ALPHAVIRUS NSP MUTANTS AS VACCINES

TECHNICAL FIELD

[0001] The disclosure generally relates to the field of immunology and virology. The present disclosure relates to polypeptides, polynucleotides, expression vectors, infectious clones, virus particles, and immunogenic compositions of recombinant alphaviruses, for use in these fields, in particular as vaccines against alphavirus infection. The present disclosure also relates to methods for eliciting an immune response against alphavirus infection using the immunogenic compositions of the present disclosure.

BACKGROUND

[0002] Alphaviruses are a genus of enveloped, RNA viruses that cause diseases in humans and animals, with symptoms such as fever, rash and arthritis. The alphavirus genome encodes nonstructural (nsP) and structural proteins. More specifically, the genome encodes four nsPs which are involved in virus replication and pathogenesis, and five structural proteins that make up the virion. Substitutions of amino acids at functionally coupled sites in the nsPs were previously shown to affect polypeptide processing and controlled the virulence of the alphavirus Semliki Forest Virus (SFV).

[0003] Another alphavirus is Chikungunya virus (CHIKV), an arthropod-borne alphavirus that causes acute febrile illness and chronic debilitating polyarthralgia. The interferon-α/β receptor (IFNAR) dependent Type 1 interferon (IFN) signaling pathway has been shown to be critical in CHIKV induced anti-viral mechanisms. Yet another alphavirus is O'nyong'nyong Virus (ONNV), which is also an arthropod-borne alphavirus that causes symptoms such as fever, rash, headache and arthralgia.

[0004] There are presently no known commercial vaccines to prevent such alphavirus infections, such as CHIKV and ONNV infections, or medicine for treating such infections. Therefore, there is a need to provide vaccines against such alphavirus infections, and methods for eliciting an immune response against the alphavirus infections.

SUMMARY

[0005] In one example, there is provided a recombinant polypeptide comprising an amino acid SEQ ID NO: 1, or a variant thereof, comprising one or more mutations selected from the group consisting of: (a) a mutation at a position equivalent to amino acid position 532 of SEQ ID NO: 1, and (b) a mutation at a position equivalent to amino acid position 1050 of SEQ ID NO: 1. In one aspect, the present disclosure refers to a recombinant polypeptide comprising an amino acid sequence SEQ ID NO: 1, or a variant thereof comprising at least 80% identity to the amino acid sequence SEQ ID NO: 1, comprising one or more mutations selected from the group consisting of: (a) substitution of R with H at a position equivalent to amino acid position 532 of SEQ ID NO: 1, and (b) substitution of E with V at a position equivalent to amino acid position 1050 of SEQ ID NO: 1, wherein the mutations are attenuating mutations.

[0006] Advantageously, mutation at a position equivalent to amino acid position 532 of SEQ ID NO: 1 alone, results in higher Type 1 IFN immune response in primary mouse fibroblasts in murine

ex-vivo and *in vivo* infection models. Additionally, when the mutation at position 532 occurs simultaneously in combination with a mutation at position 1050 of SEQ ID NO: 1, both sites being functionally coupled, not only is the Type 1 IFN immune response significantly enhanced, but alphavirus harboring these nsP mutations also exhibited lower infectivity.

[0007] In another aspect, there is provided a polynucleotide encoding the polypeptide described herein.

[0008] In another aspect, there is provided an expression vector comprising the polynucleotide sequence described herein.

[0009] In another aspect, there is provided an infectious clone comprising the polynucleotide sequence described herein.

[0010] In another aspect, there is provided a recombinant virus particle comprising the polypeptide described herein.

[0011] In another aspect, there is provided a recombinant virus particle comprising the polynucleotide described herein.

[0012] In another aspect, there is provided a virus particle derived from the infectious clone described herein.

[0013] In another aspect, there is provided an immunogenic composition comprising an infectious clone, a recombinant virus particle and/or a virus particle described herein.

[0014] In another example, there is provided a method of eliciting an immune response against alphavirus infection in a subject comprising administering an immunogenic composition described herein to the subject. In one aspect, the present disclosure refers to use of an immunogenic composition as disclosed herein in the manufacture of a medicament for eliciting an immune response against alphavirus infection in a subject. Advantageously, the method is capable of providing protection to subjects administered with the immunogenic composition against wild-type (WT) alphavirus infection, as well as re-infection upon subsequent exposure to alphavirus.

DEFINITION OF TERMS

[0015] The following words and terms used herein shall have the meaning indicated:

[0016] The term "polypeptide" refers to any polymer of amino acids (dipeptide or greater) linked through peptide bonds or modified peptide bonds, whether produced naturally or synthetically.

[0017] The term "polynucleotide" includes a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally occurring nucleotides. The terms “polynucleotide”, “nucleic acid”, “nucleic acid molecule”, and “nucleic acid sequence” are used interchangeably herein unless the context indicates otherwise.

[0018] The term “recombinant” when used in reference to a polypeptide, polynucleotide or virus refers to a polypeptide, polynucleotide or virus that is not naturally occurring, or was made

CLAIMS

1. A recombinant polypeptide comprising an amino acid sequence SEQ ID NO: 1, or a variant thereof comprising at least 80% identity to the amino acid sequence SEQ ID NO: 1, comprising one or more mutations selected from the group consisting of:
   (a) a substitution of R with H at a position equivalent to amino acid position 532 of SEQ ID NO: 1, and
   (b) a substitution of E with V at a position equivalent to amino acid position 1050 of SEQ ID NO: 1,
   wherein the mutations are attenuating mutations.

2. The recombinant polypeptide of claim 1, wherein the amino acid SEQ ID NO: 1 is derived from a Chikungunya virus strain LR2006 OPY1.

3. The recombinant polypeptide of claim 1 or 2, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and variants thereof.

4. A polynucleotide encoding the polypeptide of any one of claims 1 to 3.

5. The polynucleotide of claim 4, wherein the polynucleotide is DNA or RNA.

6. The polynucleotide of claim 4 or 5, wherein the polynucleotide is selected from the group consisting of SEQ ID NO: 5, SEQ ID NO:6 , SEQ ID NO: 7, and variants thereof .

7. The polynucleotide of claim 6, wherein the variant is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 5, SEQ ID NO:6 and SEQ ID NO: 7.

8. An expression vector comprising the polynucleotide sequence of any one of claims 4 to 7.

9. An infectious clone comprising the polynucleotide sequence of any one of claims 4 to 7.

10. A recombinant virus particle comprising the polypeptide of any one of claims 1 to 3.

11. A recombinant virus particle comprising the polynucleotide of any one of claims 4 to 7.

12. The recombinant virus particle of claim 10 or 11, wherein the virus is an alphavirus.

13. The recombinant virus particle of claim 12, wherein the alphavirus is derived from a Chikungunya virus strain.

14. The recombinant virus particle of claim 13, wherein the Chikungunya virus strain is LR2006 OPY1.

15. The recombinant virus particle of any one of claims 10 to 14, wherein the virus is a live attenuated virus.

16. A virus particle derived from the infectious clone of claim 9.

17. An immunogenic composition comprising an infectious clone of claim 9, a recombinant virus particle of any one of claims 10 to 15 and/or a virus particle of claim 16.

18. The immunogenic composition of claim 17, wherein the recombinant virus is a live attenuated Chikungunya virus.

19. The immunogenic composition of claim 17 or 18 further comprising an adjuvant, a preservative, a stabilizer and/or a pharmaceutically acceptable carrier.

20. The immunogenic composition of claim 19, wherein the adjuvant is selected from the group consisting of (a) aluminum hydroxide, (b) aluminum phosphate, (c) gamma inulin, (d) algammulin (a combination of aluminum hydroxide and gamma inulin), (e) cholecalciferol in oil, (f) an oil in water emulsion OWEM1, containing squalene, tween-80, Span-85 in 10 mM phosphate-citrate buffer, (f) oil in water emulsion OWEM2 containing squalene, tween-80, Span-85, alpha tocopherol in phosphate- citrate buffer, and (g) an oil in water emulsion OWEM3 containing squalene, tween-80, Span-85, cholecalciferol in phosphate-citrate buffer.

21. The immunogenic composition of any one of claims 17 to 20 further comprising the expression vector of claim 8.

22. Use of an immunogenic composition of any one of claims 17 to 21 in the manufacture of a medicament for eliciting an immune response against alphavirus infection in a subject.

23. The use of claim 22, wherein the subject is a human or non-human primate at risk of Chikungunya virus infection.

24. The use of claim 22, wherein the subject is a human or non-human primate at risk of O'nyong'nyong virus infection.

25. The use any one of claims 22 to 24, wherein the immunogenic composition is to be administered by a route selected from the group consisting of intramuscular, intradermal, subcutaneous, intravenous, oral, and intranasal administration.