COMPOSITIONS AND METHODS OF ENHANCING IMMUNE RESPONSES TO ENTERIC PATHOGENS

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application claims the benefit of priority of United States Provisional Patent Application No. 61/790,301, filed March 15, 2013, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

This application is being filed electronically and includes an electronically submitted Sequence Listing in .txt format. The .txt file contains a sequence listing entitled "2014-03-14_5658-00203_ST25.txt" created on March 14, 2014 and is 31,093 bytes in size. The Sequence Listing contained in this .txt file is part of the specification and is hereby incorporated by reference herein in its entirety.

INTRODUCTION

Bacterial infections still pose a significant health hazard to humans and agricultural and domesticated animals. The increase in antibiotic resistance has increased the need to move away from use of antibiotics in agriculture and the need to develop alternative methods of controlling bacterial infections and bacterial contamination of the human food supply. *Salmonella* and *E. coli* are commonly reported bacterial causes of human food-borne infections worldwide, and epidemiological evidence indicates that meat products including poultry and poultry products are a significant source of human infection. In the United States, an estimated 1.4 million cases of human *Salmonellosis* are reported annually. Of these cases, *S. enterica* serovars Enteritidis (SE) and Typhimurium (ST) are the most commonly isolated, although a number of other serovars have also been shown to cause enteritis in humans. Other gram negative bacteria responsible for significant infection rates include *Shigella* spp, *Vibrio* spp, *Erwinia* spp, *Kebsiella* spp, *Citrobacter* spp, *Yersinia* spp, *Providencia* spp and similar bacteria. Novel means to control these bacterial infections are needed.

SUMMARY

In one aspect, there is provided a vaccine vector comprising a first polynucleotide sequence encoding a PAL polypeptide consisting of a polypeptide selected from the group

consisting of SEQ ID NO: 1, an amino acid sequence having 90% or more sequence identity to SEQ ID NO: 1, an immunogenic fragment of SEQ ID NO: 1 at least eight amino acids long, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 36, SEQ ID NO: 37 and combinations thereof, wherein the PAL polypeptide is expressed on the surface of the vaccine vector.

In one aspect, there is provided a vaccine vector comprising a first polynucleotide sequence encoding a PAL polypeptide consisting of a polypeptide selected from the group consisting of SEQ ID NO: 1, an amino acid sequence having 90% or more sequence identity to SEQ ID NO: 1 and an immunogenic fragment thereof at least six amino acids long, wherein the PAL polypeptide is expressed on the surface of the vaccine vector.

In one aspect, there is provided a vaccine vector comprising a polynucleotide encoding a polypeptide selected from the group consisting of at least one of SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, a polypeptide having 90% sequence identity to SEQ ID NO: 42, a polypeptide having 90% sequence identity to SEQ ID NO: 44, and a polypeptide having 90% sequence identity to SEQ ID NO: 46.

In one aspect, there is provided a pharmaceutical composition comprising the vaccine vector as disclosed herein and a pharmaceutically acceptable carrier.

In one aspect, there is provided the use of the vaccine vector of as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for enhancing the immune response of a subject to a gram-negative bacterium.

In one aspect, there is provided the use use of the vaccine vector of as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for reducing morbidity associated with subsequent infection of a subject with a gram-negative bacterium as compared to a control subject not administered the vaccine vector.

A vaccine vector comprising a first polynucleotide sequence encoding a PAL polypeptide is disclosed. The PAL polypeptide is a heterologous, non-natively expressed,

CLAIMS

We claim:

1. A vaccine vector comprising a first polynucleotide sequence encoding a PAL polypeptide consisting of a polypeptide selected from the group consisting of SEQ ID NO: 1, an amino acid sequence having 90% or more sequence identity to SEQ ID NO: 1, an immunogenic fragment of SEQ ID NO: 1 at least eight amino acids long, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 36, SEQ ID NO: 37 and combinations thereof, wherein the PAL polypeptide is expressed on the surface of the vaccine vector.

2. The vaccine vector of claim 1, further comprising a second polynucleotide sequence encoding an immunostimulatory polypeptide, wherein the immunostimulatory polypeptide is expressed on the surface of the vaccine vector.

3. The vaccine vector of claim 2, wherein the immunostimulatory polypeptide comprises an HMGB1 polypeptide or a CD154 polypeptide capable of binding CD40.

4. The vaccine vector of claim 3, wherein the HMGB1 polypeptide comprises a polypeptide of at least one of SEQ ID NOs: 15-23.

5. The vaccine vector of claim 3, wherein the CD154 polypeptide has fewer than 50 amino acids and comprises amino acids 140-149 of a polypeptide selected from the group consisting of SEQ ID NO: 24, SEQ ID NO: 25, and a homolog thereof or a polypeptide selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

6. The vaccine vector of any one of claims 2-5, wherein the vaccine vector comprises more than one copy of the first polynucleotide and/or more than one copy of the second polynucleotide sequence.

7. The vaccine vector of any one of claims 2-6, wherein the first polynucleotide sequence is linked in the same reading frame to the second polynucleotide sequence.

8. The vaccine vector of claim 7, wherein the first polynucleotide and the second polynucleotide are linked via a spacer nucleotide sequence.

9. The vaccine vector of any one of claims 1-8, wherein the vaccine vector is selected from the group consisting of a virus, a bacterium, a yeast and a liposome.

10. The vaccine vector of claim 9, wherein the vaccine vector is selected from the group consisting of *Bacillus* spp., *Salmonella* spp., *Lactobacillus* spp., and *Escherichia* spp.

11. The vaccine vector of any one of claims 1-10, further comprising a third polynucleotide encoding a second antigenic polypeptide.

12. The vaccine vector of claim 11, wherein the second antigenic polypeptide is a polypeptide selected from the group consisting of SEQ ID NO: 7 and SEQ ID NO: 31.

13. A vaccine vector comprising a polynucleotide encoding a polypeptide selected from the group consisting of at least one of SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, a polypeptide having 90% sequence identity to SEQ ID NO: 42, a polypeptide having 90% sequence identity to SEQ ID NO: 44, and a polypeptide having 90% sequence identity to SEQ ID NO: 46.

14. A pharmaceutical composition comprising the vaccine vector of any one of claims 1-13 and a pharmaceutically acceptable carrier.

15. A use of the vaccine vector of any one of claims 1-13 or the pharmaceutical composition of claim 14 in the manufacture of a medicament for enhancing the immune response of a subject to a gram-negative bacterium.

16. The use of claim 15, wherein the enhanced immune response comprises an enhanced antibody response, an enhanced T cell response or both.

17. A use of the vaccine vector of any one of claims 1-13 or the pharmaceutical composition of claim 14 in the manufacture of a medicament for reducing morbidity associated with subsequent infection of a subject with a gram-negative bacterium as compared to a control subject not administered the vaccine vector.

18. The use of any one of claims 15-17, wherein the medicament is manufactured for administeration by a route selected from the group consisting of oral, mucosal, parenteral, sub-cutaneous, intramuscular, intraocular and *in ovo*.

19. The use of any one of claims 15-18, wherein the subject is selected from the group consisting of a member of a poultry species and a mammal.

20. The use of claim 19, wherein the subject is selected from the group consisting of a human, a chicken and a turkey.

21. The use of any one of claims 15-20, wherein the medicament is manufactured for about 10^4 to about 10^9 vector copies of the vaccine to be administered to the subject.

22. The use of any one of claims 15-21, wherein the vaccine vector is killed prior to administration to the subject or is not capable of replicating in the subject.

23. The use of any one of claims 15-22, wherein the gram-negative bacterium is selected from the group consisting of *Salmonella* spp., *Escherichia* spp., *Shigella* spp., *Vibrio* spp., *Erwinia* spp., *Kebsiella* spp., *Citrobacter* spp., *Yersinia* spp., and *Providencia* spp.