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 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,

recombinant polypeptide in the vaccine vector. The PAL polypeptide is selected from SEQ ID NO: 1, a sequence with 90% identity to SEQ ID NO: 1, such as SEQ ID NO: 6, or an immunogenic fragment thereof at least six amino acids long. The polypeptide may be expressed on the surface of the vaccine vector. The immunogenic fragment of SEQ ID NO: 1 may comprise SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 36 or SEQ ID NO: 37. The vaccine vector may also comprise a second polypeptide sequence encoding an immunostimulatory polypeptide. The immunostimulatory polypeptide may also be expressed on the surface of the vaccine vector. The immunostimulatory polypepetide may be a CD154 polypeptide capable of binding CD40 or an HMGB1 polypeptide. The CD154 polypeptides include fewer than 50 amino acids and comprise amino acids 140-149, or a homolog thereof.

Vaccines according to the present ~~invention~~ disclosure may be comprised within a vector, such as a virus, yeast, bacterium, or liposome. In one ~~aspect~~ embodiment, the vaccines include polynucleotides encoding polypeptides of SEQ ID NO: 42, 44 or 46 or a sequence having 90% identity to one of these sequences. Pharmaceutical compositions may be comprised of the vaccine vectors described herein and a pharmaceutically acceptable carrier.

In still another ~~aspect~~ embodiment, methods of enhancing the immune response against a gram-negative bacterium in a subject by administering a vaccine vector described herein to the subject are provided. The enhanced immune response may be an enhanced antibody response, an enhanced T cell response or a combination thereof.

In a still further ~~aspect~~ embodiment, methods of reducing morbidity or mortality associated with infection with a gram-negative bacterium in a subject by administering a vaccine vector as described herein to the subject are provided. The vaccine vector is capable of reducing the morbidity and mortality associated with subsequent infection with a gram-negative bacterium in subjects administered the vaccine vector as compared to controls.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the PAL sequence-specific serum IgG antibody levels as determined by ELISA with synthetic PAL-BSA as the coating antigen on day 17 post vaccination by oral gavage with either saline, or Bacillus backbone (BS BB) or PAL-vectored BS vaccine (BS PAL)candidates at 10^8 cfu/chick. The results are presented as mean S/P ratios ± SEM (n=10). Groups with different upper case letters are significantly different using an ANOVA ($P < 0.05$).

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~~homology~~ to SEQ ID NO: 1 and~~or~~ an immunogenic fragment thereof at least six amino acids long, 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~~is~~ 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 selected from the group consisting of at least one of SEQ ID NOs: 15-23 and~~or~~ a fragment 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~~or~~ 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 claim 5, wherein the CD154 polypeptide comprises SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, or SEQ ID NO: 30.~~

6~~7~~. The vaccine vector of any one of claims 1-5~~6~~, wherein the PAL polypeptide is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 32, SEQ ID NO: 36, SEQ ID NO: 37 and~~or~~ combinations thereof.

7~~8~~. The vaccine vector of any one of claims 2-6~~1~~-7, wherein the vaccine vector comprises more than one copy of the first polynucleotide and/or more than one copy of the second polynucleotide sequence.

8~~9~~. The vaccine vector of any one of claims 2-7~~8~~, wherein the first polynucleotide sequence is linked in the same reading frame to the second polynucleotide sequence.

910. The vaccine vector of claim 89, wherein the first polynucleotide and the second polynucleotide are linked via a spacer nucleotide sequence.

101. The vaccine vector of any one of claims 1-910, wherein the vaccine vector is selected from the group consisting of a virus, a bacterium, a yeast and a liposome.

112. The vaccine vector of claim 101, wherein the vaccine vector is selected from the group consisting of *Bacillus* spp., *Salmonella* spp., *Lactobacillus* spp., and~~or~~ *Escherichia* spp.

123. The vaccine vector of any one of claims 1-112, further comprising a third polynucleotide encoding a second antigenic polypeptide.

134. The vaccine vector of ~~any one of~~ claim~~s~~ 121-13, wherein the second antigenic polypeptide is a polypeptide selected from the group consisting of SEQ ID NO: 7 and~~or~~ SEQ ID NO: 31.

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

156. A pharmaceutical composition comprising the vaccine vector of any one of claims 1-145 and a pharmaceutically acceptable carrier.

167. ~~A method of enhancing the immune response against a gram-negative bacterium in a subject comprising administering to the subject~~ A use of the vaccine vector of any one of claims 1-145 or the pharmaceutical composition of claim 156 in the manufacture of a medicament for enhancing~~in an amount effective to enhance~~ the immune response of a~~the~~ subject to a~~the~~ gram-negative bacterium.

178. The use~~method~~ of claim 167, wherein the enhanced immune response comprises an enhanced antibody response, an enhanced T cell response or both.

189. ~~A method of reducing morbidity associated with infection with a gram-negative bacterium in a subject comprising administering to the subject~~ A use of the vaccine vector of any one of claims 1-145 or the pharmaceutical composition of claim 156 in the manufacture of a medicament for reducing ~~an amount effective to reduce the~~ morbidity associated with subsequent

infection of athe subject with a gram-negative bacterium as compared to a control subject not administered the vaccine vector.

1920. The usemethod of any one of claims 167-189, wherein the vaccine vector is administered by a route selected from the group consisting of oral, mucosal, parenteral, subcutaneous, intramuscular, intraocular and *in ovo*.

201. The usemethod of any one of claims 167-1920, wherein the subject is selected from the group consisting of a member of a poultry species and a mammal.

~~22. The method of claim 21, wherein the poultry species is a chicken or turkey.~~

~~23. The method of any one of claims 17-22, wherein the subject is a mammal.~~

214. The usemethod of claim 203, wherein the subject is selected from the group consisting of a human, a chicken and a turkey.

225. The usemethod of any one of claims 16-2117-24, wherein ~~from~~ about 10^4 to about 10^9 vector copies of the vaccine are administered to the subject.

~~26. The method of any one of claims 17-24, wherein from about 10^5 to about 10^7 vector copies of the vaccine are administered to the subject.~~

237. The usemethod of any one of claims 16-2217-26, wherein the vaccine vector is killed prior to administration to the subject or is not capable of replicating in the subject.

248. The usemethod of any one of claims 16-2317-27, 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.