glomerulonephritis, which likely contributes to the high rates of end-stage renal failure in many GAS endemic regions. GAS pharyngitis and impetigo are responsible for the greatest absolute number of GAS infections each year. GAS pharyngitis affects approximately 8%–15% of school-aged children per year and GAS impetigo is a very common infection in children prevalence of 10-50%. Not only are severe GAS-associated diseases a problem in developing countries, but even in developed countries particularly virulent GAS strains have emerged that are resistant to standard antibiotic therapies and cause debilitating diseases such as severe necrotizing fasciitis.

Peptide-based vaccines against group A streptococcus have focussed on M protein-derived peptides such as p145, J8 and J14 peptides. Currently, J8 peptide which comprises a twelve amino acid minimal epitope within p145 in embedded in a yeast-derived helical peptide (GCN4) amino acid sequence is a leading vaccine candidate (12). The GCN4 amino acid sequence assists maintenance of peptide helicity so as to optimize display of the minimal epitope to the immune system. This vaccine candidate also requires three doses to induce protection against skin disease (19).

SUMMARY

Surprisingly, the present inventors have discovered that the immunogenicity of a p145 peptide can be substantially increased by targeted modification of the amino acid sequence of p145. Immunization with a single dose of the modified p145 peptide, rather than multiple immunizations, can protect against group A streptococcus infection even by hypervirulent strains such as the CovR/S mutant strain. Unexpectedly, the modified p145 peptide, fragment or variant elicits a mucosal immune response upon administration to the mammal. The mucosal immune response is characterized by the production of IgG, while IgA production is substantially absent or at a relatively reduced level compared to the level of IgG produced.

The disclosure is broadly directed to a p145 peptide comprising one or more amino acid sequence modifications that substantially improve or enhance immunogenicity, particularly for eliciting mucosal immune responses. In a preferred form, the peptide comprises the amino acid sequence set forth in SEQ ID NO:1.

An example of the disclosure provides a method of eliciting an immune response to group A streptococcal bacteria in a mammal, said method including the step of administering: an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof; to the mammal to thereby elicit the immune response.

In one aspect, the present disclosure refers to use of an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof in the manufacture of a medicament for eliciting a mucosal immune response to group A streptococcal bacteria in a mammal; wherein the isolated protein, the antibody or antibody fragment, or the isolated nucleic acid is to be administered to the mammal to thereby elicit the mucosal immune response.

Another example of the disclosure provides a method of inducing immunity against a group A streptococcal bacterial infection in a mammal, said method including the step of administering: an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof; to the mammal to thereby induce immunity against the group A streptococcal bacterial infection in the mammal.

In another aspect, the present disclosure refers to use of an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof in the manufacture of a medicament for inducing mucosal immunity against a group A streptococcal bacterial infection in a mammal; wherein the isolated protein, the antibody or antibody fragment, or the isolated

nucleic acid is to be administered to the mammal to thereby induce mucosal immunity against the group A streptococcal bacterial infection in the mammal.

A further example of the disclosure provides a method of treating or preventing a group A streptococcal bacterial infection in a mammal, said method including the step of administering: an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof; to the mammal to thereby prevent or treat the group A streptococcal bacterial infection in the mammal.

In yet another aspect, the present disclosure refers to use of an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof in the manufacture of a medicament for treating or preventing a group A streptococcal bacterial infection in a mammal; wherein the isolated protein, the antibody or antibody fragment, or the isolated nucleic acid is to be administered to the mammal to thereby prevent or treat the group A streptococcal bacterial infection in the mammal, wherein administration of the isolated protein elicits a mucosal immune response and/or induces mucosal immunity to the group A streptococcal bacterial infection in the mammal.

The modified p145 peptide, fragment or variant suitably comprises an asparagine (N) amino acid at residue 13 and an arginine (R) amino acid at residue 17.

In an example, the modified p145 peptide, fragment or variant of comprises the amino acid sequence set forth in SEQ ID NO:1

In an example, the modified p145 peptide fragment comprises the amino acid sequence set forth in SEQ ID NO:2.

Preferably, the modified p145 peptide, fragment or variant elicits a mucosal immune response upon administration to the mammal.

Suitably, the mucosal immune response is characterized by the production of IgG. Typically, IgA production is substantially absent or at a relatively reduced level compared to the level of IgG produced.

Preferably, the modified p145 peptide, fragment or variant elicits a mucosal immune response upon intramuscular administration to the mammal.

Preferably, the mammal of the aforementioned examples is a human.

In a particular example of the aforementioned examples, the modified p145 peptide, fragment or variant may be administered in combination with an immunogenic fragment of a SpyCEP protein. Preferably, the fragment of SpyCEP is, or comprises an S2 peptide (SEQ ID NO:23) or a variant such as a K4S2 peptide (SEQ ID NO:24).

In yet another aspect, the present disclosure refers to a composition comprising: an isolated protein comprising: an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3, a fragment or variant thereof, an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof; and an immunogenic fragment of a SpyCEP protein or variant thereof, an antibody or antibody fragment that binds or is raised against the SpyCEP immunogenic fragment or variant thereof or an isolated nucleic acid that encodes the SpyCEP immunogenic fragment or variant thereof.

A related example of the disclosure therefore provides a composition comprising: an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3, a fragment or variant thereof, an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof; and an immunogenic fragment of a SpyCEP protein or variant thereof, an antibody or antibody fragment that binds or is raised against the SpyCEP immunogenic fragment or variant thereof or an isolated nucleic acid that encodes the SpyCEP immunogenic fragment or variant thereof.

Suitably, the modified p145 peptide amino acid sequence comprises an asparagine (N) amino acid at residue 13 and an arginine (R) amino acid at residue 17, preferably comprising the amino acid sequence set forth in SEQ ID NO:1. Preferably, the fragment of the modified p145 peptide comprises the amino acid sequence set forth in SEQ ID NO:2.

In a preferred example, the fragment of SpyCEP is, or comprises an S2 peptide comprising the amino acid sequence set forth in SEQ ID NO:23, or a variant thereof. The variant may be a K4S2 peptide comprising the amino acid sequence set forth in SEQ ID NO:24.

As used herein, the indefinite articles '*a*' and '*an*' are used here to refer to or encompass singular or plural elements or features and should not be taken as meaning or defining "one" or a "single" element or feature.

Unless the context requires otherwise, the terms "*comprise*", "*comprises*" and "*comprising*", or similar terms are intended to mean a non-exclusive inclusion, such that a recited list of elements or features does not include those stated or listed elements solely, but may include other elements or features that are not listed or stated.

By "*consisting essentially of*" in the context of an amino acid sequence is meant the recited amino acid sequence together with an additional one, two or three amino acids at the N- or C-terminus.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Designing of peptide libraries: (A) ***Recognition of various p145 variant peptides by p145 antisera*****: (A)** A peptide library consisting of 86 variants of p145 was screened with p145 antisera. The p145 antiserum was raised in B10.D2 mice and the ability of p145 antiserum to bind each peptide was determined using ELISA. The minimal epitope sequence from within the p145 is at the top of the graph with numbers of peptide sequences undergoing mutation in each amino acid are represented in the parentheses below. The vertical lines separate the peptides based on mutation in the same position. The Peptide 87 is a non-specific control peptide (pNS). **(B)** ***Identification of immunodominant peptides within the p145 variants library:*** Peptide-inhibition ELISAs were performed to identify the immunodominant peptides within the pool of p145 variants. Selected peptides at a concentration of 0.25 μg/ml were incubated with p145 antisera. The serum was then used in an ELISA against immobilised p145. Antigen (p145)-specific serum was collected from hyper-immunised B10.D2 mice. Data for each bar are Means ± SEM. **(C)** ***Designing of peptide library 2 (L2) with two alterations*****:** The selected 7 peptides were utilised to design a new peptide library with two amino acids substitutions at positions that were identified to enhance immunogenicity of parent p145. The resulting peptides demonstrating mutations at various positions within the p145 minimal epitope are shown.

Figure 2: Immune responses to doubly mutated peptides: (A) ***Immunogenicity of selected peptides in outbred Quackenbush mice showing***

CLAIMS

1. Use of an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof in the manufacture of a medicament for eliciting a mucosal immune response to group A streptococcal bacteria in a mammal; wherein the isolated protein, the antibody or antibody fragment, or the isolated nucleic acid is to be administered to the mammal to thereby elicit the mucosal immune response.

2. Use of an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof in the manufacture of a medicament for inducing mucosal immunity against a group A streptococcal bacterial infection in a mammal; wherein the isolated protein, the antibody or antibody fragment, or the isolated nucleic acid is to be administered to the mammal to thereby induce mucosal immunity against the group A streptococcal bacterial infection in the mammal.

3. Use of an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof in the manufacture of a medicament for treating or preventing a group A streptococcal bacterial infection in a mammal; wherein the isolated protein, the antibody or antibody fragment, or the isolated nucleic acid is to be administered to the mammal to thereby prevent or treat the group A streptococcal bacterial infection in the mammal, wherein administration of the isolated protein elicits a mucosal immune response and/or induces mucosal immunity to the group A streptococcal bacterial infection in the mammal.

4. The use of any preceding claim, wherein an isolated protein comprising the amino acid sequence of SEQ ID NO:1, or a fragment or variant thereof; an antibody or antibody fragment that binds or is raised against the isolated protein or fragment or variant thereof; or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof; is to be administered to the mammal intramuscularly and/or subcutaneously.

5. The use of any one of the preceding claims, wherein the mucosal immunity or the mucosal immune response is characterized by the production of mucosal IgG.

6. The use of any one of the preceding claims, wherein the mucosal immunity or the mucosal immune response is characterized by the substantial absence of mucosal IgA, or at least reduced production of IgA.

7. The use of any one of the preceding claims, wherein the modified p145 peptide amino acid sequence comprises an asparagine (N) amino acid at residue 13 and an arginine (R) amino acid at residue 17.

8. The use of Claim 7, wherein the modified p145 peptide amino acid sequence is set forth in SEQ ID NO:1.

9. The use of Claim 7, wherein the fragment comprises the amino acid sequence set forth in SEQ ID NO:2.

10. The use of any preceding claim, wherein the modified p145 peptide, variant or fragment is conjugated, coupled or otherwise linked to a carrier protein.

11. The use of Claim 10, wherein the carrier protein is diphtheria toxin, CRM protein or a fragment thereof.

12. The use of any preceding claim, which further includes administration of an immunogenic fragment of a SpyCEP protein.

13. The use of Claim 12, wherein the fragment of SpyCEP is, or comprises an S2 peptide (SEQ ID NO:23) or a variant thereof.

14. The use of Claim 13, wherein the variant is a K4S2 peptide (SEQ ID NO:24).

15. A composition comprising: an isolated protein comprising: an isolated protein comprising a modified p145 peptide amino acid sequence comprising an amino acid substitution of residues 13 and 17 of SEQ ID NO:3, a fragment or variant thereof, an antibody or antibody fragment that binds or is

raised against the isolated protein or fragment or variant thereof or an isolated nucleic acid encoding the isolated protein or fragment or variant thereof; and an immunogenic fragment of a SpyCEP protein or variant thereof, an antibody or antibody fragment that binds or is raised against the SpyCEP immunogenic fragment or variant thereof or an isolated nucleic acid that encodes the SpyCEP immunogenic fragment or variant thereof.

16. The composition of Claim 15, wherein the modified p145 peptide amino acid sequence comprises an asparagine (N) amino acid at residue 13 and an arginine (R) amino acid at residue 17.

17. The composition of Claim 16 wherein the modified p145 peptide amino acid sequence is set forth in SEQ ID NO:1.

18. The composition of Claim 17, wherein the fragment of the modified p145 peptide comprises the amino acid sequence set forth in SEQ ID NO:2.

19. The composition of any one of Claims 15-18, wherein the modified p145 peptide, variant or fragment is conjugated, coupled or otherwise linked to a carrier protein.

20. The composition of Claim 19, wherein the carrier protein is diphtheria toxin, CRM protein or a fragment thereof.

21. The composition of any one of Claims 15-20, wherein the fragment of SpyCEP is, or comprises an S2 peptide (SEQ ID NO:23) or a variant thereof.

22. The composition of Claim 21, wherein the variant is a K4S2 peptide (SEQ ID NO:24).

23. The composition of any one of Claims 15-22 which further comprises an adjuvant.

24. The composition of any one of Claims 15-23, which is suitable for intramuscular administration and/or subcutaneous administration.

25. The composition of any one of Claims 15-24, for the use according to any one of Claims 1-15.