RSV F VACCINE FORMULATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/483,119, which was filed on February 3, 2023. This application is incorporated herein in its entirety for all purposes.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The contents of the electronic sequence listing (NOVV_104_01WO_SeqList_ST26.xml; Size: 852,414 bytes; and Date of Creation: February 3, 2024) are herein incorporated by reference in its entirety.

FIELD

[0003] The present disclosure is generally related to RSV F glycoproteins and nanoparticles useful for stimulating immune responses. The nanoparticles provide antigens, for example, glycoprotein antigens, associated with a detergent core and are typically produced using recombinant approaches. The nanoparticles have improved stability and enhanced epitope presentation. The disclosure also provides compositions containing the nanoparticles, methods for producing them, and methods of stimulating immune responses.

BACKGROUND OF THE INVENTION

[0004] Infectious diseases remain a problem throughout the world. While progress has been made on developing vaccines against some pathogens, many remain a threat to human health. Each year respiratory syncytial virus (RSV) causes 6000-10,000 deaths among adults 65 and older and 100-300 deaths in children younger than 5 years old. The development of vaccines to prevent or reduce the severity of life-threatening infectious diseases like RSV is desirable.

SUMMARY

[0005] The present disclosure describes non-naturally occurring respiratory syncytial virus (RSV) fusion (F) glycoproteins suitable for inducing immune responses against respiratory syncytial virus (RSV). The disclosure also describes nanoparticles

containing the glycoproteins as well as methods of stimulating immune responses against RSV.

[0006] Described herein are RSV F glycoproteins comprising an F1 domain, an F2 domain, and p27; wherein the glycoprotein comprises one or more modifications selected from the group consisting of: (a) deletion of one or more of amino acids 137-146; (b) an inactivated primary furin cleavage site; (c) an inactivated secondary furin cleavage site; (d) S155C; (e) S290C; (f) S190F; (g) V207L; and (h) N116Q; wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330. In examples, the RSV F glycoproteins comprise S155C and S290C mutations. In examples, the RSV F glycoprotein comprise S190F and V207L mutations. In examples, the RSV F glycoproteins comprise S155C, S290C, S190F, and V207L mutations. In examples, the RSV F glycoproteins comprise a N116Q mutation. In examples, the RSV F glycoproteins comprise an inactivated primary furin cleavage site having the amino acid sequence of KKQKQQ (SEQ ID NO: 342), an S190F mutation, a V207 mutation, and deletion of amino acids 137-146. In examples, the RSV F glycoproteins comprise an inactivated primary furin cleavage site having the amino acid sequence of KKQKQQ (SEQ ID NO: 342), an S190F mutation, a V207 mutation, and deletion of amino acids 137-146, wherein the RSV F glycoprotein has at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of SEQ ID NO: 337. In examples, the RSV F glycoproteins comprise: (i) an F1 domain having an amino acid sequence with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to the F1 domain of SEQ ID NO: 352; and (ii) an F2 domain having an amino acid sequence with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to the F2 domain of SEQ ID NO: 357. In examples, the RSV F glycoprotein is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 % at least 85 %, at least 86 %, at least 87 %, at least 88 %, at least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to any one of SEQ ID NOS: 333-340 and 364-366. In examples, the RSV F glycoprotein is at least 80 %, at least 81 %, at least 82 %, at least 83 %, at least 84 % at least 85 %, at least 86 %, at least 87 %, at least 88 %, at

least 89 %, at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to the RSV F glycoprotein of SEQ ID NO: 337.

[0006a] In one aspect, the present disclosure refers to an RSV F glycoprotein, comprising an F1 domain, an F2 domain, and p27; wherein the glycoprotein comprises (i) a deletion of one or more of amino acids 137-146; (ii) an inactivated primary furin cleavage site; and (iii) one or more modifications selected from the group consisting of: (a) an inactivated secondary furin cleavage site; (b) S155C; (c) S290C; (d) S190F; (e) V207L; and (f) N116Q; wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330.

[0006b] In another aspect, the present disclosure refers to a nucleic acid comprising a nucleic acid sequence capable of encoding the RSV F glycoprotein described herein.

[0006c] In yet another aspect, the present disclosure refers to a vector comprising the nucleic acid as described herein.

[0006d] In a further aspect, the present disclosure refers to a cell expressing the RSV F glycoprotein described herein.

[0006e] In another aspect, the present disclosure refers to an immunogenic composition comprising: (i) at least one RSV F glycoprotein as described herein; and (ii) a pharmaceutically acceptable buffer.

[0006f] In yet another aspect, the present disclosure refers to a prefilled syringe containing the immunogenic composition as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] Fig. 1 shows that binding of the BV2279 glycoprotein to antibodies recognizing the site II (palivizumab), site IV (RSV.42, referred to as “RSV.42.2”), site ϕ (D25), and site VIII (hRSV90) epitopes.

[0008] Fig. 2 shows anti-RSV F IgG titers in cotton rat sera 42 days after immunization with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2.

[0009] Fig. 3 shows the RSV neutralization titers against the RSV A (Long) and RSV B (18537) strains after immunization with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2.

[0010] Fig. 4 shows competing antibody equivalent titers induced by immunization with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2.

[0011] Fig. 5 shows the RSV viral titers in cotton rat tissue four days post viral challenge. The rats were immunized with either the BV2279 (labeled “prefusion RSV F Cav1”) or BV1184 (labeled “prefusogenic RSV F”) vaccine compositions of Example 2.

[0012] Fig. 6 shows transmission electron microscopy (TEM) images of influenza HA detergent core nanoparticles alone (left), saponin adjuvant (i.e., a first iscom particle containing Fraction A of *Quillaja Saponaria* Molina and not Fraction C of *Quillaja Saponaria* Molina, and a second iscom particle containing Fraction C of *Quillaja Saponaria* Molina and not Fraction A of *Quillaja Saponaria* Molina, wherein Fraction A accounts for 85 % of the sum of the weights of fraction A of *Quillaja Saponaria* Molina and fraction C of *Quillaja Saponaria* Molina in the adjuvant, and fraction C of *Quillaja Saponaria* Molina accounts for the remainder) (middle), and Hemagglutinin Saponin Matrix Nanoparticles (HaSMaNs) (right).

[0013] Fig. 7A shows the primary structure of a wild-type SARS-CoV-2 S polypeptide, containing a signal peptide, numbered with respect to SEQ ID NO: 1. **Fig. 7B** shows the primary structure of a wild-type SARS-CoV-2 S polypeptide, without a signal peptide, numbered with respect to SEQ ID NO: 2.

CLAIMS

1. A respiratory syncytial virus (RSV) fusion (F) glycoprotein, comprising an F1 domain, an F2 domain, and p27; wherein the glycoprotein comprises (i) a deletion of one or more of amino acids 137-146; (ii) an inactivated primary furin cleavage site; and (iii) one or more modifications selected from the group consisting of:

(a) an inactivated secondary furin cleavage site;

(b) S155C;

(c) S290C;

(d) S190F;

(e) V207L; and

(f) N116Q;

wherein the modifications are numbered according to the RSV F glycoprotein of SEQ ID NO: 330.

2. The RSV F glycoprotein of claim 1, comprising a deletion of all of amino acids 137-146.

3. The RSV F glycoprotein of claim 1 or 2, wherein the inactivated primary furin cleavage site comprises an amino acid sequence selected from the group consisting of: SEQ ID NOS: 342-345.

4. The RSV F glycoprotein of claim 1 or 2, wherein the RSV F glycoprotein comprises S155C and S290C mutations, comprises S190F and V207L mutations, or comprises S155C, S290C, S190F, and V207L mutations.

5. The RSV F glycoprotein of claim 1 or 2, wherein the RSV F glycoprotein comprises a N116Q mutation.

6. The RSV F glycoprotein of claim 1, wherein the inactivated primary furin cleavage site has the amino acid sequence of KKQKQQ (SEQ ID NO: 342), and the RSV F glycoprotein comprises an S190F mutation, a V207 mutation, and deletion of amino acids 137-146.

7. The RSV F glycoprotein of claim 6, wherein the RSV F glycoprotein has at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of SEQ ID NO: 337.

8. The RSV F glycoprotein of claim 1 or 2, wherein the F1 domain is at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to a F1 domain of any one of SEQ ID NOS: 350-355, or wherein the F2 domain is at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to a F2 domain of any one of SEQ ID NOS: 356-357.

9. The RSV F glycoprotein of claim 1 or 2, wherein the p27 domain is at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to a p27 domain of any one of SEQ ID NOS: 358-359.

10. The RSV F glycoprotein of claim 1 or 2, wherein the RSV F glycoprotein is at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identical to any one of SEQ ID NOS: 333-340 and 364-366.

11. The RSV F glycoprotein of claim 1 or 2, wherein the p27 and F1 domain form a single subunit called p27-F1, and wherein p27-F1 and the F2 subunit are connected by a disulfide bond.

12. The RSV F glycoprotein of claim 1 or 2, wherein the RSV F glycoprotein comprises a signal peptide, the signal peptide comprising the sequence of MELLILKANAITTILTAVTFCFASG (SEQ ID NO: 360) or MELLIHRLSAIFLTL (SEQ ID NO: 367).

13. A nucleic acid comprising a nucleic acid sequence capable of encoding the RSV F glycoprotein of claim 1 or 2.

14. A vector comprising the nucleic acid of claim 13.

15. A cell expressing the RSV F glycoprotein of claim 1 or 2.

16. An immunogenic composition comprising:
(i) at least one RSV F glycoprotein of claim 1 or 2; and
(ii) a pharmaceutically acceptable buffer.

17. The immunogenic composition of claim 16, wherein the RSV F glycoprotein is comprised in a detergent-core nanoparticle.

18. The immunogenic composition of claim 17, wherein the detergent-core nanoparticle comprises detergent selected from the group consisting of polysorbate 20 (PS20), PS40, PS60, PS65, and PS80.

19. The immunogenic composition of claim 18, wherein the detergent comprises PS80.

20. The immunogenic composition of claim 16, wherein the pharmaceutically acceptable buffer comprises sodium phosphate, sodium chloride, and/or histidine.

21. The immunogenic composition of claim 16, wherein the pharmaceutically acceptable buffer has a pH in the range 5.8 to 7.0.

22. The immunogenic composition of claim 16, further comprising a saponin-based adjuvant.

23. The immunogenic composition of claim 22, wherein the saponin-based adjuvant comprises Fraction A and Fraction C separated from an aqueous Quillaja Saponaria Molina extract, fraction A of Quillaja Saponaria Molina and fraction C of Quillaja Saponaria Molina accounting for 85 % by weight and 15 % by weight,

respectively, of the sum of weights of fraction A of Quillaja Saponaria Molina and fraction C of Quillaja Saponaria Molina in the adjuvant.

24. The immunogenic composition of claim 23, wherein Fraction A and Fraction C are each present in the composition in the form of cage-like particles complexed with a lipid.

25. The immunogenic composition of claim 16, comprising two, three, four, five, six, seven, eight, nine, or ten different RSV F glycoproteins.

26. The immunogenic composition of claim 25, comprising
(i) a first RSV F glycoprotein with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of any one of SEQ ID NOS: 337-340; and
(ii) a second RSV F glycoprotein with at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of any one of SEQ ID NOS: 364-366.

27. The immunogenic composition of claim 26, wherein the
first RSV F glycoprotein has at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of SEQ ID NO: 337.

28. The immunogenic composition of claim 26, wherein the second RSV F glycoprotein has at least 90 %, at least 91 %, at least 92 %, at least 93 %, at least 94 %, at least 95 %, at least 96 %, at least 97 %, at least 98 %, at least 99 %, or 100 % identity to an RSV F glycoprotein of SEQ ID NO: 364.

29. The immunogenic composition of claim 16, comprising from 30 µg to 300 µg of the RSV F glycoprotein.

30. A prefilled syringe containing the immunogenic composition of claim 16.