their T cell stimulatory capacity. When DCs are matured using the pathogen-associated molecular pattern (PAMP)-containing molecule, lipopolysaccharide (LPS) and the damage-associated molecular pattern (DAMP)-containing molecule such as PGPC (1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine) they secrete IL-1β without pyroptosing, characterizing these cells as hyperactive (Zanoni et al., Science, 352(6290):1232-1236, 2016).

[0005] Accordingly, LNP formulations having the ability to hyperactivate dendritic cells are desirable for inclusion in vaccines. In particular, LNPs having the ability to induce IL-1β secretion and enhance the generation of long-lived T cell responses are needed in the art.

BRIEF SUMMARY

[0006] The present disclosure relates to lipid nanoparticles comprising a lysophosphatidylcholine (LPC) compound and at least one further lipid, and uses thereof in hyperactivating mammalian dendritic cells. The present disclosure also relates to compositions comprising lipid nanoparticles comprising a LPC and at least one further lipid, wherein the compositions further comprise one or more of a pathogen recognition receptor agonist, an antigen, and mammalian dendritic cells, as well as methods for production and use of the compositions. In one aspect, the present disclosure relates to a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, at least one further lipid, and a TLR7/8 agonist, wherein the acyl chain is a C13-C22 acyl chain or a C13-C24 acyl chain, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP).

[0007] In particular, the present disclosure provides a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, at least one further lipid, and a TLR7/8 agonist, wherein the acyl chain is a C13-C22 acyl chain or a C13-C24 acyl chain, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP). In some examples, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof. In some examples, the acyl chain is a C18-C22 acyl chain, a C21-C24 acyl chain, or a C22 acyl chain. In some examples, the composition further comprises an antigen and/or dendritic cells.

[0008] In some examples, the present disclosure provides a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, at least one further lipid, and an antigen, wherein the acyl chain is a C21-C24 acyl chain, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP). In some examples, the at least one further lipid is

selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof. In some examples, the composition further comprises dendritic cells and/or a TLR7/8 agonist. In one aspect, the present disclosure refers to a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, and at least one further lipid, wherein the acyl chain is a C21-C24 acyl chain, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP); the composition further comprising: a) an antigen; and/or b) dendritic cells; and optionally further comprising: c) a TLR7/8 agonist.

[0009] In some examples, the present disclosure provides a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, at least one further lipid, and dendritic cells, wherein the acyl chain is a C21-C24 acyl chain and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP). In some examples, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof. In some examples, the composition further comprises a TLR7/8 agonist and/or an antigen.

[0010] In some examples, the antigen is present in a biological sample obtained from an individual. In some examples, the biological sample comprises biopsy tissue. In some examples, the biological sample comprises cells. In other examples, the biological sample does not comprise cells. In some examples, the biological sample comprises pus from an abscess. In some examples, the antigen comprises a proteinaceous antigen. In some examples, the antigen comprises a tumor antigen. In some examples, the tumor antigen comprises a synthetic or recombinant neoantigen. In some examples, the tumor antigen comprises a tumor cell lysate. In some examples, the antigen comprises a microbial antigen and the microbial antigen comprises one or more of a viral antigen, a bacterial antigen, a protozoan antigen, and a fungal antigen. In some examples, the microbial antigen comprises a purified or recombinant surface protein. In some examples, the microbial antigen comprises an inactivated, whole virus.

[0011] In some examples, the composition does not comprise LPS or MPLA. In some examples, the composition does not comprise oxPAPC or a species of oxPAPC. In some examples, the composition does not comprise HOdiA-PC, KOdiA-PC, HOOA-PC, KOOA-PC, and/or PGPC. In some examples, the composition does not comprise isolated mRNA. In some examples, the composition does not comprise a surfactant (e.g., a poloxamer). In some examples, the composition does not comprise Poloxamer 407 (KP407), Poloxamer 188 (KP188), and/or Pluronic P123 (P123).

[0012] In some examples, the composition further comprises an adjuvant, wherein the adjuvant comprises an aluminum salt adjuvant, a squalene-in-water emulsion, a saponin, or combinations thereof.

[0013] In one aspect, the present disclosure provides a pharmaceutical formulation as disclosed herein and a pharmaceutically acceptable excipient. In some examples, the present disclosure provides a pharmaceutical formulation comprising the composition of any of the preceding examples and a pharmaceutically acceptable excipient. In some examples, the formulation does not comprise a surfactant (e.g., a poloxamer). In some examples, the formulation does not comprise Poloxamer 407 (KP407), Poloxamer 188 (KP188), and/or Pluronic P123 (P123).

[0014] In other examples, the present disclosure provides a method for production of hyperactivated dendritic cells, the method comprising contacting the dendritic cells with an effective amount of a composition comprising an isolated lysophosphatidylcholine (LPC) with a single C13-C22 acyl chain or a C13-C24 acyl chain, at least one further lipid, and a TLR7/8 agonist for production of hyperactivated dendritic cells, wherein the hyperactivated dendritic cells secrete IL-1beta without undergoing pyroptosis, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP). In some examples, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof. In some examples, the dendritic cells are contacted ex vivo with the composition or pharmaceutical formulation of any one of the preceding examples. In one aspect, the present disclosure provides an ex vivo method for production of hyperactivated dendritic cells, the method comprising contacting the dendritic cells with an effective amount of a composition comprising an isolated lysophosphatidylcholine (LPC) with a single C13-C22 acyl chain or a C13-C24 acyl chain, at least one further lipid, and a TLR7/8 agonist, for production of hyperactivated dendritic cells, wherein the hyperactivated dendritic cells secrete IL-1beta without undergoing pyroptosis, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP). In other examples, the dendritic cells are contacted in vivo with the pharmaceutical formulation comprising the composition of any one of the preceding examples. In another aspect, the present disclosure provides use of a composition comprising an isolated lysophosphatidylcholine (LPC) with a single C13-C22 acyl chain or a C13-C24 acyl chain, at least one further lipid, and a TLR7/8 agonist, for production of hyperactivated dendritic cells, wherein the hyperactivated dendritic cells secrete IL-1beta without undergoing pyroptosis, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP), in the manufacture of a medicament for producing hyperactivated dendritic cells. In some examples, the present disclosure provides a pharmaceutical formulation comprising a plurality of the hyperactivated dendritic cells produced

by the preceding examples, and a pharmaceutically acceptable excipient. In some examples, the plurality comprises at least 10^3, 10^4, 10^5, 10^6, 10^7 or 10^8 hyperactivated DCs. In yet another aspect, the present disclosure provides use of a formulation, wherein said formulation is the formulation as disclosed herein: a) in the manufacture of a medicament for: i) stimulating an immune response against an antigen, in an individual in need thereof; or ii) treating cancer, in an individual in need thereof ; or iii) inhibiting abnormal cell proliferation, in an individual in need thereof; or iv) treating an infectious disease, in an individual in need thereof; or b) in the manufacture of a medicament for: i) inducing an immune response against the antigen in an individual in need thereof; or ii) inducing an anti-tumor immune response in an individual in need thereof, wherein the individual is or was tumor-bearing; or iii) inducing an anti-microbe immune response in an individual in need thereof, wherein the individual is infected with the microbe or has not been exposed to the microbe.

[0014a] In one aspect, the present disclosure provides a method of preparing an immunogenic composition, the method comprising: a) obtaining a tumor cell-enriched suspension from a tumor; b) lysing cells from the tumor cell-enriched suspension to obtain a tumor cell lysate; and c) contacting the tumor cell lysate with a composition comprising an isolated lysophosphatidylcholine (LPC) having a single acyl chain, at least one further lipid, and a toll-like receptor 7/8 (TLR7/8) agonist, to obtain the immunogenic composition, wherein the acyl chain is a C13-C22 acyl chain or a C13-C24 acyl chain, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP). In another aspect, the present disclosure provides an immunogenic composition prepared by the method disclosed herein. In another aspect, the present disclosure provides use of an immunogenic composition as disclosed herein in the manufacture of a medicament for eliciting an anti-cancer immune response, in a mammalian subject with cancer. In yet another aspect, the present disclosure provides use of an immunogenic composition in the manufacture of a medicament for treating cancer, comprising: preparing said immunogenic composition comprising a tumor cell lysate, an isolated lysophosphatidylcholine (LPC) having a single acyl chain, at least one further lipid, and a toll-like receptor 7/8 (TLR7/8) agonist, wherein the tumor cell lysate is or has been prepared from a sample of a tumor obtained from the mammalian subject with cancer, the acyl chain is a C13-C22 acyl chain or a C13-C24 acyl chain, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP).

[0015] In other examples, the present disclosure provides a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, at least one further lipid, and a pathogen recognition receptor (PRR) agonist, wherein the acyl chain is a C13-C22 acyl chain or a C13-C24 acyl chain, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP). In some examples, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a

structural lipid, and mixtures thereof. In some examples, the PRR agonist is an agonist of a toll-like receptor (TLR), a NOD-like receptor (NLR), a RIG-I-like receptor (RLR), or a C-type lectin receptor (CLR). In some examples, the PRR agonist is an agonist of a cytosolic DNA sensor (CDS) or a stimulator of IFN genes (STING). In some examples, the PRR agonist comprises a TLR7/8 agonist. In some examples, the composition further comprises an antigen and/or dendritic cells.

[0016] In some examples of the preceding examples, the acyl chain is a C21-C24 acyl chain. In some examples, the acyl chain is a C22 acyl chain. In some examples, the acyl chain is fully saturated. In some examples, the LPC comprises 1-behenoyl-2-hydroxy-*sn*-glycero-3-phosphocholine [LPC(22:0)].

[0017] In some examples of the preceding examples, the TLR7/8 agonist is a small molecule with a molecule weight of 900 daltons or less. In some examples, the TLR7/8 agonist comprises an imidazoquinoline compound. In some examples, the TLR7/8 agonist comprises resiquimod (R848). In some examples, the LPC comprises LPC(22:0), and the TLR7/8 agonist comprises resiquimod (R848).

[0018] The present disclosure further provides compositions for hyperactivation of human dendritic cells, comprising an isolated lysophosphatidylcholine (LPC) compound with a single acyl chain, at least one further lipid, and a pathogen recognition receptor (PRR) agonist, wherein the acyl chain is C22 acyl chain, the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP), and the composition is effective for achieving a higher level of dendritic cell hyperactivation than a comparator composition comprising a comparator compound in place of the LPC. In some examples, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof. In some examples, the hyperactivation occurs in vitro or ex vivo. In other examples, the hyperactivation occurs in vivo. In some examples, the higher level of dendritic cell hyperactivation comprises induction of IL-1beta secretion from the mammalian (e.g., human) dendritic cells in vitro at a level that is at least 2, 3 or 4 fold higher when contacted with the composition comprising the LPC and the PRR agonist than when contacted with the comparator composition comprising the comparator compound and the PRR agonist, wherein the PRR agonist is LPS. In some

examples, the concentration of the LPC and the concentration of the comparator compound are the same concentration, optionally in a range of from about 10 μM to about 80 μM, and the LPS is present at a concentration of 1 μg/ml in both the composition and the comparator composition. In some examples, the higher level of dendritic cell hyperactivation comprises a lipid activity index for IL-1beta secretion from the mammalian (e.g., human) dendritic cells for the composition comprising the LPC and the PRR agonist that is at least 4, 5 or 6 fold higher in activity units than that of the comparator composition comprising the comparator compound and the PRR agonist. In some examples, the comparator compound is PGPC. In one aspect, the present disclosure provides use of a pharmaceutical formulation comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, at least one further lipid, and a pharmaceutically acceptable excipient, wherein the acyl chain is a C21-C24 acyl chain, the at least one further lipid is selected from the group consisting of an ionizable lipid, a cationic lipid, a further phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof, and the LPC and the at least one further lipid are part of a lipid nanoparticle (LNP), in the manufacture of a medicament for stimulating an immune response in an individual. In another aspect, the present disclosure provides use of a composition comprising a lipid nanoparticle (LNP), wherein the LNP comprises a first phospholipid, and at least one lipid selected from the group consisting of an ionizable lipid, a second phospholipid, a pegylated lipid, a structural lipid, and mixtures thereof, wherein the first phospholipid comprises a lysophosphatidylcholine (LPC) with a single acyl chain, and the acyl chain is a C13-C24 acyl chain, in the manufacture of a medicament for stimulating an immune response in an individual.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1A-1B** show IL-1β secretion by canine peripheral blood mononuclear cells (PBMCs) two days post-activation with the indicated stimuli, shown as total concentration (**FIG. 1A**) and fold change per donor relative to R848 alone (**FIG. 1B**), respectively. The results demonstrate that 22:0 LYSO PC, when combined with R848, is capable of stimulating canine PBMCs to secrete IL-1β at levels comparable or higher than DAMPs such as PGPC or LPS and Alum. **FIG. 1C** shows the relative viability of canine PBMCs two days post-activation with the indicated stimuli. The results demonstrate that canine PBMCs remain viable after treatment with 22:0 LYSO PC.

[0020] **FIG. 2A-2B** show IL-1β secretion by human PBMCs two days post-activation with the indicated stimuli, shown as total concentration (**FIG. 2A**) and fold change per donor relative to R848 alone (**FIG. 2B**). The results demonstrate that 22:0 LYSO PC, when combined with R848, is capable of stimulating human PBMCs to secrete IL-1β at levels comparable or higher than DAMPs such as PGPC or LPS and Alum. **FIG. 2C** shows the relative viability of human PBMCs two days post-activation with the indicated stimuli. The results demonstrate that human PBMCs remain viable after treatment with 22:0 LYSO PC.

[0021] **FIG. 3A-3B** show IFNγ (**FIG. 3A**) and TNFα (**FIG. 3B**) secretion by human PBMCs two days-post activation with the indicated stimuli, shown as fold change per donor relative to R848 alone. The results demonstrate that 22:0 LYSO PC, when combined with R848, is capable of stimulating human PBMCs to secrete other immunostimulatory cytokines at levels comparable or higher than DAMPs such as PGPC or LPS and Alum.