## SUMMARY

Surprisingly, the present inventors have discovered that an important factor in J8-peptide induced immunity to group A streptococcus is neutrophil activity. While *in vitro* opsonisation assays use whole blood as a source of neutrophils and complement, it was not known that neutrophils were required for in vivo protection. Furthermore, opsonisation assays demonstrate relatively modest reductions in CFU (typically less than 10-fold), whereas J8-induced protection *in vivo* can result in several log order reductions in bacterial bio-burden. More particularly, the inventors now show that neutrophil inhibitors such as SpyCEP which inactivates the neutrophil chemotactic agent interleukin 8 work against J8 in inducing immunity to group A streptococcus.

In a broad form, the invention therefore relates to restoring or enhancing neutrophil activity to thereby assist M protein-induced immunity to group A streptococcus. In one aspect, the present invention refers to use of an M protein fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity in the manufacture of a medicament for eliciting an immune response to group A streptococcal bacteria in a mammal. An ~~aspect~~ example discloses ~~of the invention provides~~ a method of eliciting an immune response to group A streptococcal bacteria in a mammal, said method including the step of administering to the mammal: an M protein fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity; to thereby elicit an immune response to group A streptococcal bacteria in the mammal. In another aspect, the present invention refers to use of an M protein fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity in the manufacture of a medicament for immunizing a mammal against group A streptococcal bacteria. Another ~~aspect~~ example discloses ~~of the invention provides~~ a method of immunizing a mammal against group A streptococcal bacteria, said method including the step of administering to the mammal: an M protein, fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity, to thereby immunize the mammal against group A streptococcal bacteria. In yet another aspect, the present invention refers to use of an M protein, fragment, variant or derivative thereof, or an antibody or antibody fragment thereto; and an agent that facilitates restoring or enhancing neutrophil activity in the manufacture of a medicament for treating or preventing a group A streptococcal bacterial infection in a mammal. Yet another ~~aspect of the invention provides~~example discloses a method of treating or preventing a group A streptococcal bacterial infection in a mammal, said method including the step of administering to the mammal an M protein fragment, variant or derivative thereof, or an antibody or antibody fragment thereto; and an agent that facilitates restoring or enhancing neutrophil activity; to thereby treat or prevent the group A streptococcal bacterial infection in the mammal. A further ~~aspect of the invention provides~~example discloses a composition suitable for administration to a mammal, said composition comprising: an M protein fragment,

variant or derivative thereof, or an antibody or antibody fragment thereto; and an agent that facilitates restoring or enhancing neutrophil activity. In a further aspect, the present invention refers to a composition comprising an M protein fragment, variant or derivative thereof, or an antibody or antibody fragment thereto; and an agent that facilitates restoring or enhancing neutrophil activity.

In another aspect, the present invention refers to an isolated peptide comprising, consisting essentially of, or consisting of an amino acid sequence selected from the group consisting of: NSDNIKENQFEDFDEDWENF (SEQ ID NO:18); SREAKKQSREAKKQVEKALKQVEKALC (SEQ ID NO:59); SREAKKQSREAKKQVEKALKQSREAKC (SEQ ID NO:60); SREAKKQVEKALKQSREAKKQVEKALC (SEQ ID NO:61); SREAKKQVEKALDASREAKKQVEKALC (SEQ ID NO:62); and a fragment, variant or derivative of any one of SEQ ID NOS:18, 59, 60, 61 or 62. In yet another aspect, the present invention refers to an antibody or antibody fragment that binds or is raised against the isolated peptide of as disclosed herein. In yet another aspect, the present invention refers to an isolated nucleic acid encoding the isolated peptide, fragment, variant or derivative as disclosed herein. In a further aspect, the present invention refers to a genetic construct comprising the isolated nucleic acid as disclosed herein. In another aspect, the present invention refers to a host cell comprising the genetic construct as disclosed herein. In yet another aspect, the present invention refers to a composition comprising one or a plurality of the isolated peptides, fragments, variants or derivatives as disclosed herein; or one or a plurality of the antibodies or antibody fragments as disclosed herein; the isolated nucleic acid as disclosed herein; the genetic construct as disclosed herein; and/or the host cell as disclosed herein. Related ~~aspects of the invention provide~~examples disclose administration of one or more isolated nucleic acids encoding an M protein fragment, variant or derivative thereof and an agent that facilitates restoring or enhancing neutrophil activity or a composition comprising same.

In a particular ~~embodiment~~ example, the M protein fragment is or comprises a conserved region of the M protein. In one ~~embodiment~~example, the fragment is an immunogenic fragment that comprises, or is contained within a p145 peptide. In a particular ~~embodiment~~example, the immunogenic fragment is within, or comprises, a J8 peptide or variant thereof. ~~On~~ In certain ~~embodiments~~examples, the variant comprises, consists essentially of consists of an amino acid sequence selected from the group consisting of: SREAKKQSREAKKQVEKALKQVEKALC (SEQ ID NO:59); SREAKKQSREAKKQVEKALKQSREAKC (SEQ ID NO:60); SREAKKQVEKALKQSREAKKQVEKALC (SEQ ID NO:61); and SREAKKQVEKALDASREAKKQVEKALC (SEQ ID NO:62); or a fragment or variant thereof.

In one broad ~~embodiment~~example, the agent that facilitates restoring or enhancing neutrophil activity is a protein, or a fragment thereof, that normally directly or indirectly inhibits or

suppresses neutrophils or neutrophil activity. Suitably, administration of the protein or a fragment thereof elicits an immune response to the protein and/or to group A streptococcus.

In another broad ~~embodiment~~example, the agent that facilitates restoring or enhancing neutrophil activity is an antibody or antibody fragment which binds a protein, or a fragment thereof, that normally directly or indirectly inhibits or suppresses neutrophils or neutrophil activity.

In a particular ~~embodiment~~example, the protein is SpyCEP, or a fragment thereof.

In a preferred ~~embodiment~~example, the fragment comprises the amino acid sequence NSDNIKENQFEDFDEDWENF (SEQ ID NO:18).

Another further ~~aspect of the invention provides~~example discloses an isolated peptide comprising, consisting essentially of, or consisting of the amino acid sequence selected from the group consisting of NSDNIKENQFEDFDEDWENF (SEQ ID NO:18); SREAKKQSREAKKQVEKALKQVEKALC (SEQ ID NO:59); SREAKKQSREAKKQVEKALKQSREAKC (SEQ ID NO:60);

SREAKKQVEKALKQSREAKKQVEKALC (SEQ ID NO:61); and SREAKKQVEKALDASREAKKQVEKALC (SEQ ID NO:62); or a fragment or variant thereof.

A related ~~aspect provides~~example discloses an isolated nucleic acid encoding the isolated peptide of the aforementioned aspect, a genetic construct comprising the isolated nucleic acid and/or a host cell comprising the genetic construct.

A further related ~~aspect provides~~example discloses an antibody or antibody fragment which binds or is raised against the isolated peptide of the aforementioned aspect.

A still further ~~aspect of the invention provides~~example discloses a composition comprising the isolated peptide, the isolated nucleic acid, the genetic construct, the host cell and/or the antibody or antibody fragment of the previous aspects.

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: Protective efficacy of J8-DT/Alum vaccination.

Figure 2: Neutrophils in J8-DT mediated immunity. E: Epidermis; D: Dermis; SC: Sub-cut layer

Figure 3: Neutrophil depletion and efficacy of J8-DT vaccination.

Figure 4: Overview of elevated gene expression in the CovR/S mutant GAS an M1T1 isolate.

Figure 5: Efficacy of J8-DT in protection against 5448AP.

Figure 6: IL-8 degradation by various GAS strains.

Figure 7: MIP-2 degradation by various GAS strains.

Figure 8: KC degradation by various GAS strains.

CLAIMS

1. Use of an M protein fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity in the manufacture of a medicament for ~~A method of~~ eliciting an immune response to group A streptococcal bacteria in a mammal ~~said method including the step of administering to the mammal: an M protein fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity; to thereby elicit an immune response to group A streptococcal bacteria in the mammal~~.

2. Use of an M protein fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity in the manufacture of a medicament for ~~A method of~~ immunizing a mammal against group A streptococcal bacteria~~, said method including the step of administering to the mammal: an M protein fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity; to thereby immunize the mammal against group A streptococcal bacteria~~.

3. Use of an M protein, fragment, variant or derivative thereof, or an antibody or antibody fragment thereto; and an agent that facilitates restoring or enhancing neutrophil activity in the manufacture of a medicament for~~A method of~~ treating or preventing a group A streptococcal bacterial infection in a mammal~~, said method including the step of administering to the mammal: an M protein, fragment, variant or derivative thereof, or an antibody or antibody fragment thereto; and an agent that facilitates restoring or enhancing neutrophil activity; to thereby treat or prevent the group A streptococcal bacterial infection in the mammal~~.

4. The ~~method~~ use of any one of Claims 1 to 3, which includes ~~administration of~~ one or more isolated nucleic acids encoding, the M protein fragment, variant or derivative thereof and encoding the agent that facilitates restoring or enhancing neutrophil activity or a composition comprising same.

5. A ~~composition suitable for administration to a mammal, said~~ composition comprising an M protein fragment, variant or derivative thereof, or an antibody or antibody fragment thereto; and an agent that facilitates restoring or enhancing neutrophil activity.

6. The composition of Claim 5, which comprises one or more isolated nucleic acids encoding the M protein fragment, variant or derivative thereof and the agent that facilitates restoring or enhancing neutrophil activity or a composition comprising same.

7. The ~~method~~ use of any one of Claims 1 to 4 or the composition of Claim 5 or Claim 6, wherein the agent that facilitates restoring or enhancing neutrophil activity is a protein, or a fragment thereof, normally directly or indirectly inhibits or suppresses neutrophils or neutrophil activity.

8. The ~~method~~ use of any one of Claims 1 to 4 or the composition of Claim 5 or Claim 6, wherein the agent that facilitates restoring or enhancing neutrophil activity is an antibody or antibody fragment which binds a protein, or a fragment thereof, that normally directly or indirectly inhibits or suppresses neutrophils or neutrophil activity.

9. The ~~method~~ use or composition of Claim 7 or Claim 8, wherein the neutrophil activity is neutrophil chemotaxis.

10. The ~~method~~ use or composition of Claim 9, wherein the protein is a protease, or a fragment thereof, that proteolytically inactivates interleukin 8.

11. The ~~method~~ use or composition of Claim 10, wherein the protease is SpyCEP.

12. The ~~method~~ use or composition of Claim 11, wherein the fragment of SpyCEP comprises, consists essentially of or consists of the amino acid sequence NSDNIKENQFEDFDEDWENF (SEQ ID NO:18) or a variant having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:18.

13. The ~~method~~ use or composition of any preceding claim wherein the M protein fragment is of a conserved C-terminal region of the M protein.

14. The ~~method~~ use or composition of Claim 13, wherein the M protein fragment is, comprises or is contained within, a p145 amino acid sequence.

15. The ~~method~~ use or composition of Claim 14, wherein the M protein fragment is present in an amino acid sequence of a J8 peptide.

16. The ~~method~~ use or composition of Claim 13, wherein the M protein fragment is, or comprises or is contained within a J8 variant peptides.

17. The ~~method~~ use or composition of Claim 16, wherein the J8 variant peptide comprises, consists essentially of or consists of an amino acid sequence selected from the group consisting of: SREAKKQSREAKKQVEKALKQVEKALC (SEQ ID NO:59); SREAKKQSREAKKQVEKALKQSREAKC (SEQ ID NO:60); SREAKKQVEKALKQSREAKKQVEKALC (SEQ ID NO:61); and SREAKKQVEKALDASREAKKQVEKALC(SEQ ID NO:62).

18. The ~~method~~ use of any one of Claims 1-4 or 7-17 or the composition of any one of Claims 5-17, wherein the variant or derivative comprises one or a plurality of lysine residues at an N and/or C-terminus thereof.

19.The ~~method~~ use or composition of any preceding claim wherein the mammal is a human.