

post boost, lung tissues were collected for CD8 T cell analysis by flow cytometry, and BALF and blood were collected for antibody measurement.

[0028] **FIG. 7B** shows the quantification of total Tetramer⁺ CD8 T cells, CD69⁺CD103⁻ Tetramer⁺ CD8 T cells, or CD69⁺CD103⁺Tetramer⁺ CD8 T cells in lung tissues from naïve, IM Prime, or Prime and Spike mice.

[0029] **FIG. 7C** shows measurement of SCV2 spike S1 subunit-specific BALF IgA (**E**), BALF IgG (**F**), serum IgA (**G**), and serum IgG (**H**) in naïve, IM Prime, or Prime and Spike mice.

[0030] **FIG. 8A** illustrates an experiment in which K18-hACE2 mice were IM primed with 1 µg of mRNA-LNP, followed by boosting with 1 µg of mRNA-LNP IM, or 1 µg of SCV2 spike IN (IN Spike) 14 days post IM Prime. Forty-five days post boost, lung tissues were collected for T cell analysis by flow cytometry, and BALF and blood were collected for antibody measurement.

[0031] **FIG. 8B** shows the quantification of total Tetramer⁺ CD8 T cells, CD69⁺CD103⁻ Tetramer⁺ CD8 T cells, or CD69⁺CD103⁺Tetramer⁺ CD8 T cells in lung tissues from naïve, mRNA-LNP Prime/Boost, or Prime and Spike mice.

[0032] **FIG. 8C** shows Measurement of SARS-CoV-2 spike S1 subunit specific BALF IgA (**E**), BALF IgG (**F**), serum IgA (**G**), and serum IgG (**H**) in naïve, mRNA-LNP Prime/Boost, or Prime and Spike mice. (**I,J**) Measurement of neutralization titer against SCV2 spike-pseudotyped VSV.

[0033] **FIG. 9** shows the length and integrity of extracted mRNA was analyzed using agarose gel electrophoresis. Extracted mRNA was mixed with SYBR Safe stain before being loaded onto a 1% agarose gel, let run in the TAE buffer, and imaged with a gel imaging system.

[0034] **FIGS. 10A- 10B** shows (**FIG. 10A**) Gating strategies to identify extravascular antigen-specific CD8 T cells and polyclonal activated CD4 T cells. (**FIG. 10B**) Gating strategies to identify extravascular antigen-specific and polyclonal B cell subsets.

SUMMARY

[0034a] In one aspect, the present disclosure refers to a use of a pharmaceutical composition comprising an antigen or a nucleic acid encoding the antigen and a lipid nanoparticle (LNP), wherein the lipid nanoparticle (LNP) comprises at least one cationic lipid

and/or a poly(amine-co-ester) (PACE) polymer, in the manufacture of a medicament for enhancing an immune response to the antigen in a human in need thereof, wherein the medicament is to be administered at a mucosal site, and wherein the human has been previously vaccinated against or infected by a virus.

[0034b] In another aspect, the present disclosure refers to a use of a pharmaceutical composition comprising an antigen in the manufacture of a medicament for enhancing an immune response to the antigen in a human in need thereof, wherein the medicament is to be administered at a mucosal site, and wherein the human has elevated antibodies, memory B cells, effector CD4⁺ and/or CD8⁺ T cells, wherein the elevated antibodies, memory B cells, effector CD4⁺ and/or CD8⁺ T cells are caused by a previous vaccination against a virus, or by a previous infection of the virus.

[0034c] In another aspect, the present disclosure refers to a use of a pharmaceutical composition comprising at least one mRNA and a lipid nanoparticle (LNP), wherein the lipid nanoparticle (LNP) comprises at least one cationic lipid and/or a poly(amine-co-ester) (PACE) polymer, in the manufacture of a medicament for enhancing an immune response to SARS-CoV-2 in a human in need thereof, wherein the medicament is to be administered at a mucosal site, and wherein the human has been previously vaccinated against or infected by a virus.

[0035] The disclosure encompasses a method of enhancing an immune response to an antigen in a human in need thereof, the method comprises administering to the subject an effective amount of a pharmaceutical composition comprising the antigen or a nucleic acid encoding the

antigen at a mucosal site, wherein the human has been previously vaccinated against or infected by a virus. In some embodiments, the human has elevated antibodies, memory B cells and effector CD4⁺ and CD8⁺ T cells. In some embodiments, the elevated antibodies, memory B cells and effector CD4⁺ and CD8⁺ T cells are caused by a previous vaccination against a virus.

[0036] In some examples, the elevated antibodies, memory B cells and effector CD4⁺ and CD8⁺ T cells are caused by a previous infection of a virus.

[0037] In some examples, the mucosal site is selected from the group consisting of rectal, vaginal, bladder, ocular, oral, sublingual, esophageal, nasal, gastrointestinal, pulmonary and aural mucosal sites.

[0038] In some examples, the antigen comprises a protein or polypeptide.

[0039] In some examples, the antigen is multivalent antigen.

[0040] In some examples, the antigen comprises a nucleic acid encoding a protein or a polypeptide.

[0041] In some examples, the nucleic acid is DNA or RNA.

[0042] In some examples, the nucleic acid is mRNA.

[0043] In some examples, the antigen is derived from a microbial pathogen.

[0044] In some examples, the microbial pathogen is a mycobacterium, bacterium, fungus, virus, parasite, or prion.

[0045] In some examples, the virus is selected from the group consisting of rotavirus, norovirus, adenovirus, astrovirus, variants thereof, and any combination thereof.

[0046] In some examples, the virus is selected from the group consisting of influenza virus, respiratory syncytial virus, parainfluenza viruses, metapneumovirus, rhinovirus, coronavirus, adenovirus, bocavirus, variants thereof, and any combination thereof.

[0047] In some examples, the virus is selected from the group consisting of herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), human papillomavirus (HPV), variants thereof, and any combination thereof.

[0048] In some examples, the virus is selected from the group consisting of human immunodeficiency virus (HIV), hepatitis A, hepatitis B, hepatitis C, herpes virus, adenovirus, poliomyelitis, Japanese encephalitis, smallpox, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, coronavirus, respiratory syncytial virus (RSV), mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, human T-lymphotropic virus

(HTLV), dengue virus, human papillomavirus (HPV), molluscum virus, poliovirus, rabies virus, JC virus, arboviral encephalitis virus, SARS-CoV-2, Henoch-Schonlein purpura (HSP), an RNA virus, a DNA virus, variants thereof, and any combination thereof.

[0049] In some examples, the RNA virus selected from the group consisting of common cold, influenza, SARS, MERS, Covid-19 , Dengue Virus, hepatitis C, hepatitis E, West Nile fever, Ebola virus disease, rabies, polio, mumps, measles, variants thereof, and any combination thereof .

[0050] In some examples, the DNA virus selected from the group consisting of herpes simplex virus, cytomegalo virus, varicella zoster virus, Epstein-Barr virus, roseolo virus, human herpesvirus-7, Kaposi's sarcoma-associated virus, variants thereof, and any combination thereof .

[0051] In some examples, the pharmaceutical composition is administered by mucosal delivery.

[0052] In some examples, the mucosal delivery is selected from the group consisting of rectal delivery, buccal delivery, pulmonary delivery, ocular delivery, nasal delivery, intranasal delivery, vaginal delivery and oral delivery.

[0053] In some examples, the pharmaceutical composition is administered to a mucosal tissue of the human subject.

[0054] In some examples, the mucosal tissue is selected from the group consisting of anterior nostril, nasal sinus, rectal, vaginal, esophagus, urethral, sublingual and buccal.

[0055] In some examples, the pharmaceutical composition is administered orally, intravenously, intramuscularly, intradermally, subcutaneously, intranasally, or by inhalation.

[0056] In some examples, the pharmaceutical composition is administered by intranasal spray.

[0057] In some examples, the pharmaceutical composition does not comprise an adjuvant.

[0058] In some examples, the pharmaceutical composition comprises an adjuvant.

[0059] In some examples, the pharmaceutical composition comprises a lipid nanoparticle (LNP).

[0060] In some examples, the antigen is encapsulated within the lipid nanoparticle (LNP).

[0061] In another example, the disclosure relates to a method of enhancing an immune response to SARS-CoV-2 in a human in need thereof; the method comprises administering to the subject an effective amount of a pharmaceutical composition comprising at least one mRNA at a mucosal site, wherein the human has been previously vaccinated against or infected by SARS-CoV-2. In some examples, the mRNA encodes the spike protein of SARS-CoV-2 or a fragment thereof.

[0062] In some examples, the pharmaceutical composition does not comprise an adjuvant.

[0063] In some examples, the pharmaceutical composition comprises an adjuvant.

[0064] In some examples, the pharmaceutical composition further comprises a lipid nanoparticle (LNP).

[0065] In some examples, the mRNA is encapsulated within the lipid nanoparticle (LNP).

[0066] In some examples, the lipid nanoparticle (LNP) comprises at least one cationic lipid.

[0067] In some examples, the at least one cationic lipid comprises 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine (DMEPC), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and/or 1,2-dioleoyl-3-trimethylammonium propane (DOTAP).

[0068] In some examples, the lipid nanoparticle (LNP) further comprise at least one phospholipid.

[0069] In some examples, the at least phospholipid comprises 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol (Chol), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholin (POPC) and/or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC).

[0070] In some examples, the lipid nanoparticle has an average diameter in the range of from about 50 nm to about 1000 nm.

[0071] In some examples, the lipid nanoparticle has an average diameter in the range of from about 50 nm to about 400 nm, from about 50 nm to about 200 nm, from about 200 nm to about 1000 nm, from about 200 nm to about 800 nm, or from about 300 nm to about 600 nm.

[0072] In some examples, the immune response is a mucosal immune response.

[0073] In some examples, the mucosal immune response is an antigen-specific IgA antibody production.

[0074] In some examples, the mucosal immune response is an antigen-specific IgG antibody production.

[0075] In some examples, the human has elevated IgA antibody.

[0076] In some examples, the human has elevated IgG antibody.

DETAILED DESCRIPTION OF THE INVENTION

Definition

[0077] The articles “a” and “an” as used herein and in the appended claims are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article unless the context clearly indicates otherwise. By way of example, “an element” means one element or more than one element.

[0078] The term "variant" means a polypeptide or a nucleotide including an alteration, i.e., a substitution, insertion, and/or deletion, at one or more (e.g., several) positions. In some embodiments, the term “variant” refers to a SARS-CoV-2 virus variant.

[0079] The term “spike”, “boost” or “booster” are used interchangeably.

[0080] As used herein, the term "immunogenic agent" encompasses any substance, composition of matter, or composition of organic material as for example a suspension of cells or cell components, the immunogenic agent being capable of conferring a substantial immune response to a coronavirus in a human subject, when administered in a suitable amount and in admixture with suitable substances.

[0081] The term "immunologically equivalent" means that the polypeptide is functionally equivalent to the polypeptide having the amino acid sequence of any S protein with respect to its ability of eliciting an immune response.

[0082] As used herein, the term "polypeptide" encompasses both short peptides with a length of 2-10 amino acid residues, oligopeptides (11-100 amino acid residues), and longer peptides (the usual interpretation of polypeptide, i.e. more than 100 amino acid residues in length) as well as proteins (the functional entity comprising at least one peptide, oligopeptide, or polypeptide

Claims

1. Use of a pharmaceutical composition comprising an antigen or a nucleic acid encoding the antigen and a lipid nanoparticle (LNP), wherein the lipid nanoparticle (LNP) comprises at least one cationic lipid and/or a poly(amine-co-ester) (PACE) polymer, in the manufacture of a medicament for enhancing an immune response to the antigen in a human in need thereof, wherein the medicament is to be administered at a mucosal site, and wherein the human has been previously vaccinated against or infected by a virus.
2. The use of claim 1, wherein the human has been parenterally vaccinated against the virus.
3. The use of claim 1, wherein the antigen is a multivalent antigen.
4. Use of a pharmaceutical composition comprising an antigen in the manufacture of a medicament for enhancing an immune response to the antigen in a human in need thereof, wherein the medicament is to be administered at a mucosal site, and wherein the human has elevated antibodies, memory B cells, effector CD4⁺ and/or CD8⁺ T cells, wherein the elevated antibodies, memory B cells, effector CD4⁺ and/or CD8⁺ T cells are caused by a previous vaccination against a virus, or by a previous infection of the virus.
5. The use of claim 4, wherein the elevated antibodies are immunoglobulin G (IgG), IgM, IgA, or combinations thereof.
6. The use of claim 1, wherein the mucosal site is nasal.
7. The use of claim 1, wherein the antigen comprises at least a protein or polypeptide.
8. The use of claim 1, wherein the nucleic acid is DNA or RNA, preferably wherein the nucleic acid is mRNA; preferably wherein the mRNA is N1-methyl-pseudouridine-modified mRNA or pseudouridine-modified mRNA.
9. The use of claim 1, wherein the antigen is derived from a microbial pathogen, preferably wherein the microbial pathogen is a mycobacterium, bacterium, fungus, virus, parasite, or prion.
10. The use of claim 9, wherein the virus is selected from the group consisting of rotavirus, norovirus, adenovirus, astrovirus, variants thereof, and any combination thereof, or wherein the virus is selected from the group consisting of influenza virus, respiratory syncytial virus, parainfluenza viruses, metapneumovirus, rhinovirus, coronavirus, adenovirus, bocavirus, variants thereof, and any combination thereof, or wherein the virus is selected from the group consisting of herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), human

papillomavirus (HPV), variants thereof, and any combination thereof, or wherein the virus is selected from the group consisting of human immunodeficiency virus (HIV), hepatitis A, hepatitis B, hepatitis C, herpes virus, adenovirus, poliomyelitis, Japanese encephalitis, smallpox, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, coronavirus, respiratory syncytial virus (RSV), mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, human T-lymphotropic virus (HTLV), dengue virus, human papillomavirus (HPV), molluscum virus, poliovirus, rabies virus, JC virus, arboviral encephalitis virus, SARS-CoV-2, Henoch-Schonlein purpura (HSP), an RNA virus, a DNA virus, variants thereof, and any combination thereof, preferably wherein the RNA virus is selected from the group consisting of common cold, influenza, SARS, MERS, Covid-19, Dengue Virus, hepatitis C, hepatitis E, West Nile fever, Ebola virus disease, rabies, polio, mumps, measles, variants thereof, and any combination thereof, preferably wherein the DNA virus is selected from the group consisting of herpes simplex virus, cytomegalo virus, varicella zoster virus, Epstein-Barr virus, roseolo virus, human herpesvirus-7, Kaposi's sarcoma-associated virus, variants thereof, and any combination thereof.

11. The use of claim 1, wherein the medicament comprising the antigen is to be administered by mucosal delivery, preferably wherein the medicament comprising the antigen is to be administered to a mucosal tissue of the human subject, preferably wherein the mucosal tissue is selected from the group consisting of anterior nostril, nasal sinus, rectal, vaginal, esophagus, urethral, sublingual and buccal, preferably wherein the medicament is to be administered intranasally.
12. The use of claim 1, wherein the medicament comprises an adjuvant or does not comprise an adjuvant.
13. The use of claim 1, wherein the antigen is encapsulated within the lipid nanoparticle (LNP).
14. The use of claim 1, wherein the at least one cationic lipid comprises 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine (DMEPC), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and/or 1,2-dioleoyl-3-trimethylammonium propane (DOTAP).
15. The use of claim 1, wherein the lipid nanoparticle (LNP) further comprise at least one phospholipid, preferably wherein the at least phospholipid comprises 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE),

cholesterol (Chol), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholin (POPC) and/or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC).

16. The use of claim 1, wherein the human has been vaccinated against or infected by the virus about one week ago, two weeks ago, three weeks ago, one month ago, two months ago, three months ago, four months ago, five months ago, six months ago, seven months ago, eight months ago, nine months ago, ten months ago, eleven months ago, or twelve months ago.
17. Use of a pharmaceutical composition comprising at least one mRNA and a lipid nanoparticle (LNP), wherein the lipid nanoparticle (LNP) comprises at least one cationic lipid and/or a poly(amine-co-ester) (PACE) polymer, in the manufacture of a medicament for enhancing an immune response to SARS-CoV-2 in a human in need thereof, wherein the medicament is to be administered at a mucosal site, and wherein the human has been previously vaccinated against or infected by a virus.
18. The use of claim 17, wherein the human has been previously vaccinated with one or more COVID-19 vaccines selected from the group consisting of BNT162b2 (Pfizer/BioNTech), mRNA-1273 (Moderna), AZD1222/ChAdOx1 (AstraZeneca/Oxford Univ), Ad5-vectored COVID-19 vaccine (CanSino Biologies), CoronaVac (Sinovac), NVX-CoV2373 (Novavax), and combinations thereof.
19. The use of claim 17, wherein the at least one mRNA encodes the spike protein of SARS-CoV-2 or variants thereof or a fragment thereof.
20. The use of claim 17, wherein the medicament comprises mRNAs encoding two or more different antigens, preferably wherein the two or more antigens are spike proteins of SARS-CoV-2 or variants thereof or a fragment thereof.
21. The use of claim 20, wherein the two or more antigens comprise at least one mutation listed in Table 1.
22. The use of claim 17, wherein the medicament comprises an adjuvant or does not comprise an adjuvant.
23. The use of claim 17, wherein the at least one mRNA is encapsulated within the lipid nanoparticle (LNP).
24. The use of claim 17, wherein the at least one cationic lipid comprises 1,2-dimyristoyl-sn-glycero-3-ethylphosphocholine (DMEPC), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and/or 1,2-dioleoyl-3-trimethylammonium propane (DOTAP).

25. The use of claim 17, wherein the lipid nanoparticle (LNP) further comprise at least one phospholipid, preferably wherein the at least phospholipid comprises 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol (Chol), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholin (POPC) and/or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC).
26. The use of claim 17, wherein the lipid nanoparticle has an average diameter in the range of from about 50 nm to about 1000 nm, preferably wherein the lipid nanoparticle has an average diameter in the range of from about 50 nm to about 400 nm, from about 50 nm to about 200 nm, from about 200 nm to about 1000 nm, from about 200 nm to about 800 nm, or from about 300 nm to about 600 nm.
27. The use of claim 1, wherein the immune response is a mucosal immune response, preferably wherein the mucosal immune response is an antigen-specific IgA antibody production, an antigen-specific IgG antibody production, or an antigen-specific IgM antibody production.
28. The use of claim 17, wherein the human has elevated neutralizing antibody levels caused by a previous vaccination against the virus or a previous infection of the virus selected from the group consisting of MERS-CoV, SARS-CoV -1, SARS-Cov-2, and variants thereof.
29. The use of claim 28, wherein the elevated neutralizing antibody is IgG, IgM, IgA, or combinations thereof.
30. The use of claim 29, wherein the human has elevated IgG, IgM, or IgA antibody caused by a previous vaccination against the virus or a previous infection of the virus selected from the group consisting of MERS-CoV, SARS-CoV -1, SARS-Cov-2, and variants thereof.
31. The use of claim 17, wherein the human has been vaccinated against or infected by the virus about one week ago, two weeks ago, three weeks ago, one month ago, two months ago, three months ago, four months ago, five months ago, six months ago, seven months ago, eight months ago, nine months ago, ten months ago, eleven months ago, or twelve months ago.
32. The use of claim 17, wherein the SARS-CoV-2 variant is selected from the group consisting of Alpha (B.1.1.7 and Q lineages), Beta (B.1.351 and descendent lineages), Gamma (P.1 and descendent lineages), Delta (B.1.617.2 and AY lineages), Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), 1.617.3, Mu (B.1.621, B.1.621.1), Zeta (P.2), Mu (B.1.621, B.1.621.1), Omicron (Pango lineages B.1.1.529, BA.1, BA.1.1, BA.2, BA.3), and combinations thereof.

33. The use of claim 17, wherein the at least one mRNA is N1-methyl-pseudouridine-modified mRNA, or pseudouridine-modified mRNA.