

IMMUNOGENIC CORONAVIRUS FUSION PROTEINS AND RELATED METHODS

BACKGROUND

[0001] Coronaviruses (CoV) are a large family of viruses that cause human illness ranging from the common cold to more severe diseases, such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). Coronaviruses are zoonotic, meaning they can be transmitted between animals and humans. Coronaviruses are large, enveloped, single-stranded RNA viruses having a characteristic crown, or corona, around the virions, due to the surface of the virus particle being covered in well-separated, petal-shaped glycoprotein “spikes,” having a diameter of 80-160 nm, that project from the virions. Spike glycoprotein is a Class I viral fusion protein located on an outer envelope of the virion. Spike protein plays an important role in viral infection by interacting with host cell receptors.

[0002] Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the strain of coronavirus that causes so-called coronavirus disease 2019 (COVID-19), a respiratory illness. SARS-CoV-2 has spread throughout the world and has already resulted in over 16 million cases of COVID-19 and over 600 thousand deaths. SARS-CoV-2 can enter eukaryotic cells via endosomes or plasma membrane fusion. In both routes, spikes on the virion surface bind to the membrane-bound protein Angiotensin-converting enzyme 2 (ACE2) and mediate attachment to the membrane of and entry into a host cell. SARS-CoV-2 is highly infectious and primarily spreads between people through close contact and via respiratory droplets. Long-term control of SARS-CoV-2 will require an effective vaccine that can be made widely available across the globe.

SUMMARY

[0003] The terms “invention,” “the invention,” “this invention” and “the present invention,” as used in this document, are intended to refer broadly to all of the subject matter of this patent application and the claims below. Statements containing these terms should be understood not to limit the subject matter described herein or to limit the meaning or scope of the patent claims below. Covered ~~embodiments~~ examples of the invention-disclosure are defined by the claims, not this summary. This summary is a high-level overview of various aspects of the ~~invention-disclosure~~ and introduces some of the concepts that are described and illustrated in the present document and the accompanying figures. This summary is not intended to identify key or essential

features of the claimed subject matter, nor is it intended to be used in isolation to determine the scope of the claimed subject matter. The subject matter should be understood by reference to appropriate portions of the entire specification, any or all figures and each claim. Some of the exemplary ~~embodiment~~examples of the present ~~invention~~disclosure are discussed below.

[0003a] In one aspect, included among the aspects of the present disclosure is a fusion protein of an artificially modified amino acid sequence of a Spike protein of a coronavirus and an amino acid sequence of a ferritin subunit polypeptide, wherein the artificially modified amino acid sequence of the Spike protein is a sequence with at least 90% sequence identity to SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:14, or SEQ ID NO:15.

[0003b] In another aspect, provided herein is a nanoparticle comprising an oligomer of the fusion protein of the disclosure.

[0003c] In another aspect, provided herein is a nucleic acid encoding the fusion protein of the disclosure.

[0003d] In another aspect, provided herein is vector comprising the nucleic acid of the disclosure.

[0003e] In another aspect, provided herein is a cell comprising the nucleic acid of the disclosure or the vector of the disclosure.

[0003f] In another aspect, provided herein is an immunogenic composition comprising the fusion protein of the disclosure, the nanoparticle of the disclosure, the nucleic acid of the disclosure, or the vector of the disclosure.

[0003g] In another aspect, provided herein is an immunogenic composition comprising two or more different fusion proteins of the disclosure, two or more different nanoparticles of the disclosure, two or more different nucleic acids of the disclosure, or two or more different vectors of the disclosure.

[0003h] In another aspect, provided herein is a kit comprising the immunogenic composition of the disclosure and one or more of: a device for administering the immunogenic composition, and an excipient.

[0003i] In another aspect, provided herein is a use of the immunogenic composition of the disclosure in the manufacture of a medicament for inducing an immune response in a subject.

[0003j] In another aspect, provided herein is a method of producing the fusion protein, comprising: introducing into a cell the nucleic acid of the disclosure or the vector of the

disclosure; incubating the cell under conditions allowing for expression of the fusion protein; and, isolating the fusion protein.

[0003k] In another aspect, provided herein is a method of producing a nanoparticle, comprising: introducing into a cell the nucleic acid of the disclosure or the vector of the disclosure; incubating the cell under conditions allowing for expression of the fusion protein and self-assembly of the nanoparticle; and, isolating the nanoparticle.

[0004] Included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are fusion proteins of an artificially modified amino acid sequence of a Spike protein of a coronavirus and an amino acid sequence of a ferritin subunit polypeptide. In some exemplary ~~embodiment~~examples, the artificially modified amino acid sequence of the Spike protein is an artificially modified amino acid sequence of an ectodomain of the Spike protein with at least 90% sequence identity to SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:14, or SEQ ID NO:15. In some exemplary ~~embodiment~~examples, the coronavirus is SARS-CoV-2. In some exemplary ~~embodiment~~examples, the artificially modified amino acid sequence of the Spike protein of the coronavirus comprises a C-terminal deletion of at least an amino acid sequence of heptad repeat 2 (HR2). In some exemplary ~~embodiment~~examples, the artificially modified amino acid sequence of the Spike protein of the coronavirus comprises a mutation eliminating a furin recognition site. In some exemplary ~~embodiment~~examples, the artificially modified amino acid sequence of the Spike protein of the coronavirus comprises one or more mutations stabilizing the Spike protein a pre-fusion conformation. The ferritin subunit polypeptide can be *Helicobacter pylori* ferritin subunit polypeptide. In some exemplary ~~embodiment~~examples, the amino acid sequence of the ferritin subunit polypeptide is a sequence with at least 90% sequence identity to SEQ ID NO:2. In some exemplary ~~embodiment~~examples, the ferritin subunit polypeptide contains one or more (i.e. at least one) artificial glycosylation sites. In some exemplary ~~embodiment~~examples, the artificially modified amino acid sequence of the Spike protein of the coronavirus is joined to the amino acid sequence of the ferritin subunit polypeptide by a linker amino acid sequence. In some exemplary ~~embodiment~~examples, the amino acid sequence of the fusion protein is a sequence with at least 90% sequence identity to SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:33, or SEQ ID NO:34.

[0005] Also included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are nanoparticles comprising oligomers of the fusion proteins according to the ~~embodiment~~examples of the present ~~invention~~disclosure. The nanoparticles according to the ~~embodiment~~examples of the present ~~invention~~disclosure comprise surface-exposed trimers of an ectodomain of the Spike

protein of the coronavirus. In some exemplary ~~embodiment~~examples, each nanoparticle comprises eight of the surface-exposed trimers of the ectodomain of the Spike protein of the coronavirus. Also included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are nucleic acids encoding the fusion protein according to the ~~embodiment~~examples of the present ~~invention~~disclosure. The nucleic acids according to the ~~embodiment~~examples of the present ~~invention~~disclosure can be DNA or RNA. Also included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are vectors comprising the nucleic acids according to the ~~embodiment~~examples of the present ~~invention~~disclosure. Also included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are cells comprising the nucleic acids according to the ~~embodiment~~examples of the present ~~invention~~disclosure, or the vectors according to the ~~embodiment~~examples of the present ~~invention~~disclosure. Also included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are methods of producing fusion proteins according to the ~~embodiment~~examples of the present ~~invention~~disclosure. A method of producing a fusion proteins can comprise the steps of: introducing into a cell a nucleic acid according to the ~~embodiment~~examples of the present ~~invention~~disclosure, or a vector according to the ~~embodiment~~examples of the present ~~invention~~disclosure; incubating the cell under conditions allowing for expression of the fusion protein; and, isolating the fusion protein. Also included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are methods of producing nanoparticles according to the ~~embodiment~~examples of the present ~~invention~~disclosure. A method of producing a nanoparticle can comprise the steps of: introducing into a cell a nucleic acid according to the ~~embodiment~~examples of the present ~~invention~~disclosure, or a vector according to the ~~embodiment~~examples of the present ~~invention~~disclosure; incubating the cell under conditions allowing for expression of a fusion protein according to the ~~embodiment~~examples of the present ~~invention~~disclosure and self-assembly of the nanoparticle; and, isolating the nanoparticle.

[0006] Also included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are immunogenic compositions comprising the fusion proteins according to the ~~embodiment~~examples of the present ~~invention~~disclosure, the nanoparticles according to the ~~embodiment~~examples of the present ~~invention~~disclosure, the nucleic acids according to the ~~embodiment~~examples of the present ~~invention~~disclosure, or the vectors according to the ~~embodiment~~examples of the present ~~invention~~disclosure. In some exemplary ~~embodiment~~examples, an immunogenic composition comprises two or more different fusion proteins according to the ~~embodiment~~examples of the present ~~invention~~disclosure, two or more different nanoparticles according to the ~~embodiment~~examples of the present ~~invention~~disclosure, two or more different nucleic acids according to the ~~embodiment~~examples of the present ~~invention~~disclosure, or two or more different

vectors according to the ~~embodiment~~examples of the present ~~invention~~disclosure. The immunogenic compositions can further comprise one or more adjuvants (i.e. at least one), which may comprise alum. In some exemplary ~~embodiment~~examples, the immunogenic compositions are lyophilized. Also included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are kits comprising an immunogenic composition according to one or more of the ~~embodiment~~examples of the present ~~invention~~disclosure, and one or more of: a device for administering the immunogenic composition, and an excipient.

[0007] Also included among the ~~embodiment~~examples of the present ~~invention~~disclosure and described in the present disclosure are methods of inducing an immune response in a subject, the method comprising the step of administering to the subject an immunogenic composition according to the ~~embodiment~~examples of the present ~~invention~~disclosure. In such methods, an immunogenic composition can be administered in an amount capable of eliciting a protective immune response against the coronavirus in the subject. The immune response can comprise production of neutralizing antibodies against the coronavirus in the subject. In methods of inducing an immune response in a subject according to the ~~embodiment~~examples of the present ~~invention~~disclosure, the subject can be a human.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The present disclosure includes the following figures. The figures are intended to illustrate certain embodiments and/or features of the compositions and methods, and to supplement any description(s) of the compositions and methods. The figures do not limit the scope of the compositions and methods, unless the written description expressly indicates that such is the case.

[0009] FIGURE 1A is a schematic illustration of SARS-CoV-2 Spike protein antigen polypeptide constructs according to certain aspects of this disclosure.

[0010] FIGURE 1B is a schematic illustration of three-dimensional structures of SARS-CoV-2 Spike protein antigen polypeptides according to certain aspects of this disclosure, which are based on the structures of Spike trimers determined by cryogenic electron microscopy (cryo-EM) and the structure of *Helicobacter pylori* ferritin nanoparticles determined by X-ray crystallography.

[0011] FIGURE 2A shows a photographic image of the Western blot illustrating the results of expression and characterization of SARS-CoV-2 Spike protein antigens according to certain aspects of this disclosure.

WHAT IS CLAIMED IS:

1. A fusion protein of an artificially modified amino acid sequence of a Spike protein of a coronavirus and an amino acid sequence of a ferritin subunit polypeptide, wherein the artificially modified amino acid sequence of the Spike protein is a sequence with at least 90% sequence identity to SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:14, or SEQ ID NO:15.
2. The fusion protein of claim 1, wherein the coronavirus is SARS-CoV-2.
3. The fusion protein of claim 1 or 2, wherein the artificially modified amino acid sequence of the Spike protein of the coronavirus comprises a C-terminal deletion of at least an amino acid sequence of heptad repeat 2 (HR2).
4. The fusion protein of any one of claims 1 to 3, wherein the artificially modified amino acid sequence of the Spike protein of the coronavirus comprises a mutation eliminating a furin recognition site.
5. The fusion protein of any one of claims 1 to 4, wherein the artificially modified amino acid sequence of the Spike protein of the coronavirus comprises one or more mutations stabilizing the Spike protein a pre-fusion conformation.
6. The fusion protein of any one of claims 1 to 5, wherein the ferritin subunit polypeptide is *Helicobacter pylori* ferritin subunit polypeptide.
7. The fusion protein of any one of claims 1 to 6, wherein the amino acid sequence of the ferritin subunit polypeptide is a sequence with at least 90% sequence identity to SEQ ID NO:2.
8. The fusion protein of any one of claims 1 to 7, wherein the ferritin subunit polypeptide contains one or more artificial glycosylation sites.

9. The fusion protein of any one of claims 1 to 8, wherein the artificially modified amino acid sequence of the Spike protein of the coronavirus is joined to the amino acid sequence of the ferritin subunit polypeptide by a linker amino acid sequence.
10. The fusion protein of any one of claims 1 to 9, wherein the amino acid sequence of the fusion protein is a sequence with at least 90% sequence identity to SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:33, or SEQ ID NO:34.
11. A nanoparticle comprising an oligomer of the fusion protein of any one of claims 1-10.
12. The nanoparticle of claim 11, wherein the nanoparticle comprises surface-exposed trimers of an ectodomain of the Spike protein of the coronavirus.
13. The nanoparticle of claim 12, wherein the nanoparticle comprises eight of the surface-exposed trimers of the ectodomain of the Spike protein of the coronavirus.
14. A nucleic acid encoding the fusion protein of any one of claims 1 to 10.
15. The nucleic acid of claim 14, wherein the nucleic acid is DNA or RNA.
16. A vector comprising the nucleic acid of claim 14 or 15.
17. A cell comprising the nucleic acid of claim 14 or 15 or the vector of claim 14.
18. An immunogenic composition comprising the fusion protein of any one of claims 1-9, the nanoparticle of any one of claims 10 to 12, the nucleic acid of claim 13 or 14, or the vector of claim 15.
19. An immunogenic composition comprising two or more different fusion proteins of any one of claims 1 to 9, two or more different nanoparticles of any one of claims

10 to 12, two or more different nucleic acids of claim 13 or 14, or two or more different vectors of claim 15.

20. The immunogenic composition of claim 18 or 19, further comprising one or more adjuvants.

21. The immunogenic composition of any one of claims 18 to 20, wherein the one or more adjuvants comprise alum.

22. The immunogenic composition of claim 18 or 20, wherein the immunogenic composition is lyophilized.

23. A kit comprising the immunogenic composition of any one of claims 18 to 22 and one or more of: a device for administering the immunogenic composition, and an excipient.

24. ~~A method of inducing an immune response in a subject, the method comprising administering to the subject~~Use of the immunogenic composition of any one of claims 18 to 22 in the manufacture of a medicament for inducing an immune response in a subject.

25. The ~~method-use~~ of claim 24, wherein the immunogenic composition is to be administered in an amount capable of eliciting a protective immune response against the coronavirus in the subject.

26. The ~~method-use~~ of claim 25, wherein the protective immune response comprises production of neutralizing antibodies against the coronavirus in the subject.

27. The ~~method-use~~ of any one of claim 24 to 26, wherein the subject is a human.

28. A method of producing the fusion protein, comprising:

introducing into a cell the nucleic acid of claim 14 or 15 or the vector of claim 15;

incubating the cell under conditions allowing for expression of the fusion protein;
and,

isolating the fusion protein.

29. A method of producing a nanoparticle, comprising:

introducing into a cell the nucleic acid of claim 14 or 15 or the vector of claim 15;

incubating the cell under conditions allowing for expression of the fusion protein and
self-assembly of the nanoparticle; and,

isolating the nanoparticle.