effector function of $CD8^+$ T cells. In addition, IL-1 activates intrinsic IL-1 receptor (IL-1R) signaling in pre-committed T helper (TH) cell lineages, and acts as a signal for increased T effector cytokine production. IL-1 usage has been suggested as an adjuvant in weak or inefficient vaccines, to increase vaccination protection activity. Accordingly, inflammation-activators such as oxidized phospholipids have been examined as inducers of hyperactive dendritic cells *in vivo* capable of enhancing hyperactivation of dendritic cells and inducing an improved antigen specific immune response when the oxidized lipids are administered to a patient with an immunogen.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

The present disclosure provides methods and compositions for inducing the *in vitro* or *ex vivo* maturation of immature dendritic cells (DC) to produce hyperactive mature dendritic cells, with a combination of agents that simultaneously provide broad immune stimulation (*i.e.,* a dendritic cell maturation agent with or without Interferon γ in combination with an inflammation-activating lipid), and for priming those cells for an enhanced immune response, including an enhance T cell response, an enhanced Th1 response, and/or antigen-specific cytotoxic T cell response.

In one aspect, an *in vitro* or *ex vivo* method is provided for producing a cell population enriched for hyperactive mature dendritic cells that produce an enhanced immune response to a predetermined antigen, the method comprising: providing immature dendritic cells; and contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent, with or without Interferon gamma (IFNγ), and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells for a time period sufficient to form mature dendritic cells; wherein the mature dendritic cell population produces an enhanced Th1 response to the antigen when contacted with naïve T cells.

In another aspect, an *in vitro* or *ex vivo* method is provided for producing an enhanced immune response to an antigen, the method comprising: providing immature dendritic cells; contacting the immature dendritic cells with an effective amount of a

dendritic cell maturation agent, with or without Interferon gamma (IFNγ), and a predetermined antigen under culture conditions suitable for maturation of the immature dendritic cells to form the mature dendritic cell population; wherein the maturing dendritic cells take up and process antigen during maturation; formulating the mature dendritic cells for administration to an individual; wherein the mature dendritic cell formulation is to be administered together with an effective amount of an inflammation-activating lipid to produce an enhanced Th1 immune response to the predetermined antigen as compared with the formulated dendritic cell composition administered without the inflammation-activating lipid.

In another aspect, an *in vitro* or *ex vivo* method is provided for producing a hyperactivated mature dendritic cell population, comprising: providing a cell population enriched for immature dendritic cells; and contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent; with or without Interferon gamma (IFNγ); and an inflammation activating lipid under culture conditions suitable for maturation of the immature dendritic cells to a time period sufficient to form a hyperactivated mature dendritic cell population. In another aspect, an *in vitro* method is provided for producing activated antigen specific T cells, comprising: providing immature dendritic cells; contacting the immature dendritic cells with a predetermined antigen; contacting the immature dendritic cells with an effective concentration of a dendritic cell maturation agent; with or without IFNγ; and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells for a time period sufficient to form a hyperactive mature dendritic cell population; and contacting the hyperactive mature dendritic cells with naïve T cells to form activated antigen specific T cells producing an increased amount of IFNγ as compared with T cells activated with a dendritic cell maturation agent with or without IFNγ, and without the inflammation-activating lipid.

In another aspect, a use of hyperactivated T cells in the manufacture of a medicament for producing an enhanced immune response in an animal is provided, wherein

the use comprises: providing immature dendritic cells; contacting the immature dendritic cells with effective amounts of a dendritic cell maturation agent; with or without IFNγ; and an inflammation-activating lipid, and a predetermined antigen under culture conditions sufficient for maturation of the immature dendritic cells to form mature dendritic cells; contacting the mature dendritic cells with naïve T cells to form activated T cells producing an increased amount of IFNγ and/or IL-1β as compared with T cells activated with a dendritic cell maturation agent and IFNγ without the inflammation-activating lipid; and wherein the enhanced immune response is preferably a Th1 response.

In another aspect, an *in vitro* method is provided for producing an anti-tumor immune response comprising: providing immature dendritic cells; contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent; with or without IFNγ; under culture conditions suitable for inducing maturation of the immature dendritic cells; and isolating the maturing dendritic cells prior to full maturation; formulating the maturing dendritic cells for administration; wherein the maturing dendritic cells are to be administered intratumorally; and wherein an inflammation-activating lipid is to be administered either intratumorally or systemically.

In another aspect, an *in vitro* method is provided for producing a mature dendritic cell population that produces an enhanced Th1 response to an antigen, the method comprising: providing immature dendritic cells; and contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent; with or without Interferon gamma (IFNγ); and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells for a sufficient time to form the mature dendritic cell population; wherein the mature dendritic cell population produces an enhanced Th1 response to the antigen when contacted with naïve T cells.

In another aspect, a use of mature dendritic cells in the manufacture of a medicament for producing an anti-tumor immune response in an individual is provided, the use comprising: providing immature dendritic cells; contacting the immature dendritic cells with a predetermined antigen, an effective amount of a dendritic cell maturation agent; with

or without IFNγ; and an inflammation-activating lipid, under culture conditions suitable for inducing maturation of the immature dendritic cells and for a time period sufficient for the dendritic cells to uptake and process the predetermined antigen and to mature the dendritic cells; isolating the mature dendritic cells; and formulating the isolated mature dendritic cells for administration to the individual.

In another aspect, a use of maturing dendritic cells in the manufacture of a medicament for producing an enhanced anti-tumor immune response in an individual is provided, the use comprising: providing immature dendritic cells; contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent; with or without IFNγ under culture conditions suitable for inducing maturation of the immature dendritic cells; isolating the maturing dendritic cells prior to full maturation; formulating the isolated maturing dendritic cells for administration to the individual; and wherein the maturing dendritic cells are to be co-administered to the individual with an inflammation-activating lipid.

In another aspect, a composition for activating antigen specific T cells is provided, comprising: a cell population enriched for dendritic cells matured with an effective concentration of a dendritic cell maturation agent, with or without IFNγ, and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells and for a time period sufficient to form hyperactive mature dendritic cells; and a predetermined antigen; wherein the cell population enriched for hyperactive mature dendritic cells produces an enhanced T cell response as compared with mature dendritic cells matured with the effective concentration of a dendritic cell maturation agent; with or without IFNγ; and without the inflammation-activating lipid, preferably wherein the enhanced T cell response is a Th1 response.

In another aspect, an isolated, hyperactive mature dendritic cell population, comprising: isolated hyperactive mature dendritic cells, produced by contacting immature dendritic cells with an effective concentration of a dendritic cell maturation agent; with or without IFNγ; and an inflammation-activating lipid under culture conditions suitable for

maturation of the immature dendritic cells for a time period sufficient to form the hyperactive mature dendritic cells; wherein the hyperactive mature dendritic cells produce an enhanced T cell response, preferably wherein the enhanced T cell response is an increase in effector and memory T cell generation when contacted with naïve T cells; an increase in terminal differentiation and/or activation of CD4+ and CD8+ T cells; and an increase in the secretion of IL1β.

In another aspect, a composition comprising isolated or enriched hyperactive mature dendritic cells and a pharmaceutically acceptable carrier is provided, wherein the hyperactive mature dendritic cells produce more IL-1β, produce an increase in effector and memory T cells; and produce an increase in terminal differentiation and/or activation of CD4+ and CD8+ T cells than mature dendritic cells prepared by maturation of immature dendritic cells with a composition comprising effective concentrations of the dendritic cell maturation agent; with or without IFNγ; without the inflammation-activating lipid under conditions suitable for the maturation of the dendritic cells.

In one example, a method is provided for producing a mature hyperactive dendritic cell population, including providing immature dendritic cells; and contacting the immature dendritic cells *in vitro* or *ex vivo* with an effective concentration of dendritic cell maturation agent with or without Interferon γ and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells to form a hyperactive mature dendritic cell population. The mature hyperactive dendritic cell population produces an increased level of IL-1, in particular, IL-1β, an increase in effector and memory T cell generation, and an increase in terminal differentiation and activation of $CD4^+$ and $CD8^+$ T cells than induced by a mature dendritic cell population contacted *in vitro* or *ex vivo* with only the dendritic cell maturation agent with or without Interferon γ and without the inflammation-activating lipid. Similarly, the hyperactivated DCs produce greater amounts of Th-1 stimulating cytokines and smaller amounts of Th-2 stimulating cytokines than induced by a mature

dendritic cell population contacted *in vitro* or *ex vivo* with only the dendritic cell maturation agent in combination with or without Interferon γ and without the inflammation-activating lipid.

The immature dendritic cells can be contacted *in vitro* or *ex vivo* with a predetermined antigen prior to, simultaneously with, or subsequent to contact with the dendritic cell maturation agent with or without Interferon γ and the inflammation-activating lipid. Where the predetermined antigen is contacted with the dendritic cells after maturation, or when antigen uptake has been substantially reduced, the antigen can be internalized by known means such as, for example, osmotic loading.

The predetermined antigen can be, for example, a tumor specific antigen, a tumor associated antigen, a viral antigen, a bacterial antigen, tumor cells, bacterial cells, transformed or transfected cells expressing a recombinant antigen, such as a tumor associated tumor antigen, or tumor specific antigen, a cell lysate, such as a bacterial or a tumor cell lysate, a bacterial or tumor cell membrane preparation, a recombinantly produced tumor associated or tumor specific antigen, a peptide antigen (*e.g.,* a synthetic tumor associated, or tumor specific peptide associated or tumor specific antigen), or an isolated antigen, for example, an isolated bacterial, viral, tumor associated or tumor specific antigen.

In certain examples, the method can optionally further include isolating or enriching for monocytic dendritic cell precursors; and culturing the precursors *in vitro* or *ex vivo* in the presence of a dendritic cell differentiation agent to form a population of immature dendritic cells. Suitable dendritic cell differentiation agents include, for example, GM-CSF, or a combination of GM-CSF and Interleukin 4 (IL-4), Interleukin 7 (IL-7), Interleukin 13 (IL-13), or Interleukin 15 (IL-15). The monocytic dendritic cell precursors can be isolated from a human subject.

In another example, an *in vitro* or *ex vivo* method for producing a mature hyperactive dendritic cell population is provided. The method generally includes providing immature dendritic cells; and contacting the immature dendritic cells *in vitro* or *ex vivo* with an effective amount of a dendritic cell maturation agent with or without Interferon γ and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells to form the mature hyperactive dendritic cell population. The resulting mature hyperactive dendritic cell population produces an enhanced immune response. The enhanced immune response can be an enhanced

type 1 immune response. The immature dendritic cells can be contacted *in vitro* or *ex vivo* with a predetermined antigen prior to, simultaneously with, or subsequent contacting the immature dendritic cells with the dendritic cell maturation agent in combination with or without Interferon γ and the inflammation-activating lipid. When the antigen is contacted with the dendritic cell after full maturation or when antigen uptake is substantially reduced, antigen can be transported into the dendritic cell by known means, such as, for example, osmotic loading.

The predetermined antigen can be, for example, a tumor specific antigen, a tumor associated antigen, a viral antigen, a bacterial antigen, tumor cells, bacterial cells, a transfected or transformed cell expressing a recombinant antigen, such as a tumor associated or tumor specific antigen, a bacterial antigen, or a viral antigen, a cell lysate, such as a tumor cell or bacterial cell lysate, a membrane preparation, such as a tumor or bacterial cell membrane preparation, a recombinantly produced antigen, a peptide antigen (*i.e.,* a synthetic peptide), or an isolated antigen, wherein the antigen is a bacterial, viral, tumor associated, or tumor specific antigen.

In certain examples, the *in vitro* or *ex vivo* method can optionally further include isolating or providing a cell population enriched for monocytic dendritic cell precursors; and culturing the precursors *in vitro* or *ex vivo* in the presence of a dendritic cell differentiation agent to form the immature dendritic cells. Suitable dendritic cell differentiation agents include, for example, GM-CSF, or a combination of GM-CSF and IL-4, IL-13 or IL-15. The monocytic dendritic cell precursors can be isolated from a human subject.

In still another example, compositions for activating T cells are provided. The compositions can include a dendritic cell population matured *in vitro* or *ex vivo* with an effective concentration of a dendritic cell maturation agent with or without Interferon γ (IFNγ) and the inflammation-activating lipid under suitable conditions for maturation; and a predetermined antigen. The dendritic cell population can produce an increased level of IL-1, in particular, IL-1β, an enhanced immune response, and preferably an increase in a Th1 response, and more preferably an increase in effector and memory T cell generation, and an increase in terminal differentiation and/or activation of $CD4^+$ and $CD8^+$ T cells than induced by a mature dendritic cell population contacted *in vitro* or *ex vivo* with only the dendritic cell maturation agent with or without Interferon γ and without the inflammation-activating lipid.

In another example, a composition is provided that comprises isolated, immature dendritic cell population. The cell population includes immature monocytic dendritic cells, and an effective concentration of dendritic cell maturation agent with or without Interferon γ and an inflammation-activating lipid to induce maturation of the immature dendritic cells. The resulting mature hyperactive dendritic cells produces an increased level of IL-1, in particular, IL-1β, an increase in effector and memory T cell generation, and an increase in terminal differentiation and/or of $CD4^+$ and $CD8^+$ T cells than induced by a mature dendritic cell population contacted *in vitro* or *ex vivo* with only the dendritic cell maturation agent with or without Interferon γ and without the inflammation-activating lipid. The cell population can optionally include a predetermined antigen and/or isolated T cells, such as naïve T cells. The T cell can optionally be present in a preparation of isolated lymphocytes.

An *in vitro* or *ex vivo* method for producing activated T cells is also provided. The method generally includes providing immature dendritic cells; contacting the immature dendritic cells *in vitro* or *e vivo* with a predetermined antigen; and contacting the immature dendritic cells with an effective concentration of dendritic cell maturation agent with or without Interferon γ and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells to form mature hyperactive dendritic cells. The mature hyperactive dendritic cells can be contacted with naïve T cells to form activated T cells producing enhanced levels of IFNγ and/or polarized for a type 1 (Th-1) response. Suitable antigens include, for example, a tumor specific antigen, a tumor associated antigen, a viral antigen, a bacterial antigen, tumor cells, bacterial cells, transformed or transfected cells expressing a recombinant antigen, such as a bacterial, viral, tumor associated, or tumor specific antigen, a cell lysate, such as a bacterial or tumor cell lysate, a membrane preparation, such as a bacterial or tumor cell membrane preparation, a recombinantly produced antigen, a peptide antigen *(e.g.,* a synthetic peptide antigen), or an isolated antigen, wherein the antigen is a bacterial, viral, tumor associated or tumor specific antigen.

The immature dendritic cells can be contacted simultaneously with the predetermined antigen, the dendritic cell maturation agent with or without Interferon γ and the inflammation-activating lipid, or the cells can be contacted with the predetermined antigen prior to contacting with the dendritic cell maturation agent with or without Interferon γ and the inflammation-activating lipid. The dendritic cells can

also be contacted with the antigen subsequent to full maturation and antigen uptake enhanced using a known method, such as, for example, osmotic loading. In certain examples, the *in vitro* or *ex vivo* method can further include isolating monocytic dendritic cell precursors; and culturing the precursors *in vitro* or *ex vivo* in the presence of a dendritic cell differentiation agent to induce the formation of the immature dendritic cells. Suitable dendritic cell differentiation agents include, for example, GM-CSF, or a combination of GM-CSF and IL-4, IL-13, or IL-15. The monocytic dendritic cell precursors can optionally be isolated from a human subject. In a particular example the immature dendritic cells and T cells are autologous or heterologous to each other.

Isolated mature hyperactive dendritic cells producing more IL-12 are also provided. The mature dendritic cells can be provided by maturing of immature dendritic cells *in vitro* or *ex vivo* with a composition comprising effective concentrations of dendritic cell maturation agent with or without Interferon γ and an inflammation-activating lipid under conditions suitable for the maturation of the immature dendritic cells. A predetermined antigen can optionally be included with the isolated, mature dendritic cells. Isolated mature hyperactive dendritic cells loaded with a predetermined antigen are also provided. The dendritic cells can produce more Interleukin 12 (IL-12) and IL-1β than a similar immature dendritic cell population cultured in the presence of the bacteria, the dendritic cell maturation agent with or without Interferon γ and without the inflammation-activating lipid during maturation.

A method for producing a type 1 (Th-1) immune response in an animal is also provided. The method generally includes providing immature dendritic cells; contacting the immature dendritic cells *in vitro* or *ex vivo* with an effective amount of dendritic cell maturation agent with or without Interferon γ and an inflammation-activating lipid, and a predetermined antigen under *in vitro* or *ex vivo* culture conditions suitable for maturation of the immature dendritic cells to form mature hyperactive dendritic cells. The mature hyperactive dendritic cells can either be administered to an animal or can be contacted *in vitro* or *ex vivo* with naïve T cells to form activated T cells characterized by the production of enhanced levels of Interferon gamma (IFNγ) and/or tumor necrosis factor α. (TNFα.). The activated T cells can be administered to the animal in need of stimulation of a cytotoxic T cell response to the specific antigen.

Suitable antigens include, for example, a tumor specific antigen, a tumor

associated antigen, a viral antigen, a bacterial antigen, tumor cells, bacterial cells, transformed or transfected cells expressing a recombinant antigen, wherein the antigen is a bacterial antigen, a viral antigen, a tumor associated or tumor specific antigen, a cell lysate, wherein the cell lysate is a bacterial cell lysate or a tumor cell lysate, a membrane preparation, wherein the membrane preparation is a bacterial cell or tumor cell membrane preparation, or a recombinantly produced antigen, a peptide antigen (*e.g.,* a synthetic peptide antigen), or an isolated antigen, wherein the antigen is a bacterial, viral, tumor associated or tumor specific antigen. The immature dendritic cells can be simultaneously contacted with the predetermined antigen, the dendritic cell maturation agent with or without Interferon γ and the inflammation-activating lipid, or the immature dendritic cells can be contacted with the predetermined antigen prior to of subsequent contacting the cells with the dendritic cell maturation with or without Interferon γ and the inflammation-activating lipid.

In certain examples, the *in vitro* or *ex vivo* method can further include isolating monocytic dendritic cell precursors from the animal; and culturing the precursors in the presence of a dendritic cell differentiation agent to form the immature dendritic cells. The dendritic cell differentiation agent can be, for example, GM-CSF, or a combination of GM-CSF and IL-4, IL-13, or IL-15.

The immature dendritic cells and T cells can be autologous to the animal, or allogenic to the animal. Alternatively, the immature dendritic cells and T cells can have the same MHC haplotype as the animal or share an MHC marker. In certain examples, the animal can be human, or can be a non-human animal.

In another example an *in vitro* or *ex vivo* method is provided for producing maturing isolated hyperactivated dendritic cells that can uptake and process antigen, and that can induce an enhanced anti-tumor response subsequent to administration to an individual. The method comprises providing non-activated immature dendritic cells; contacting the immature dendritic cells with an effective amount of dendritic cell maturation agent with or without Interferon γ, and an inflammation-activating lipid under *in vitro* or *ex vivo* culture conditions suitable for inducing maturation of the immature dendritic cells; and isolating the maturing dendritic cells prior to full maturation.

In certain examples of the above methods the inflammation-activating lipid used in the method can be one of more of oxidized 1-palmitoyl-2-arachidonyl-*sn*-

glycero-3-phosphorylcholine (oxPAPC), 1-palmityl-2-arachidonyl-*sn*-glycero-3-phosphorylcholine (PAPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine (PGPC), and 1-palmitoyl-2-[5-oxovaleroyl]-sn-glycero-3-phosphorylcholine (POV-PC), and species of oxPAPC (*e.g.*, 1-palmitoyl-2-(5-hydroxy-8-oxo-6-octenedioyl) *sn*-glycero-3-phosphocholine (HOdiA-PC), 1-palmitoyl-2-(5-keto-6-octene-dioyl)-*sn*-glycero-3-phosphocholine (KOdiA-PC), 1-palmitoyl-2-(5-hydroxy-8-oxooct-6-enoyl)-*sn*-glycero-3-phosphocholine (HOOA-PC), 1-palmitoyl-(5-keto-8-oxo-6-octenoyl)-*sn*-glycero-3-phosphocholine (KOOA-PC). The method can further comprise the steps of formulating the isolated maturing dendritic cells prior to full maturation followed by administration of the maturing dendritic cells to the individual. The method can also optionally include cryopreserving the maturing dendritic cells and thawing the maturing dendritic cells prior to formulation and administration.

In another example an *in vitro* or *ex vivo* method is provided for producing maturing isolated hyperactivated dendritic cells that can uptake and process antigen, and that can induce an enhanced anti-tumor response subsequent to administration to an individual. The method comprises providing non-activated immature dendritic cells; contacting the immature dendritic cells *in vitro* or *ex vivo* with an effective amount of a dendritic cell maturation agent in combination with or without Interferon γ under culture conditions suitable for inducing maturation of the immature dendritic cells; and isolating the maturing dendritic cells prior to full maturation. The maturing dendritic cells are formulated for administration to an individual, for example, an individual with a solid tumor, together with an inflammation-activating lipid. The maturing dendritic cells can be combined with the inflammation-activating lipid in either the same formulation or it can be formulated separately. When formulated separately, the maturing dendritic cells and the inflammation-activating lipid can be administered either simultaneously or sequentially in either order. In certain examples, the inflammation-activating lipid can be formulated for topical administration and can be applied to the area of, for example, the skin or mucosa where the formulated maturing dendritic cells is to be administered.

In certain examples of the *in vitro* or *ex vivo* methods above the dendritic cell maturation agent is a Toll-like Receptor agonist, such as an agonist of TLR3, TLR4, TLR7 and/or TLR8, or TLR9 such as for example, a bacterial LPS, BCG, an imidazoquinoline compound, *e.g.,* a imidazoquinoline-4-amine compound, such as

4-amino-2-ethoxymethyl-α,α-dimethyl-1H-imidazol[4,5-c]quinolin-1-ethanol (R848) or 1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine (837, imiquimod), and their derivatives, a synthetic double stranded polyribonucleotide, *e.g.,* poly I:C, or its derivative, poly[I]:poly[C(12)U], and the like, a sequence of nucleic acids containing unmethylated CpG motifs known to induce the maturation of DC, and the like, or any combination thereof.

The method can use as the inflammation-activating lipid an oxidized lipid, including for example, one or more of a bioactive oxidized phospholipid which can contain fragmentation products of polyunsaturated fatty acid (PUFA), such as 1-palmitoyl-2-oxovaleroyl-*sn*-glycero-3-phosphorylcholine and 9-keto-10-dodecendioic acid ester of 2-lyso-phosphatidyl choline (KOdiA-PC). Chromatographic separation of many products formed by oxidation of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC) led to the identification of 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphatidylcholine (POVPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphatidylcholine (PGPC) and 1-palmitoyl-2-(5,6-epoxyisopropane E_2)-*sn*-glycero-3-phosphatidylcholine (PEIPC) as potent lipid mediators of inflammation. In certain examples of the disclosure the oxidized lipid can include, but is not limited to oxPAPC which is a mixture of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PAPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine (PGPC), and 1-palmitoyl-2-[5-oxovaleroyl]-sn-glycero-3-phosphorylcholine (POVPC), and species of oxPAPC (*e.g.*, 1-palmitoyl-2-(5-hydroxy-8-oxo-6-octenedioyl) *sn*-glycero-3-phosphocholine (HOdiA-PC), 1-palmitoyl-2-(5-keto-6-octene-dioyl)-*sn*-glycero-3-phosphocholine (KOdiA-PC), 1-palmitoyl-2-(5-hydroxy-8-oxooct-6-enoyl)-*sn*-glycero-3-phosphocholine (HOOA-PC), 1-palmitoyl-(5-keto-8-oxo-6-octenoyl)-*sn*-glycero-3-phosphocholine (KOOA-PC), as well as Rhodo LPS (LPS-RS or LPS from *Rhodobacter sphaeroides*, which has been previously demonstrated to activate a caspase 11-dependent inflammasome).

Predetermined antigen useful in the present method include a tumor specific antigen, a tumor associated antigen, a viral antigen, a bacterial antigen, a tumor cell, a bacterial cell, a transformed or transfected cell expressing a recombinant protein, wherein the recombinant protein is a bacterial, viral, tumor associated or tumor specific protein, a bacterial or tumor cell lysate, a bacterial or tumor cell membrane preparation, and a recombinantly produced antigen, a peptide antigen, or an isolated antigen,

wherein the antigen, peptide antigen or isolated antigen can be a bacterial, viral, tumor associated, or tumor specific antigen. In certain examples, the recombinantly produced antigen, peptide antigen or isolated antigen is a bacterial, a viral, a tumor associated, or a tumor specific antigen, peptide antigen, or isolated antigen. In these examples none of the predetermined antigens are intended to be dendritic cell maturation agents.

In an optional example, the method can further comprise isolating monocytic dendritic cell precursors; and culturing the precursors *in vitro* or *ex vivo* in the presence of a dendritic cell differentiating agent to form the immature dendritic cells. The dendritic cell differentiating agent can be GM-CSF when used alone with a high concentration of an animal protein, for example, a bovine, goat, horse, monkey, ape, and/or human protein, particularly when used in combination with a culture media supplemented with a high protein concentration, or a combination of GM-CSF and Interleukin 4 (IL-4), Interleukin 7 (IL-7), Interleukin 13 (IL-13), or Interleukin 15 (IL-15). The monocytic dendritic cell precursors can be isolated from a human subject.

In yet another example the present disclosure provides an *in vitro* method for producing an enhanced Th1 immune response to an antigen. The method comprises: providing immature dendritic cells; contacting the immature dendritic cells *in vitro* or *ex vivo* with an effective amount of a dendritic cell maturation agent, such as a TLR agonist, with or without Interferon gamma (IFNγ), and a predetermined antigen under culture conditions suitable for maturation of the immature dendritic cells to form the mature dendritic cell population; wherein the maturing dendritic cells take up and process antigen during maturation; formulating the mature dendritic cells for administration to an individual; and administering the mature dendritic cell formulation together with an effective amount of an inflammation-activating lipid to produce an enhanced Th1 immune response to the predetermined antigen as compared with the formulated dendritic cell composition administered without the inflammation-activating lipid.

In this example, the dendritic cell maturation agent can be one of, or a combination of Toll-like receptor (TLR) agonists, such as, agonists of TLR-3, TLR-4, TLR-7 and/or TLR-8, and TLR9, for example and not for limitation, a bacterial LPS, BCG, an imidazoquinoline compound, *e.g.,* a imidazoquinoline-4-amine compound, such as 4-amino-2-ethoxymethyl-α,α-dimethyl-1H-imidazol[4,5-c]quinolin-1-ethanol

(R848) or 1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine (R837, Imiquimod), and their derivatives, a synthetic double stranded polyribonucleotide, *e.g.,* poly I:C; or its derivative poly[I]:poly[C(12)U], a sequence of nucleic acids containing unmethylated CpG motifs known to induce the maturation of DC, for example, ODN 2216 (5'-ggGGGACGA:TCGTCgggggg-3'), and ODN2336 (5'-gggGGACGAC:GTCG TGgggggg-3'), and the like, or any combination thereof.

Inflammation-activating lipids useful in this method can be a bioactive phospholipid which can contain fragmentation products of , one or more of a polyunsaturated fatty acid (PUFA), such as 1-palmitoyl-2-oxovaleroyl-*sn*-glycero-3-phosphorylcholine and 9-keto-10-dodecendioic acid ester of 2-lyso-phosphatidyl choline (KOdiA-PC). Chromatographic separation of many products formed by oxidation of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC) led to the identification of 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphatidylcholine (POV-PC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphatidylcholine (PGPC) and 1-palmitoyl-2-(5,6-epoxyisopropane E_2)-*sn*-glycero-3-phosphatidylcholine (PEIPC) as potent lipid mediators of inflammation. In certain examples of the disclosure the oxidized lipid can include, but is not limited to oxPAPC which is a mixture of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PAPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine (PGPC), and 1-palmitoyl-2-[5-oxovaleroyl]-sn-glycero-3-phosphorylcholine (POV-PC), and species of oxPAPC (*e.g.*, 1-palmitoyl-2-(5-hydroxy-8-oxo-6-octenedioyl) *sn*-glycero-3-phosphocholine (HOdiA-PC), 1-palmitoyl-2-(5-keto-6-octene-dioyl)-*sn*-glycero-3-phosphocholine (KOdiA-PC), 1-palmitoyl-2-(5-hydroxy-8-oxooct-6-enoyl)-*sn*-glycero-3-phosphocholine (HOOA-PC), 1-palmitoyl-(5-keto-8-oxo-6-octenoyl)-*sn*-glycero-3-phosphocholine (KOOA-PC).

In addition, the predetermined antigen can be a tumor specific antigen, a tumor associated antigen, a viral antigen, a bacterial antigen, a tumor cell, a bacterial cell, a transformed or transfected cell producing a recombinant protein, wherein the recombinant protein is a bacterial protein, a viral protein, a tumor specific, or tumor associate protein, a bacterial or tumor cell lysate, a bacterial or tumor cell membrane preparation, a recombinantly produced antigen, a peptide antigen, or an isolated antigen. The recombinantly produced antigen, peptide antigen or isolated antigen can

be a bacterial, a tumor associated, or a tumor specific antigen, peptide antigen, or isolated antigen.

The *in vitro* or *ex vivo* method for producing an enhanced immune response above, can further comprise isolating monocytic dendritic cell precursors and culturing the precursors *in vitro* or *ex vivo* in the presence of a dendritic cell differentiating agent to form the immature dendritic cells. Dendritic cell differentiating agents useful in the method include, for example, GM-CSF, or a combination of GM-CSF and Interleukin 4 (IL-4), Interleukin 7 (IL-7), Interleukin 13 (IL-13), or Interleukin 15 (IL-15). The monocytic dendritic cell precursors can be isolated from a human subject.

In certain examples, the formulated mature dendritic cells and the inflammation-activating lipid are administered simultaneously or sequentially in any order.

In another example the *in vitro* or *ex vivo* method comprises maturation of immature dendritic cells (DC) and for activating those cells without the use of a dendritic cell maturation agent. The activated DC can be used for inducing an antigen specific T cell response. Methods can also comprise the addition of a directional maturation agent, such as interferon gamma (IFNγ), to induce a Th-1 and/or Th-2 bias in the response obtained. Unlike prior methods, activation of the immature dendritic cells in the method of the present disclosure is initiated or triggered by contacting isolated immature dendritic cells with a new, clean and unused tissue culture substrate and a dendritic cell differentiation inducing agent under conditions suitable for adherence of the dendritic cell to the tissue culture surface. In a typical method of the present disclosure, activation is achieved without the addition of a maturation agent. The immature dendritic cells are removed from a prior culture substrate or purification media and isolated from the prior culture media. The isolated immature dendritic cells are then counted and frozen for later use or combined with a fresh culture media without adding a dendritic cell maturation factor for the remainder of the process.

In one alternative method, a directional maturation agent can be added during activation to bias the dendritic cells so that they can polarize a T cell response to a Th-l or a Th-2 response. As an example, but not as a limitation to the disclosed methods, the agent used is IFNγ which can be added to bias the T cell response toward a Th-l response. Interferon gamma (IFNγ) added *in vitro* or *ex vivo* to monocytic dendritic

cell precursors or immature DCs by itself does not induce DC differentiation and/or maturation.

Tissue culture substrates useful in the methods of this example can comprise a tissue culture well, flask, bottle, bag, or any matrix used in a bioreactor, such as fiber, beads, plates, and the like. Typically, tissue culture substrates comprise plastics, such as polystyrene, Teflon® (polytetrafluoroethylene, PTFE), and the like. These substrates are uncoated with proteins or other substituents to increase the binding of various cells to the substrate. Those tissue culture substrates most often used for the *ex vivo* culture of dendritic cells for immunotherapy comprise tissue culture-flasks, -bags, or cell fractions which are comprised of multiple stacked layers of plastic, and the like.

Fully mature dendritic cells differ qualitatively and quantitatively from immature DCs. Fully mature DCs express higher levels of MHC class I and class II antigens, and of T cell costimulatory molecules, *i.e.,* CD80 and CD86. These changes typically increase the capacity of the dendritic cells to activate T cells, for example, by increasing antigen density on the cell surface, as well as the T cell activation signal through the counterparts of the costimulatory molecules on the T cells, *e.g.,* like CD28. In addition, mature DC produce large amounts of cytokines, which stimulate and direct the T cell response. Two of these cytokines are Interleukin 10 (IL-10) and Interleukin (IL-12). These cytokines have opposing effects on the direction of the induced T cell response. IL-10 production results in the induction of a Th-2 type response, while IL-12 production results in a Th-1 type response.

In one example, the hyperactive mature dendritic cells produced by any of the above methods are used for producing a medicament for treating cancer, particularly a solid tumor, a bacterial infection, or a viral infection. The medicament can be a formulation of the hyperactive mature dendritic cells comprising a therapeutically effective amount of the cells. The formulation can comprise greater than 10^2 cells, greater than 10^3 cells, greater than 10^4 cells, greater than 10^5 cells, greater than 10^6 cells, greater than 10^7 cells, greater than 10^8 cells, greater than 10^9 cells, greater than 10^{10} cells, or even greater than 10^{11}. Certain example of the compositions and formulations thereof can be adapted for administration by, for example, injection, infusion, perfusion, or lavage and can more particularly include administration through one or more means such as, for example, intravenous,

intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, and/or subcutaneous infusions and/or bolus injections.

These and other examples of the present disclosure will become more readily apparent to those possessing ordinary skill in the art when reference is made to the following detailed description

DETAILED DESCRIPTION

The present disclosure provides *in vitro* or *ex vivo* methods for inducing maturation of immature dendritic cells (DC) and for priming those cells for an enhanced antigen-specific cytotoxic T cell response (Th-1 response). The present disclosure also provides dendritic cell populations useful for activating and for preparing T cell populations polarized towards enhanced production of type 1 cytokines *(e.g.,* IFNγ, TNFα, IL-1β, and/or IL2). Such dendritic cell populations include immature monocytic dendritic cells contacted *in vitro* or *ex vivo* with a Toll-like Recentor (TLR) agonist, such as, for example, a bacterial lipopolysaccharide, such as *E. coli* LPS, BCG, Poly I:C and its less toxic derivatives, and/or R848 or R837, with or without Interferon γ, and an inflammation-activating lipid. Optionally the DCs can also be contacted with a predetermined antigen under suitable maturation conditions. The immature dendritic cells can be contacted with the antigen either simultaneous with, prior or subsequent to maturation. Where contact with the predetermined antigen takes place after maturation or where the ability to take up antigen has been substantially reduced, antigen uptake can be enhanced using known methods, such as osmotic loading.

Alternatively, immature monocytic dendritic cells, already exposed to antigen (*e.g., in vivo*), can be contacted with the TLR agonist with or without Interferon γ, and the inflammation-activating lipid under suitable maturation conditions. The resulting hyperactivated mature dendritic cells are primed to activate an immune response and to activate and potentially polarize T cells (*e.g.*, naïve T cells) towards a type 1 response. A type 1 response includes production of type 1 cytokines (*e.g.,* IFNγ, and/or IL-2), production of more IL-12 p70, a cytotoxic T cell response, production of Th-1 cells, an increase of effector and memory T cells, the production of an increase in terminal differentiation and/or activation of $CD4^+$ and $CD8^+$ T cells, and the production of certain types of antibodies. The upregulation of IL-1β and tumor

CLAIMS

1. An *in vitro* or *ex vivo* method for producing a cell population enriched for hyperactive mature dendritic cells that produce an enhanced immune response to a predetermined antigen, the method comprising:

providing immature dendritic cells; and

contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent, with or without Interferon gamma (IFNγ), and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells for a time period sufficient to form mature dendritic cells;

wherein the mature dendritic cell population produces an enhanced Th1 response to the antigen when contacted with naïve T cells.

2. An *in vitro* or *ex vivo* method for producing an enhanced immune response to an antigen, the method comprising:

providing immature dendritic cells;

contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent, with or without Interferon gamma (IFNγ), and a predetermined antigen under culture conditions suitable for maturation of the immature dendritic cells to form the mature dendritic cell population;

wherein the maturing dendritic cells take up and process antigen during maturation;

formulating the mature dendritic cells for administration to an individual; and

wherein the mature dendritic cell formulation is formulated for administration together with an effective amount of an inflammation-activating lipid to produce an enhanced Th1 immune response to the predetermined antigen as compared with the formulated dendritic cell composition administered without the inflammation-activating lipid.

3. An *in vitro* or *ex vivo* method for producing a hyperactivated mature dendritic cell population, comprising:

providing a cell population enriched for immature dendritic cells; and

contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent; with or without Interferon gamma (IFNγ); and an inflammation-activating lipid under culture conditions suitable for maturation of the

immature dendritic cells to a time period sufficient to form a hyperactivated mature dendritic cell population.

4. An *in vitro* method for producing activated antigen specific T cells, comprising:

providing immature dendritic cells;

contacting the immature dendritic cells with a predetermined antigen;

contacting the immature dendritic cells with an effective concentration of a dendritic cell maturation agent; with or without IFNγ; and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells for a time period sufficient to form a hyperactive mature dendritic cell population; and

contacting the hyperactive mature dendritic cells with naïve T cells to form activated antigen specific T cells producing an increased amount of IFNγ as compared with T cells activated with a dendritic cell maturation agent with or without IFNγ, and without the inflammation-activating lipid.

5. Use of hyperactivated T cells in the manufacture of a medicament for producing an enhanced immune response in an animal, wherein the use comprises:

providing immature dendritic cells;

contacting the immature dendritic cells with effective amounts of a dendritic cell maturation agent; with or without IFNγ; and an inflammation-activating lipid, and a predetermined antigen under culture conditions sufficient for maturation of the immature dendritic cells to form mature dendritic cells;

contacting the mature dendritic cells with naïve T cells to form activated T cells producing an increased amount of IFNγ and/or IL-1β as compared with T cells activated with a dendritic cell maturation agent and IFNγ without the inflammation-activating lipid; and

wherein the enhanced immune response is preferably a Th1 response.

6. An *in vitro* method for producing an anti-tumor immune response comprising:

providing immature dendritic cells;

contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent; with or without IFNγ; under culture conditions suitable for inducing maturation of the immature dendritic cells; and

isolating the maturing dendritic cells prior to full maturation;

formulating the maturing dendritic cells for intratumoral administration; and

wherein the maturing dendritic cells is to be administered with an inflammation-activating lipid either intratumorally or systemically.

7. An *in vitro* method for producing a mature dendritic cell population that produces an enhanced Th1 response to an antigen, the method comprising:

providing immature dendritic cells; and

contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent; with or without Interferon gamma (IFNγ); and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells for a sufficient time to form the mature dendritic cell population;

wherein the mature dendritic cell population produces an enhanced Th1 response to the antigen when contacted with naïve T cells.

8. Use of mature dendritic cells in the manufacture of a medicament for producing an anti-tumor immune response in an individual, the use comprising:

providing immature dendritic cells;

contacting the immature dendritic cells with a predetermined antigen, an effective amount of a dendritic cell maturation agent; with or without IFNγ; and an inflammation-activating lipid, under culture conditions suitable for inducing maturation of the immature dendritic cells and for a time period sufficient for the dendritic cells to uptake and process the predetermined antigen and to mature the dendritic cells;

isolating the mature dendritic cells; and

formulating the isolated mature dendritic cells for administration to the individual.

9. Use of maturing dendritic cells in the manufacture of a medicament for producing an enhanced anti-tumor immune response in an individual, the use comprising:

providing immature dendritic cells;

contacting the immature dendritic cells with an effective amount of a dendritic cell maturation agent; with or without IFNγ under culture conditions suitable for inducing maturation of the immature dendritic cells;

isolating the maturing dendritic cells prior to full maturation;

formulating the isolated maturing dendritic cells for administration to the individual; and

wherein the maturing dendritic cells are to be co-administered to the individual with an inflammation-activating lipid.

10. The method of claims 1 to 4, 6 to 7, or the use of claims 5, 8, or 9, of any one of the preceding claims, wherein:

a) the dendritic cell maturation agent is a Toll-like Receptor (TLR) agonist, preferably wherein the TLR agonist is a TLR2 agonist, a TLR3 agonist, a TLR4 agonist, a TLR7 agonist ,and/or a TLR8 agonist, or a TLR9, more preferably wherein the TLR4 agonist is LPS, or BCG, the TLR7 and/or TLR8 agonist is an imidazoquinoline compound, preferably, a imidazoquinoline-4-amine compound, more preferably 4-amino-2-ethoxymethyl-α,α-dimethyl-1H-imidazol[4,5-c]quinolin-1-ethanol (R848) or 1-(2-methylpropyl)-lH-imidazo[4,5-c]quinolin-4-amine (R837), and their derivatives, the TLR3 agonist is a synthetic double stranded polyribonucleotide, preferably poly I:C or poly[I]:poly[C(12)U], or the TLR9 agonist is a sequence of nucleic acids containing unmethylated CpG motifs known to induce the maturation of DC, or any combination thereof;

b) wherein the inflammation-activating lipid is one or more of oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (oxPAPC), 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphatidylcholine (POV-PC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphatidylcholine (PGPC) and 1-palmitoyl-2-(5,6-epoxyisopropane E_2)-*sn*-glycero-3-phosphatidylcholine (PEIPC), 1-palmitoyl-2-(5-hydroxy-8-oxo-6-octenedioyl) *sn*-glycero-3-phosphocholine (HOdiA-PC), 1-palmitoyl-2-(5-keto-6-octene-dioyl)-*sn*-glycero-3-phosphocholine (KOdiA-PC), 1-palmitoyl-2-(5-hydroxy-8-oxooct-6-enoyl)-*sn*-glycero-3-phosphocholine (HOOA-PC), 1-palmitoyl-(5-keto-8-oxo-6-octenoyl)-*sn*-glycero-3-phosphocholine (KOOA-PC);

c) wherein the immature dendritic cells are contacted with the dendritic cell maturation agent for at least about 3 hours prior to the addition of the inflammation-activating lipid, and the dendritic cells are cultured for an additional about 1 to 24 hours, or more;

d) wherein the method comprises contacting the maturing dendritic cells with a predetermined antigen prior to, simultaneously with, or subsequent contacting the immature dendritic cells with the dendritic cell maturation agent, with or without IFNγ, and the oxidized inflammation-activating lipid; preferably

wherein the predetermined antigen is a tumor specific antigen, a tumor associated antigen, a viral antigen, a bacterial antigen, a tumor cell, a bacterial cell, a bacterial or tumor cell lysate, a bacterial or tumor cell membrane preparation, a recombinantly produced antigen, a peptide antigen, or an isolated antigen; preferably

wherein the recombinantly produced antigen, peptide antigen or isolated antigen is a bacterial, a tumor associated, or a tumor specific antigen, peptide antigen, or isolated antigen;

e) the method further comprises:

obtaining a cell population enriched for monocytic dendritic cell precursors; and

culturing the precursors in the presence of a dendritic cell differentiating agent to form the immature dendritic cells; preferably

wherein the dendritic cell differentiating agent is GM-CSF, or a combination of GM-CSF and Interleukin 4 (IL-4), Interleukin 7 (IL-7), Interleukin 13 (IL-13), or Interleukin 15 (IL-15);

or wherein the monocytic dendritic cell precursors are isolated from a human subject; and/or

f) wherein the enhanced immune response is a Th1 response.

11. The method of any one of claims 2 or 10, wherein the formulated mature or maturing dendritic cells and the inflammation-activating lipid are to be administered simultaneously or sequentially in any order.

12. The method of claim 3 or 10, further comprising contacting the immature dendritic cells with a predetermined antigen prior to or simultaneously contacting with the dendritic cell maturation agent; with or without Interferon gamma (IFNγ); or the inflammation-activating lipid; and/or

wherein the activity of the mature dendritic cells is measured by an increase in a Th1 response, preferably wherein the increase in Th1 is measured by an increase in effector and memory T cell generation induced by the mature dendritic cells; an increase in terminal differentiation and/or activation of $CD4^+$ an $CD8^+$ T cells induced by the mature dendritic cells; and an increase in the secretion of IL1β, IL-2, and/or TNFα, by the mature dendritic cells.

13. The method of any one of claims 4 or 10, wherein the immature dendritic cells and T cells are autologous to each other.14. Use of the hyperactivated dendritic cells produced by any of the methods of the preceding claims, in the manufacture of a medicament for treating cancer, a bacterial infection, or a viral infection.

15. A composition for activating antigen specific T cells, comprising:

a cell population enriched for dendritic cells matured with an effective concentration of a dendritic cell maturation agent, with or without IFNγ, and an inflammation-activating lipid under culture conditions suitable for maturation of the immature dendritic cells and for a time period sufficient to form hyperactive mature dendritic cells; and

a predetermined antigen;

wherein the cell population enriched for hyperactive mature dendritic cells produces an enhanced T cell response as compared with mature dendritic cells matured with the effective concentration of a dendritic cell maturation agent; with or without IFNγ; and without the inflammation-activating lipid, preferably wherein the enhanced T cell response is a Th1 response.

16. An isolated, hyperactive mature dendritic cell population, comprising:

isolated hyperactive mature dendritic cells, produced by contacting immature dendritic cells with an effective concentration of a dendritic cell maturation agent; with or without IFNγ; and an inflammation-activating lipid under culture conditions suitable for

maturation of the immature dendritic cells for a time period sufficient to form the hyperactive mature dendritic cells;

wherein the hyperactive mature dendritic cells produce an enhanced T cell response, preferably wherein the enhanced T cell response is an increase in effector and memory T cell generation when contacted with naïve T cells; an increase in terminal differentiation and/or activation of $CD4^+$ and $CD8^+$ T cells; and an increase in the secretion of IL1β.

17. A composition comprising isolated or enriched hyperactive mature dendritic cells and a pharmaceutically acceptable carrier, wherein the hyperactive mature dendritic cells produce more IL-1β, produce an increase in effector and memory T cells; and produce an increase in terminal differentiation and/or activation of $CD4^+$ and $CD8^+$ T cells than mature dendritic cells prepared by maturation of immature dendritic cells with a composition comprising effective concentrations of the dendritic cell maturation agent; with or without IFNγ; without the inflammation-activating lipid under conditions suitable for the maturation of the dendritic cells.

18. The composition of any one of claims 15-17, wherein:

a) the cell population enriched for hyperactive mature dendritic cells produces an enhanced antigen specific Th1 response when measured *in vitro* or *in vivo* and wherein the enhanced Th cell response is an increase in effector and memory T cell generation; an increase in terminal differentiation and/or activation of $CD4^+$ and $CD8^+$ T cells; and/or an increase in the secretion of IL1β, IL-2, and/or TNFα;

b) wherein the dendritic cell maturation agent is a Toll-like Receptor (TLR) agonist, preferably wherein the TLR agonist is a TLR2 agonist, a TLR3 agonist, a TLR4 agonist, a TLR7 agonist ,and/or a TLR8 agonist, or a TLR9, more preferably wherein the TLR4 agonist is LPS, or BCG, the TLR7 and/or TLR8 agonist is an imidazoquinoline compound, preferably, a imidazoquinoline-4-amine compound, more preferably 4-amino-2-ethoxymethyl-α,α-dimethyl-1H-imidazol[4,5-c]quinolin-1-ethanol (R848) or 1-(2-methylpropyl)-lH-imidazo[4,5-c]quinolin-4-amine (R837), and their derivatives, the TLR3 agonist is a synthetic double stranded polyribonucleotide, preferably poly I:C or poly[I]:poly[C(12)U], or the TLR9 agonist is a sequence of nucleic acids containing

unmethylated CpG motifs known to induce the maturation of DC, or any combination thereof;

c) wherein the inflammation-activating lipid is one or more of oxidized 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphorylcholine (oxPAPC), 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphatidylcholine (POV-PC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphatidylcholine (PGPC) and 1-palmitoyl-2-(5,6-epoxyisopropane E_2)-*sn*-glycero-3-phosphatidylcholine (PEIPC), 1-palmitoyl-2-(5-hydroxy-8-oxo-6-octenedioyl) *sn*-glycero-3-phosphocholine (HOdiA-PC), 1-palmitoyl-2-(5-keto-6-octene-dioyl)-*sn*-glycero-3-phosphocholine (KOdiA-PC), 1-palmitoyl-2-(5-hydroxy-8-oxooct-6-enoyl)-*sn*-glycero-3-phosphocholine (HOOA-PC), 1-palmitoyl-(5-keto-8-oxo-6-octenoyl)-*sn*-glycero-3-phosphocholine (KOOA-PC); and/or

d) the predetermined antigen is a tumor specific antigen, a tumor associated antigen, a viral antigen, a bacterial antigen, a tumor cell, a bacterial cell, a recombinant cell expressing an antigen, a bacterial or tumor cell lysate, a bacterial or tumor cell membrane preparation, a recombinantly produced antigen, a peptide antigen, or an isolated antigen, wherein the recombinantly produced antigen, peptide antigen or isolated antigen are a bacterial, a viral, a tumor associated, or a tumor specific antigen.

19. The cell population of claim 16, further comprising a predetermined antigen, isolated T cells or isolated lymphocytes, optionally

wherein the T cells are naïve T cells.

20. The use of claim 5, wherein:

a) the immature dendritic cells and T cells are autologous or allogeneic to the animal;

b) wherein the immature dendritic cells and T cells have the same MHC haplotype;

c) wherein the animal is human;

d) wherein the administration is parenteral; optionally

wherein parenteral administration is intravenous, intradermal, subcutaneous, oral, transdermal, transmucosal, intratumoral, or rectal; or

e) wherein the inflammation-activating lipid is to be applied to the site of administration.

21. The method of claim 6, wherein the dendritic cells are obtained from skin, spleen, bone marrow, thymus, lymph node, umbilical cord blood, or peripheral blood; or

wherein the dendritic cells are obtained from an individual to be treated or from a healthy individual HLA-matched to the individual to be treated.

22. The use of claim 8, wherein:

a) the dendritic cells are obtained from skin, spleen, bone marrow, thymus, lymph node, umbilical cord blood, or peripheral blood or

wherein the dendritic cells are obtained from the individual to be treated or from a healthy individual HLA-matched to the individual to be treated; and/or

b) wherein the mature dendritic cells are cryopreserved and are thawed prior to administration.

23. The use of claim 9, wherein:

a) the dendritic cells are obtained from skin, spleen, bone marrow, thymus, lymph node, umbilical cord blood, or peripheral blood; or

wherein the dendritic cells are obtained from the individual to be treated or from a healthy individual HLA-matched to the individual to be treated; or

the isolated maturing dendritic cells are cryopreserved and are thawed prior to administration; and/or

b) wherein the isolated maturing dendritic cells and the inflammation-activating lipid are to be administered simultaneously or sequentially in any order; optionally

wherein the inflammation-activating lipid is in the same composition, or in separate compositions optionally

wherein the inflammation-activating lipid is in a separate composition and is to be applied topically to the site of administration.

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