COMPOSITIONS AND METHODS OF ENHANCING IMMUNE RESPONSES TO EIMERIA OR LIMITING EIMERIA INFECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application claims the benefit of priority of United States Provisional Patent Application No. 61/764,681, filed February 14, 2013, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

This application is being filed electronically via EFS-Web and includes an electronically submitted Sequence Listing in .txt format. The .txt file contains a sequence listing entitled "2014-02-13 5658-00201_ST25.txt" created on February 13, 2014 and is 40.3 kilobytes 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

Coccidiosis, an infectious disease of poultry, swine, and cattle caused by apicomplexan protozoan parasites (*Eimeria* spp. and related parasites) presents problems worldwide. Coccidiosis is among the top ten infectious diseases of poultry in terms of its economic impact on the poultry industry with production losses estimated to be up to $2 billion annually. Other apicomplexan parasites also cause disease, including *Plasmodium*, *Cryptosporidium* and *Toxoplasma*, which are the causative agents of malaria, cryptosporidiosis and toxoplasmosis, respectively.

Typical signs of coccidiosis include rapid loss of appetite, reduction in weight, diarrhea and acute mortality. Outbreaks in a flock occur upon exposure to high levels of pathogen and in most cases, coccidiosis predisposes birds to secondary bacterial infections. Traditional methods of disease control include the administration of antibiotics and chemotherapeutic agents. However, with continuous usage, this has led to resistance issues. Antibiotic use also decreases social acceptance of poultry meat. Vaccination is a rational approach because of its ability to confer long-term protection, typically for the entire lifespan of commercial chickens.

Most commercially available vaccines against *Eimeria* are based on controlled low dosage of essentially fully virulent but drug-sensitive *Eimeria* parasites. Vaccination with

current *Eimeria*-based vaccines produces substantial vaccine-reaction morbidity and economic losses in vaccinated flocks. Thus an effective low-virulence vaccine against *Eimeria* is needed. An effective vaccine for *Eimeria* based on conserved immunogenic targets may also prove useful as a vaccine against other apicomplexan parasites.

SUMMARY

A vaccine vector comprising a first polynucleotide sequence encoding an Apicomplexan Rhomboid polypeptide and methods of using the same are provided herein.

In one aspect, a vaccine vector comprising a first polynucleotide encoding an Apicomlexan Rhomboid polypeptide or an immunogenic fragment thereof and a second polypeptide sequence encoding an immunostimulatory polypeptide is disclosed. The Apicomplexan Rhomboid polypeptide and the immunostimulatory polypeptide are suitably expressed on the surface of the vaccine vector. The Apicomplexan Rhomboid polypeptide may comprise SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 37, SEQ ID NO: 38, an immunogenic fragment of at least one of SEQ ID NO: 1-4, 37-38 or combinations of SEQ ID NO: 1-4 and 37-38. The immunostimulatory polypeptide 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 of CD154 or a homolog thereof.

In another aspect, a vaccine vector comprising a first polynucleotide encoding an Apicomlexan Rhomboid polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 37, SEQ ID NO: 38, an immunogenic fragment of at least one of SEQ ID NO: 1-4 or 37-38 or combinations of SEQ ID NO: 1-4 or 37-38. The Apicomplexan Rhomboid polypeptide may be expressed on the surface of the vaccine vector.

Vaccine vectors according to the present invention may be a virus, yeast, bacterium, or liposome vector. Pharmaceutical compositions may be comprised of the vaccine vectors described herein and a pharmaceutically acceptable carrier.

In still another aspect, methods of enhancing the immune response against an Apicomplexan parasite 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, methods of reducing morbidity and mortality associated with infection with an apicomplexan parasite 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 an apicomplexan parasite in subjects administered the vaccine vector as compared to controls.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation showing the homology of the MPP sequence among several Apicomplexan parasites. The consensus MPP sequence is highly similar in amino acid sequences in the Apicomplexans. Positions that are not identical are indicated with an X in the consensus sequence which is shown on the top line of the figure and is SEQ ID NO: 38. The *Toxoplasma gondii* sequences (the first four lines below the consensus) share 100% identity to the MPP sequence of SEQ ID NO: 2 from *Eimeria maxima*. The bottom two sequences are the homolog from *Neospora caninum* (SEQ ID NO: 3) and *Eimeria tenella* (SEQ ID NO: 4), respectively.

Figure 2 is a schematic representation of the vaccine vector constructs described in the Examples.

Figure 3 is a bar graph showing the body weight (grams) of the chickens eight days post-infection with *Eimeria maxima* after inoculation with the indicated vaccine vector expressing the indicated sequences. Significant differences ($p < 0.05$) between treatment groups are indicated by different letters.

Figure 4 is a bar graph showing the body weight (grams) of the surviving chickens 29 days post-challenge infection with *Eimeria maxima* after inoculation with the indicated vaccine vector expressing the indicated sequences. Significant differences ($p < 0.05$) between treatment groups are indicated by actual p values and an asterisk (*).

Figure 5 is a bar graph showing the percent mortality in the face of a virulent challenge infection with *Eimeria maxima* at eight days post-challenge infection with *Eimeria maxima* after inoculation with the indicated vaccine vector expressing the indicated sequences. Significant differences ($p < 0.05$) are indicated with an asterisk (*).

DETAILED DESCRIPTION

Conventional vaccines against coccidiosis are generally based on live/attenuated parasites that are delivered in controlled numbers. However, the risk of infection is not eliminated because the parasites are viable and capable of causing disease. Additionally, production costs for these types of vaccine are extremely high because it involves passing the

parasites through live birds, collecting them at regular intervals and ensuring an uninterrupted cold transit chain from production to use at the hatchery or on the farm. With vaccination being a critical control method, the use of recombinant vaccines may improve the overall efficacy of coccidiosis-based vaccines while decreasing the production costs.

Species of *Eimeria* are highly immunogenic and are capable of stimulating robust host immune responses. The wide repertoire of antigens that are part of this eukaryote are highly specialized in function and are suitable targets for recombinant vaccine development. Sporozoites and merozoites are the most motile stages of the parasite and are responsible for initiating and sustaining an active infection. Invasion of these stages into intestinal epithelial cells is an essential process for the parasite to continue its life-cycle within host cells. A highly specialized set of organelles located at the anterior (apical) end of the parasite is involved in transporting the numerous proteins required for the translocation of these motile stages from the intestinal lumen into the epithelial layer. This apical complex consists of a variety of secretory organelles including a large number of micronemes that transport a milieu of proteins to the surface of motile apicomplexan zoites in support of the essential function of motility.

Among several well-described microneme-associated proteins, thrombospondin-related adhesive protein (TRAP) has been used as a successful recombinant antigen in *Salmonella* recombinant and *Bacillus*-vectored systems as a vaccine candidate. See U.S. Publication No. 2011/0111015, which is incorporated herein by reference in its entirety. Many microneme proteins have a similar mode of action in that they are released from the microneme complex at the anterior end of the sporozoite as they approach a host cell and act as a link between the parasite and whatever substrate they are upon. The microneme protein is then translocated across the surface of the parasite posteriorly, thereby moving the parasite closer to the host cell. This gliding form of motility is typical of all apicomplexan parasites. When the microneme protein has been translocated to the posterior end of the parasite, it needs to be cleaved and released from the surface of the parasite in order to successfully complete the invasion process. This function is performed by a family of proteases that are constitutively expressed within or on the parasite cell membrane. The cleavage process occurs intracellularly and is an absolute requirement for propagating the infection.

A novel approach to recombinant vaccine design involves targeting this protease and interfering with the cleavage/invasion process. The family of proteases that are involved in the cleavage process are called rhomboid proteases and are extremely well-described in

Toxoplasma species with homologues in *Eimeria* and other Apicomplexa. Rhomboid proteases (ROM4 and ROM5, MPP) are centrally implicated in the cleavage of microneme proteins and share good homology among different apicomplexan parasites. Our hypothesis was based on the premise that if we are able to immunologically target the protease, antibody binding would interfere with the cleavage process and thereby impair sporozoite/merozoite mobility. For successful infection to occur, intracellular development of the parasite is essential and our approach may curtail cell invasion thus, interfering with establishment of the life-cycle. One advantage of targeting MPP is that the conserved nature of this protein across many apicomplexan species makes it a suitable target not only for *Eimeria*, but other Apicomplexa as well.

Predicted antigenic regions of MPP (ROM5) were aligned and checked for homology among six different Apicomplexa (Figure 1). The seven sequences compared are as follows: *Eimeria tenella* ROM4 (JN558353), *Toxoplasma gondii* ME49 ROM5 (XP_002370238), *Toxoplasma gondii* ROM5 (AAT84606), *Toxoplasma gondii* ROM5 (AY587208), *Toxoplasma gondii* RH ROM5 (AM055942), *Toxoplasma gondii* (AY634626), and the MPP insert from *Eimeria maxima* of SEQ ID NO: 2. Suitable Apicomplexan parasites include, but are not limited to: *Eimeria* species, including but not limited to *Eimeria tenella*, *Eimeria maxima*, and *Eimeria brunetti*; *Toxoplasma gondii*; *Neospora caninum*; *Cryptosporidium* species; and *Plasmodium* species, including but not limited to *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium knowlesi*, and *Plasmodium vivax*.

Recombinant DNA technologies enable relatively easy manipulation of many yeast, bacterial and viral species. Some microorganisms are mildly pathogenic or non-pathogenic, but are capable of generating a robust immune response. These microorganisms make attractive vaccine vectors for eliciting an immune response to antigens recombinantly expressed in the vector. Vaccines vectored by microorganisms may mimic a natural infection, help produce robust and long lasting mucosal immunity, and may be relatively inexpensive to produce and administer. In addition, such vectors can often carry more than one antigen and have potential to provide protection against multiple infectious agents.

In one aspect, a vaccine vector comprising a first polynucleotide sequence encoding an Apicomplexan Rhomboid polypeptide of SEQ ID NO: 1-4, 37-38, an immunogenic fragment thereof or combinations thereof is provided. In another embodiment, the vaccine vector may include a first polynucleotide encoding an Apicomplexan Rhomboid polypeptide and a second polynucleotide encoding an immunostimulatory polypeptide is provided. The

Rhomboid polypeptide and the optional immunostimulatory polypeptide are expressed on the surface of the vaccine vector. The Rhomboid polypeptide may comprise the full-length protein (SEQ ID NO: 39) or an immunogenic fragment such as those provided in SEQ ID NO: 1-4 and 37-38. For example, the Rhomboid polypeptide may comprise, may consist essentially of or may consist of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 37, SEQ ID NO: 38 or an immunogenic fragment of any of these SEQ ID NOs. Combinations of these fragments may also be used in a vaccine vector. A vaccine vector may include SEQ ID NO: 1-4 or 37-38. A single vaccine vector may include multiple copies of a single fragment as well.

The immunogenic fragment of a Rhomboid polypeptide may be a sequence that is at least 5, 6, 7, 8, 10, 12, 14, 16, 18 or 20 amino acids long and has at least 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% percent identity to the fragments of SEQ ID NO: 1-4 or 37-38 provided herein. Without being limited by theory, the vaccine vectors provided herein are believed to be reducing morbidity and mortality associated with *Eimeria* infection by inducing an antibody response that is capable of blocking invasion of the parasites into cells. Those of skill in the art are aware that B cells epitopes are often hydrophilic in nature and this information can be used to generate immunogenic fragments to the polypeptides of SEQ ID NO: 1-4 and 37-38 provided herein. A hydrophilicity plot of SEQ ID NO: 2 reveals three hydrophilic areas of the peptide and three potential B cell epitopes including amino acids 1-11, 18-27 and 31-43 of SEQ ID NO: 2. These amino acid fragments correspond to amino acids 7-16 of SEQ ID NO: 3 and 37 and amino acids 12-21 of SEQ ID NO: 4. As shown by the two consensus sequences of SEQ ID NO: 1 and SEQ ID NO: 38, amino acids corresponding to 18-27 of SEQ ID NO: 2 are highly conserved across species and genera of Apicomplexan parasites. An immune response to such a highly conserved epitope may allow for cross species or even cross genera immunity from a single vaccine.

A vaccine includes any composition comprising a polynucleotide encoding an antigenic polypeptide that is capable of eliciting an immune response to the polypeptide. A vaccine vector is a composition that can be engineered to carry antigens or immunostimulatory polypeptides and may also comprise an adjuvant or be administered with an adjuvant to further increase the immune response to the parasite and provide better protection from morbidity and mortality associated with a subsequent infection. The use of vectors, such as bacterial vectors, for vaccination and generation of immune responses against *Eimeria* or other apicomplexan parasites such as *Plasmodium* (the causative agent of

malaria), *Toxoplasma* and *Cryptosporidium* is disclosed. The immune responses after administration of the vaccine vector need not be fully protective, but may decrease the morbidity or percentage mortality (i.e. likelihood of mortality) associated with subsequent infection.

Polynucleotides encoding Rhomboid polypeptide antigens of SEQ ID NO: 1-4, 37-38 or fragments thereof and other antigens from any number of pathogenic organisms may be inserted into the vector and expressed in the vector. The expression of these polynucleotides by the vector will allow generation of antigenic polypeptides following immunization of the subject. The polynucleotides may be inserted into the chromosome of the vector or encoded on plasmids or other extrachromosomal DNA. Those of skill in the art will appreciate that numerous methodologies exist for obtaining expression of polynucleotides in vectors such as *Salmonella* or *Bacillus*. The polynucleotides may be operably connected to a promoter (e.g., a constitutive promoter, an inducible promoter, etc.) by methods known to those of skill in the art. Suitably, polynucleotides encoding the Rhomboid antigens are inserted into a vector, e.g., a bacterial vector, such that the polynucleotide is expressed.

The polynucleotides encoding the Rhomboid antigens may be inserted in frame in a polynucleotide encoding a transmembrane protein. The polynucleotide encoding the Rhomboid antigen is inserted into the vector polynucleotide sequence to allow expression of the Rhomboid antigen on the surface of the vector. For example, the polynucleotide encoding Rhomboid antigen may be inserted in frame into the vector polynucleotide in a region encoding an external loop region of a transmembrane protein such that the vector polynucleotide sequence remains in frame. In one embodiment, the first polynucleotide encoding the Rhomboid polypeptide may be inserted into loop 9 of the *lamB* gene of *Salmonella*.

In another embodiment, the first polynucleotide is inserted into or at a surface exposed end of a protein that is attached to the cell wall, but is not a transmembrane protein. The protein may be a secreted protein that is anchored or attached to the cell wall via a protein or lipid anchor. In the Examples, the MPP (SEQ ID NO: 2) polypeptide is inserted at the 3' end of the fibronectin binding protein (FbpB) of *Bacillus subtilis*. Alternatively, the first polynucleotide encoding the Rhomboid antigen may be inserted into a polynucleotide encoding a secreted polypeptide.

Those of skill in the art will appreciate that the polynucleotide encoding the Rhomboid antigen could be inserted in a wide variety of vector polynucleotides to provide

expression and presentation of the Rhomboid antigen to the immune cells of a subject treated with the vaccine. The polynucleotide encoding the Rhomboid antigen may be included in a single copy or more than one copy. The multiple copies may be inserted in a single location or more than one location. Alternatively, multiple epitopes such as combinations of the Rhomboid antigens provided herein as SEQ ID NO: 1-4 and 37-38 or combinations of this epitope with other apicomplexan epitopes such as TRAP or epitopes from other pathogens may be inserted into the vector at the same or more than one location.

Suitably the first polynucleotide encodes a portion of the Rhomboid polypeptide, the entire Rhomboid polypeptide or more than one epitope from the Rhomboid polypeptide. The combination of epitopes from more than one polypeptide from a single parasite or pathogen or the combination of epitopes from related pathogens is specifically contemplated. The polynucleotide may be inserted into the vector and may be inserted as a fusion protein containing more than a single epitope. In the Examples, SEQ ID NOs: 2 and 15 (MPP-HMGB1) or SEQ ID NOs: 2, 40 and 15 (MPP-TRAP-HMGB1) were incorporated into a *Bacillus* vector. Suitably, the portion of the Rhomboid polypeptide inserted into the vector is an antigenic fragment. An antigenic fragment is a peptide or polypeptide capable of eliciting a cellular or humoral immune response or capable of reducing the morbidity or mortality associated with subsequent infection with the parasite.

An antigenic polypeptide or epitope includes any polypeptide that is immunogenic. The antigenic polypeptides include, but are not limited to, antigens that are pathogen-related, allergen-related, tumor-related or disease-related. Pathogens include viral, parasitic, fungal and bacterial pathogens as well as protein pathogens such as the prions. The antigenic polypeptides may be full-length proteins or portions thereof. It is well established that immune system recognition of many proteins is based on a relatively small number of amino acids, often referred to as the epitope. Epitopes may be only 4-8 amino acids long. Thus, the antigenic polypeptides described herein may be full-length proteins, four amino acid long epitopes or any portion between these extremes. In fact the antigenic polypeptide may include more than one epitope from a single pathogen or protein. The antigenic polypeptides may have at least 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% percent identity to the SEQ ID NOs provided herein. Suitably, an antigenic fragment of the Rhomboid antigen or polypeptide may be four, five, six, seven, eight, nine, 10 or more amino acids, 15 or more amino acids or 20 or more amino acids of the full-length protein sequence.

Multiple copies of the same epitope or multiple epitopes from the same or different proteins may be included in the vaccine vector. The epitopes in the vaccine vector may be related and homologous to allow targeting of multiple related pathogens with a single vaccine vector. It is envisioned that several epitopes or antigens from the same or different pathogens or diseases may be administered in combination in a single vaccine vector to generate an enhanced immune response against multiple antigens. Recombinant vaccine vectors may encode antigens from multiple pathogenic microorganisms, viruses or tumor associated antigens. Administration of vaccine vectors capable of expressing multiple antigens has the advantage of inducing immunity against two or more diseases at the same time, providing broader protection against multiple strains of a single pathogen or a more robust immune response against a single pathogen.

In the examples, the MPP antigen (SEQ ID NO: 2) was co-expressed in several of the vectors with a second antigenic polypeptide. A high molecular mass, asexual stage antigen from *Eimeria maxima* (EmTFP250) was demonstrated to be a target for maternal antibodies produced by breeding hens infected with this protozoan parasite. Analysis of the amino acid sequence of the antigen revealed a novel member of the TRAP (thrombospondin-related anonymous protein) family, containing 16 thrombospondin type-1 repeats and 31 epidermal growth factor-like calcium binding domains. See U.S. Patent Publication No. 2011/0111015. EmTFP250 or TRAP also contains two low complex, hydrophilic regions rich in glutamic acid and glycine residues, and a transmembrane domain/cytosolic tail associated with parasite gliding motility that is highly conserved within apicomplexan microneme proteins. Several potential epitopes were selected and are identified in SEQ ID NO: 1-3 and 11 of U.S. Patent Publication No. 2011/0111015 which are reproduced herein as SEQ ID NO: 5-8. SEQ ID NO: 40 was used in the Examples provided herein and is referred to as a TRAP antigen as well. SEQ ID NO: 40 and SEQ ID NO: 6 vary by a single amino acid. A proline at position 6 of SEQ ID NO: 6 is changed to an arginine at the same position 6 of SEQ ID NO: 40. This change was made to make the epitope more flexible and hydrophilic with the goal of making it a better antigen. Those of skill in the art may make other single amino acids changes to improve antigenicity within the scope of this invention. Due to the conserved nature of this antigen, expression of these epitopes by a vector may induce protective immunity against multiple apicomplexan parasites and administration of a vaccine vector comprising two distinct antigenic polypeptides may induce a more robust immune response.

Those of skill in the art will appreciate that the antigenic polypeptides from other pathogens may be used in the vaccine vectors to enhance the immune response against more than one pathogen by a single vaccine. It would be advantageous to administer a single vaccine directed against multiple pathogens. A vaccine capable of eliciting an immune response to an Apicomplexan parasite, such as *Eimeria*, in combination with Influenza, Salmonella, Campylobacter or other pathogens is envisioned.

For example, the second antigenic polypeptide may be an Influenza polypeptide, suitably it is an Influenza H5N1 polypeptide or a polypeptide associated with multiple strains of the Influenza virus such as a polypeptide of the Influenza M2 protein. The ectodomain of the Influenza A virus M2 protein, known as M2e, protrudes from the surface of the virus. The M2e portion of the M2 protein contains about 24 amino acids. The M2e polypeptide varies little from one isolate to the next within Influenza. In fact, only a few naturally occurring mutations in M2e have been isolated from infected humans since the 1918 flu epidemic. In addition, influenza viruses isolated from avian and swine hosts have different, yet still conserved, M2e sequences. For reviews of the M2e polypeptide sequences isolated from human, avian and swine hosts see Liu et al., Microbes and Infection 7:171-177 (2005) and Reid et al., J. Virol. 76:10717-10723 (2002) each of which are incorporated herein by reference in its entirety. Suitably the entire M2e polypeptide may be inserted into the vaccine vector or only a portion may be used. An eight amino acid polypeptide (LM2 having amino acid sequence: EVETPIRN, SEQ ID NO: 9 or its variant M2eA having amino acid sequence EVETPTRN, SEQ ID NO: 10) was incorporated into a vaccine vector and demonstrated to produce an antibody response after administration to chickens. See U.S. Publication No. 2011/0027309 which is incorporated herein by reference in its entirety.

Other suitable epitopes for inclusion in an Influenza A vaccine vector include, but are not limited to, polypeptides of the hemagglutinin (HA) or the nuclear protein (NP) of Influenza A. For example, the peptides of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 or SEQ ID NO: 14 may be included in a vaccine vector. One of skill in the art will appreciate that any of these sequences may be used in combination with any other epitope including epitopes derived from other pathogens or antigens.

Immunostimulatory molecules included as part of the vaccine vector could potentially activate parts of the immune system critical to long-lasting protection or provide an adjuvant effect. Immunostimulatory polypepetides may be polypeptides capable of stimulating a naïve or adaptive immune response. The immunostimulatory polypeptides are not natively

associated with the vaccine vector and are polypeptides natively associated with a vertebrate immune system, such as that of the subject to which the vaccine will be administered. Two immunostimulatory polypeptides are described herein, namely CD154 and High Mobility Group Box 1 (HMGB1) polypeptides, but one of skill in the art will appreciate that other immunostimulatory polypeptides could be used or alternatively could be used in combination with those described herein.

Additional polynucleotides encoding polypeptides involved in triggering the immune system may also be included in a vaccine vector. The polynucleotides may encode immune system molecules known for their stimulatory effects, such as an interleukin, Tumor Necrosis Factor, interferon, complement, or another polynucleotide involved in immune-regulation. The vaccine may also include polynucleotides encoding peptides known to stimulate an immune response, such as the CD154 or HMGB1 polypeptides described herein.

HMGB1 is secreted by activated macrophages and damaged cells, and acts as a cytokine mediator of inflammation, affecting the innate immune response. Portions of the HMGB1 sequence have been included in the vaccine vectors described in the Examples. The HMGB1 (High Mobility Group Box-1) protein was first identified as a DNA-binding protein critical for DNA structure and stability. It is a ubiquitously expressed nuclear protein that binds DNA with no sequence specificity. The protein is highly conserved and found in plants to mammals. The zebrafish, chicken and human HMGB1 amino acid sequences are provided in SEQ ID NO: 23, SEQ ID NO: 15 and SEQ ID NO: 22, respectively. The sequence throughout mammals is highly conserved with 98% amino acid identity and the amino acid changes are conservative. Thus an HMGB1 protein from one species can likely substitute for that from another species functionally. The full-length HMGB1 protein or a portion thereof may be used as the HMGB1 polypeptide in the vaccine vectors described herein. HMGB1 has two DNA binding regions termed A box as shown in SEQ ID NO: 16 and 17 and B box as shown in SEQ ID NO: 18 and 19. See Andersson and Tracey, Annu. Rev. Immunol. 2011, 29:139-162, which is incorporated herein by reference in its entirety.

HMGB1 is a mediator of inflammation and serves as a signal of nuclear damage, such as from necrotic cells. HMGB1 can also be actively secreted by cells of the monocyte/macrophage lineage in a process requiring acetylation of the protein, translocation across the nucleus and secretion. Extracellular HMGB1 acts as a potent mediator of inflammation by signaling via the Receptor for Advanced Glycated End-products (RAGE) and via members of the Toll-like Receptor family (TLR), in particular TLR4. The RAGE

binding activity has been identified and requires the polypeptide of SEQ ID NO: 20. TLR4 binding requires the cysteine at position 106 of SEQ ID NO: 15, which is found in the B box region of HMGB1.

The inflammatory activities of HMGB1 do not require the full-length protein and functional fragments have been identified. The B box has been shown to be sufficient to mediate the pro-inflammatory effects of HMGB1 and thus SEQ ID NO: 18 and 19 are HMGB1 polypeptides or functional fragments thereof within the context of the present invention. In addition, the RAGE binding site and the pro-inflammatory cytokine activity have been mapped to SEQ ID NO: 20 and SEQ ID NO: 21, respectively. Thus, these polypeptides are functional fragments of HMGB1 polypeptides in the context of the present invention.

Those of skill in the art are capable of identifying HMGB1 polypeptides and fragments thereof capable of stimulating pro-inflammatory cytokine activity, using methods such as those in International Publication No. WO02/092004, which is incorporated herein by reference in its entirety. Suitably, the HMGB1 polypeptide includes the RAGE binding domain at amino acids 150-183 of SEQ ID NO:15 (SEQ ID NO: 20 or a homolog thereof) and the pro-inflammatory cytokine activity domain between amino acids 89-109 of SEQ ID NO: 15 (SEQ ID NO: 21 or a homolog thereof). In particular, HMGB1 polypeptides and functional fragments or homologs thereof include polypeptides identical to, or at least 99% identical, at least 98% identical, at least 97% identical, at least 96% identical, at least 95% identical, at least 90% identical, at least 85% identical, or at least 80% identical to the HMGB1 polypeptides of SEQ ID NOs: 15 or 16-23.

As described in more detail below, a vaccine vector may include a CD154 polypeptide that is capable of binding CD40 in the subject and stimulating the subject to respond to the vector and its associated antigen. Involvement of dendritic cells (DCs) is essential for the initiation of a powerful immune response as they possess the unique ability to activate naïve T cells, causing T cell expansion and differentiation into effector cells. It is the role of the DC, which is an antigen presenting cell (APC) found in virtually all tissues of the body, to capture antigens, transport them to associated lymphoid tissue, and then present them to naïve T cells. Upon activation by DCs, T cells expand, differentiate into effector cells, leave the secondary immune organs, and enter peripheral tissues. Activated cytotoxic T cells (CTLs) are able to destroy virus-infected cells, tumor cells or even APCs infected with intracellular parasites (e.g., *Salmonella*) and have been shown to be critical in the protection

against viral infection. CD40 is a member of the TNF-receptor family of molecules and is expressed on a variety of cell types, including professional antigen-presenting cells (APCs), such as DCs and B cells. Interaction of CD40 with its ligand CD154 is extremely important and stimulatory for both humoral and cellular immunity. Stimulation of DCs via CD40, expressed on the surface of DCs, can be simulated by anti-CD40 antibodies. In the body, however, this occurs by interaction with the natural ligand for CD40 (i.e. CD154) expressed on the surface of activated T-cells. Interestingly, the CD40-binding regions of CD154 have been identified. The CD40-binding region of CD154 may be expressed on the surface of a vector, such as a *Salmonella* or *Bacillus* vector, and results in an enhanced immune response against a co-presented peptide sequence as shown in the Examples provided herein and in U.S. Patent Publication No. 2011/0027309, which is incorporated herein by reference in its entirety. A CD154 polypeptide may be a portion of CD154 full-length protein or the entire CD154 protein. Suitably, the CD154 polypeptide is capable of binding CD40.

As discussed above, a CD154 polynucleotide encoding a CD154 polypeptide that is capable of enhancing the immune response to the antigen may be included in the vaccine. Suitably, the CD154 polypeptide is fewer than 50 amino acids long, more suitably fewer than 40, fewer than 30 or fewer than 20 amino acids in length. The polypeptide may be between 10 and 15 amino acids, between 10 and 20 amino acids or between 10 and 25 amino acids in length. The CD154 sequence and CD40 binding region are not highly conserved among the various species. The CD154 sequences of chicken and human are provided in SEQ ID NO: 24 and SEQ ID NO: 25, respectively.

The CD40 binding regions of CD154 have been determined for a number of species, including human, chicken, duck, mouse and cattle and are shown in SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30, respectively. Although there is variability in the sequences in the CD40 binding region between species, the human CD154 polypeptide was able to enhance the immune response in chickens. Therefore, one may practice the invention using species specific CD154 polypeptides or a heterologous CD154 polypeptide. Thus the CD154 polypeptides of SEQ ID NO: 24-30 may be included in a vaccine vector or a polypeptide at least 99, 98, 97, 96, 95, 93, 90 or 85% identical to the sequences of SEQ ID NO: 24-30 may be included in a vaccine vector.

The polypeptide from CD154 stimulates an immune response at least in part by binding to its receptor, CD40. A polypeptide homologous to the CD154 polypeptide which is expressed on immune cells of the subject and which is capable of binding to the CD40

receptor on macrophages and other antigen presenting cells. Binding of this ligand-receptor complex stimulates macrophage (and macrophage lineage cells such as dendritic cells) to enhance phagocytosis and antigen presentation while increasing cytokine secretions known to activate other local immune cells (such as B-lymphocytes). As such, molecules associated with the CD154 peptide are preferentially targeted for immune response and expanded antibody production.

The antigenic polypeptides and the immunostimulatory polypeptides are delivered via a vaccine vector. The vaccine vectors may be bacterial, yeast, viral or liposome-based vectors. Potential vaccine vectors include, but are not limited to, *Bacillus* (*Bacillus subtilis*), *Salmonella* (*Salmonella enteritidis*), *Shigella*, *Escherichia* (*E. coli*), *Yersinia*, *Bordetella*, *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Vibrio* (*Vibrio cholerae*), *Listeria*, yeast such as *Saccharomyces*, or *Pichia*, adenovirus, poxvirus, herpesvirus, alphavirus, and adeno-associated virus. Live bacterial, yeast or viral vaccine vectors may still pose risks to immunocompromised individuals and require additional regulatory scrutiny. Thus use of vectors that are killed or inactivated or qualify as Generally Recognized As Safe (GRAS) organisms by the Food and Drug Administration (FDA) is desirable. The problem is generating a robust immune response using such vectors. Methods of inactivating or killing bacterial, yeast or viral vaccine vectors are known to those of skill in the art and include, but are not limited to methods such as formalin inactivation, antibiotic- based inactivation, heat treatment and ethanol treatment. By including an immunostimulatory polypeptide such as HMGB1 (high mobility group box 1) polypeptide on the surface of the vaccine vector we can generate a robust immune response against an apicomplexan parasite using a *Bacillus* spp. vector. In fact, the Examples demonstrate that this vector can be inactivated such that it cannot replicate and still elicit a robust immune response after administration. The vaccine vectors may be wild-type bacteria, yeasts or viruses that are not pathogenic. Alternatively the vectors may be attenuated such that the vector has limited ability to replicate in the host or is not capable of growing without supplemented media for more than a few generations. Those of skill in the art will appreciate that there are a variety of ways to attenuate vectors and means of doing so.

At least a portion of the antigenic polypeptide and at least a portion of the immunostimulatory polypeptide are present or expressed on the surface of the vaccine vector. Present on the surface of the vaccine vector includes polypeptides that are comprised within an external loop of a transmembrane protein, interacting with, e.g., covalently or chemically

cross-linked to, a transmembrane protein, a membrane lipid or membrane anchored carbohydrate or polypeptide. A polypeptide can be comprised within a transmembrane protein by having the amino acids comprising the polypeptide linked via a peptide bond to the N-terminus, C-terminus or anywhere within the transmembrane protein (i.e. inserted between two amino acids of the transmembrane protein or in place of one or more amino acids of the transmembrane protein (i.e. deletion-insertion)). Suitably, the polypeptides may be inserted into an external loop of a transmembrane protein. Suitable transmembrane proteins are *srtA*, *cotB* and *lamB*, but those of skill in the art will appreciate many suitable transmembrane proteins are available. Polypeptides may be linked to a membrane or cell wall anchored protein or lipid such that the antigenic polypeptide and the immunostimulatory polypeptide are expressed on the surface of the vaccine vector.

As described above, polynucleotides encoding the antigenic or immunostimulatory polypeptides may be inserted into the chromosome of the vector or maintained extrachromosomally (e.g., on a plasmid, BAC or YAC). Those of skill in the art will appreciate that these polynucleotides can be inserted in frame in a variety of polynucleotides and expressed in different parts of the vector or may be secreted. The polynucleotide encoding the immunostimulatory polypeptide capable of enhancing the immune response to the antigenic polypeptide may also encode the antigenic polypeptide. The polynucleotide encoding the antigenic polypeptide may be linked to the polynucleotide encoding the immunostimulatory polypeptide, such that in the vector, the two polypeptides are portions of the same polypeptide, such as in a fusion protein. In the Examples, a polynucleotide encoding the antigenic polypeptide also encodes the immunostimulatory polypeptide. In one embodiment, the two polynucleotides encoding the polypeptides are both inserted in frame in loop 9 of the *lamB* gene of *Salmonella enteritidis* or another vaccine vector. Those of skill in the art will appreciate that bacterial polynucleotides encoding other transmembrane proteins and other loops of the *lamB* gene may also be used.

Alternatively, the polynucleotide encoding the antigenic polypeptide and/or the immunostimulatory polypeptide may be inserted into a secreted polypeptide that is displayed or presented on the surface of the vaccine vector through association with a protein, lipid or carbohydrate on the surface of the vaccine vector. Those of skill in the art will appreciate that the polynucleotide encoding the antigenic polypeptide and/or the immunostimulatory polypeptide could be inserted in a wide variety of vaccine vector polynucleotides to provide expression and presentation of the antigenic polypeptide and/or the immunostimulatory

polypeptide to the immune cells of a subject treated with the vaccine vector by expression on the surface of the vaccine vector. The coding region of the Apicomplexan Rhomboid polypeptide and the immunostimulatory polypeptide can be fused to the C-terminus of the *Staphylococcus aureus* fibronectin binding protein containing a sorting motif for sortase from *Listeria*. This allows the secreted proteins to be anchored on the cell wall of gram positive bacteria such as *Bacillus*. See Nguyen and Schumann, J Biotechnol (2006) 122: 473-482, which is incorporated herein by reference in its entirety. This system was used in the Examples to allow expression of the Rhomboid polypeptide linked to HMGB1 on the surface of Bacillus. Other similar methods may also be used.

Alternatively, the polypeptides may be covalently or chemically linked to proteins, lipids or carbohydrates in the membrane, cell wall, or capsid if a viral vector is being used through methods available to persons of skill in the art. For example, di-sulfide bonds or biotin – avidin cross-linking could be used to present the antigenic and immunostimulatory polypeptides on the surface of a vaccine vector. Suitably, the antigenic polypeptide and the immunostimulatory polypeptide are part of a fusion protein. The two polypeptides may be directly linked via a peptide bond or may be separated by a linker, spacer, or a section of a third protein into which they are inserted in frame. In the Examples, an amino acid spacer was used between the polypeptides. A spacer may be between 2 and 20 amino acids, suitably between 4 and 10 amino acids, suitably between 6 and 8 amino acids. Suitably the amino acids in the spacer have a small side chain and are not charged, such as glycine, alanine or serine. In the Examples, a spacer including two glycine residues, two serine residues and arginine and two more serine residues was used. Those of skill in the art will appreciate other spacers could be used.

In the Examples, the vaccine vectors have the antigenic polypeptides (MPP and/or TRAP polypeptides) and the immunostimulatory polypeptide (either CD154 or HMGB1 or both) encoded on the same polynucleotide and in frame with each other. In alternative embodiments, the immunostimulatory polypeptide and the antigenic polypeptide may be encoded by distinct polynucleotides. Those of skill in the art will appreciate that a variety of methods may be used to obtain expression of the antigenic polypeptide and the HMGB1 polypeptide on the surface of the vaccine vector. Such methods are known to those skilled in the art.

Compositions comprising the vaccine vector and a pharmaceutically acceptable carrier are also provided. A pharmaceutically acceptable carrier is any carrier suitable for *in*

vivo administration. Suitably, the pharmaceutically acceptable carrier is acceptable for oral, nasal or mucosal delivery. The pharmaceutically acceptable carrier may include water, buffered solutions, glucose solutions or bacterial culture fluids. Additional components of the compositions may suitably include excipients such as stabilizers, preservatives, diluents, emulsifiers and lubricants. Examples of pharmaceutically acceptable carriers or diluents include stabilizers such as carbohydrates (e.g., sorbitol, mannitol, starch, sucrose, glucose, dextran), proteins such as albumin or casein, protein-containing agents such as bovine serum or skimmed milk and buffers (e.g., phosphate buffer). Especially when such stabilizers are added to the compositions, the composition is suitable for freeze-drying or spray-drying. The vaccine vector in the compositions may not be capable of replication, suitably the vaccine vector is inactivated or killed prior to addition to the composition.

Methods of enhancing immune responses in a subject by administering a vaccine vector are also provided. The vaccine vector may contain a first polynucleotide encoding an Aplicomplexan Rhomboid polypeptide and a second polynucleotide encoding an immunostimulatory polypeptide. The immunostimulatory polypeptide is suitably a polypeptide natively associated with a vertebrate immune system and involved in stimulating an immune response. The immunostimulatory polypeptide may stimulate the native or adaptive immune response of the subject. Suitably a HMGB1 polypeptide or a CD154 polypeptide as described more fully above may be used as the immunostimulatory polypeptide. In the methods provided herein, the vaccine vector comprising an Apicomplexan Rhomboid polypeptide and an immunostimulatory polypeptide is administered to a subject in an amount effective to enhance or effect an immune response of the subject to the vaccine vector and in particular to the antigenic Rhomboid polypeptide and suitably to the apicomplexan parasite. The enhanced immune response may include the antibody or T cell response. Suitably the immune response is a protective immune response, but the immune response may not be fully protective, but may be capable of reducing the morbidity or mortality associated with infection. The immunostimulatory polypeptides may be used to enhance the immune response in the subject to any foreign antigen or antigenic polypeptide present in the vaccine vector in addition to the Rhomboid polypeptide. One of skill in the art will appreciate that the immunostimulatory polypeptide could be used to enhance the immune response to more than one antigenic polypeptide present in a vaccine vector. Enhancing an immune response includes, but is not limited to, inducing a therapeutic or prophylactic effect that is mediated by the immune system of the subject. Specifically,

enhancing an immune response may include, but is not limited to, enhanced production of antibodies, enhanced class switching of antibody heavy chains, maturation of antigen presenting cells, stimulation of helper T cells, stimulation of cytolytic T cells or induction of T and B cell memory.

Suitably, the vaccine vector contains a polynucleotide encoding a polypeptide including amino acids 150-183 and 89-109 of the HMGB1 polypeptide (SEQ ID NO: 15) or a homolog thereof. In the Examples, a 190 amino acid polypeptide of HMGB1 was used. Suitably, the polynucleotide encodes a HMGB1 polypeptide from the same species as the subject. Heterologous combinations of HMGB1 polypeptides and subjects (e.g. a human HMGB1 polypeptide for use in a chicken vaccine) may be useful in the methods of the invention because HMGB1 is highly conserved through a wide number of species. The HMGB1 polypeptide may be used to enhance the immune response to more than one antigenic polypeptide present in a vaccine vector. The polypeptide from HMGB1 stimulates an immune response at least in part by activating dendritic cells and macrophages and thus stimulating production of cytokines such as IL-1, IL-6, IFN-γ and TNF-α. In the Examples, a polypeptide of HMGB1 was expressed on the surface of the vaccine vector.

The vaccine vector may suitably contain a CD154 polypeptide capable of binding to CD40 and activating CD40. The vaccine comprising the polynucleotide encoding a CD154 polypeptide capable of binding to CD40 is administered to a subject in an amount effective to enhance or affect the immune response of the subject to the vaccine. Suitably, the vaccine contains a polynucleotide encoding a polypeptide including amino acids 140-149 of the human CD154 polypeptide (SEQ ID NO: 25) or a homolog thereof. As noted above, a homologue of amino acid 140-149 derived from one species may be used to stimulate an immune response in a distinct species. Suitably, the polynucleotide encodes a CD154 polypeptide from the same species as the subject. Suitably, a polynucleotide encoding the polypeptide of SEQ ID NO: 26 is used in human subjects, a polynucleotide encoding the polypeptide of SEQ ID NO: 27 is used in chickens, a polynucleotide encoding the polypeptide of SEQ ID NO: 28 is used in ducks, a polynucleotide encoding the polypeptide of SEQ ID NO: 29 is used in mice, and a polynucleotide encoding the polypeptide of SEQ ID NO: 30 is used in cows. The human CD154 polypeptide (SEQ ID NO: 26) has been used in a chicken vaccine and was demonstrated to enhance the immune response to a foreign antigen. Thus other heterologous combinations of CD154 polypeptides and subjects may be useful in the methods of the invention.

In addition, methods of enhancing an immune response against an apicomplexan parasite and methods of reducing morbidity associated with subsequent infection with an apicomplexan parasite are disclosed. Briefly, the methods comprise administering to a subject an effective amount of a vaccine vector comprising a first polynucleotide sequence encoding an Apicomplexan Rhomboid polypeptide. The vaccine vector may also include a second polynucleotide encoding an immunostimulatory polypeptide in an effective amount. The Rhomboid polypeptides may include SEQ ID NO: 1-4, 37, 38 or combinations or fragments thereof. The insertion of the Rhomboid polypeptides into the vector may be accomplished in a variety of ways known to those of skill in the art, including but not limited to the scarless site-directed mutation system described in BMC Biotechnol. 2007 Sept, 17: 7(1): 59, Scarless and Site-directed Mutagenesis in *Salmonella* Enteritidis chromosome, which is incorporated herein by reference in its entirety and the method used herein as described in Nguyen and Schumann J Biotechnol 2006 122: 473-482, which is incorporated herein by reference in its entirety. The vector may also be engineered to express the Rhomboid polypeptides in conjunction with other antigenic polypeptides from apicomplexan parasites such as TRAP or from other pathogens including viruses such as Influenza M2e or bacteria such as *Salmonella* or *E. coli*. In particular, a polypeptide of CD154 capable of binding CD40 or HMGB1 may be expressed by the vector to enhance the immune response of the subject to the Rhomboid polypeptide.

The compositions containing antigenic polypeptides may also be used to decrease the morbidity associated with subsequent infection by an apicomplexan parasite. The compositions may prevent the parasite from causing disease or may limit or reduce any associated morbidity in a subject to which the compositions or vaccine vectors described herein were administered. The compositions and vaccine vectors described herein may reduce the severity of subsequent disease by decreasing the length of disease, weight loss, severity of symptoms of the disease, decreasing the morbidity or mortality associated with the disease or reducing the likelihood of contracting the disease. The compositions may also reduce the spread of the parasite by inhibiting transmission. The morbidity or mortality associated with the disease after administration of the vaccine vectors described herein may be reduced by 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or even 100% as compared to similar subjects not provided the vaccine vector.

For administration to animals or humans, the compositions may be administered by a variety of means including, but not limited to, intranasally, mucosally, by spraying,

intradermally, parenterally, subcutaneously, intraperitonelly, intravenously, intracrannially, orally, by aerosol or intramuscularly. Eye-drop administration, oral gavage or addition to drinking water or food is additionally suitable. For poultry, the compositions may be administered *in ovo*.

Some embodiments of the invention provide methods of enhancing immune responses in a subject. Suitable subjects may include, but are not limited to, vertebrates, suitably mammals, suitably a human, and birds, suitably poultry such as chickens or turkeys. Other animals such as cows, cats, dogs or pigs may also be used. Suitably, the subject is non-human and may be an agricultural animal.

The useful dosage of the vaccine to be administered will vary depending on the age, weight and species of the subject, the mode and route of administration and the type of pathogen against which an immune response is sought. The composition may be administered in any dose sufficient to evoke an immune response. It is envisioned that doses ranging from 10^3 to 10^{10} vector copies (i.e. colony forming units or plaque forming units), from 10^4 to 10^9 vector copies, or from 10^5 to 10^7 vector copies are suitable.

The composition may be administered only once or may be administered two or more times to increase the immune response. For example, the composition may be administered two or more times separated by one week, two weeks, three weeks, 1 month, 2 months, 3 months, 6 months, 1 year or more. The vaccine vector may comprise viable microorganisms prior to administration, but in some embodiments the vector may be killed prior to administration. In some embodiments, the vector may be able to replicate in the subject, while in other embodiments the vector may not be capable of replicating in the subject. Methods of inactivating microorganisms used as vectors are known to those of skill in the art. For example a bacterial vaccine vector may be inactivated using formalin, ethanol, heat exposure, or antibiotics. Those of skill in the art may use other methods as well.

It is envisioned that several epitopes or antigens from the same or different pathogens may be administered in combination in a single vaccine to generate an enhanced immune response against multiple antigens. Recombinant vaccines may encode antigens from multiple pathogenic microorganisms, viruses or tumor associated antigens. Administration of vaccine capable of expressing multiple antigens has the advantage of inducing immunity against two or more diseases at the same time. For example, live attenuated bacteria provide a suitable vector for eliciting an immune response against multiple antigens from a single

pathogen, e.g., TRAP (SEQ ID NO: 6) and MPP from *Eimeria* (SEQ ID NO: 2); or against multiple antigens from different pathogens, e.g., *Eimeria* and Influenza or *Salmonella*.

Vaccine vectors may be constructed using exogenous polynucleotides encoding antigens which may be inserted into the vaccine vector at any non-essential site or alternatively may be carried on a plasmid or other extra chromosomal vehicle (e.g. a BAC or YAC) using methods well known in the art. One suitable site for insertion of polynucleotides is within external portions of transmembrane proteins or coupled to sequences that target the exogenous polynucleotide for secretory pathways and/or allow attachment to the cell wall. One example of a suitable transmembrane protein for insertion of polynucleotides is the *lamB* gene. One suitable method of cell wall attachment is provided in the Examples

Exogenous polynucleotides include, but are not limited to, polynucleotides encoding antigens selected from pathogenic microorganisms or viruses and include polynucleotides that are expressed in such a way that an effective immune response is generated. Such polynucleotides may be derived from pathogenic viruses such as influenza (e.g., M2e, hemagglutinin, or neuraminidase), herpesviruses (e.g., the genes encoding the structural proteins of herpesviruses), retroviruses (e.g., the gp160 envelope protein), adenoviruses, paramyxoviruses, coronaviruses and the like. Exogenous polynucleotides can also be obtained from pathogenic bacteria, e.g., genes encoding bacterial proteins such as toxins, outer membrane proteins or other highly conserved proteins. Further, exogenous polynucleotides from parasites, such as other Apicomplexan parasites are attractive candidates for use in a vector vaccine.

The present disclosure is not limited to the specific details of construction, arrangement of components, or method steps set forth herein. The compositions and methods disclosed herein are capable of being made, practiced, used, carried out and/or formed in various ways that will be apparent to one of skill in the art in light of the disclosure that follows. The phraseology and terminology used herein is for the purpose of description only and should not be regarded as limiting to the scope of the claims. Ordinal indicators, such as first, second, and third, as used in the description and the claims to refer to various structures or method steps, are not meant to be construed to indicate any specific structures or steps, or any particular order or configuration to such structures or steps. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language

(e.g., "such as") provided herein, is intended merely to facilitate the disclosure and does not imply any limitation on the scope of the disclosure unless otherwise claimed. No language in the specification, and no structures shown in the drawings, should be construed as indicating that any non-claimed element is essential to the practice of the disclosed subject matter. The use herein of the terms "including," "comprising," or "having," and variations thereof, is meant to encompass the elements listed thereafter and equivalents thereof, as well as additional elements. Embodiments recited as "including," "comprising," or "having" certain elements are also contemplated as "consisting essentially of" and "consisting of" those certain elements. The terms "a", "an" and "the" may mean one or more than one unless specifically delineated.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure. Use of the word "about" to describe a particular recited amount or range of amounts is meant to indicate that values very near to the recited amount are included in that amount, such as values that could or naturally would be accounted for due to manufacturing tolerances, instrument and human error in forming measurements, and the like. All percentages referring to amounts are by weight unless indicated otherwise.

The following examples are meant only to be illustrative and are not meant as limitations on the scope of the invention or of the appended claims. All references, included patents, patent publications and non-patent literature, cited herein are hereby incorporated by reference in their entirety. Any conflict between statements in references and those made herein should be resolved in favor of the statements contained herein.

EXAMPLES

Example 1. Construction of vaccine vectors

Multiple combinations of vaccine were constructed for the purpose of testing efficacy and determining the influence of each on protection against *Eimeria maxima* challenge. A cartoon showing the constructs used in the examples is shown as Figure 2. The TRAP MPP HMGB1, and MPP HMGB1 sequences were synthesized and inserted into pNDH10 plasmid for cell surface expression. Each sequence was synthesized with a BamHI restriction site at the 5' end and an AatII restriction site at the 3' end immediately adjacent to the fibronectin binding protein B (*fbpB*). Expression of the vaccine sequence and *fbpB* is regulated by a *xyl* operon previously inserted into pNDH10 plasmid [1]. The *fbpB* included a sorting motif that was recognized by sortase A that anchors the *fbpB* to the cell surface of a sortase A expressing bacterium [1]. Thus, the vaccine sequences are placed upstream and in frame with the *fbpB* sequence such that when the *fbpB* is anchored to sortase A on the cell wall the vaccine vector sequence will be expressed on the surface of the bacteria. Plasmid pNDH10 containing the vaccine sequence, *fbpB*, and *xyl* operon was transformed into *Bacillus subtilis* 1A857 expressing sortase A [2]. Each plasmid was transformed into 1A857 by adding 0.6 µg insert/plasmid into a competent 1A857 culture with 0.1 M ethylene glycol tetraacetic acid (EGTA). After transformation, 1A857 expressing pNDH10 were selected on LB agar containing 5 µg/mL chloramphenicol to select only cells that carried antibiotic resistance conferred by the plasmid via a *cat* sequence that encodes chloramphenicol acetyl transferase. *Bacillus subtilis* 1A857 transformed with MPP HMGB1 (SEQ ID NO: 33), or TRAP MPP HMGB1 (SEQ ID NO: 31) pNDH10 plasmids were confirmed by plasmid extraction followed by PCR. Each 1A857/pNDH10/insert construct was grown and induced in 0.6% xylose in LB broth +0.1% glucose with 5 µg/mL chloramphenicol for 9 h at 37°C while shaking. MPP-HMGB1 (SEQ ID NO: 34) and TRAP-MPP-HMGB1 (SEQ ID NO: 32) protein expression were confirmed by Western blot and indirect fluorescence microscopy with rabbit anti-HMGB1 antibodies.

Example 2. Reduced morbidity and mortality of chicks after *Eimeria* infection

Vectored vaccines MPP HMGB1 and TRAP MPP HMGB1 were tested for ability to provide protection against an *Eimeria maxima* challenge when administered through the

drinking water in conjunction with a modified chitosan adjuvant. Broiler chicks were vaccinated at 4 and 14 days of age with the respective vaccine in the drinking water at a dilution of 1:128 (5 X 10^5 cfu/chick) for 24h. At 21d of age, all groups were weighed and challenged with 4x10^4 sporulated oocysts of *E. maxima* by oral gavage. At 28d of age, body weight (BW) and body weight gain of survivors (BWG) were recorded during the challenge period. Additionally, mortality was documented to determine vaccine candidate efficacy. Eight days post-challenge BW was significantly higher in chicks vaccinated with TRAP-MPP-HMGB1 and MPP-HMGB1 when compared with non-vaccinated chicks (Figure 3). BWG was significantly higher for all vaccinated groups 8d post-challenge when compared to controls (Figure 4). Mortality was also significantly lower in the TRAP-MPP-HMGB1 and MPP-HMGB1 vaccinated groups with the unvaccinated group (Figure 5).

[1] Kim L, Mogk A, Schumann W. A xylose-inducible *Bacillus subtilis* integration vector and its application. Gene 1996 Nov 28;181(1-2):71-6.

[2] Nguyen HD, Schumann W. Establishment of an experimental system allowing immobilization of proteins on the surface of *Bacillus subtilis* cells. Journal of biotechnology 2006 Apr 20;122(4):473-82.

CLAIMS

We claim:

1. A vaccine vector comprising a first polynucleotide sequence encoding an Apicomplexan Rhomboid polypeptide optionally expressed on the surface of the vaccine vector, wherein the Rhomboid polypeptide is or has greater than 90% sequence identity to at least one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 37, SEQ ID NO: 38, an immunogenic fragment of SEQ ID NO: 1, an immunogenic fragment of SEQ ID NO: 2, an immunogenic fragment of SEQ ID NO: 3, an immunogenic fragment of SEQ ID NO: 4, an immunogenic fragment of SEQ ID NO: 37, an immunogenic fragment of SEQ ID NO: 38 or combinations thereof.

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 is an HMGB1 polypeptide.

4. The vaccine vector of claim 3, wherein the HMGB1 polypeptide comprises a polypeptide selected from at least one of SEQ ID NOs: 15-23, a fragment of at least one of SEQ ID NOs: 15-23, a polypeptide having at least 95% sequence identity to SEQ ID NO: 15-23 and combinations thereof.

5. The vaccine vector of any one of claims 2-4, wherein the immunostimulatory polypeptide is a CD154 polypeptide capable of binding CD40, the CD154 polypeptide having fewer than 50 amino acids and comprising amino acids 140-149 of SEQ ID NO: 24, SEQ ID NO: 25 or a homolog thereof.

6. The vaccine vector of claim 5, wherein the CD154 polypeptide is SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30 or has at least 90% sequence identity to at least one of SEQ ID NO: 26-30.

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

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

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

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

11. The vaccine vector of claim 10, wherein the vaccine vector is a *Bacillus* spp.

12. The vaccine vector of any one of claims 1-11, further comprising a third polynucleotide encoding a TRAP polypeptide.

13. The vaccine vector of claim 12, wherein the TRAP polypeptide is at least 95% identical to SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 40, an immunogenic fragment of SEQ ID NO: 5, an immunogenic fragment of SEQ ID NO: 6, an immunogenic fragment of SEQ ID NO: 7 or an immunogenic fragment of SEQ ID NO: 40.

14. The vaccine vector of claim 1, wherein the vaccine vector comprises the polypeptide of SEQ ID NO: 32, SEQ ID NO: 34 or a polypeptide having 95% sequence identity to SEQ ID NO: 32 or SEQ ID NO: 34.

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

16. A method of enhancing the immune response against an apicomplexan parasite in a subject comprising administering to the subject the vaccine vector of any one of claims 1-14 or the pharmaceutical composition of claim 15 in an amount effective to enhance the immune response of the subject to the apicomplexan parasite.

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

18. A method of reducing morbidity associated with infection with an apicomplexan parasite in a subject comprising administering to the subject the vaccine vector of any one of claims 1-14 or the pharmaceutical composition of claim 15 in an amount effective to reduce the morbidity associated with subsequent infection of the subject with an apicomplexan parasite as compared to a control subject not administered the vaccine vector.

19. The method of any one of claims 16-18, wherein the vaccine vector is administered by a route selected from the group consisting of oral, mucosal, parenteral, sub-cutaneous, intramuscular, intraocular and *in ovo*.

20. The method of any one of claims 16-19, wherein the subject is member of a poultry species.

21. The method of claim 18, wherein the poultry species is a chicken or turkey.

22. The method of any one of claims 16-21, wherein the subject is a mammal.

23. The method of claim 22, wherein the subject is a human, swine or cow.

24. The method of any one of claims 16-23, wherein from about 10^8 to about 10^9 vector copies of the vaccine are administered to the subject.

25. The method of any one of claims 16-23, wherein from about 10^5 to about 10^7 vector copies of the vaccine are administered to the subject.

26. The method of any one of claims 16-25, wherein the vaccine vector is killed prior to administration to the subject or is not capable of replicating in the subject.

27. The method of any one of claims 16-26, wherein the apicomplexan parasite is selected from the group consisting of *Eimeria, Plasmodium, Toxoplasma, Neospora* and *Cryptosporidium*.