vivo; iii) retain their tolerogenic phenotype upon stimulation with at least one TLR agonist ex vivo (and in some embodiments, increase expression of costimulatory molecules with the same stimulus); and/or iv) do not transiently increase their oxygen consumption rate upon stimulation with at least one TLR agonist ex vivo. In some embodiments, the itDCs have at least 2 of the above properties. In some embodiments, the itDCs have at least 3 of the above properties. In yet some embodiments, the itDCs have all 4 of the above properties. Induced tolerogenic DCs that convert naïve T cells to Foxp3+ T regulatory cells are itDCs that induce expression of the transcription factor Foxp3 in naïve T cells, e.g., in the absence of cell division, such that naïve T cells that did not previously express Foxp3 are induced to express Foxp3 and become T reg cells. In addition to expression of Foxp3, T regulatory cells (Treg cells) express CD25 and are capable of sustained suppression of effector T cell responses.

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

In embodiments, antigens may be presented by the itDCs and are combined with the itDCs 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 can include any of the antigens provided herein. Such antigens include those described above including antigens associated with an inflammatory disease, autoimmune disease, allergy, organ or tissue rejection, graft versus host disease, or a transplantable graft.

In some embodiments, the itDCs are combined with a transplantable graft, and such compositions are provided herein. In other embodiments, the itDCs are administered prior to, concomitantly with or after the administration of a transplantable graft, antigen, 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., polydimethylsiloxone), 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 itDCs and related compositions, and some aspects provide methods of using the itDCs provided herein. The itDCs may be produced from itDCs generated by the methods provided herein. Antigen-specific itDCs may also be produced from itDCs generated by the methods provided herein.

In some embodiments, the itDCs are combined with an antigen as provided herein to produce antigen-specific itDCs. The antigen-specific itDCs may also be produced from itDCs generated according to the methods provided in PCT Publication, WO201 1/109833.

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 naïve T cells into FoxpP3+ CD4+ regulatory T cells, and/or ii) deleting effector T cells. In another embodiment, a protocol employs at least one agent which tolerogenically locks dendritic cells or dendritic cell precursors ex vivo to

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produce induced tolerogenic DCs capable of antigen specific tolerance induction by, for example, i) converting naive T cells into Foxp3+ CD4+ regulatory T cells, and/or ii) deleting effector T cells.

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

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

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

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

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

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

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

The tolerogenic stimuli includes substances which, alone or in combination, induce a dendritic cell or a dendritic cell precursor to become tolerogenic, e.g., by inducing the dendritic cell to become capable of increasing the proportion of antigen specific Treg cells to antigen specific Teff cells in a cell population. More specifically, induced tolerogenic dendritic cells are produced by one or more agents which induce a tolerogenic phenotype in the DCs characterized by, for example, at least one of the following properties i) induced tolerogenic DCs are capable of converting 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 which include inhibitors of mammalian Target of Rapamycin (mTOR), agonists of TGFβ 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, TGFβ 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 TGFβ agonist; ii) a statin; iii) an mTOR inhibitor and a statin; iv) an mTOR inhibitor, a TGFβ agonist, and a statin; v) a purinergic receptor antagonist; vi) a purinergic receptor antagonist and a statin; vii) a purinergic receptor antagonist and an mTOR inhibitor; viii) a purinergic receptor antagonist, an mTOR inhibitor and a TGFβ agonist; ix) a purinergic receptor antagonist, an mTOR inhibitor, a TGFβ 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 PI, P2X, P2X7, and P2Y. In some embodiments, the purinergic receptor antagonist is oxidized ATP.

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

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

1. mTOR Inhibitors
In some exemplary embodiments, a tolerogenic stimulus for use in the instant invention comprises or consists of an mTOR inhibitor. mTOR inhibitors suitable for practicing the invention include inhibitors or antagonists of mTOR or mTOR-induced signaling. mTOR inhibitors include rapamycin and analogs, portions, or derivatives thereof, e.g., Temsirolimus (CCI-779), everolimus (RAD001) and deforolimus (AP23573). Additional rapamycin derivatives include 42- and/or 31-esters and ethers of rapamycin, which are disclosed in the following patents, all hereby incorporated by reference in their entirety: alkyl esters (U.S. Pat. No. 4,316,885); aminoalkyl esters (U.S. Pat. No. 4,650,803); fluorinated esters (U.S. Pat. No. 5,100,883); amide esters (U.S. Pat. No. 5,118,677); carbamate esters (U.S. Pat. No. 5,118,678); silyl ethers (U.S. Pat. No. 5,120,842); aminoesters (U.S. Pat. No. 5,130,307); acetics (U.S. Pat. No. 5,141,413); aminodiesters (U.S. Pat. No. 5,162,333); sulfonate and sulfate esters (U.S. Pat. No. 5,177,203); esters (U.S. Pat. No. 5,221,670); alkoxyesters (U.S. Pat. No. 5,233,036); O-aryl, -alkyl, -alkenyl, and -alkynyl ethers (U.S. Pat. No. 5,258,389); carbonate esters (U.S. Pat. No. 5,260,300); arylcarbonyl and alkoxy carbonyl carbamates (U.S. Pat. No. 5,262,423); carbomates (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 carbamate esters (U.S. Pat. No. 5,463,048); carbamate esters (U.S. Pat. No. 5,480,988); carbamate esters (U.S. Pat. No. 5,480,989); carbamate esters (U.S. Pat. No. 5,489,680); hindered N-oxide esters (U.S. Pat. No. 5,491,231); biotin esters (U.S. Pat. No. 5,504,091); O-alkyl ethers (U.S. Pat. No. 5,665,772); and PEG esters of rapamycin (U.S. Pat. No. 5,780,462). The preparation of these esters and ethers are disclosed in the patents listed above. 27-esters and ethers of rapamycin are disclosed in U.S. Pat. No. 5,256,790, which is hereby incorporated by reference in its entirety. Oximes, hydrazones, and hydroxylamines of rapamycin are disclosed in U.S. Pat. Nos. 5,373,014, 5,378,836, 5,023,264, and 5,563,145, which are hereby incorporated by reference in their entirety. The preparation of these oximes, hydrazones, and hydroxylamines are disclosed in the foregoing patents. The preparation of 42-oxorapamycin is disclosed in U.S. Pat. No. 5,023,263, which is hereby incorporated by reference in its entirety.

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

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

2. TGFP Pathway Agonists

In some exemplary embodiments, a tolerogenic stimulus for use in the instant invention comprises or consists of one or more TGFP agonists. TGFP agonists suitable for practicing the invention include substances that stimulate or potentiate responses induced by TGFP signaling. In some embodiments, a TGFP pathway agonist is acts by modulating TGFP receptor-mediated signaling. In some embodiments, a TGFP pathway agonist is a TGFP 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 TGFP receptor agonist useful for practicing the invention is TGFP, including TGFPi, TGFP2, TGFP3, variants thereof, and mixtures thereof. Additional TGFP agonists are described in Patent Publication No. US20090143394A1, the entire contents of which are incorporated herein by reference.

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

3. Statins

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

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


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

6. Combinations of Agents

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

In some exemplary embodiments, the tolerogenic stimulus comprises or consists of a combination of agents selected from the group consisting of: i) an mTOR inhibitor (e.g., rapamycin or a variant or derivative thereof); a TGFP agonist (e.g., TGFP); ii) a statin; an mTOR inhibitor (e.g., rapamycin or a variant or derivative thereof), a TGFP 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, and ranges therein.

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

In some embodiments, a TGFP agonist is used as a tolerogenic stimulus at a concentration of 1 pg/mL and 10 mg/mL, for example, 1 pg/mL, 10 pg/mL, 100 pg/mL, 200 pg/mL, 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL, 1 ng/mL, 10 ng/mL, 20 ng/mL, 30 ng/mL, 50 ng/mL, 75 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL, 800 µg/mL, 900 µg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 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 pM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 nM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 mM, and ranges therein. In some exemplary embodiments, TGFP is used as a tolerogenic stimulus at a concentration of 20 ng/mL.

In some embodiments, a purinergic receptor antagonist (e.g., oATP) is used as a tolerogenic stimulus at a concentration of 1 pg/mL and 10 mg/mL, for example, 1 pg/mL, 10
pg/mL, 100 pg/mL, 200 pg/mL, 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL,
800 pg/mL, 900 pg/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL,
500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, 1 µg/mL, 10 µg/mL, 100
µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL, 800
µg/mL, 900 µg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL,
8 mg/mL, 9 mg/mL, or 10 mg/mL, and ranges therein. In some embodiments, a purinergic
receptor antagonist is used at a concentration of 1 pM to 10 mM, for example, 1, 10, 25, 50,
100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 pM, about 1, 10, 25, 50, 100, 200, 300,
400, 500, 600, 700, 800, 900 or 1000 nM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600,
700, 800, 900 or 1000 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. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least eight hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least nine hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least ten hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least eleven hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least twelve hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least thirteen hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least fourteen hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least fifteen hours and less than ten hours. In some embodiments, cells are contacted with at least one tolerogenic stimulus for at least sixteen hours and less than ten hours.
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, \( \text{O}_2 \) consumption (the oxygen consumption rate; OCR) by cells can be measured. For example, induced tolerogenic dendritic cells can be tested to ensure that \( \text{O}_2 \) consumption decreases or does not increase. OCR can be measured, e.g., using an analyzer such as the Seahorse XF24 flux analyzer of Clark electrode. In some embodiments, a different assay can also be used to confirm the effect of an agent on mitochondrial function. For example, in some embodiments, mRNA levels of the expression of one or more of PGC-1α, PGC-1β, PRC, or other molecules involved in mitochondrial function, such as estrogen-related receptor α, NRF-1, NRF-2, Sp1, YY1, CREB and MEF-2/E-box factors can be measured. For example, induced tolerogenic dendritic cells exposed to a tolerogenic stimulus can be tested to ensure that levels of PGC-1α mRNA do not increase or decrease. Other methods of testing mitochondrial function which are known in the art can also be used for this purpose.

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

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

To make antigen-specific itDCs, the itDCs are contacted, or "loaded," with an antigen of interest. Alternatively, precursor, 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 embodiment, 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. The antigen of interest may be provided in the form as elsewhere described herein, e.g., by contacting the itDCs with an antigen or a cell that expresses the antigen. Accordingly, in some embodiments, prior to, during, and/or following treatment with a tolerogenic stimulus,
the cells are exposed to antigen. In some embodiments, before the cells have been induced with a tolerogenic stimulus, the cells are exposed to antigen. In some embodiments, after the cells have been induced with a tolerogenic stimulus, the cells are exposed to antigen. The antigen may be provided as a population of cells, processed forms thereof, a crude preparation comprising many proteins, polypeptides, and/or peptides (e.g., a lysate or extract) or may comprise one or more purified proteins, polypeptides, or peptides. Such proteins, polypeptides, or peptides can be naturally occurring, chemically synthesized, or expressed recombinantly.

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

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

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

In some embodiments, the antigen comprises or consists of a polypeptide that can be endocytosed, processed, and presented by dendritic cells. In some embodiments, the antigen comprises or consists of a short peptide that can be presented by dendritic cells without the need for processing. Short peptide antigens can bind to MHC class II molecules on the surface of dendritic cells. In some embodiments, short peptide antigens can displace antigens previously bound to MHC class II molecules on the surface of dendritic cells. Thus, the
antigen may be processed by the dendritic cells and presented or maybe 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 (e.g., class II 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 cells). Other methods are described elsewhere herein.

In some embodiments, cells are contacted with an antigen comprising more than one protein or more than one polypeptide or more than one peptide and the antigen is not purified to remove irrelevant or unwanted proteins, polypeptides, or peptides and the cells present those antigens which are processed and displayed. In some embodiments, the antigen used to contact dendritic cells comprises or consists of a single short peptide or polypeptide or mixture of peptides or polypeptides that are substantially pure, e.g., isolated from contaminating peptides or polypeptides. Likewise, the antigen can be a single polypeptide or peptide that is substantially pure and isolated from contaminating polypeptides or peptides. Such short peptides and polypeptides can be obtained by suitable methods known in the art. For example, short peptides or polypeptides can be recombinantly expressed, purified from a complex protein antigen, or produced synthetically.

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

Other methods of loading antigen onto dendritic cells will be apparent to one of ordinary skill in the art (See, e.g., Dieckman et al. Int. Immunol. (May 2005) 17(5):621-635).
In some embodiments, the antigen is associated with allergic responses. In such embodiments, the antigen with which the dendritic cells are contacted with can comprise one or more allergens (e.g., one or more polypeptides or peptides derived therefrom). In some embodiments, the antigen is a complex antigen, such as: a food protein (e.g., one or more proteins peptides or polypeptides derived from food, such as eggs, milk, wheat, soy, nuts, seeds, fish, shellfish, or gluten), pollen, mold, dust mites, or particular cell types or cells modified by exposure to a drug or chemical.

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

In some embodiments, the antigen comprises or consists of one or more peptides or polypeptides derived from food. In still some embodiments, the antigen comprises one or more peptides or polypeptides derived pollen. In some embodiments, the antigen comprises one or more peptides or polypeptides derived dust mites. In some embodiments, the antigen comprises one or more peptides or polypeptides derived 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 itDCs. For example, in some embodiments, cells are contacted with antigen at concentrations ranging between 1 pg/mL and 10 mg/mL. In exemplary embodiments, cells are contacted with antigen at 1 pg/mL, 10 pg/mL, 100 pg/mL, 200 pg/mL, 300 pg/mL, 400 pg/mL, 500 pg/mL, 600 pg/mL, 700 pg/mL, 800 pg/mL, 900 pg/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL, 500 ng/mL, 600 ng/mL, 700 ng/mL, 800 ng/mL, 900 ng/mL, 1 µg/mL, 10 µg/mL, 30 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL, 700 µg/mL, 800 µg/mL, 900 µg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 10 mg/mL, and ranges therein. In some embodiments, cells are contacted with 100 µg/mL of antigen. In some embodiments, cells are contacted with antigen at a concentration of 1 pM to 10 mM, for example, 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 pM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 nM, about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 µM, or about 1, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 mM, and ranges therein.

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

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

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

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, Sciarr and Cutie, "Aerosols," in Remington's Pharmaceutical Sciences, 18th edition, 1990, pp. 1694-1712; incorporated by reference).

Other agents can 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 itDCs and/or varying amounts of antigens and/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 cells, 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, 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 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 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, Breg cell, B cell, Treg 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, 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 3/4, about 2/3, about 1/2, about v3, about v4, about v8, about 1/10, about 1/20, about 1/25, about 1/50, about 1/100, about 1/1,000, about Vio.000, about Vio0.000, or about 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 iTDCs is undertaken e.g., prior to administration of a 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 transplantable graft or exposure to an allergen. In addition or alternatively, iTDCs can be administered to a subject concomitantly with or following administration of a transplantable graft or exposure to an allergen. In exemplary embodiments, 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 transplantable graft or exposure to an allergen.

In some embodiments, the use of 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 compositions 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.

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 thrombocytopenic purpura, idiopathic feucopenia, Wegener's granulomatosis and polydermatomyositis.

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</td>
<td>ribonucleoproteins</td>
<td>Primary Sjogren's syndrome</td>
</tr>
<tr>
<td>autoantibodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>Anti-centromere antibodies</td>
<td>centromere</td>
<td>CREST syndrome</td>
<td></td>
</tr>
<tr>
<td>Anti-neuronal nuclear antibody-2</td>
<td>Ri [disambiguation needed]</td>
<td>Opsonoclonus</td>
<td></td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>double-stranded DNA</td>
<td>SLE</td>
<td></td>
</tr>
<tr>
<td>Anti-Jol</td>
<td>histidine-tRNA ligase</td>
<td>Inflammatory myopathy</td>
<td></td>
</tr>
<tr>
<td>Anti-Smith</td>
<td>snRNP core proteins</td>
<td>SLE</td>
<td></td>
</tr>
<tr>
<td>Anti-topoisomerase antibodies</td>
<td>Type I topoisomerase</td>
<td>Systemic sclerosis (anti-Scl-70 antibodies)</td>
<td></td>
</tr>
<tr>
<td>Anti-histone antibodies</td>
<td>histones</td>
<td>SLE and Drug-induced LE[2]</td>
<td></td>
</tr>
<tr>
<td>Anti-splOO antibodies [4]</td>
<td>SplOO nuclear antigen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-glycoprotein-210 antibodies [5]</td>
<td>nucleoparin 210kDa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-transglutaminase antibodies</td>
<td>Anti-tTG</td>
<td>Coeliac disease</td>
<td></td>
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<tr>
<td></td>
<td>Anti-cTG</td>
<td>Dermatitis herpetiformis</td>
<td></td>
</tr>
<tr>
<td>Anti-ganglioside antibodies</td>
<td>ganglioside GQ1B</td>
<td>Miller-Fisher Syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ganglioside GD3</td>
<td>Acute motor axonal neuropathy (AMAN)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ganglioside GM1</td>
<td>Multifocal motor neuropathy with conduction block (MMN)</td>
<td></td>
</tr>
<tr>
<td>Anti-actin antibodies</td>
<td>actin</td>
<td>Coeliac disease anti-actin antibodies correlated with the level of intestinal damage [6][7]</td>
<td></td>
</tr>
<tr>
<td>Liver kidney microsomal type 1 antibody</td>
<td></td>
<td>Autoimmune hepatitis. [8]</td>
<td></td>
</tr>
<tr>
<td>Lupus anticoagulant antibodies</td>
<td>Anti-thrombin antibodies</td>
<td>thrombin Systemic lupus erythematosus</td>
<td></td>
</tr>
<tr>
<td>Anti-neutrophil cytoplasmic</td>
<td>phospholipid</td>
<td>Antiphospholipid syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c-ANCA</td>
<td>Wegener's granulomatosis</td>
<td></td>
</tr>
<tr>
<td>Antibody</td>
<td>Target</td>
<td>Associated Disease</td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Antibody</td>
<td>Neutrophil cytoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-ANCA</td>
<td>Neutrophil perinuclear</td>
<td>Microscopic polyangiitis, Churg-Strauss syndrome, systemic vasculitides (non-specific)</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>IgG</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></td>
<td>Exosome complex</td>
<td>Scleromyositis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nicotinic acetylcholine receptor</td>
<td>Myasthenia gravis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Muscle-specific kinase (MUSK)</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></td>
<td>Thyroid peroxidase (microsomal)</td>
<td>Hashimoto's thyroiditis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSH receptor</td>
<td>Graves' disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hu</td>
<td>Paraneoplastic cerebellar syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yo (cerebellar Purkinje Cells)</td>
<td>Paraneoplastic cerebellar syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amphiphysin</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></td>
<td>Basal ganglia neurons</td>
<td>Sydenham's chorea, paediatric autoimmune neuropsychiatric disease associated with Streptococcus (PANDAS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-methyl-D-aspartate receptor (NMDA)</td>
<td>Encephalitis</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 erythematosus, 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, Aniklosing spondylitis, sarcoidosis, scleroderma, Goodpasture's syndrome, Guillain-Barre syndrome, Graves' disease, glomerulonephritis, psoriasis, pemphigus vulgaris, pemphigoid, excema, bulous pemphigous, sympathetic ophthalmia, 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, t-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 O2 consumption of the cell populations.

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

**Example 3: Antigen-loading of itDCs (Prophetic)**

Cultures of itDCs are contacted with an antigen of interest for 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 to Antigen In Vivo (Prophetic)**

Balb/c mice are immunized with an antigen in incomplete Freund's adjuvant to induce cytokine production, the level of which is assessed. Subsequently, a composition of the invention is administered in a dose-dependent manner. The same mice are then again exposed to the antigen, and the level of cytokine production is again assessed. Changes in the cytokine production are then monitored with a reduction in cytokine production upon subsequent challenge with the antigen indicating a tolerogenic immune response.
Example 5: Administration to a Subject to Suppress an Undesired Immune Response
(Prophetic)
A composition comprising 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 6: Administration to a Subject to Suppress an Undesired Immune Response Against an Antigen (Prophetic)
Antigen-specific itDCs are formulated into a dosage form suitable for human administration. The composition is administered to the subject as an injectable cell suspension.

Example 7: Evaluating Tolerogenic Immune Response In Vivo (Prophetic)
An inflammatory cytokine level in a mouse model of inflammatory disease is assessed. Subsequently, a composition of the invention is administered in a dose-dependent manner. Cytokine production in the same mice is again assessed. Cytokine production by various T cells such as Tregs and iNKT cells can be measured by intracellular cytokine staining in a flow cytometric assay. Cytokine production by immune cells can be measured in various organs such as liver, lung or in blood. Cytokine levels in serum can be measured by ELISA. Changes in the level of cytokine production are then monitored with a reduction indicating a tolerogenic immune response.

Example 8: Administration to a Subject to Suppress an Undesired Immune Response
(Prophetic)
Antigen-specific itDCs are generated according to methods described herein and formulated for administration to a human subject having a chronic inflammatory disease. The subject exhibits chronically elevated systemic inflammatory cytokine levels. The elevated cytokine levels are pathologically high. Briefly, the antigen-specific itDCs are generated by combining itDCs with an antigen associated with the chronic systemic inflammatory cytokine production in the subject before administering the itDCs to the subject. Antigen-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 the subject. A decrease in the levels of the chronically elevated systemic inflammatory cytokines 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 the chronic systemic inflammation. For one year after administration of the initial dose of itDCs, the subject receives a bi-monthly maintenance dose of 10^6 antigen-specific itDCs (a total of 6 maintenance doses). At the end of this treatment schedule, the subject is expected to show no pathological elevation of systemic inflammatory cytokine levels.

**Example 9: Administration to NOD Animals to Reduce Production of Systemic Chronic Cytokines**

NOD animals have a spontaneous propensity to acquire type 1 diabetes. The immunological process leading to disease starts by 4 weeks of age and continue until diabetes onset around week 12. About 60% of male animals become diabetic.

In this study, NOD animals were treated weekly from week 12 for a total of 7 i.v. injections with control vehicle (PBS), itDC loaded with Insulin or itDC* loaded with pancreatic islet cell extract. Production of itDCs and antigen-loaded itDCs are described herein. The pancreatic islet cell extract was prepared as follows. Islet cell suspensions were frozen at -80°C and disrupted by four freeze-thaw cycles and vigorously pipetted. For the removal of crude debris the lysate was centrifuged for 10 min at 300x g and the supernatant was collected and passed through a 40um filter. The protein concentration was determined by sampling lysate with UV-Vis spectroscopy at 260nm. Samples were then stored at -80°C in polypropylene tubes. Total cell lysate that was incubated with DCs was 10ug/ml.

Animals were bled at 25 weeks of age and the levels of IL-6, IL-12p70 and MCP-1 were determined using a Cytometric Bead Array Mouse Inflammation Kit (CBA, BD Biosciences). The results, which show a decrease in the level of these potent cytokines/chemokines with the antigen-loaded itDCs, are shown in **Fig. 1**.
What is claimed is:

**CLAIMS**

1. A method comprising:
   administering to a subject induced tolerogenic dendritic cells (itDCs) in an amount effective to reduce the generation of systemic inflammatory cytokines in the subject, wherein the subject is experiencing or is at risk of experiencing chronic systemic inflammatory cytokine production at pathological levels.

2. A method comprising:
   reducing the generation of chronically elevated systemic inflammatory cytokines in a subject by administering itDCs to the subject.

3. A method comprising:
   administering itDCs to a subject according to a protocol that was previously shown to reduce the generation of systemic inflammatory cytokines in one or more test subjects, wherein the subject is experiencing or is at risk of experiencing chronic systemic inflammatory cytokine production at pathological levels.

4. The method of any of claims 1-3, wherein the subject is experiencing or is at risk of experiencing chronic systemic or chronic local inflammation.

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

6. The method of any of claims 1-5, wherein the amount effective is effective to reduce the generation of IFN-γ, TNF-a, IL-2, IL-5, IL-6, IL-8, IL-9, IL-12, IL-13, IL-17, IL-18, IL-21, IL-22, IL-23, IL-1β, GM-CSF, M-CSF, C reactive protein, acute phase proteins, MCP-1, RANTES, MIP-1cc, MIP-1β, MIG, ITAC and/or IP-10 in the subject.

7. The method of any of claims 1-6, wherein the reduced generation of systemic inflammatory cytokines is chronic reduced generation of systemic inflammatory cytokines.
8. The method of claim 7, wherein the generation of systemic inflammatory cytokines is reduced in the subject for at least 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months or 1 year.

9. The method of any of claims 1-8, wherein the itDCs have been combined with an antigen associated with the chronic systemic inflammatory cytokine production before administering the itDCs to the subject.

10. The method of claim 9, wherein the antigen is a protein, polypeptide, lipoprotein, glycolipid, polynucleotide or is contained or expressed in, by or on a cell.

11. The method of claim 9 or 10, wherein the antigen is an allergen, or is associated with an inflammatory disease, an autoimmune disease, organ or tissue rejection or graft versus host disease.

12. The method of any of claims 1-11, wherein the antigen is an autoantigen.

13. The method of claim 12, wherein the autoantigen comprises myelin basic protein, collagen, human cartilage gp 39, gp130-RAPS, proteolipid protein, fibrillarin, a nuclear protein, a nucleolar protein, thyroid stimulating factor receptor, a histone, glycoprotein gp 70, a ribosomal protein, pyruvate dehydrogenase dehydrodipoamide acetyltransferase, a hair follicle antigen, human tropomyosin isoform 5, a mitochondrial protein, a pancreatic β-cell protein, a pancreatic islet cell protein, myelin oligodendrocyte glycoprotein, insulin, gluten or GAD.

14. The method of any of claims 1-13, wherein the itDCs have not been combined with an antigen associated with the chronic systemic inflammatory cytokine production before administering the itDCs to the subject.

15. The method of any of claims 1-14, wherein the method further comprises assessing the generation of systemic inflammatory cytokines in the subject prior to and/or after the administration of the itDCs.
16. The method of any of claims 1-15, wherein one or more maintenance doses of the itDCs are administered to the subject.

17. The method of any of claims 1-16, wherein the generation of an undesired antigen-specific immune response is also reduced in the subject.

18. The method of any of claims 1-17, wherein the method further comprises administering a transplantable graft.

19. The method of any of claims 1-18, wherein the subject has or is at risk of having an inflammatory disease, an autoimmune disease, an allergy or graft versus host disease.

20. The method of any of claims 1-18, wherein the subject has undergone or will undergo transplantation.

21. The method of any of claims 1-18, wherein the subject suffers from obesity.

22. The method of any of claims 1-18, wherein the subject suffers from type I diabetes.

23. The method of claim 22, wherein the antigen is insulin or a pancreatic islet cell protein.

24. The method of any of claims 1-23, wherein the administering of the itDCs or transplantable graft 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.

25. The method of any of claims 1-23, wherein the administering is by subcutaneous, intrathecal, intraventricular, intramuscular, intraperitoneal, intracoronary, intrapancreatic, intrahepatic or bronchial injection.
26. A composition comprising induced tolerogenic dendritic cells (itDCs) for use in a method of:
   (a) reducing the generation of systemic inflammatory cytokines in a subject;
   (b) reducing the generation of chronically elevated systemic inflammatory cytokines in a subject;
   (c) treatment or prophylaxis of chronic systemic inflammatory cytokine production at pathological levels;
   (d) treatment or prophylaxis of chronic local inflammatory cytokine production at pathological levels;
   (e) treatment or prophylaxis of chronic local inflammation;
   (f) treatment or prophylaxis of chronic systemic inflammation;
   (g) treatment or prophylaxis of an inflammatory disease, an autoimmune disease, an allergy, organ or tissue rejection or graft versus host disease;
   (h) treatment or prophylaxis of obesity;
   (i) treatment of prophylaxis in a subject according to a protocol that was previously shown to reduce the generation of systemic inflammatory cytokines in one or more test subjects; or
   (j) treatment or prophylaxis as defined in any one of claims 1-25.

27. Use of a composition comprising induced tolerogenic dendritic cells (itDCs) for the manufacture of a medicament for use in a method as defined in claim 26.

28. The composition of claim 26 or use according to claim 27, wherein the method comprises chronic reduction of systemic inflammatory cytokines.

29. The composition or use according to claim 28, wherein the generation of systemic inflammatory cytokines is reduced for at least 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 9 months or 1 year.

30. The composition or use according to any one of claims 26-29, wherein:
   (a) the composition comprises itDCs as defined in any one of claims 9-14; and/or
(b) the composition is for use in a method of therapy or prophylaxis comprising administration by the routes as defined in claim 24 or 25.
Fig. 1
PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT
(PCT Article 17(2)(a), Rules 13ter.l(c) and (d) and 39)

Applicant's or agent's file reference
S1681.70034

IMPORTANT DECLARATION

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Applicant
SELECTA BIOSCIENCES, INC. et al

This International Searching Authority hereby declares, according to Article 17(2)(a), that no international search report will be established on the international application for the reasons indicated below.

1. The subject matter of the international application relates to:
   a. [ ] scientific theories.
   b. [ ] mathematical theories.
   c. [ ] plant varieties.
   d. [ ] animal varieties.
   e. [ ] essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
   f. [ ] schemes, rules or methods of doing business.
   g. [ ] schemes, rules or methods of performing purely mental acts.
   h. [ ] schemes, rules or methods of playing games.
   i. [ ] methods for treatment of the human body by surgery or therapy.
   j. [ ] methods for treatment of the animal body by surgery or therapy.
   k. [ ] diagnostic methods practised on the human or animal body.
   l. [ ] mere presentation of information.
   m. [ ] computer programs for which this International Searching Authority is not equipped to search prior art.

2. The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:
   [ ] the description
   [ ] the claims
   [ ] the drawings

3. A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:
   [ ] furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
   [ ] furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
   [ ] pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b)

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