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 which is a SpyCEP protein fragment comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:18 or a variant having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:18 in the manufacture of a medicament for eliciting an immune response to group A streptococcal bacteria in a mammal. In one example, there is disclosed 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 example discloses 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 which is a SpyCEP protein fragment comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:18 or a variant having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:18 in the manufacture of a medicament for immunizing a mammal against group A streptococcal bacteria. In one example, there is disclosed 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 example discloses 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 which is a SpyCEP protein fragment comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:18 or a variant having at least 80% sequence identity to the amino

acid sequence of SEQ ID NO:18 in the manufacture of a medicament for treating or preventing a group A streptococcal bacterial infection in a mammal. In one example, there is disclosed 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 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 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 which is a SpyCEP protein fragment comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:18 or a variant having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:18. In one example, there is disclosed 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 example, there is disclosed 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. 3a another example, there is disclosed an antibody or antibody fragment that binds or is raised against the isolated peptide of as disclosed herein. In yet another example, there is disclosed an isolated nucleic acid encoding the isolated peptide, fragment, variant or derivative as disclosed herein. In a further example, there is disclosed a genetic construct comprising the isolated nucleic acid as disclosed herein. In another example, there is disclosed a host cell comprising the genetic construct as disclosed herein. In yet another example, there is disclosed 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 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 example, the M protein fragment is or comprises a conserved region of the M protein. In one example, the fragment is an immunogenic fragment that comprises, or is contained within a p145 peptide. In a particular example, the immunogenic fragment is within, or comprises, a J8 peptide or variant thereof. In certain 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 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 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 example, the protein is SpyCEP, or a fragment thereof.

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

Another further 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);

CLAIMS

1. Use of an M protein fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity which is a SpyCEP protein fragment comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:18 or a variant having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:18 in the manufacture of a medicament for eliciting an immune response to group A streptococcal bacteria in a mammal.

2. Use of an M protein fragment, variant or derivative thereof; and an agent that facilitates restoring or enhancing neutrophil activity which is a SpyCEP protein fragment comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:18 or a variant having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:18 in the manufacture of a medicament for immunizing a 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 which is a SpyCEP protein fragment comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:18 or a variant having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:18 in the manufacture of a medicament for treating or preventing a group A streptococcal bacterial infection in a mammal.

4. The use of any one of Claims 1 to 3, which includes 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 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 which is a SpyCEP protein fragment comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:18 or a variant having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:18.

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 which is a SpyCEP protein fragment comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:18 or the variant having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:18.

7. The use or composition of any one of Claims 1-6, wherein the agent that facilitates restoring or enhancing neutrophil activity is a variant having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:18.

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

9. The use or composition of Claim 8, wherein the M protein fragment is, comprises or is contained within, a p145 amino acid sequence (SEQ ID NO:56).

10. The use or composition of Claim 8, wherein the M protein fragment is present in an amino acid sequence of a J8 peptide (SEQ ID NO:58).

11. The use or composition of Claim 8, wherein the M protein fragment is, or comprises or is contained within a J8 variant peptide.

12. The use or composition of Claim 11, 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).

13. The use of any one of Claims 1-4 or 7-12 or the composition of any one of Claims 5-12, wherein the variant or derivative comprises one or a plurality of lysine residues at an N and/or C-terminus thereof.

14. The use or composition of any preceding claim wherein the mammal is a human.