inventors have found that these antigens aggregate and precipitate at a concentration of sodium chloride as low as 150 mM. Consequently, the stability of immunogenic compositions comprising M72 related antigens can surprisingly be improved by a reduction in the concentration of sodium chloride.

Accordingly, the present invention provides an immunogenic composition comprising an M72 related antigen, which comprises a sequence having at least 90% identity of SEQ ID No. 1, wherein:

(i) the conductivity of the composition is 13 mS/cm or lower; and/or

(ii) the concentration of salts in said composition is 130 mM or lower; and/or

(iii) the concentration of sodium chloride in said composition is 130mM or lower.

Additionally provided is an immunogenic composition comprising an M72 related antigen, wherein the concentration of salts in said composition is 130 mM or lower.

The present invention also provides an immunogenic composition comprising an M72 related antigen, wherein the concentration of sodium chloride in said composition is 130 mM or lower.

Brief description of the drawings

Figure 1. QS21 lytic activity curve

Figure 2. Percentage of each 3D-MPL congener in the different ASA formulations

Figure 3. DLS of immunogenic compositions with varied pH and NaCl concentrations after storage

Figure 4. Nepholometry of immunogenic compositions with varied pH and NaCl concentrations after storage

Figure 5. Antigenic stability of immunogenic compositions with varied pH and NaCl concentrations following after storage

Figures 6a-6d. SEC-HPLC analysis of immunogenic compositions with varied pH and NaCl concentrations after storage

Figure 7. Antigenicity of immunogenic compositions with varied pH and NaCl concentrations after storage

Figure 8. Conductivity of NaCl standard solutions

Figure 9. Induction of CD4 T cell responses in mice using immunogenic compositions of the invention

Figure 10. Induction of CD8 T cell responses in mice using immunogenic compositions of the invention

Figure 11. Nepholometry of immunogenic compositions with varied pH and NaCl concentrations after storage

Figure 12. DLS of immunogenic compositions with varied pH and NaCl concentrations after storage

Figure 13. Antigenicity of immunogenic compositions with varied NaCl concentrations after storage

Brief description of sequence identifiers

CLAIMS

1. An immunogenic composition comprising an M72 related antigen which comprises a sequence having at least 90% identity to SEQ ID No: 1, wherein:
(i) the conductivity of the composition is 13 mS/cm or lower; and/or
(ii) the concentration of salts in said composition is 130 mM or lower; and/or
(iii) the concentration of sodium chloride in said composition is 130 mM or lower.

2. The immunogenic composition according to claim 1, wherein the conductivity of the composition is 10 mS/cm or lower.

3. The immunogenic composition according to claim 2, wherein the conductivity of the composition is 3 mS/cm or lower.

4. The immunogenic composition according to any one of claims 1 to 3, wherein the concentration of salts in said composition is 100 mM or lower.

5. The immunogenic composition according to claim 4, wherein the concentration of salts in said composition is 40 mM or lower.

6. The immunogenic composition according to any one of claims 1 to 5, wherein the concentration of sodium chloride in said composition is 100 mM or lower.

7. The immunogenic composition according to claim 6, wherein the concentration of sodium chloride in said composition is 40 mM or lower.

8. The immunogenic composition according to any one of claims 1 to 7, wherein the concentration of $CaCl_2$ in the immunogenic composition is 30 mM or lower.

9. The immunogenic composition according to any one of claims 1 to 8, wherein the concentration of $MgSO_4$ in the immunogenic composition is 60 mM or lower.

10. The immunogenic composition according to any one of claims 1 to 9, total concentration of NH_4^+, Mg^{2+} and Ca^{2+} ions is 40 mM or lower.

11. The immunogenic composition according to claim any one of claims 1 to 10, further comprising a non-ionic tonicity agent.

12. The immunogenic composition according to claim 11, wherein the non-ionic tonicity agent is a polyol.

13. The immunogenic composition according to claim 12, wherein the polyol is sorbitol.

14. The immunogenic composition according to claim 13, wherein the concentration of sorbitol is between about 4 and about 6% (w/v).

15. The immunogenic composition according to any one of claims 1 to 14, wherein the concentration of sucrose is between about 4 and about 6% (w/v).

16. The immunogenic composition according to any one of claims 1 to 15, further comprising one or more immunostimulants.

17. The immunogenic composition according to claim 16, wherein the immunostimulant is a saponin and/or a TLR4 agonist.

18. The immunogenic composition according to claim 17, wherein the immunostimulant(s) is/are in the form of liposomes.

19. The immunogenic composition according to either claim 17 or 18, wherein the saponin is QS21.

20. The immunogenic composition according to any one of claims 17 to 19, wherein the TLR4 agonist is 3-de-O-acylated monophoshoryl lipid A.

21. The immunogenic composition according to any one of claims 1 to 20, wherein the osmolality is 250 to 750 mOsm/kg.

22. The immunogenic composition according to any one of claims 1 to 21, wherein the composition is provided as a unit dose of between 50 ul and 1 ml.

23. The immunogenic composition according to claim 22, wherein the unit dose is between 100 ul and 750 ul.

24. The immunogenic composition according to claim 22 or 23, wherein a unit dose contains 1 to 100 ug of M72 related protein.

25. The immunogenic composition according to claim 24, wherein a unit dose contains 5 to 50 ug of M72 related protein.

26. The immunogenic composition according to any one of claims 22 to 25, wherein the unit dose is a human dose and contains between 1 and 100 ug of 3D-MPL.

27. The immunogenic composition according to any one of claims 22 to 26, wherein the unit dose is a human dose and contains between 1 and 100 ug of QS21.

28. The immunogenic composition according to any one of claims 1 to 25, wherein the pH of said composition is in the range 7.0 to 9.0.

29. The immunogenic composition according to claim 1, wherein the M72 related antigen consists of a sequence having at least 90% identity to SEQ ID No: 1.

30. The immunogenic composition according to any one of claims 1 to 29, wherein the M72 related antigen comprises the amino acid sequence of SEQ ID No: 1.

31. The immunogenic composition according to any one of claims 1 to 29, wherein the M72 related antigen comprises the amino acid sequence of SEQ ID No: 3.

32. The immunogenic composition according to claim 30, wherein the M72 related antigen consists of the amino acid sequence of SEQ ID No: 1.

33. The immunogenic composition according to claim 31, wherein the M72 related antigen consists of the amino acid sequence of SEQ ID No: 3.

34. The immunogenic composition according to any one of claims 1 to 33, for use in medicine.

35. Use of an immunogenic composition according to any one of claims 1 to 33 in the manufacture of a medicament.

36. A process for the making an immunogenic composition according to any one of claims 1 to 33 comprising the steps:

a) lyophilising an M72 related antigen which comprises a sequence having at least 90% identity to SEQ ID No: 1; and

b) reconstituting the lyophilised M72 related antigen of step a) with an aqueous solution wherein:

(i) the conductivity of the solution is 13 mS/cm or lower; and/or

(ii) the concentration of salts in said solution is 130 mM or lower; and/or

(iii) the concentration of sodium chloride in said solution is 130 mM or lower.

37. A kit comprising:

a) a lyophilised M72 related antigen which comprises a sequence having at least 90% identity to SEQ ID No: 1; and

b) an aqueous solution wherein:

(i) the conductivity of the solution is 13 mS/cm or lower; and/or

(ii) the concentration of salts in said solution is 130 mM or lower; and/or

(iii) the concentration of sodium chloride in said solution is 130 mM or lower.

38. The process according to claim 36 or kit according to claim 37 wherein the aqueous solution comprises an immunostimulant.

39. The process or kit according to claim 38, wherein the aqueous solution comprises a saponin and a TLR4 agonist, in a liposomal formulation, and a non-ionic tonicity agent.

40. The process or kit according to claim 39, wherein the non-ionic tonicity agent is sorbitol.

41. The process or kit according to claim 40, wherein the concentration of sorbitol is between about 2.5 and about 15% (w/v).

42. The process or kit according to any one of claims 39 to 41, wherein the saponin is QS21.

43. The process or kit according to any one of claims 39 to 42, wherein the TLR4 agonist is 3-de-O-acylated monophoshoryl lipid A.

CLAIMS

1. An immunogenic composition comprising an M72 related antigen which comprises a sequence having at least 90% identity to SEQ ID No: 1, wherein:
(i) the conductivity of the composition is 13 mS/cm or lower; and/or
(ii) the concentration of salts in said composition is 130 mM or lower; and/or
(iii) the concentration of sodium chloride in said composition is 130 mM or lower.

2. The immunogenic composition according to claim 1, wherein the conductivity of the composition is 10 mS/cm or lower.

3. The immunogenic composition according to claim 2, wherein the conductivity of the composition is 5 mS/cm or lower.

4. The immunogenic composition according to claim 3, wherein the conductivity of the composition is 3 mS/cm or lower.

5. The immunogenic composition according to any one of claims 1 to 4, wherein the concentration of salts in said composition is 100 mM or lower.

6. The immunogenic composition according to claim 5, wherein the concentration of salts in said composition is 40 mM or lower.

7. The immunogenic composition according to any one of claims 1 to 6, wherein the concentration of sodium chloride in said composition is 100 mM or lower.

8. The immunogenic composition according to claim 7, wherein the concentration of sodium chloride in said composition is 40 mM or lower.

9. The immunogenic composition according to any one of claims 1 to 8, wherein the concentration of $CaCl_2$ in the immunogenic composition is 30 mM or lower.

10. The immunogenic composition according to any one of claims 1 to 9, wherein the concentration of $MgSO_4$ in the immunogenic composition is 60 mM or lower.

11. The immunogenic composition according to any one of claims 1 to 10, total concentration of NH_4^+, Mg^{2+} and Ca^{2+} ions is 40 mM or lower.

12. The immunogenic composition according to claim any one of claims 1 to 11, further comprising a non-ionic tonicity agent.

13. The immunogenic composition according to claim 12, wherein the non-ionic tonicity agent is a polyol.

14. The immunogenic composition according to claim 13, wherein the polyol is sorbitol.

15. The immunogenic composition according to claim 14, wherein the concentration of sorbitol is between about 4 and about 6% (w/v).

16. The immunogenic composition according to any one of claims 1 to 15, wherein the concentration of sucrose is between about 4 and about 6% (w/v).

17. The immunogenic composition according to any one of claims 1 to 16, further comprising one or more immunostimulants.

18. The immunogenic composition according to claim 17, wherein the immunostimulant is a saponin and/or a TLR4 agonist.

19. The immunogenic composition according to claim 18, wherein the immunostimulant(s) is/are in the form of liposomes.

20. The immunogenic composition according to either claim 18 or 19, wherein the saponin is QS21.

21. The immunogenic composition according to any one of claims 18 to 20, wherein the TLR4 agonist is a lipopolysaccharide.

22. The immunogenic composition according to any one of claims 18 to 21, wherein the TLR4 agonist is 3-de-O-acylated monophoshoryl lipid A.

23. The immunogenic composition according to any one of claims 1 to 22, wherein the osmolality is 250 to 750 mOsm/kg.

24. The immunogenic composition according to any one of claims 1 to 23, wherein the composition is provided as a unit dose of between 50 ul and 1 ml.

25. The immunogenic composition according to claim 24, wherein the unit dose is between 100 ul and 750 ul.

26. The immunogenic composition according to claim 24 or 25, wherein a unit dose contains 1 to 100 ug of M72 related protein.

27. The immunogenic composition according to claim 26, wherein a unit dose contains 5 to 50 ug of M72 related protein.

28. The immunogenic composition according to any one of claims 24 to 27, wherein the unit dose is a human dose and contains between 1 and 100 ug of 3D-MPL.

29. The immunogenic composition according to any one of claims 24 to 28, wherein the unit dose is a human dose and contains between 1 and 100 ug of QS21.

30. The immunogenic composition according to any one of claims 1 to 29, wherein the pH of said composition is in the range 7.0 to 9.0.

31. The immunogenic composition according to any one of claims 1 to 30, wherein the pH of said composition is in the range 7.5 to 8.5.

32. The immunogenic composition according to claim 1, wherein the M72 related antigen consists of a sequence having at least 90% identity to SEQ ID No: 1.

33. The immunogenic composition according to any one of claims 1 to 32, wherein the M72 related antigen comprises the amino acid sequence of SEQ ID No: 1.

34. The immunogenic composition according to any one of claims 1 to 32, wherein the M72 related antigen comprises the amino acid sequence of SEQ ID No: 3.

35. The immunogenic composition according to claim 33, wherein the M72 related antigen consists of the amino acid sequence of SEQ ID No: 1.

36. The immunogenic composition according to claim 34, wherein the M72 related antigen consists of the amino acid sequence of SEQ ID No: 3.

37. The immunogenic composition according to any one of claims 1 to 36, for use in medicine.

38. Use of an immunogenic composition according to any one of claims 1 to 36 in the manufacture of a medicament.

39. The immunogenic composition for use according to either claim 37 or 38, for administration to a human.