

CLAIMS

1. An immunogenic composition comprising
 - a. a first Group A Streptococcus (GAS) polypeptide antigen;
 - b. a second GAS polypeptide antigen; and
 - c. at least one polypeptide-polysaccharide conjugate, wherein the conjugate polypeptide is a third GAS polypeptide antigen or a non-GAS carrier polypeptide and comprises at least one non-natural amino acid, and wherein the conjugate polysaccharide is a GAS polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.
2. The immunogenic composition of claim 1, wherein the first and second GAS polypeptide antigens are independently selected from C5a peptidase, streptolysin O (SLO), Sib35, Sfb1, and SpyAD.
3. The immunogenic composition of claim 1, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO.
4. The immunogenic composition of any one of claims 1-3 wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.
5. The immunogenic composition of any one of claims 1-3 wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the C5a peptidase polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.
6. The immunogenic composition of any one of claims 1-5, wherein the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31 or SEQ ID NO: 32.
7. The immunogenic composition of any one of claims 1-4, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide

antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

8. The immunogenic composition of any one of claims 1-5, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 32.

9. The immunogenic composition of claim 3, wherein the SLO polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 31 or SEQ ID NO: 32.

10. The immunogenic composition of any one of claims 1-4, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

11. The immunogenic composition of any one of claims 1-4, wherein the first and second GAS polypeptide antigens are C5a peptidase and SLO, and the SLO polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

12. The immunogenic composition claims 1 or 4, wherein the first and second GAS polypeptide antigens are C5a peptidase and SpyAD.

13. The immunogenic composition of any one of claims 1-3, 8-9, or 12, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 30.

14. The immunogenic composition of any one of claims 1-3, 8-9 or 12, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

15. The immunogenic composition of any one of claims 1-3, 8-9, 12, or 14, wherein the C5a peptidase polypeptide antigen consists of the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 30.

16. The immunogenic composition of any one of claims 1-15, wherein the third GAS polypeptide antigen or non-GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), SEQ ID NO: 25, and Protein D.

17. The immunogenic composition of any one of claims 1-15, wherein the third GAS polypeptide antigen or non-GAS polypeptide antigen is selected from Streptococcus pyogenes Adhesion and Division (SpyAD) polypeptide, arginine deiminase (ADI), SEQ ID NO: 25, Protein D, and SLO.

18. The immunogenic composition of any one of claims 1-17, wherein the nnAA comprises a click chemistry reactive group.

19. The immunogenic composition of any one of claims 1-18, wherein the nnAA is selected from 2-amino-3-(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid.

20. The immunogenic composition of claim 18 or 19, wherein the nnAA is pAMF.

21. The immunogenic composition of any one of claims 16-20, wherein the conjugate polypeptide is a third GAS protein, which is a SpyAD polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33.

22. The immunogenic composition of any one of claims 16-21, wherein the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 33.

23. The immunogenic composition of any one of claims 16-22, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

24. The immunogenic composition of claim 22, wherein the SpyAD polypeptide consisting of the amino acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

25. The immunogenic composition of any one of claims 16-20, wherein the conjugate polypeptide is a third GAS polypeptide, which is an ADI polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36.

26. The immunogenic composition of any one of claims 16-20 or 25, wherein the ADI polypeptide comprises the amino acid sequence of SEQ ID NO: 27, SEQ ID NO: 37 or SEQ ID NO: 38.

27. The immunogenic composition of any one of claims 16-20 or 25, wherein the ADI polypeptide consist of the amino acid sequence of SEQ ID NO: 27, SEQ ID NO: 37 or SEQ ID NO: 38.

28. The immunogenic composition of any one of claims 17-20, wherein the conjugate polypeptide is a third GAS polypeptide, which is a SLO polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, or SEQ ID NO: 53.

29. The immunogenic composition of claim 28, wherein the SLO polypeptide comprises 3 or 4 pAMF substitutions at positions selected from K98, K112, R151, K189, K272, K323, K357, K375, K407, or K464.

30. The immunogenic composition of claim 29, wherein the SLO polypeptide comprises the amino acid sequence of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

31. The immunogenic composition of claim 29, wherein the SLO polypeptide consists of the amino acid sequence of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, or SEQ ID NO: 64.

32. The immunogenic composition of claim 28, wherein the SLO polypeptide comprises the amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, or SEQ ID NO: 76.

33. The immunogenic composition of claim 28, wherein the SLO polypeptide consists of the amino acid sequence of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, or SEQ ID NO: 76.

34. The immunogenic composition of any one of claims 18-20, wherein the conjugate polypeptide is a ferritin polypeptide and comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21.

35. The immunogenic composition of claim 34, wherein the ferritin polypeptide comprises a pAMF substitution at position I5 of SEQ ID NO: 21.

36. The immunogenic composition of any one of claims 1-35, wherein the conjugate polysaccharide lacks the immunodominant GlcNAc side chain.

37. The immunogenic composition of any one of claims 1-36, wherein the conjugate polysaccharide has an average molecular weight of about 10 kDa to about 45 kDa.

38. An immunogenic composition comprising

- a. a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;
- b. a GAS streptolysin O (SLO) polypeptide antigen; and
- c. a polypeptide-polysaccharide conjugate comprising a Streptococcus pyogenes Adhesion and Division (SpyAD) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

39. The immunogenic composition of claim 38, wherein the SpyAD protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33 and comprises a pAMF substitution at positions K64, K287, K386, and K657.

40. The immunogenic composition of claims 38 or 39, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

41. The immunogenic composition of claims 1 or 38, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

42. The immunogenic composition of claims 1 or 38, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 52; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

43. The immunogenic composition of claims 1 or 38, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 30; a SLO polypeptide antigen of SEQ ID NO: 32; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

44. The immunogenic composition of claims 1 or 38, comprising a C5a peptidase polypeptide antigen of SEQ ID NO: 29; a SLO polypeptide antigen of SEQ ID NO: 53; and a polypeptide-polysaccharide conjugate comprising a SpyAD conjugate polypeptide of SEQ ID NO: 34 and a GAS conjugate polysaccharide.

45. An immunogenic composition comprising
- a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;
 - a GAS streptolysin O (SLO) polypeptide antigen; and
 - a polypeptide-polysaccharide conjugate comprising an arginine deiminase (ADI) conjugate polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

46. The immunogenic composition of claim 45, wherein the ADI polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 35 or SEQ ID NO: 36 and comprises a pAMF substitution at positions K15, K193, and K316.

47. An immunogenic composition comprising
- a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;
 - a GAS streptolysin O (SLO) polypeptide antigen; and
 - a polypeptide-polysaccharide conjugate comprising a ferritin polypeptide and a GAS conjugate polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

48. The immunogenic composition of claim 47, wherein the ferritin protein comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 21 and comprises a pAMF substitution at position I5.

49. The immunogenic composition of any one of claims 1-48, wherein the polypeptide-polysaccharide conjugate has an average molecular mass of about 10 kDa to about 5000 kDa.

50. A method of inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject comprising administering the immunogenic composition of any one of claims 1-49 to the subject.

51. The method of claim 50, wherein the immunogenic composition induces an antibody response in the subject against the Group A Streptococcus (GAS) bacterium and does not induce an antibody response in the subject against human tissue.

52. The method of claims 50 or 51, wherein the immunogenic composition induces a protective immune response against a Shigella bacterium in the subject, wherein the Shigella bacterium comprises a polysaccharide with a polyrhmanose backbone.

53. A method of inducing a protective immune response against a Shigella bacterium in a subject comprising administering the immunogenic composition of any one of claims 1-49 to the subject.

54. Use of the immunogenic composition of any one of claims 1-49 for inducing a protective immune response against a Shigella bacterium in a subject.

55. Use of the immunogenic composition of any one of claims 1-49 in the manufacture of a medicament for inducing a protective immune response against a Shigella bacterium in a subject.

56. Use of the immunogenic composition of any one of claims 1-49 in the manufacture of a medicament for inducing a protective immune response against a GAS bacterium in a subject.

57. Use of the immunogenic composition of any one of claims 1-82 for inducing a protective immune response against a GAS bacterium in a subject.