CLAIMS

We claim:

1. Use of a composition comprising an mRNA encapsulated in a lipid nanoparticle (LNP), in the manufacture of a medicament for stimulating an immune response in an individual, wherein the mRNA comprises a coding region of an antigen, and 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.

2. A composition comprising an mRNA and a TLR7/8 agonist encapsulated in a lipid nanoparticle (LNP), wherein the mRNA comprises a coding region of an antigen, and 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.

3. A composition comprising an mRNA encapsulated in a lipid nanoparticle (LNP), and a TLR7/8 agonist, wherein the mRNA comprises a coding region of an antigen, and 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.

4. The use of claim 1 or the composition of claim 2 or 3:

I) wherein the at least one lipid comprises an ionizable lipid, a second phospholipid, a pegylated lipid, and a structural lipid; optionally wherein:

a) the ionizable lipid comprises:

i) 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]-octanoic acid, 1-octylnonyl ester (SM-102) or analogs or derivatives thereof; and/or

ii) 6-((2-hexyldecanoyl)oxy)-N-(6-((2-hexyldecanoyl)oxy)hexyl)-N-(4-hydroxybutyl)hexan-1-aminium (ALC-0315) or analogs or derivatives thereof;

and/or

b) the pegylated lipid is:

i) selected from the group consisting of a PEG-modified phosphatidyiethanolamine, a PEG-modified phosphatide acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, a PEG-modified dialkylglyerol, and combinations thereof; or

ii) comprises polyethylene glycol [PEG] 2000 dimyristoyl glycerol [DMG];

and/or

c) the structural lipid is:

i) selected from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol, and combinations thereof; or

ii) comprises cholesterol;

and/or

d) the second phospholipid:

i) comprises:

I) a hydrophilic head moiety selected from the group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and sphingomyelin; and

II) one or more fatty acid tail moieties selected from the group consisting of lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, arachidic acid, arachidonic acid, phytanoic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid; or

ii) is selected from the group consisting of
1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC),
1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC),
1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC),
1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC),
1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC),
1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC),
1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC),
1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine,
1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine,
1,2-dilinolenoyl-sn-glycero-3-phosphocholine,
1,2-diarachidonoyl-sn-glycero-3-phosphocholine,
1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine,
1,2-dioleoyl-sn-glycero-3-phosphoethanola mine (DOPE),
1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine,
1,2-distearoyl-sn-glycero-3-phosphoethanolamine,
1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine,
1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine,
1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine,
1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine,
1,2-dioleoyl-sn-glycero-3-phospho-rac-(1 -glycerol) sodium salt (DOPG),
sphingomyelin, and
combinations thereof;

and/or

e) the second phospholipid comprises 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC);

and/or

II) further comprising at least one excipient, optionally wherein the excipient comprises sucrose.

5. Use of a composition comprising:

i) an mRNA complexed with one or more lipids (RNA-Lipoplex); and

ii) a lysophosphatidylcholine (LPC) with a single C13-C24 acyl chain,

wherein the mRNA comprises a coding region of an antigen, and the one or more lipids comprise a first lipid and a second lipid, in the manufacture of a medicament for stimulating an immune response in an individual.

6. A composition comprising:

i) an mRNA complexed with one or more lipids (RNA-Lipoplex);

ii) a lysophosphatidylcholine (LPC) with a single C13-C24 acyl chain; and

iii) a TLR7/8 agonist,

wherein the mRNA comprises a coding region of an antigen, and the one or more lipids comprise a first lipid and a second lipid.

7. The use of claim 5 or the composition of claim 6, wherein the first lipid is a cationic lipid, and the second lipid is a neutral or anionic lipid.

8. The use or the composition of claim 7, wherein the cationic lipid comprises one or both of:

i) 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) or analogs or derivatives thereof; and

ii) 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) or analogs or derivatives thereof;

and/or

the neutral or anionic lipid comprises:

i) 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE) or analogs or derivatives thereof; and/or

ii) cholesterol or analogs or derivatives thereof; and/or

iii) 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) or analogs or derivatives thereof.

9. The use of any one of claims 1, 4-5 and 7-8 or the composition of any one of claims 2-3, 4 and 6-8, wherein

a) the acyl chain of the LPC is a C21-C24 acyl chain, or the acyl chain of the LPC is a C22 acyl chain;

and/or

b) the acyl chain of the LPC is fully saturated, optionally wherein the LPC comprises 1-behenoyl-2-hydroxy-sn-glycero-3-phosphocholine [LPC(22:0)];

and/or

c) the TLR7/8 agonist:

i) is a small molecule with a molecule weight of 900 daltons or less,

or

ii) comprises an imidazoquinoline compound, optionally wherein the TLR7/8 agonist comprises resiquimod (R848);

and/or

d) wherein:

i) the LPC comprises LPC(22:0), and the TLR7/8 agonist comprises resiquimod (R848); or

ii) the antigen is a tumor antigen; optionally wherein the tumor antigen is a neoantigen; or

iii) the antigen comprises a microbial antigen, optionally wherein the microbial antigen comprises:

I) a viral antigen, a bacterial antigen, a protozoan antigen, or a fungal antigen; or

II) a surface antigen;

and/or

e) the mRNA comprises a 5’ untranslated region (5’UTR) at the 5’ end of the coding region and a 3’ untranslated region (3’UTR) at the 3’ end of the coding region, optionally wherein the mRNA comprises a 5' cap structure, further optionally wherein the mRNA comprises a polyA tail;

and/or

f) the composition does not comprise lipopolysaccharide (LPS) or monophosphoryl lipid A (MPLA);

and/or

g) the composition does not comprise oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC) or a species of oxPAPC.

10. A pharmaceutical formulation comprising the composition of any one of claims 2-3, 4 and 6-9, and a pharmaceutically acceptable excipient.

11. Use of the composition of any one of claims 2-3, 4 and 6-9 in the manufacture of a medicament for producing hyperactivated dendritic cells, wherein the hyperactivated dendritic cells secrete IL-1beta without undergoing cell death within about 48 hours of exposure, wherein the dendritic cells are to be contacted in vivo with the composition.

12. An ex vivo method for production of hyperactivated dendritic cells, the method comprising contacting the dendritic cells with an effective amount of the composition of any one of claims 2-3, 4 and 6-9 to produce hyperactivated dendritic cells, wherein the hyperactivated dendritic cells secrete IL-1beta without undergoing cell death within about 48 hours of exposure.

13. A pharmaceutical formulation comprising at least 10^3, 10^4, 10^5 or 10^6 of the hyperactivated dendritic cells produced by the method of claim 12, and a pharmaceutically acceptable excipient.

14. Use of a pharmaceutical formulation, wherein:

a) the pharmaceutical formulation is that of claim 10 or claim 13, in the manufacture of a medicament for stimulating an immune response against an antigen in an individual in need thereof; or

b) the pharmaceutical formulation is that of claim 10 or claim 13, in the manufacture of a medicament for treating cancer in an individual in need thereof; or

c) the pharmaceutical formulation is that of claim 10 or claim 13, in the manufacture of a medicament for inhibiting abnormal cell proliferation in an individual in need thereof; or

d) the pharmaceutical formulation is that of claim 10, in the manufacture of a medicament for treating or preventing an infectious disease in an individual in need thereof; optionally wherein the infectious disease is a viral disease and/or a bacterial disease.

15. The composition of any one of claims 2-3, 4 and 6-9, formulation of claim 10 or 13, or method of claim 12, or use of any one of claims 1, 4-5 and 7-9, 11 and 14, wherein:

i) the composition does not comprise a protein; and/or

ii) the LNP has an effective diameter of less than about 500 nanometers, optionally from about 5 to about 500 nanometers, optionally from about 10 to about 400 nanometers, optionally from about 20 to about 300 nanometers, or optionally from about 25 to about 250 nanometers; optionally wherein the LNP has an effective diameter of less than about 250 nanometers, optionally less than about

125 nanometers, optionally wherein the LNP has an effective diameter of from about 20 to about 120 nanometers.