LPS and PGPC for hyperactivation of mammalian DCs is desirable. In particular, while LPS (endotoxin) is a potent PAMP, it is contraindicated for use in humans as it can lead to septic shock.

SUMMARY

[0006] The present disclosure relates to lysophosphatidylcholine (LPC) compounds and uses thereof in hyperactivating mammalian dendritic cells, such as human dendritic cells or canine dendritic cells. The present disclosure also relates to compositions comprising a LPC and one or more of a pathogen recognition receptor agonist, an antigen, and human or canine dendritic cells, as well as methods for production and use of the compositions.

[0007] In particular, the present disclosure provides a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, and a TLR7/8 agonist, wherein the acyl chain is a C13-C22 acyl chain or a C13-C24 acyl chain. In one aspect, the present disclosure refers to a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, and a TLR7/8 agonist, wherein the acyl chain is a C13-C24 acyl chain. 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, and an antigen, wherein the acyl chain is a C21-C24 acyl chain. In some examples, the composition further comprises dendritic cells and/or a TLR7/8 agonist.

[0009] In some examples, the present disclosure provides a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, and dendritic cells, wherein the acyl chain is a C21-C24 acyl chain. 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 liposomes. 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.

[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 some examples, the present disclosure provides a pharmaceutical formulation comprising the composition of any of the preceding examples and a pharmaceutically acceptable excipient. In one aspect, the present disclosure refers to a pharmaceutical formulation comprising the composition as disclosed herein and a pharmaceutically acceptable excipient.

[0014] In other examples, the present disclosure provides a method for production of hyperactivated dendritic cells, the method comprising contacting the dendritic cells with a composition comprising effective amounts of an isolated lysophosphatidylcholine (LPC) with a single C13-C22 acyl chain or a C13-C24 acyl chain, and a TLR7/8 agonist for production of hyperactivated dendritic cells, wherein the hyperactivated dendritic cells secrete IL-1beta without undergoing pyroptosis. In one aspect, the present disclosure refers to a method for production of hyperactivated dendritic cells, the method comprising contacting the dendritic cells with an effective amount of the composition as disclosed herein or the formulation as disclosed herein for production of hyperactivated dendritic cells, wherein the hyperactivated dendritic cells secrete IL-1beta without undergoing pyroptosis. In some examples, the dendritic cells are contacted ex vivo with the composition or pharmaceutical formulation of any one of the preceding examples. 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 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 one aspect, the present disclosure refers to a pharmaceutical formulation comprising at least 10^3, 10^4, 10^5 or 10^6 of the hyperactivated dendritic cells produced by the method as disclosed herein, and a pharmaceutically acceptable excipient.

[0015] In other examples, the present disclosure provides a composition comprising an isolated lysophosphatidylcholine (LPC) with a single acyl chain, and a pathogen recognition receptor (PRR) agonist, wherein the acyl chain is a C13-C22 acyl chain or a C13-C24 acyl chain. 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 aspects, 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-s*n*-glycero-3-phosphocholine [LPC(22:0)].

[0017] In some examples of the preceding aspects, 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, and a pathogen recognition receptor (PRR) agonist, wherein the acyl chain is C22 acyl chain, and wherein the composition is effective for achieving a higher level of dendritic cell hyperactivation than a comparator composition comprising PGPC in place of the LPC. 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 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 PGPC and the PRR agonist, wherein the PRR agonist is LPS. In some examples, the concentration of the LPC and the concentration of the PGPC 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 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 LPC and the PRR agonist.

[0018a] In one aspect, the present disclosure refers to use of the formulation as disclosed herein in the manufacture of a medicament for inducing an immune response against the antigen in an individual in need thereof. In one aspect, the present disclosure refers to use of the formulation as disclosed herein in the manufacture of a medicament for inducing an anti-tumor immune response in an individual in need thereof, wherein the individual is or was tumor-bearing. In one aspect, the present disclosure refers to use of the formulation as disclosed herein in the manufacture of a medicament for inducing an anti-microbe immune response in an individual in need thereof.

[0018b] In one aspect, the present disclosure refers to a method of preparing an immunogenic composition, the method comprising: a) preparing a tumor cell-enriched suspension from a tumor obtained from a mammalian subject; b) lysing cells from the tumor cell-enriched suspension to obtain a tumor cell lysate; and c) contacting the tumor cell lysate with the composition as disclosed herein or the formulation as disclosed herein. In one aspect, the present disclosure refers to an immunogenic composition prepared by the method as disclosed herein.

[0018c] In one aspect, the present disclosure refers to use of the immunogenic composition as disclosed herein in the manufacture of a medicament for eliciting an anti-cancer immune response in a mammalian subject with cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1** is a cartoon showing the effects of various stimuli on dendritic cell function. Depending on the stimuli, dendritic cells remain quiescent, or become activated, pyroptotic, or hyperactivated.

[0020] **FIG. 2** shows IL-1β secretion by human monocyte-derived dendritic cells activated with 1 μg/mL LPS in the presence of 82.5 μM of various lysophosphatidylcholine compounds (LPCs). The results demonstrate that acyl chain length affects the ability of LPCs to promote IL-1β secretion by DCs.

[0021] **FIG. 3** shows IL-1β secretion by human monocyte-derived dendritic cells activated with 1 μg/mL LPS in the presence of 82.5 μM of various lipids. The results demonstrate that lysophosphatidylcholine compounds (LPCs) with an acyl chain length of at least 12 carbons (12:0 Lyso PC, No. 29) are capable of stimulating human DCs to secrete IL-1β at levels comparable to or better (22:0 Lyso PC, No. 42) than DAMPs such as PGPC, which had been identified by screening murine DCs.

[0022] **FIG. 4** shows a lipid activity index of various compounds. The index was calculated by multiplying the reciprocal of the lowest concentration at which IL-1β secretion by human monocyte-derived dendritic cells was 2-fold higher than the LPS only control by the highest IL-1β signal observed at any concentration.

[0023] **FIG. 5** shows IL-1β secretion by human monocyte-derived dendritic cells contacted with various PRR agonists in the presence or absence of 22:0 Lyso PC.

[0024] **FIG. 6A-6B** show IL-1β secretion by canine peripheral blood mononuclear cells (PBMCs) two days post-activation with the indicated stimuli, shown as total concentration (**FIG. 6A**) and fold change per donor relative to R848 alone (**FIG. 6B**), respectively. The results demonstrate that 22:0 LYSO PC, when combined with R848, is capable of stimulating canine