

It is claimed:

1. An immunogen, comprising:
a recombinant Mumps virus (MuV) F ectodomain trimer stabilized in a prefusion conformation by one or more amino acid substitutions in protomers of the trimer, the amino acid substitutions comprising cysteine substitutions that form a non-natural disulfide bond to stabilize the MuV F ectodomain trimer in the prefusion conformation.
2. The immunogen of claim 1, wherein the cysteine substitutions are located at one or more of MuV F positions 86 and 215, positions 155 and 161, positions 165 and 231, positions 206 and 223, positions 209 and 214, and positions 221 and 255.
3. The immunogen of claim 1 or claim 2, wherein the recombinant MuV F ectodomain trimer is stabilized in the prefusion conformation by a non-natural disulfide bond between the cysteine substitutions in protomers of the trimer at MuV F positions 206 and 223.
4. The immunogen of any one of the prior claims, wherein the cysteine substitutions at:
MuV F positions 86 and 215 are N86C and A215C substitutions;
MuV F positions 155 and 161 are K155C and L161C substitutions;
MuV F positions 165 and 231 are V165C and M231C substitutions;
MuV F positions 206 and 223 are V206C and A223C substitutions;
MuV F positions 209 and 214 are P209C and P214C substitutions; and
MuV F positions 221 and 255 are I221C and M255C substitutions.
5. The immunogen of any one of the prior claims, wherein the protomers of the recombinant MuV F ectodomain trimer comprise a F₂ protein comprising or consisting of MuV F positions 20-100 and a F₁ ectodomain comprising or consisting of MuV F positions 104-469, 104-476, or 104-483.
6. The immunogen of any one of the prior claims, wherein the protomers of the trimer further comprise a mutation to remove a F1/F2 furin cleavage site of the MuV F ectodomain and optionally an a first residue of a fusion peptide of a F1 ectodomain.
7. The immunogen of claim 6, wherein the mutation to remove the F1/F2 furin cleavage site and the first residue of the fusion peptide comprise a deletion of MuV F positions 101-103 with positions 100 and 104 fused by a peptide linker.
8. The immunogen of claim 7, wherein the peptide linker is a Gly-Gly-Gly linker.
9. The immunogen of any one of the prior claims, wherein the positioning of the amino acid substitutions is according to a reference MuV F proteins sequence set forth as SEQ ID NO: 1.

10. The immunogen of any one of the prior claims, wherein the protomers of the MuV F ectodomain trimer comprise an amino acid sequence at least 90% identical to
residues 20-483 of any one of SEQ ID NOs: 3-8,
residues 20-476 of any one of SEQ ID NOs: 11-16, 26, or 51, or
residues 20-469 of any one of SEQ ID NOs: 19-24; and
wherein the protomers comprise the one or more amino acid substitutions that stabilize the MuV F ectodomain trimer in the prefusion conformation.

11. The immunogen of claim 10, wherein the protomers of the MuV F ectodomain trimer comprise or consist of the amino acid sequence set forth as
residues 20-483 of any one of SEQ ID NOs: 3-8,
residues 20-476 of any one of SEQ ID NOs: 11-16, 26, or 51, or
residues 20-469 of any one of SEQ ID NOs: 19-24.

12. The immunogen of any one of the prior claims, wherein the protomers of the recombinant MuV F ectodomain trimer are fused C-terminally to a trimerization domain.

13. The immunogen of claim 12, wherein the trimerization domain comprises a GCN4 trimerization domain, a T4 fibrin trimerization domain, or both.

14. The immunogen of claim 13, wherein
the GCN4 trimerization domain comprises an amino acid sequence set forth as
IEDKIEEILSKIYHIENEIARIKKLIGGEAP (SEQ ID NO: 33).
the T4 fibrin trimerization domain comprises an amino acid sequence set forth as
GYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 34).
the trimerization domain comprising both the GCN4 and T4 fibrin trimerization domains
comprises an amino acid sequence set forth as
IEDKIEEILSKIYHIENEIARIKKLIGGEAPGSGYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 35).

15. The immunogen of any one of claims 12-14, wherein the protomers of the MuV F ectodomain trimer fused to the trimerization domain comprise an amino acid sequence at least 90% identical to
residues 20-513 of any one of SEQ ID NOs: 3-8,
residues 20-506 of any one of SEQ ID NOs: 11-16, 26, or 51, or
residues 20-499 of any one of SEQ ID NOs: 19-24; and
wherein the protomers comprise the one or more amino acid substitutions that stabilize the MuV F ectodomain trimer in the prefusion conformation.

16. The immunogen of claim 15, wherein the protomers of the MuV F ectodomain trimer fused to the trimerization domain comprise or consist of the amino acid sequence set forth as

residues 20-513 of any one of SEQ ID NOs: 3-8,
residues 20-506 of any one of SEQ ID NOs: 11-16, 26, or 51, or
residues 20-499 of any one of SEQ ID NOs: 19-24.

17. The immunogen of any one of the prior claims, wherein the protomers of the recombinant MuV F ectodomain trimer are linked to a heterologous protein.

18. The immunogen of claim 17, wherein the heterologous protein is an ectodomain head or ectodomain stalk and head of a MeV H protein or a MuV HN protein.

19. The immunogen of claim 18, wherein the ectodomain head or ectodomain stalk and head of the MeV H protein or the MuV HN protein is fused C-terminally to a trimerization domain that is fused C-terminally to the protomer of the recombinant MuV F ectodomain trimer.

20. The immunogen of claim 19, wherein the protomers of the MuV F ectodomain trimer linked to the trimerization domain and the ectodomain of the MeV H protein or the ectodomain of a MuV HN protein comprise an amino acid sequence set forth as residues 20-966 of SEQ ID NO: 27, residues 21-981 of SEQ ID NO: 28, or residues 20-1006 of SEQ ID NO: 29.

21. An immunogen, comprising:

a recombinant Measles virus (MeV) F ectodomain trimer stabilized in a prefusion conformation by one or more amino acid substitutions in protomers of the trimer, the amino acid substitutions comprising one or more of the following:

cysteine substitutions at one or more of MeV F positions 48 and 284, positions 90 and 225, positions 141 and 270, positions 165 and 171, positions 173 and 245, positions 175 and 241, positions 212 and 236, positions 216 and 233, positions 219 and 224, positions 99 and 117, positions 100 and 117, positions 101 and 117, positions 102 and 117, and positions 103 and 117 that form a non-natural disulfide bond;

a phenylalanine substitution at MeV F position 175; and

a proline substitution at MeV F position 194.

22. The immunogen of claim 21, wherein the recombinant MeV F ectodomain trimer is stabilized in the prefusion conformation by a non-natural disulfide bond between the cysteine substitutions in protomers of the trimer at MeV F positions 165 and 171.

23. The immunogen of claim 21 or claim 22, wherein:

the cysteine substitutions at:

MeV F positions 48 and 284 are R48C and A284C substitutions;

MeV F positions 90 and 225 are A90C and I225C substitutions;

MeV F positions 141 and 270 are M141C and T270C substitutions;

MeV F positions 165 and 171 are R165C and M171C substitutions;

MeV F positions 173 and 245 are L173C and V245C substitutions;

MeV F positions 175 and 241 are V175C and D241C substitutions;

MeV F positions 212 and 236 are E212C and Y236C substitutions;

MeV F positions 216 and 233 are L216C and A233C substitutions;

MeV F positions 219 and 224 are P219C and P224C substitutions;

MeV F positions 99 and 117 are R99C-V117C substitutions;

MeV F positions 100 and 117 are P100C-V117C substitutions;

MeV F positions 101 and 117 are V101C-V117C substitutions;

MeV F positions 102 and 117 are Q102C-V117C substitutions;

MeV F positions 103 and 117 are S103C-V117C substitutions;

the phenylalanine substitution at MeV F position 175 is a V175F substitution; and

the proline substitution at MeV F position 194 is a S194P substitution.

24. The immunogen of any one of claims 21-23, wherein the protomers of the recombinant MeV F ectodomain trimer comprise a F₂ protein comprising or consisting of MeV F positions 24-110 and a F₁ ectodomain comprising or consisting of MeV F positions 114-486.

25. The immunogen of any one of claims 21-24, wherein the protomers of the trimer further comprise a mutation to remove a F1/F2 furin cleavage site of the MeV F ectodomain and optionally a first residue of a fusion peptide of a F1 ectodomain.

26. The immunogen of claim 25, wherein the mutation to remove the F1/F2 furin cleavage site and the first residue of the fusion peptide comprise a deletion of MeV F positions 111-113 with positions 110 and 114 fused by a peptide linker.

27. The immunogen of claim 26, wherein the peptide linker is a Gly-Gly-Gly linker.

28. The immunogen of any one of claims 21-27, wherein the positioning of the amino acid substitutions is according to a reference MeV F proteins sequence set forth as SEQ ID NO: 36.

29. The immunogen of any one of claims 21-28, wherein the protomers of the MeV F ectodomain trimer comprise an amino acid sequence at least 90% identical to residues 21-483 of any one of SEQ ID NOs: 37-43, 53-55, 62-68, or 72-80 or residues 21-490 of any one of SEQ ID NOs: 70-72; and wherein the protomers comprise the one or more amino acid substitutions that stabilize the MeV F ectodomain trimer in the prefusion conformation.

30. The immunogen of claim 29, wherein the protomers of the MeV F ectodomain trimer comprise or consist of the amino acid sequence set forth as 21-483 of any one of SEQ ID NOs: 37-43, 53-55, 62-68, or 72-80 or residues 21-490 of any one of SEQ ID NOs: 70-72.

31. The immunogen of any one claims 21-30, wherein the protomers of the recombinant MeV F ectodomain trimer are fused C-terminally to a trimerization domain.

32. The immunogen of claim 31, wherein the trimerization domain comprises a GCN4 trimerization domain, a T4 fibrin trimerization domain, or both.

33. The immunogen of claim 32, wherein
the GCN4 trimerization domain comprises an amino acid sequence set forth as
IEDKIEEILSKIYHIENEIARIKKLIGEAP (SEQ ID NO: 33).
the T4 fibrin trimerization domain comprises an amino acid sequence set forth as
GYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 34).
the trimerization domain comprising both the GCN4 and T4 fibrin trimerization domains
comprises an amino acid sequence set forth as
IEDKIEEILSKIYHIENEIARIKKLIGEAPSGYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 34).

34. The immunogen of any one of claims 31-33, wherein the protomers of the MeV F ectodomain trimer fused to the trimerization domain comprise an amino acid sequence at least 90% identical to residues 21-513 of any one of SEQ ID NOs: 37-43, 53-55, 62-68, or 72-80 or residues 21-520 of any one of SEQ ID NOs: 70-72; and wherein the protomers comprise the one or more amino acid substitutions that stabilize the MeV F ectodomain trimer in the prefusion conformation.

35. The immunogen of claim 34, wherein the protomers of the MeV F ectodomain trimer fused to the trimerization domain comprise or consist of the amino acid sequence set forth as residues 21-513 of any one of SEQ ID NOs: 37-43, 53-55, 62-68, or 72-80 or residues 21-520 of any one of SEQ ID NOs: 70-72.

36. The immunogen of any one of claims 21-35, wherein the protomers of the recombinant MeV F ectodomain trimer are linked to a heterologous protein.

37. The immunogen of claim 36, wherein the heterologous protein is an ectodomain head or ectodomain stalk and head of a MeV H protein or a MuV HN protein.

38. The immunogen of claim 37, wherein the ectodomain head or ectodomain stalk and head of the MeV H protein or the MuV HN protein is fused C-terminally to a trimerization domain that is fused C-terminally to the protomer of the recombinant MuV F ectodomain trimer.

39. The immunogen of claim 38, wherein the protomers of the MeV F ectodomain trimer linked to the trimerization domain and the ectodomain of the MeV H protein or the MuV HN protein comprise an amino acid sequence set forth as residues 21-959 of SEQ ID NO: 56 or 21-973 of SEQ ID NO 57 or residues 21-988 of SEQ ID NO: 81.

40. The immunogen of any one of the prior claims, wherein the protomers of the recombinant MeV F ectodomain trimer or the recombinant MuV F ectodomain trimer further comprise one or more additional amino acid substitutions.

41. An isolated immunogen, comprising:

a trimer of fusion proteins, each fusion protein comprising or consisting of, in an N- to C-terminal direction, a trimerization domain, an optional peptide linker, and one or more copies of a MuV HN ectodomain head or a MeV H ectodomain head; or

a trimer of fusion proteins, each fusion protein comprising or consisting of, in an N- to C-terminal direction, one or more copies of a MuV HN ectodomain head or a MeV H ectodomain head, an optional peptide linker, a trimerization domain, an optional peptide linker, and one or more copies of a MuV HN ectodomain head or a MeV H ectodomain head; or

a trimer of fusion proteins, each fusion protein comprising or consisting of, in an N- to C-terminal direction, one or more copies of a MuV HN ectodomain head or stalk and head or a MeV H ectodomain head or stalk and head, an optional peptide linker, a trimerization domain, an optional peptide linker, and one or more copies of a MuV HN ectodomain head or stalk and head or a MeV H ectodomain head or stalk and head.

42. The immunogen of claim 41, wherein

the MuV HN ectodomain head comprises or consists of an amino acid sequence set forth as residues 59-510 of SEQ ID NO: 58, or a sequence at least 90% identical to residues 59-510 of SEQ ID NO: 58 ;

the MuV HN ectodomain stalk and head comprises residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93, or a sequence at least 90% identical to residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93;

the MeV H ectodomain head comprises or consists of an amino acid sequence set forth as residues 59-496 of SEQ ID NO: 59, or a sequence at least 90% identical to residues 59-496 of SEQ ID NO: 59; or

the MeV H ectodomain stalk and head comprises residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89, or a sequence at least 90% identical to residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89.

43. The immunogen of claim 41 or claim 42, wherein

the GCN4 trimerization domain comprises an amino acid sequence set forth as

IEDKIEEILSKIYHIENEIARIKKLIGEAP (SEQ ID NO: 33).

the T4 fibrin trimerization domain comprises an amino acid sequence set forth as

GYIPEAPRDGQAYVRKDGWVLLSTFL SEQ ID NO: 34).

the trimerization domain comprising both the GCN4 and T4 fibrin trimerization domains comprises an amino acid sequence set forth as

IEDKIEEILSKIYHIENEIARIKKLIGEAPSGYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 35).

44. The immunogen of any one of claims 41-43, wherein the fusion proteins in the trimer comprise or consist of residues 24-510 of SEQ ID NO: 58, residues 24-496 of SEQ ID NO: 59, residues 22-950 of SEQ ID NO: 82, residues 25-985 of SEQ ID NO: 83, residues 22-948 of SEQ ID NO: 84, or residues 22-981 of SEQ ID NO: 85, or a sequence at least 90% identical to any one of residues 24-510 of SEQ ID NO: 58, residues 24-496 of SEQ ID NO: 59, residues 22-950 of SEQ ID NO: 82, residues 25-985 of SEQ ID NO: 83, residues 22-948 of SEQ ID NO: 84, or residues 22-981 of SEQ ID NO: 85.

45. An isolated immunogen, comprising:

a dimer of a MeV H ectodomain head;

a dimer of a MeV H ectodomain stalk and head;.

a dimer of a MuV HN ectodomain head; or

a dimer of a MuV HN ectodomain stalk and head.

46. The immunogen of claim 45, wherein

the MeV H ectodomain head comprises or consists of the amino acid sequence set forth as residues 22-459 of SEQ ID NO: 60, or a sequence at least 90% identical to residues 22-459 of SEQ ID NO: 60;

the MeV H ectodomain stalk and head comprises residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89, or a sequence at least 90% identical to residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89.

the MuV H ectodomain head comprises or consists of the amino acid sequence set forth as SEQ ID NO: 30, or a sequence at least 90% identical to SEQ ID NO: 30; or

the MuV HN ectodomain stalk and head comprises or consists of residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93, or a sequence at least 90% identical to residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93.

47. The immunogen of any one of the prior claims, conjugated to a heterologous carrier.
48. The immunogen of any one of the prior claims, wherein the immunogen is soluble.
49. The immunogen of any one of prior claims, wherein the protomers of the recombinant MeV F ectodomain trimer, the protomers of the recombinant MuV F ectodomain trimer, the trimer of fusion proteins, or the dimer of the MeV H ectodomain head are fused to a transmembrane domain by a peptide linker, or directly fused to the transmembrane domain.
50. The immunogen of claim 49, wherein the protomers of the recombinant MeV F ectodomain trimer or recombinant MuV F ectodomain trimer comprise a full-length F₁ protein.
51. A virus-like particle comprising the immunogen of any one or the prior claims.
52. A self-assembling protein nanoparticle comprising the immunogen of any one or the prior claims.
53. A nucleic acid molecule encoding the immunogen of any one of the prior claims.
54. The nucleic acid molecule of claim 53, operably linked to a promoter.
55. A vector comprising the nucleic acid molecule of claim 54.
56. The vector of claim 55, wherein the vector is an RNA vector.

57. A method of producing an immunogen, comprising:
expressing the nucleic acid molecule of any one of claims 55-56 in a host cell; and
purifying the immunogen.
58. The immunogen produced by the method of claim 57.
59. An immunogenic composition, comprising the immunogen, the nucleic acid molecule, the vector, or the virus like particle, of any one of claims 1-56 and 58, and a pharmaceutically acceptable carrier.
60. A method of eliciting an immune response to MuV F, MeV F, MuV NH, and/or MeV H in a subject, comprising administering to the subject an effective amount of the immunogenic composition of claim 59 to elicit the immune response.
61. The method of claim 60, wherein the immune response inhibits MuV and/or MeV infection in the subject.
62. The method of claim 60 or 61, wherein the subject is an adult who was previously immunized with a live attenuated vaccine to MeV and/or MuV and the administration boosts the immune response to the MuV and/or MeV.