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(54) **IMMUNOGENIC PROTEIN CONJUGATES AND METHOD FOR MAKING AND USING THE SAME**

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C12P 21/02 (2006.01)

(52) **U.S. Cl.**

CPC ***A61K 47/4833*** (2013.01); ***C12P 21/02*** (2013.01)
USPC **424/190.1**; 435/68.1

(57) **ABSTRACT**

Production of protein conjugate vaccines by use of transpeptidase enzymes, such as sortase enzymes. For example, homogenous immunoconjugates (e.g., a population of molecules having the same structure) formed by conjugating an antigenic polypeptide and a bacterial capsule component are provided. In certain aspects, methods for generating an immune response to *B. anthracis* by use of protective antigen-PDGA immunoconjugates are provided.

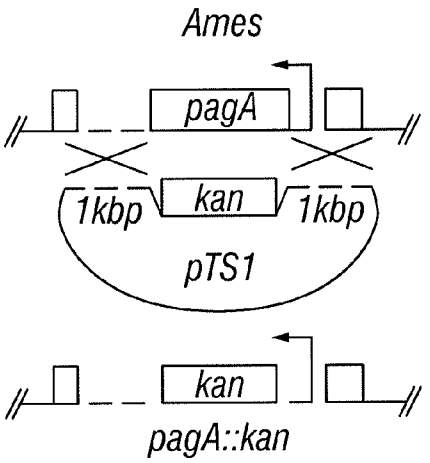

FIG. 1A

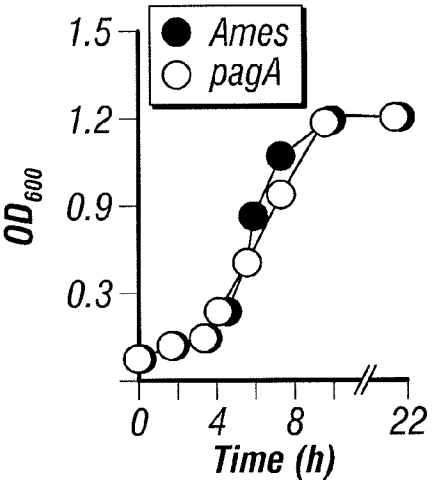

FIG. 1B

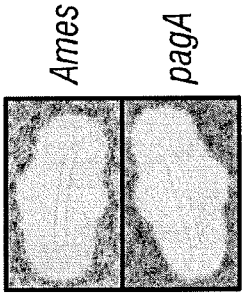

FIG. 1C

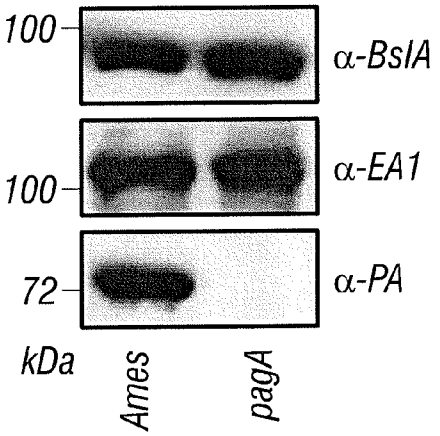

FIG. 1D

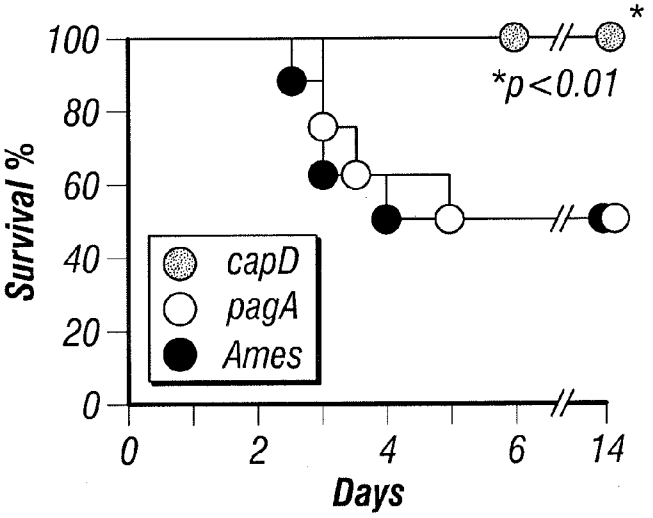

FIG. 1E

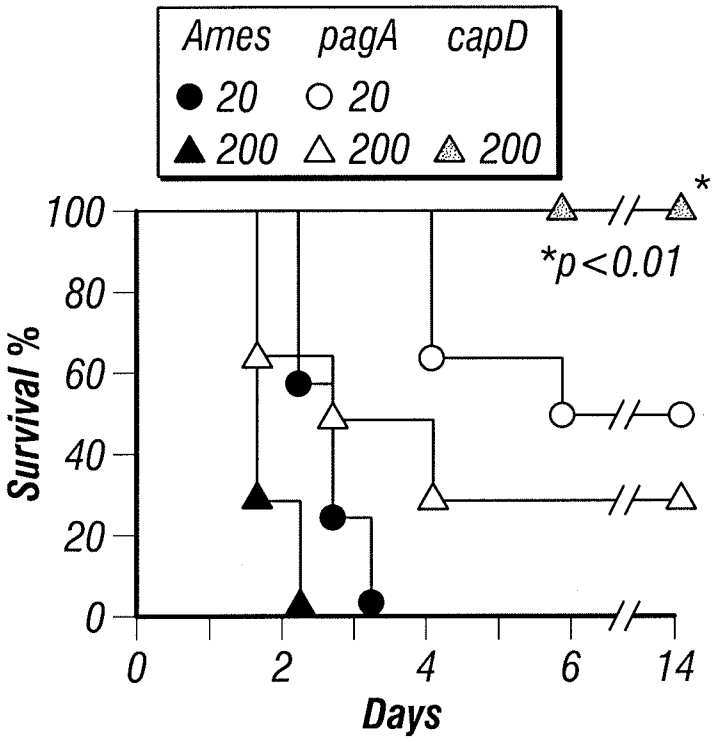
Ames pagA capD
20 20
200 200 200
*p<0.01
Survival %
100
80
60
40
20
0
0 2 4 6 14
Days

FIG. 2A

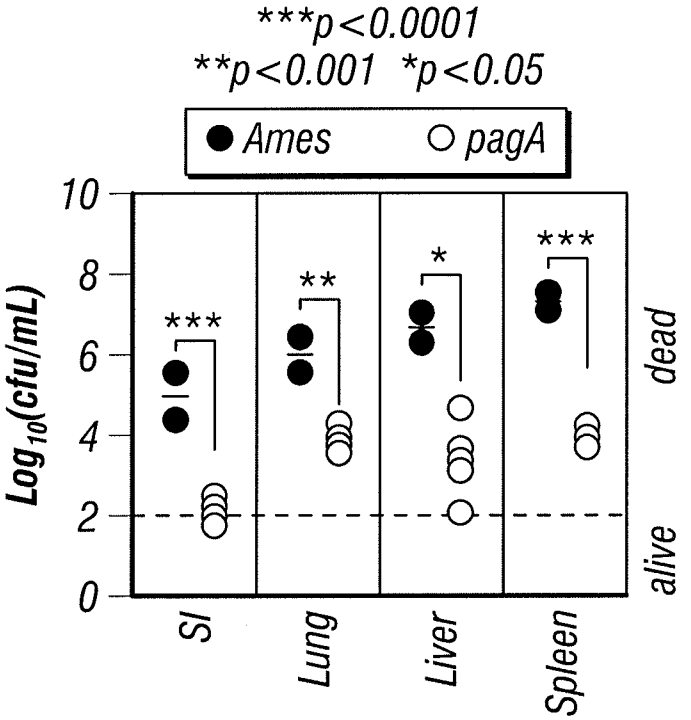
***p<0.0001
**p<0.001 *p<0.05
Ames pagA
Log10(cfu/mL)
10
8
6
4
2
0
dead
alive
SI
Lung
Liver
Spleen

FIG. 2B

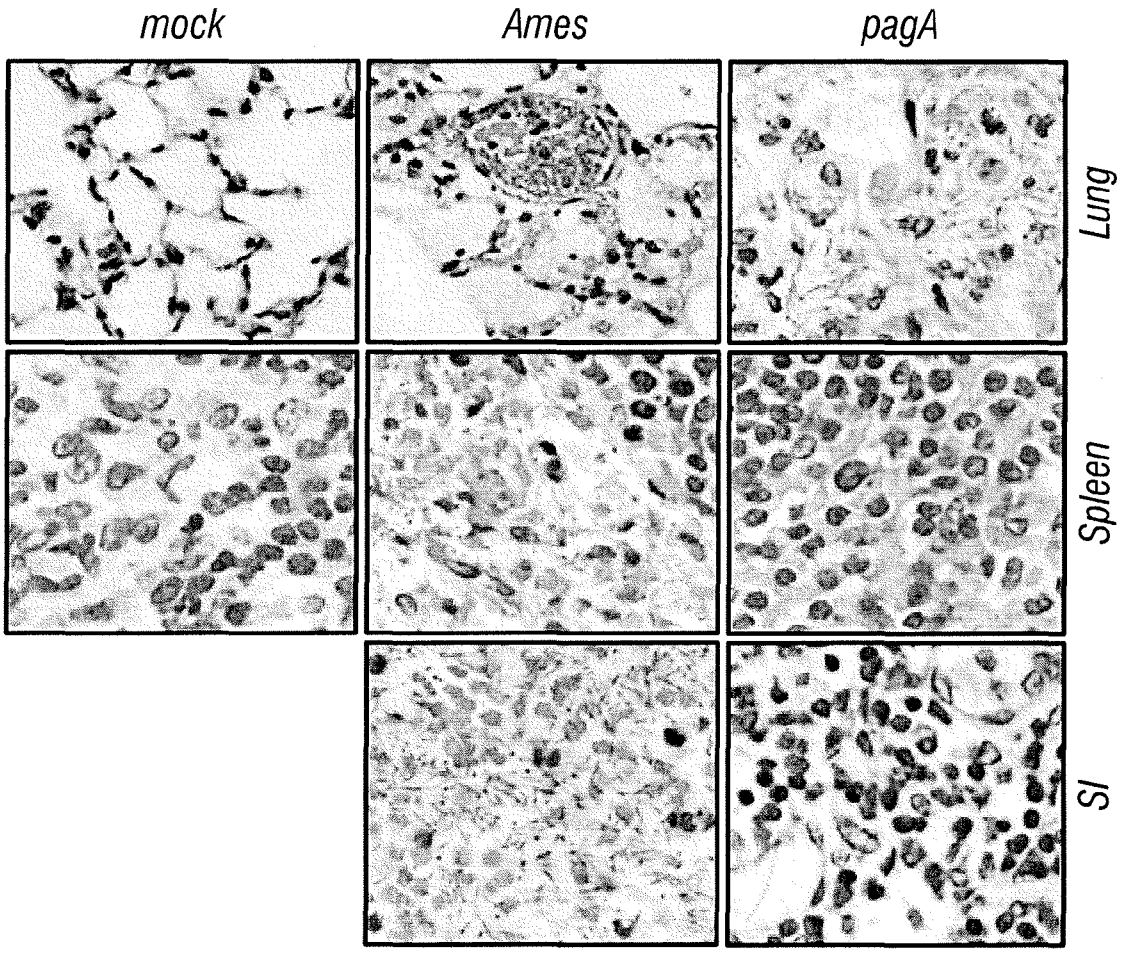

FIG. 2C

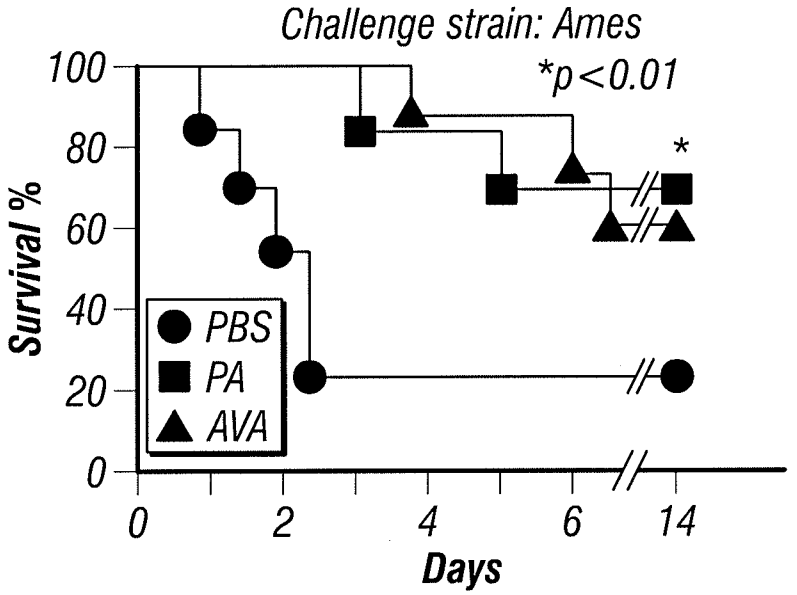
Challenge strain: Ames
*p<0.01
100
80
60
40
20
0
Survival %
PBS
PA
AVA
*
0
2
4
6
14
Days

FIG. 3A

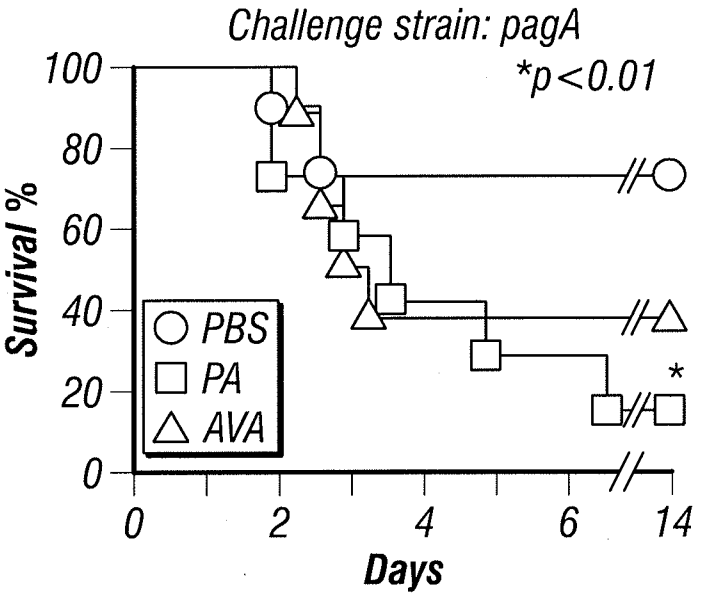
Challenge strain: pagA
*p<0.01
100
80
60
40
20
0
Survival %
PBS
PA
AVA
*
0
2
4
6
14
Days

FIG. 3B

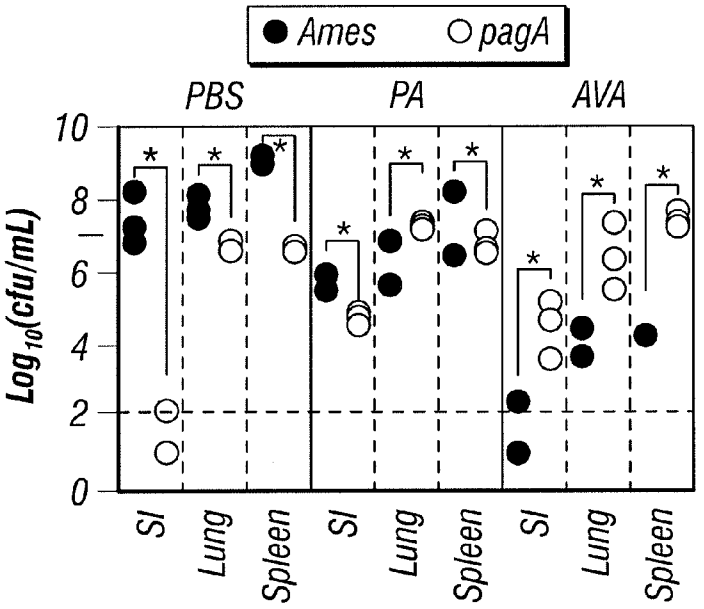

FIG. 3C

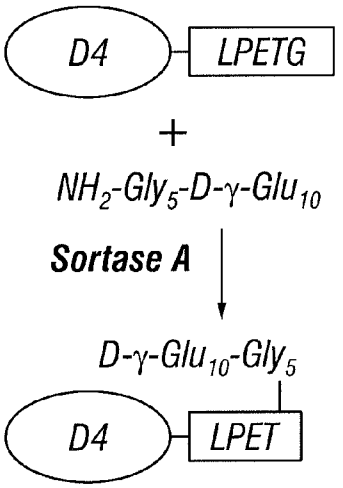

FIG. 4A

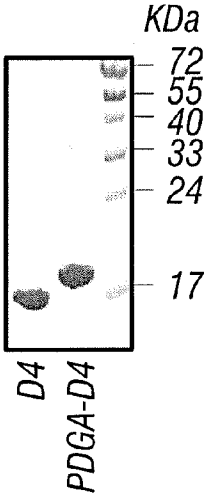

FIG. 4B

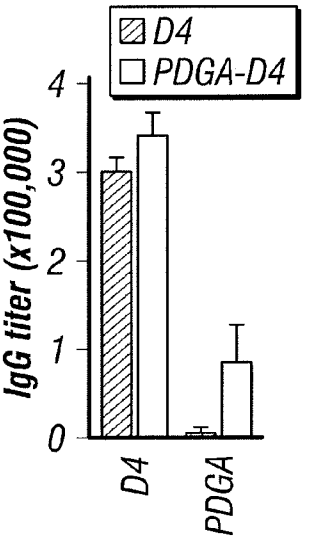

FIG. 4C

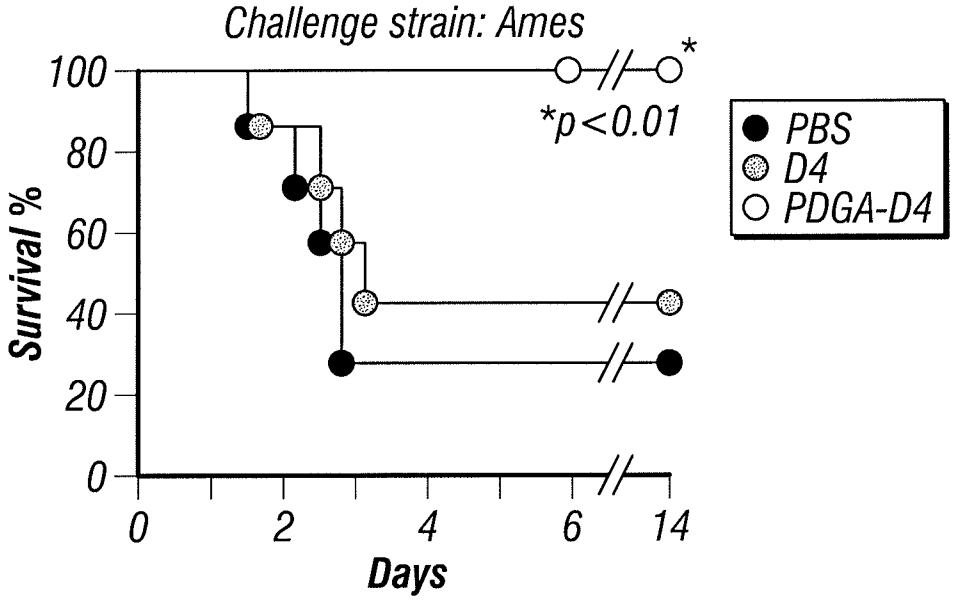
Challenge strain: Ames
100
80
60
40
20
0
Survival %
0
2
4
6
14
Days
*p<0.01
*
PBS
D4
PDGA-D4

FIG. 5A

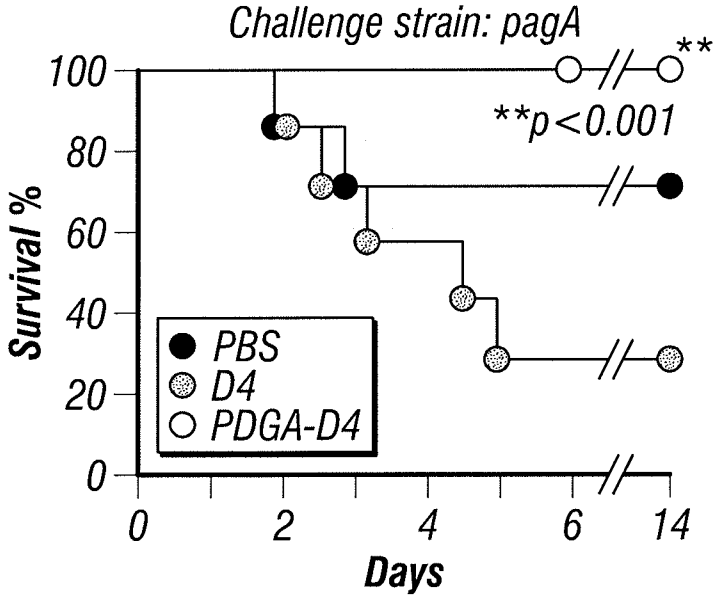
Challenge strain: pagA
100
80
60
40
20
0
Survival %
0
2
4
6
14
Days
**p<0.001
**
PBS
D4
PDGA-D4

FIG. 5B

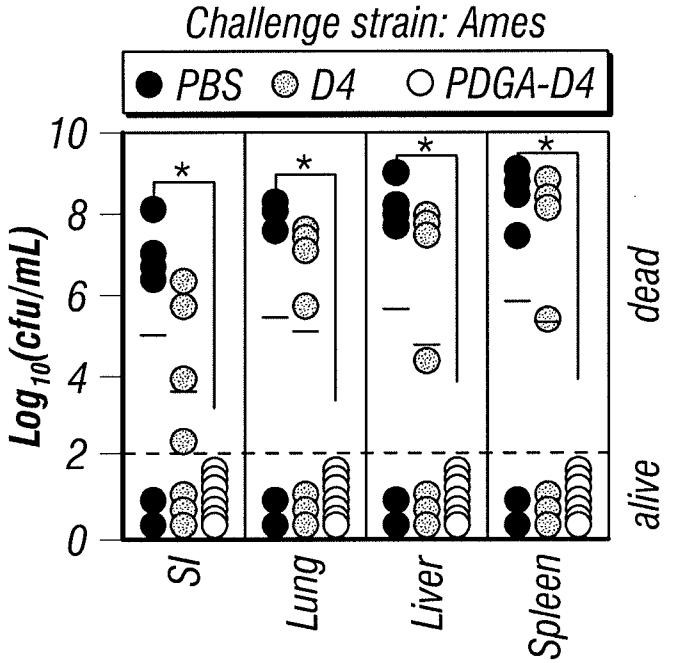
Challenge strain: Ames
PBS
D4
PDGA-D4
Log10(cfu/mL)
10
8
6
4
2
0
*
SI
Lung
Liver
Spleen
dead
alive

FIG. 5C

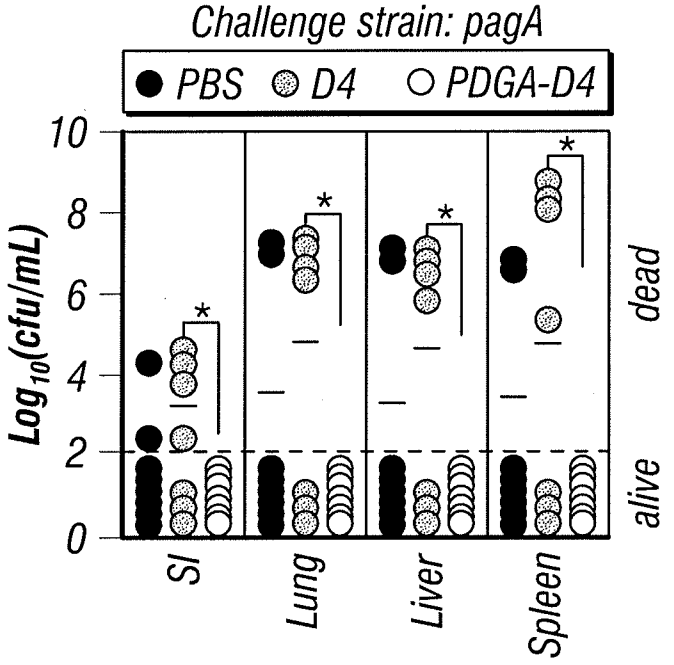
Challenge strain: pagA
PBS
D4
PDGA-D4
Log10(cfu/mL)
10
8
6
4
2
0
*
SI
Lung
Liver
Spleen
dead
alive

FIG. 5D

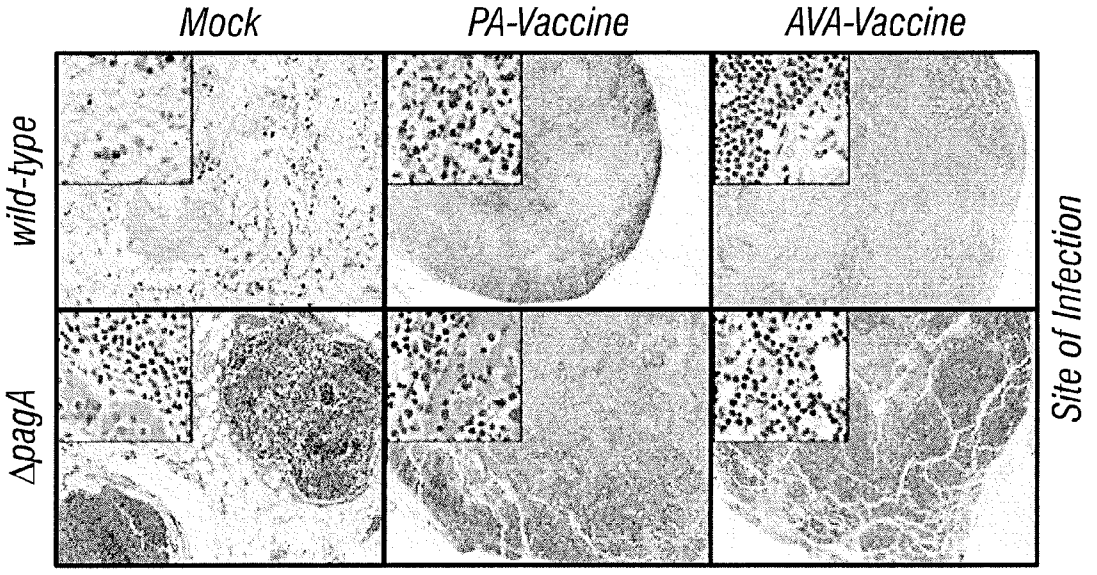
Mock
PA-Vaccine
AVA-Vaccine
wild-type
ΔpagA
Site of Infection

FIG. 6A

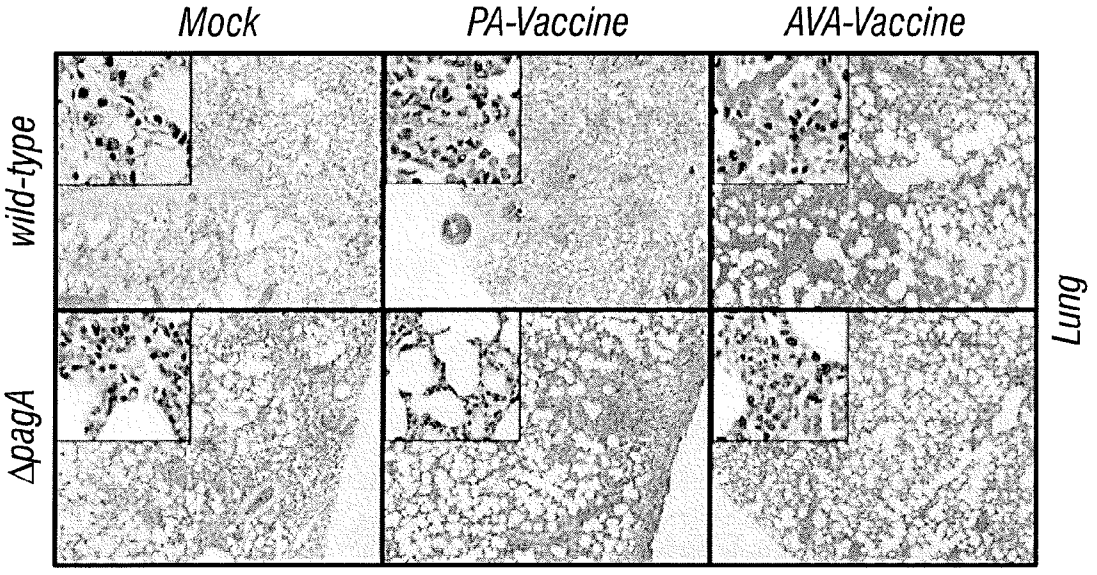
Mock
PA-Vaccine
AVA-Vaccine
wild-type
ΔpagA
Lung

FIG. 6B

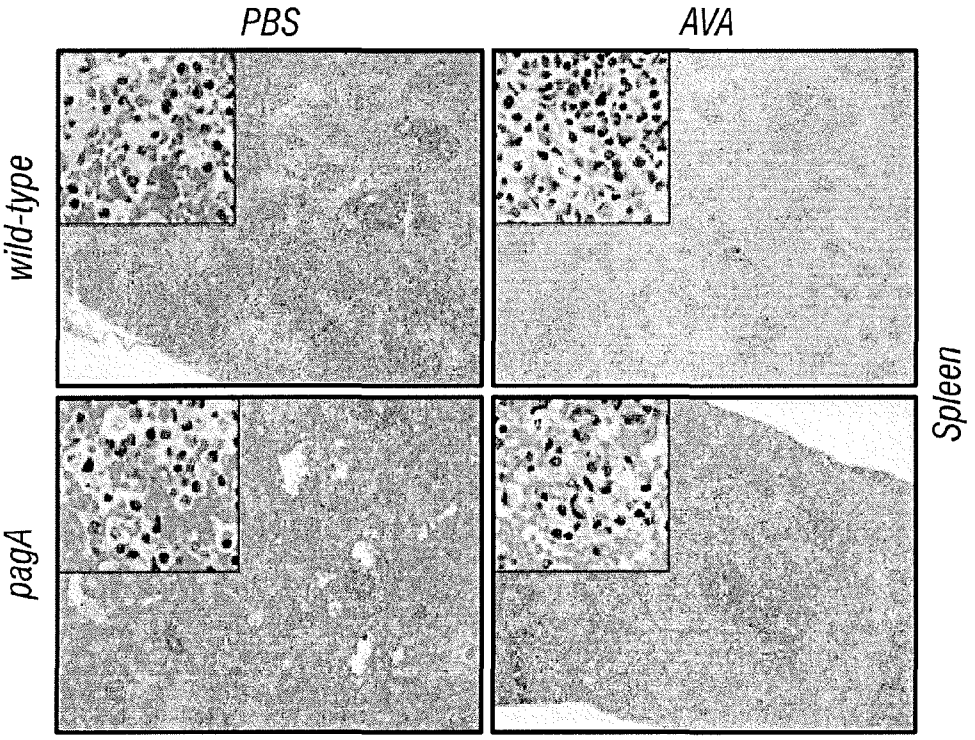
PBS
AVA
wild-type
pagA
Spleen

FIG. 7

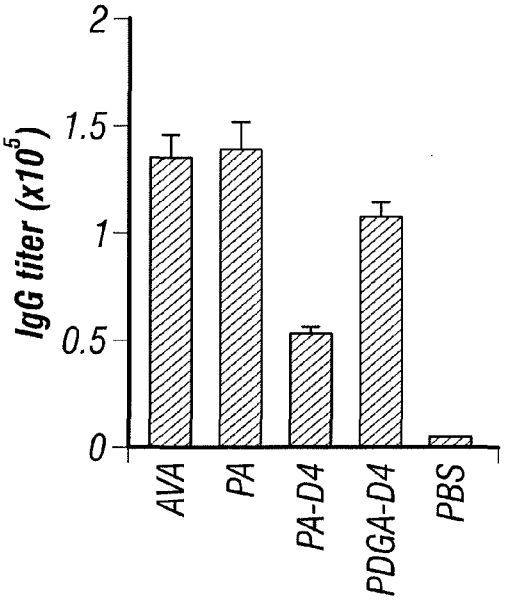
IgG titer (x10^5)
2
1.5
1
0.5
0
AVA
PA
PA-D4
PDGA-D4
PBS

FIG. 8

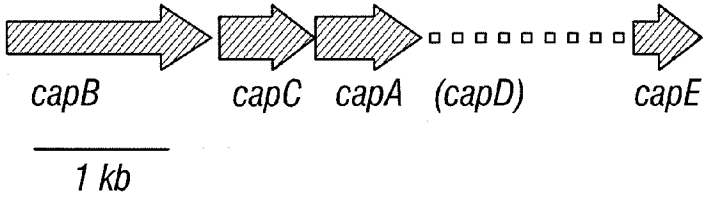

FIG. 9A

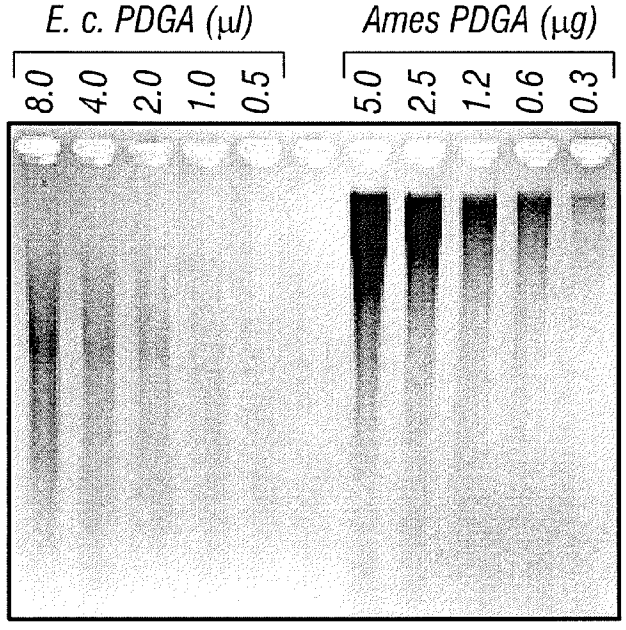

FIG. 9B

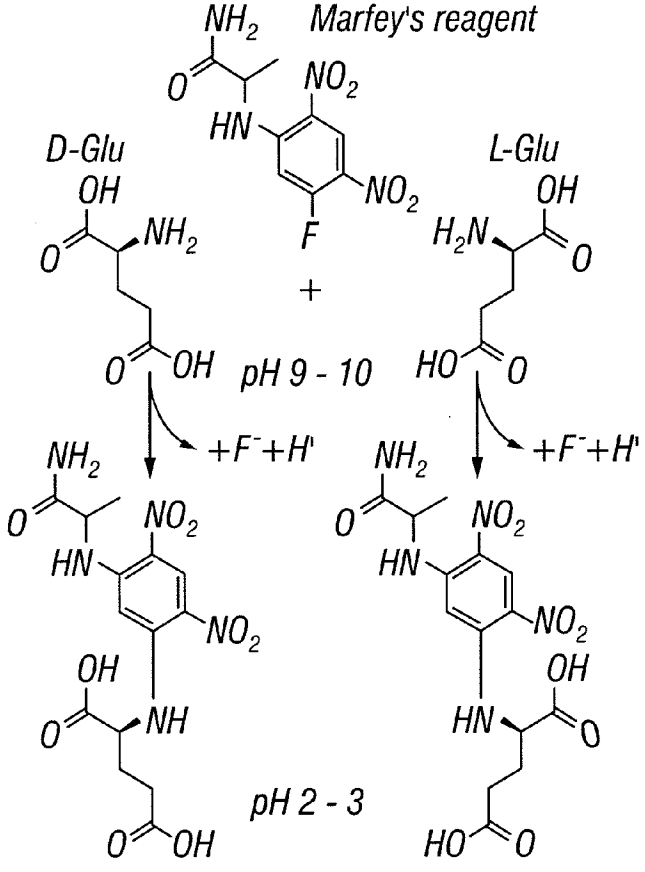

FIG. 9C

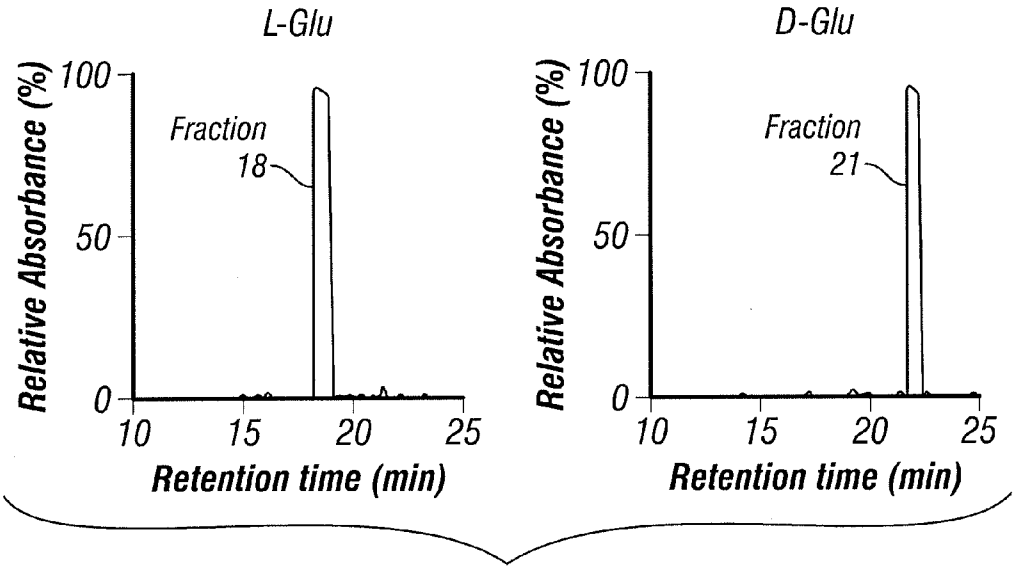
L-Glu
D-Glu
Relative Absorbance (%)
100
50
0
Fraction 18
Fraction 21
10
15
20
25
Retention time (min)

FIG. 9D

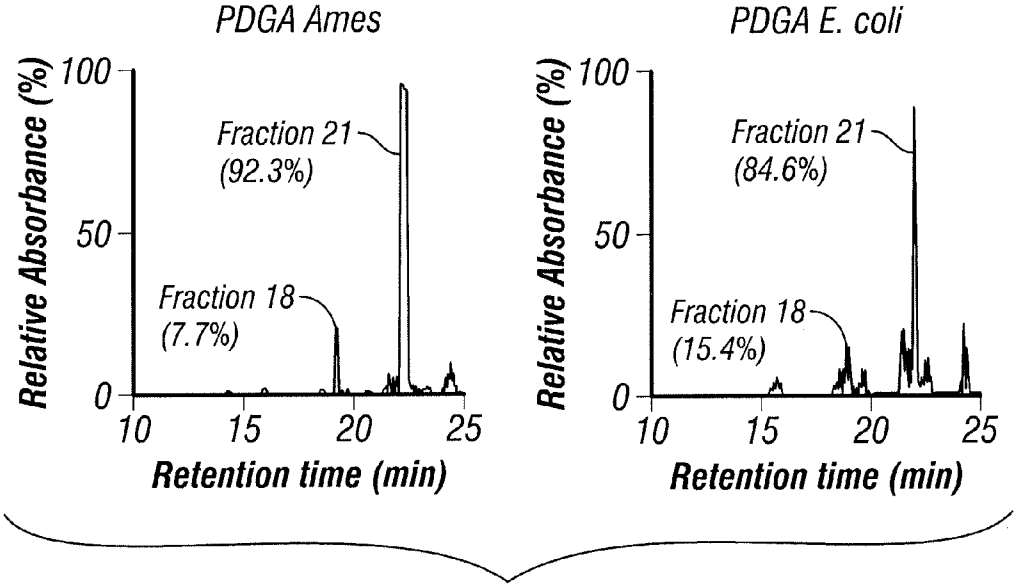
PDGA Ames
PDGA E. coli
Relative Absorbance (%)
100
50
0
Fraction 21
(92.3%)
Fraction 18
(7.7%)
Fraction 21
(84.6%)
Fraction 18
(15.4%)
10
15
20
25
Retention time (min)

FIG. 9E

ATGAAAAAACGAAAAGTGTTAATACCATTAATGGCATTGTCTACGATATTAGTTTCA
AGCACAGGTAATTTAGAGGTGATTCAGGCAGAAGTTAAACAGGAGAACCGGTTATT
AAATGAATCAGAATCAAGTTCCCAGGGGTTACTAGGATACTATTTTAGTGATTTGAA
TTTTCAAGCACCCATGGTGGTTACCTCTTCTACTACAGGGGATTTATCTATTCCTAGT
TCTGAGTTAGAAAATATTCCATCGGAAAACCAATATTTTCAATCTGCTATTTGGTCA
GGATTTATCAAAGTTAAGAAGAGTGATGAATATACATTTGCTACTTCCGCTGATAAT
CATGTAACAATGTGGGTAGATGACCAAGAAGTGATTAATAAAGCTTCTAATTCTAAC
AAAATCAGATTAGAAAAAGGAAGATTATATCAAATAAAAATTCAATATCAACGAGA
AAATCCTACTGAAAAAGGATTGGATTTCAAGTTGTACTGGACCGATTCTCAAAATAA
AAAAGAAGTGATTTCTAGTGATAACTTACAATTGCCAGAATTAAAACAAAAATCTTC
GAACTCAAGAAAAAAGCGAAGTACAAGTGCTGGACCTACGGTTCCAGACCGTGACA
ATGATGGAATCCCTGATTCATTAGAGGTAGAAGGATATACGGTTGATGTCAAAAAT
AAAAGAACTTTTCTTTCACCATGGATTTCTAATATTCATGAAAAGAAAGGATTAACC
AAATATAAATCATCTCCTGAAAAATGGAGCACGGCTTCTGATCCGTACAGTGATTTC
GAAAAGGTTACAGGACGGATTGATAAGAATGTATCACCAGAGGCAAGACACCCCCT
TGTGGCAGCTTATCCGATTGTACATGTAGATATGGAGAATATTATTCTCTCAAAAAA
TGAGGATCAATCCACACAGAATACTGATAGTCAAACGAGAACAATAAGTAAAAATA
CTTCTACAAGTAGGACACATACTAGTGAAGTACATGGAAATGCAGAAGTGCATGCG
TCGTTCTTTGATATTGGTGGGAGTGTATCTGCAGGATTTAGTAATTCGAATTCAAGTA
CGGTCGCAATTGATCATTCACTATCTCTAGCAGGGGAAAGAACTTGGGCTGAAACA
ATGGGTTTAAATACCGCTGATACAGCAAGATTAAATGCCAATATTAGATATGTAAAT
ACTGGGACGGCTCCAATCTACAACGTGTTACCAACGACTTCGTTAGTGTTAGGAAAA
AATCAAACACTCGCGACAATTAAAGCTAAGGAAAACCAATTAAGTCAAATACTTGC
ACCTAATAATTATTATCCTTCTAAAAACTTGGCGCCAATCGCATTAAATGCACAAGA
CGATTTCAGTTCTACTCCAATTACAATGAATTACAATCAATTTCTTGAGTTAGAAAA
AACGAAACAATTAAGATTAGATACGGATCAAGTATATGGGAATATAGCAACATACA
ATTTTGAAAATGGAAGAGTGAGGGTGGATACAGGCTCGAACTGGAGTGAAGTGTTA
CCGCAAATTCAAGAAACAACTGCACGTATCATTTTTAATGGAAAAGATTTAAATCTG
GTAGAAAGGCGGATAGCGGCGGTTAATCCTAGTGATCCATTAGAAACGACTAAACC
GGATATGACATTAAAAGAAGCCCTTAAAATAGCATTTGGATTTAACGAACCGAATG
GAAACTTACAATATCAAGGGAAAGACATAACCGAATTTGATTTTAATTTCGATCAAC
AAACATCTCAAAATATCAAGAATCAGTTAGCGGAATTAAACGCAACTAACATATAT
ACTGTATTAGATAAAATCAAATTAAATGCAAAAATGAATATTTTAATAAGAGATAA
ACGTTTTCATTATGATAGAAATAACATAGCAGTTGGGGCGGATGAGTCAGTAGTTAA
GGAGGCTCATAGAGAAGTAATTAATTCGTCAACAGAGGGATTATTGTTAAATATTGA
TAAGGATATAAGAAAAATATTATCAGGTTATATTGTAGAAATTGAAGATACTGAAG
GGCTTAAAGAAGTTATAAATGACAGATATGATATGTTGAATATTTCTAGTTTACGGC
AAGATGGAAAAACATTTATAGATTTTAAAAAATATAATGATAAATTACCGTTATATA
TAAGTAATCCCAATTATAAGGTAAATGTATATGCTGTTACTAAAGAAAACACTATTA
TTAATCCTAGTGAGAATGGGGATACTAGTACCAACGGGATCAAGAAAATTTTAATCT
TTTCTAAAAAAGGCTATGAGATAGGATAA

FIG. 10A

P13423[625-764], Protective antigen, Bacillus anthracis

10 20 30 40 50 60
MKKRKVLIPL MALSTILVSS TGNLEVIQAE VKQENRLLNE SESSSQGLLG
YYFSDLNFQA

70 80 90 100 110 120
PMVVTSSTTG DLSIPSSELE NIPSENQYFQ SAIWSGFIKV KKSDEYTFAT
SADNHVTMWV

130 140 150 160 170 180
DDQEVINKAS NSNKIRLEKG RLYQIKIQYQ RENPTEKGLD FKLYWTDSQN
KKEVISSDNL

190 200 210 220 230 240
QLPELKQKSS NSRKKRSTSA GPTVPDRDND GIPDSLEVEG YTVDVKNKRT
FLSPWISNIH

250 260 270 280 290 300
EKKGLTKYKS SPEKWSTASD PYSDFEKVTG RIDKNVSPEA RHPLVAAYPI
VHVDMENIIL

310 320 330 340 350 360
SKNEDQSTQN TDSQTRTISK NTSTSRTHTS EVHGNAEVHA SFFDIGGSVS
AGFSNSNSST

370 380 390 400 410 420
VAIDHSLSLA GERTWAETMG LNTADTARLN ANIRYVNTGT APIYNVLPTT
SLVLGKNQTL

430 440 450 460 470 480
ATIKAKENQL SQILAPNNYY PSKNLAPIAL NAQDDFSSTP ITMNYNQFLE
LEKTKQLRLD

490 500 510 520 530 540
TDQVYGNIAT YNFENGRVRV DTGSNWSEVL PQIQETTARI IFNGKDLNLV
ERRIAAVNPS

FIG. 10B

550 560 570 580 590 600
DPLETTKPDM TLKEALKIAF GFNEPNGNLQ YQGKDITEFD FNFDQQTSQN
IKNQLAELNA

610 620 630 640 650 660
TNIYTVLDKI KLNAKMNILI RDKRFHYDRN NIAVGADESV VKEAHREVIN
SSTEGLLLNI

670 680 690 700 710 720
DKDIRKILSG YIVEIEDTEG LKEVINDRYD MLNISSLRQD GKTFIDFKKY NDKLPLYISN

730 740 750 760
PNYKVNVYAV TKENTIINPS ENGDTSTNGI KKILIFSKKG YEIG

FIG. 10B (cont'd)

TTGAGCCTTGATAGTGCGAGAAGACATATGAAAAACATAAAAATTGTAAGAATATT
GAAACATGATGAGGCAATACGCATTGAACATAGGATTTCAGAATTATACTCAGATG
AATTCGGTGTTGTATATGCAGGGAACCACCTAATTTTTAATTGGTATCAACGACTCT
ACTTAAGTCGAAATATCTTAATAAGCAAGAAATCGAAAAGCAGGAAGGGATTAATA
CAGATGATCTTCATAATAGGTATATGTACAGTGTTTTTGATTATTTATGGTATATGGG
AACAACGTTGCCATCAGAAAAGGCTCAATTCTATCCCAATTCGAGTAAACATAAATG
GAATTCGAGGTAAATCTACCGTTACAAGACTAATTACAGGTGTTGTACAAGAAGCG
AAATATAAGACTGTAGGGAAAACAACTGGTACATCTGCGCGAATGATATATTGGTTT
ACTGACGAGGAGCAACCGATTAAGCGCCGTAAAGAAGGTCCTAATATCGGTGAGCA
ACGCAGGGTAGTTAAAGAGGCTGCTGATTTAGAAGCAGAAGCACTTATTTGTGAAT
GTATGGCAGTTCAACCCGATTATCAAATTATCTTCCAAAATAAAATGATTCAAGCAA
ATGTTGGAGTGATTGTAAATGTTTTAGAAGATCATATGGATGTTATGGGACCTACAC
TTGACGAAGTAGCTGAAGCTTTCACTGCTACCATTCCATATAATGGACATTTAGTCA
CTATTGAAAGTGAATACTTGGATTACTTTAAAGAGGTTGCAGAAGAGAGAAATACA
AAAGTGATTGTTGCGGATAATTCTAGAATTTCAGAAGAATTCTTACGAAAATTTGAT
TACATGGTCTTCCCAGATAATGCATCGCTTGCTTTAGCGGTAGCAGAGGCTCTTGGG
ATTGATGAGGAAACAGCATTCCGTGGTATGTTGAATGCTCATCCGGATCCAGGAGCA
ATGAGAATTACACGTTTTGCTGACCAATCTAAGCCTGCGTTCTTCGTAAATGGTTTTG
CAGCGAATGATCCCTCATCAACATTACGTATTTGGGAACGTGTGGATGATTTTGGAT
ATAGTAATCTAGCTCCAATTGTAATTATGAATTGCCGCCCTGACCGCGTTGATCGTA
CTGAGCAGTTTGCTAGGGATGTTTTGCCATATATTAAAGCGGAAATAGTTATTGCGA
TTGGAGAAACGACTGCACCTATTACAAGTGCTTTTGAAAAAGGAGATATTCCAACGC
AAGAGTATTGGAACTTAGAAGGCTGGTCAACAAGTGAAATTATGTCTCGTATGCGTC
CATATTTAAAAAATCGGATTGTATATGGAGTGGGTAATATTCATGGTGCAGCTGAGC
CATTAATCGATATGATTATGGAAGAACAAATTGGCAAAAAGCAAGCAAAAGTGATT
TAAGTGGAGGGACAGGAATGTTTGGATCAGATTTATATATTGCATTAGTATTAGGAG
TTACACTGAGCCTTATTTTTACAGAAAGAACAGGTATTTTACCTGCAGGTTTAGTTGT
ACCTGGTTATTTAGCACTCGTTTTTAATCAGCCCGTATTTATGTTGGTTGTTTTATTTA
TCAGTATTTTAACATATGTAATCGTTACGTATGGTGTTTCAAGATTCATGATTTTATA
TGGCCGTAGAAAATTTGCGGCAACGCTAATTACAGGTATTTGTTTAAAACTTTTATTT

FIG. 11

capD mutant variant of cap operon of Bacillus anthracis (pXO2); nucleotides 1 to 4873

GATTATTGTTATCCTGTTATGCCATTTGAGATTTTTGAATTCCGTGGTATTGGAGTTA
TTGTTCCAGGATTAATTGCAAATACAATTCAAAGACAAGGGTTACCATTAACAATTG
GAACTACAATTTTGTTAAGTGGTGCAACATTTGCAATCATGAATATTTATTACTTATT
TTAAGGTGAGGTAGAATGAGACGAAAATTGACATTTCAAGAAAAGTTACTGATCTTT
ATTAAGAAAACCAAGAAAAAAAATCCTCGTTATGTAGCAATCGTATTACCTCTTATC
GCAGTTATATTAATAGCTGCGACATGGGTACAACGTACAGAAGCAGTAGCACCAGT
AAAACATCGTGAGAACGAAAAATTGACGATGACGATGGTTGGTGACATTATGATGG
GACGTCACGTAAAAGAGATTGTTAATCGTTACGGTACAGATTATGTTTTTCGTCATG
TTTCGCCATATTTAAAAAACTCAGATTACGTAAGTGGGAATTTCGAACATCCTGTTTT
GTTAGAAGATAAAAAGAATTATCAAAAAGCAGATAAGAATATTCACTTAAGTGCAA
AAGAAGAAACAGTTAAGGCAGTAAAAGAAGCCGGATTTACAGTATTAAATTTGGCG
AATAACCATATGACGGATTATGGTGCTAAGGGAACTAAAGATACAATAAAGGCCTT
TAAAGAAGCTGATCTTGACTATGTGGGTGCTGGTGAAAATTTCAAAGATGTAAAAA
ATATTGTGTATCAAAATGTAAATGGTGTTAGGGTTGCTACTCTTGGATTTACAGATG
CATTTGTAGCAGGAGCTATTGCAACGAAAGAACAACCAGGTTCGTTAAGTATGAAC
CCAGATGTATTACTTAAGCAAATTAGTAAGGCAAAGGATCCTAAAAAAGGTAATGC
TGATCTTGTCGTAGTAAATACGCACTGGGGGGGAAGAATACGATAATAAACCGAGTC
CTAGACAGGAAGCCTTAGCAAAAGCAATGGTTGATGCAGGGGCAGATATTATTGTG
GGACACCATCCGCATGTACTTCAATCTTTTGATGTGTATAAGCAAGGGATTATCTTCT
ATAGTTTAGGTAACTTTGTGTTTGACCAAGGATGGACAAGAACAAAAGATAGTGCA
CTTGTGCAATATCATTTACGTGATAATGGTACTGCAATTCTTGATGTTGTACCTTTAA
ATATTCAAGAGGGATCACCAAAACCAGTTACCAGTGCATTGGATAAAAATCGTGTG
TATCGTCAATTAACAAAAGATACATCCAAGGGTGCTCTATGGAGTAAAAAAGATGA
TAAATTGGAAATCAAATTAAATCATAAACATGTTATTGAAAAAATGAAAAAGAGGG
AAAAGCAAGAGCATCAAGATAAGCAAGAAAAAGAAAATCAAGTATCAGTGGAGAC
AACAACTTGAATTCCTTTAAATGGGGAAAGAAGATAATTCTTTTCTGTTTGATAGTC
AGCTTAATGGGGGGTATCGGGGTATCCTGTTCTTTCAATAAAATAAAAGACAGTGTT
AAGCAAAAAATTGATAGTATGGGTGATAAAGGAACTTATGGAGTGAGTGCCTCTCA
CCCCCTTGCGGTTGAGGAAGGTATGAAAGTATTAAAGAACGGTGGAAGTGCAGTAG
ATGCAGCGATTGTGGTCTCATATGTTTTAGGCGTTGTAGAACTGCATGCCTCAGGAA
TAGGTGGGGGCGGTGGAATGCTCATTATATCTAAAGATAAAGAAACCTTTATTGATT

FIG. 11 (cont'd)

ATCGTGAAACAACTCCGTACTTTACAGGAAACCAAAAGCCACATATTGGAGTACCC
GGATTTGTGGCTGGAATGGAGTATATTCATGATAATTATGGTTCATTACCGATGGGT
GAGTTATTACAACCAGCCATTAATTATGCGGAAAAAGGGTTCAAGGTAGATGATTCC
TTAACAATGCGATTAGACCTTGCGAAGCCACGTATTTATTCTGATAAGCTAAGTATC
TTCTATCCGAATGGTGAACCTATTGAAACTGGAGAAACACTTATCCAGACAGATTTA
GCGAGAACCTTAAAGAAGATTCAAAAAGAAGGGGCTAAAGGCTTTTATGAAGGAGG
AGTCGCTAGGGCAATCAGTAAAACTGCAAAAATATCGTTAGAAGATATAAAAGGAT
ATAAAGTAGAGGTACGTAAACCAGTAAAAGGTAACTACATGGGATATGATGTTTAT
ACCGCTCCACCACCTTTTTCAGGAGTTACTTTATTACAAATGTTGAAATTAGCTGAA
AAGAAAGAAGTATATAAAGATGTAGATCATACGGCAACTTATATGTCTAAAATGGA
AGAGATTTCAAGGATTGCCTATCAAGATAGAAAGAAAAACCTAGGGGATCCAATTA
CGTTAATATGGATCCAAATAAAATGGTGAGTGACAAATATATATCAACAATGAAGA
ATGAGAATGGTGATGCGCTTTCGGAAGCAGAGCATGAAAGCACAACGCATTTTGTT
ATCATTGATAGAGATGGAACGGTTGTCTCTTCAACTAATACACTAAGCAATTTCTTT
GGAACAGGAAAGTACACAGCAGGGTTCTTCTTAAATAATCAATTGCAGAACTTTGG
AAGTGAGGGATTTAATAGTTATGAACCTGGTAAACGTTCACGAACGTTTATGGCCCC
CACTGTATTAAAGAAAGATGGGGAAACGATCGGCATTGGGTCACCAGGTGGTAACC
GTATTCCGCAAATTTTAACCCCAATATTGGATAAATATACGCATGGTAAGGGTAGCT
TGCAAGACATTATCAATGAATACCGTTTTACTTTTGAAAAAAATACAGCGTATACAG
AGATTCAGCTAAGTTCAGAAGTGAAAAATGAGTTATCTAGAAAAGGATTGAACGTA
AAGAAGAAAGTATCCCCTGCCTTTTTTGGTGGGGTACAGGCCTTAATTAAAGACGAG
AGAGATAATGTTATCACCGGCGCTGGAGATGGCAGAAGAAATGGAACTTGGAAATC
AAATAAATAGGAGGTAATGGAGAAATGGTTAAAAAAGTTTTTGGATGGATTATGCC
GATTTTAATTGTAGGTTTATTACTTGTAACAATGGGGACCTTTAAACGTTCGGAAAC
ATTAACGACTGATGAGCAGAAGAAGATTAGTGATTATCTACAGGCTAACCCCTAA

FIG.11 (cont'd)

IMMUNOGENIC PROTEIN CONJUGATES AND METHOD FOR MAKING AND USING THE SAME

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application Ser. No. 61/515,733, filed Aug. 5, 2011, hereby incorporated by reference in its entirety.

[0002] This invention was made with government support under 1-U54-AI-057153 and R01-AI069227 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] A. Field of the Invention

[0004] Embodiments of this invention are directed generally to microbiology, medicine and immunology. In certain aspects, the invention is directed to immunoconjugate production and the treatment or prevention of *Bacillus anthracis* infection.

[0005] B. Background

[0006] The Gram-positive, spore forming bacterium *Bacillus anthracis* is the causative agent of anthrax, which is primarily a disease of herbivores. Following ingestion of infectious spores, *B. anthracis* germinate in host tissues and replicate as chains of vegetative bacilli, enclosed by a large poly-D-γ-glutamic acid (PDGA) capsule to prevent their clearance by phagocytes. Bacilli secrete three proteins—lethal factor (LF), edema factor (EF) and protective antigen (PA)—that assemble into the binary lethal (LF and PA) and edema (EF and PA) toxins. PA interacts with anthrax toxin receptors to translocate LF and EF into host cells, where toxins exert their zinc protease (LF) and adenylate cyclase (EF) functions. The two virulence strategies of *B. anthracis*, toxin secretion and capsule formation, are encoded by two large virulence plasmids pXO1 and pXO2. Loss of virulence plasmid occurs under laboratory conditions and prompted the development of attenuated vaccine strains, Pasteur (pXO1−, pXO2+) and Sterne (pXO1+, pXO2−).

[0007] Human infections with *B. anthracis* spores occurs following contact, ingestion or inhalation, giving rise to the cutaneous, gastrointestinal or respiratory forms of disease. Owing to ease of preparation and dissemination, *B. anthracis* spores have been used as biological weapons. To counter the catastrophic consequences of an anthrax aerosol attack, the United States stockpiles vaccine and its military personnel are immunized with anthrax vaccine adsorbed (AVA, BioThrax®) (15). AVA is the aluminum hydroxide adsorbed precipitate of *B. anthracis* V63340 77/-NP1—R (pXO1+, pXO2−) culture supernatants. PA is the principal immunogen of AVA and purified PA is being pursued as a next generation human anthrax vaccine. However, conventional *B. anthracis* vaccines require numerous boosters and a long period of administration. Thus, the deficiency in current *B. anthracis* vaccine compositions highlights the need for improved immunogens to combat, not only *B. anthracis*, but a wide range other infectious diseases.

SUMMARY OF THE INVENTION

[0008] In a first embodiment, a method is provided for producing an antigenic composition comprising contacting an antigenic polypeptide and a second molecule comprising a reactive amino group with a sortase enzyme to produce an immunoconjugate. Administration of the immunoconjugate to a subject, can for instance, produce an immune response to the second molecule or an immune response to the second molecule and the antigenic polypeptide in the subject. For instance, the immune response to the second molecule can be enhanced relative to the immune response produced by administration of the second molecule alone. In certain aspects, the second molecule is non-antigenic when administered alone.

[0009] In a further embodiment an antigenic composition is provided comprising an isolated and essentially homogenous population (e.g., a population of molecules having the same structure) of antigenic polypeptide covalently linked to a bacterial capsule component wherein the covalent linkage comprises a peptide bond at the carboxyl-terminus of the antigenic polypeptide. For example, the covalent linkage between the antigenic polypeptide and the bacterial capsule component can consist of a peptide bond at the carboxyl-terminus of the antigenic polypeptide. In certain aspects, the carboxyl-terminus of the antigenic polypeptide, prior to linkage with the bacterial capsule component, comprises a recognition motif for a sortase enzyme, such as a sortase A, B, C or D recognition motif. Such an antigenic polypeptide can, in certain aspects, be engineered to comprise a sortase recognition motif that is not present in the native polypeptide. Thus, in certain aspects, the antigenic composition comprises the sequence LPXT, wherein X is any amino acid, at the carboxyl-terminus of the antigenic polypeptide (e.g., the polypeptide can comprise the sequence LPET). In some aspects, the antigenic polypeptide and the bacterial capsule component comprise the amino acid sequence LPXT (e.g., LPET) at the covalent linkage.

[0010] In still a further embodiment there is provided a method of producing an immune response in a subject comprising administering an antigenic composition or an immunoconjugate of the embodiments. For example, a method of the embodiments can be defined as a method of producing a protective or sterilizing immune response in a subject.

[0011] In yet a further embodiment there is provided a method of inducing a protective immune response in a subject comprising administering a composition comprising an antigenic portion of protective antigen (e.g., a portion of PA comprising the D4 domain) from *B. anthracis* conjugated to a poly-D-γ-glutamic acid (PDGA) capsule component, wherein administration of the composition provides resistance to *B. anthracis*-induced infection or mortality in the subject. In certain aspects, the PA and PDGA are chemically conjugated, such as by use of succinimidyl 3-(bromoacetamide) propionate (SBAP), 2-iminothiolane (ITL) or succinimidyl 4-formylbenzoate (SFB). In further aspects, PA and PDGA are enzymatically conjugated, for example, such that the carboxyl-terminus of the antigenic portion of PA is covalently attached to PDGA via a peptide bond. In certain cases, a method of the embodiments provides resistance to infection or mortality induced by *B. anthracis* that lacks a functional PagA gene product (e.g., a pagA mutant bacteria). In certain cases a method of the embodiments provides sterilizing immunity to *B. anthracis*, such that *B. anthracis* bacteria cannot be detected in the subject following bacterial challenge.

[0012] Antigenic polypeptides for use according the embodiments (e.g., for use in immunoconjugates) include, but are not limited, to antigenic polypeptides from a virus, bacteria, parasite or fungus or a polypeptide with adjuvant properties. For example, the antigenic polypeptide can be

from a bacteria, such as a bacterial virulence factor. Such bacterial virulence factors include, without limitation, a virulence factor from *Staphylococcus aureus* (e.g., alpha hemolysin, a non-toxigenic form of alpha hemolysin, coagulase, von Willebrand factor binding protein, protein A, a non-toxigenic form of protein A, clumping factor A, clumping factor B, IsdA, IsdB, IsdH, FhuD2, EsxA or EsxB); *Bordetella pertussis* (such as pertussis toxin); *Vibrio cholera* (such as cholera toxin subunit A or B); *Corynebacterium* diphtheria (such as diphtheria toxin); *Clostridium tetani* (such as tetanus toxin); *Pseudomonas aeruginosa* (such as exotoxin A); *Streptococcus pneumonia* (such as pneumolysin); *Streptococcus pyogenes* (such as the C5a peptidase, M protein or T protein); *Streptococcus agalactiae* (such as, Rib, alpha-C, beta-C or BipA); *Neisseria meningitides* (such as a *N. meningitides* outer membrane protein); or *H. influenza* (such as *H. influenzae*-derived protein D). In certain aspects, the antigenic polypeptide is an antigen that, in vivo, may be anchored to or displayed on the cell wall of a Gram-positive bacteria. In certain aspects, the antigenic polypeptide is a sortase A, sortase B, sortase C, or sortase D substrate. In certain specific aspects, the antigenic polypeptide is from *B. anthracis*, such a *B. anthracis* protective antigen (PA) or an immunogenic fragment thereof. For example, the immunogenic fragment of PA can comprise the PA D4 domain (e.g., a portion of the protein corresponding to the amino acids encoded by a nucleic acid isolatable using PCR with primers having the sequence of SEQ ID NOs: 5 and 6).

[0013] In certain embodiments, a second molecule comprising a reactive amino group is used to produce an immunoconjugate. For example, the second molecule can be a peptide, a hapten, a peptidoglycan, a bacterial capsule component or a carbohydrate with a reactive amino group. The second molecule can, for instance, be a bacterial capsule component such as a capsular polysaccharide from *Streptococcus pneumoniae, Neisseria meningitides, Staphylococcus aureus, Hemophilus influenza* or *Streptococcus agalactiae*. In some aspects, the bacterial capsule component comprises a peptide chain such as a poly-γ-glutamic acid or the poly-D-γ-glutamic acid capsule of *B. anthracis*. In still further aspects, bacterial capsule component for use according to the embodiments can be chemically modified to add a reactive amino group.

[0014] In further aspects of the embodiments an antigenic polypeptide and a second molecule (e.g., a bacterial capsule component) of an immunoconjugate can be from the same or different organisms. For example, the antigenic polypeptide and the second molecule can both be from *B. anthracis*, such as PA and PDGA. Even in this situation, the immunoconjugate may be described as heterologous in the context of a linker because the PA and PDGA are not attached to one another in nature that way.

[0015] A sortase enzyme for use according to the embodiments can, in certain aspects, be an isolated sortase enzyme. For example the sortase enzyme can be a purified or recombinant sortase enzyme. Thus, in a further aspect, there is provided an immunoconjugate produced by the methods of the embodiments. Certain aspects of the embodiments concern sortase enzymes, such as a sortase A, sortase B, sortase C or sortase D enzyme. Sortase enzymes can be from a bacterial source, including but not limited to, sortase enzymes from *Staphylococcus aureus, Bacillus anthracis* or *Bacillus cereus*. For example, the sortase enzyme can be a sortase A from *Staphylococcus aureus* or *Bacillus anthracis*. Certain sortase enzymes and method for using the same are described in U.S. Pat. No. 7,238,489, incorporated herein by reference in its entirety.

[0016] In some embodiments, there is an immunogenic composition comprising a bacterial capsular component conjugated to a polypeptide via a linker comprising at least four amino acids of a sortase binding site, wherein the bacterial capsular component is immunogenic. In certain embodiments, the polypeptide is an antigenic polypeptide. In other embodiments, the polypeptide is a non-antigenic polypeptide. In further embodiments, the antigenic polypeptide comprises an antigenic amino acid sequence from a microbe. Some aspects concern a bacterial capsular component that is a bacterial polysaccharide. It is contemplated that the linker may be conjugated to the bacterial capsule component. In some cases, the conjugation occurred through transpeptidation.

[0017] Other embodiments concern a composition comprising an immunoconjugate comprising: a) a truncated anthrax protective antigen (PA) that comprises a D4 domain, wherein the PA is conjugated to a resulting sortase binding site, wherein the resulting sortase binding site has been previously cleaved by sortase; and, b) a bacterial capsular polysaccharide component. In some embodiments, the bacterial capsular polysaccharide component is a peptidoglycan. The peptidoglycan is PDGA in some embodiments.

[0018] Other embodiments of the invention are discussed throughout this application. Any embodiment discussed with respect to one aspect of the invention applies to other aspects of the invention as well and vice versa. The embodiments in the Example section are understood to be embodiments of the invention that are applicable to all aspects of the invention.

[0019] The terms "inhibiting," "reducing," or "prevention," or any variation of these terms, when used in the claims and/or the specification includes any measurable decrease or complete inhibition to achieve a desired result.

[0020] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0021] It is contemplated that any embodiment discussed herein can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions and kits of the invention can be used to achieve methods of the invention.

[0022] Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0023] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." It is also contemplated that anything listed using the term "or" may also be specifically excluded.

[0024] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0025] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE DRAWINGS

[0026] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0027] FIG. **1**A-E: Protective antigen (pagA) deficient *Bacillus anthracis* Ames are not attenuated in mice. (FIG. **1**A) Deletion of the pXO1-encoded pagA gene of *B. anthracis* Sterne via allelic replacement with the kanamycin (kan) resistance cassette. (FIG. **1**B) Growth of wild-type and pagA mutant *B. anthracis* Ames in LB broth was monitored as the absorbance at 600 nm light. (FIG. **1**C) *B. anthracis* wild-type (Ames) and pagA mutant strains were grown in the presence of carbon dioxide and stained with India ink to reveal the poly-D-γ-glutamic acid (PDGA) capsule. (FIG. **1**D) Immunoblotting of *B. anthracis* culture supernatants with rabbit antibodies specific for Bs1A, EA1 and protective antigen (PA). (FIG. **1**E) Wild-type *B. anthracis* (Ames), pagA and capD mutant spores were injected into the peritoneal cavity of C57Bl/6 mice and animal morbidity and mortality monitored. Statistical significance was examined with the log-rank test (* indicates $P<0.01$). Data are representative of two independent determinations.

[0028] FIG. **2**A-C: Protective antigen (pagA) deficient *Bacillus anthracis* Ames are attenuated in the guinea pig model of anthrax disease. (FIG. **2**A) Wild-type *B. anthracis* (Ames), pagA and capD mutant spores were injected into inguinal fold of guinea pigs and animal morbidity and mortality monitored. (FIG. **2**B) Bacterial replication at the site of infection (SI) or dissemination into lung, liver and spleen was enumerated by plating homogenized tissues on agar media and incubation for colony formation. (FIG. **2**C) Tissue from the site of infection (SI) of guinea pigs challenged with *B. anthracis* Ames or the pagA mutant strain as well as lung or spleen tissues were fixed, thin-sectioned, stained with hematoxylin-eosin and light microscopy images captured. Tissues from mock infected animals are included as a control. Statistical significance was examined with the unpaired student's t-test (* indicates $P<0.01$, ** $P<0.001$, *** $P<0.0001$). Data are representative of two independent determinations.

[0029] FIG. **3**A-C: AVA immunized guinea pigs are not protected against anthrax challenge with pagA mutant spores. (FIG. **3**A) Guinea pigs (n=10) were immunized with a prime-two booster schedule with either AVA, PA adsorbed to Alhydrogel or mock (PBS/Alhydrogel) control in 14 day intervals. Animals were challenged by subcutaneous inoculation with *B. anthracis* Ames spores. (FIG. **3**B) Immunized guinea pigs were challenged with pagA mutant spores. (FIG. **3**C) Bacterial replication at the site of infection (SI) or dissemination into lung, liver and spleen was enumerated by plating homogenized guinea pig tissues on agar for colony formation. Statistical significance was examined with the unpaired student's t-test (* indicates $P<0.01$, ** $P<0.001$).

[0030] FIG. **4**A-C: Sortase conjugation generates the PDGA-D4 vaccine. (FIG. **4**A) The sortase reaction scheme shows that capsule peptide is ligated onto PA-D4 LPETG sorting sequence through the attachment of N-terminal pentaglycine. (FIG. **4**B) Sortase conjugation of PDGA to D4 (PDGA-D4) results in a mobility shift on 15% Coomassie-stained SDS-PAGE. (FIG. **4**C) Using a prime-two booster schedule, guinea pigs were immunized with either D4 or PDGA-D4 adsorbed to Alhydrogel and serum IgG analyzed for immune reactivity to either D4 or PDGA antigen.

[0031] FIG. **5**A-D: PDGA-D4 protects guinea pigs from wild-type as well as pagA mutant *B. anthracis* spore challenge. (FIG. **5**A) Guinea pigs (n=7) were immunized with a prime-two booster schedule with either PDGA-D4 or D4 adsorbed to Alhydrogel or mock (PBS/Alhydrogel) control. Animals were challenged by subcutaneous inoculation with *B. anthracis* Ames spores. (FIG. **5**B) Immunized guinea pigs (n=7) were challenged with pagA mutant spores. All guinea pigs were monitored for 14 days for disease and survival. The log-rank test was used to determine significance between PDGA-D4 and PBS mock immunized guinea pigs for wild-type *B. anthracis* AMES challenge (* $P<0.01$). For pagA challenge, PDGA-D4 offers significant protection when compared to D4 alone (* $P<0.01$). (FIG. **5**C, D) Bacterial replication at the site of infection (SI) or dissemination into lung, liver and spleen from wild-type (FIG. **5**C) or pagA (FIG. **5**D) infected animals was enumerated by plating homogenized guinea pig tissues on agar for colony formation. After the 14 days of animal monitoring, all the survival guinea pigs were killed and tissues removed. No bacteria were detected in tissue homogenates from wild-type animas who survived the challenge (* $P<0.01$).

[0032] FIG. **6**A-B: Histopathology of AVA or PA vaccinated guinea pigs following challenge with *B. anthracis* spores. (FIG. **6**A) Guinea pigs (n=10) were immunized with a prime-two booster schedule with either AVA or PA adsorbed to Alhydrogel or a mock (PBS/Alhydrogel) control and challenged by subcutaneous inoculation with *B. anthracis* Ames (wild-type) or pagA mutant spores. Moribund animals were killed and the inguinal site of infection removed during necropsy. Tissues were fixed, thin-sectioned, stained with hematoxylin-eosin and light microscopy images captured. Tissues from mock infected animals are included as a control. (FIG. **6**B) AVA or PA immunized guinea pigs (n=10) were challenged with wild-type or pagA mutant spores and thin sectioned hematoxylin-eosin stained lung tissues analyzed for histopathology.

[0033] FIG. **7**: Spleen pathology of AVA or PA vaccinated guinea pigs challenged with wild-type or pagA mutant *B. anthracis* spores. Guinea pigs (n=10) were immunized with a prime-two booster schedule with either AVA or PA adsorbed to Alhydrogel or a mock (PBS/Alhydrogel) control and challenged by subcutaneous inoculation with *B. anthracis* Ames (wild-type) or pagA mutant spores. Moribund animals were killed and the spleen removed during necropsy. Spleen tissues were fixed, thin-sectioned, stained with hematoxylin-eosin and light microscopy images captured.

[0034] FIG. **8**: Serum IgG immune response to anthrax vaccines. Guinea pigs (n=10) were immunized with a prime-two booster schedule in 14 day intervals using either AVA, PA, D4 or PDGA-D4 adsorbed to Alhydrogel or a mock

(PBS/Alhydrogel) control. PA-specific serum IgG titers of animals were analyzed on day 36 of the immunization schedule.

[0035] FIG. **9**A-E. Production and composition of recombinant PDGA. (A) Schematic representation of cap operon used for expression in *E. coli*. (B) High-molecular weight PDGA isolated from *E. coli* carrying operon shown in (A) and *B. anthracis* Ames. Samples were separated by agarose gel electrophoresis and stained with methylene blue (recombinant PDGA, left lanes; Ames PDGA, right lanes). (C) Schematic overview of derivatization reaction of D- and L-glutamate using Marfey's reagent. (D) HPLC chromatograms of derivatizied D- and L-form standards of glutamate were recorded at 350 nm. (E) HPLC chromatograms (350 nm) of processed PDGA samples. Relative amounts of D-form (fraction 21) and L-form (fraction 18) are indicated in percent.

[0036] FIG. **10**A-B. (A) Nucleotide sequence pagA gene (gene pXO1-110 Sterne). Primers used to amplify D4 are underlined—D4 nt sequence is in red. (B) PagA Protective antigen full-length precursor. P13423[625-764], Protective antigen, *Bacillus anthracis*.

[0037] FIG. **11**. capD mutant variant of cap operon of *Bacillus anthracis* (pXO2); nucleotides 1 to 4873 (SEQ ID NO:9).

DETAILED DESCRIPTION OF THE INVENTION

[0038] The AVA vaccine has been subjected to preclinical efficacy trials in mice, rats, guinea pigs and non-human primates challenged with wild-type *B. anthracis* spores. However, *B. anthracis* Ames variants lacking the pXO1-encoded pagA gene remained virulent following either subcutaneous or respiratory spore challenge of mice. These studies suggest that AVA immunization or PA-specific antibodies are unlikely to protect mice against challenge with pagA mutant spores. Thus, new vaccine compositions are needed to provide protection against such strains.

[0039] Studies detailed here demonstrate that by conjugating antigenic portions of PA to PDGA a robust immunogen can be produced. Administration of the conjugates to guinea pigs elicited a robust antibody response. More importantly, the conjugate protected the animals from both virulent *B. anthracis* Ames variants and pagA mutant strains, that were not protected by PA vaccines alone. Moreover, the response to these immunoconjugates was sufficient to provide sterilizing immunity to the animals. Thus, PA-PDGA conjugates offer a significant advance relative to previously available *B. anthracis* immunogens in both overall efficacy and spectrum of immunity (i.e., effective immunity to pagA mutant strains).

[0040] The studies here likewise show the efficacy of new method for antigenic conjugation. Specifically, conjugation of a polypeptide and a second molecule, such as a bacterial capsule component, having a reactive amine can be accomplished by treatment with a sortase enzyme. The resulting conjugate comprises the antigenic polypeptide and a second molecule in a direct 1:1 ratio and the covalent linkage is at the same position relative to the two components (i.e., the carboxyl-terminus of the antigen polypeptide) in all conjugates. Thus, the antigenic polypeptide can effectively display the second molecule and facilitate a robust immune response to the molecule. The immune response to the antigenic polypeptide can also be enhanced in the immunoconjugate relative to the isolated antigenic polypeptide. Unlike chemical conjugation, all immunoconjugates produced by the method described herein are homogenous in their structure and thus offer compositions with a high and reproducible specific activity. Thus, the new conjugation procedures can be applied to a wide range of antigens to provide improved and highly active vaccine compositions.

[0041] I. Pharmaceutical Compositions and Methods

[0042] In some embodiments, pharmaceutical compositions are administered to a subject. Different aspects involve administering an effective amount of a composition to a subject. In some embodiments of the present invention, a composition comprising an immunoconjugate may be administered to the subject or patient to protect against or treat infection. Additionally, such compounds can be administered in combination with an adjuvant or an antibacterial therapy. In an embodiment, the adjuvant can comprise an aluminum salt adjuvant, such as aluminum hydroxide. Such compositions will generally be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

[0043] The active immunogens of the present invention can be formulated for parenteral administration, e.g., formulated for injection via the intravenous, intramuscular, subcutaneous, or even intraperitoneal routes. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified. In addition to the compounds formulated for parenteral administration, other pharmaceutically acceptable forms include, e.g., aerosolizable, inhalable, or instillable formulations; tablets or other solids for oral administration; time release capsules; creams; lotions; mouthwashes; and the like. The preparation of such formulations will be known to those of skill in the art in light of the present disclosure.

[0044] In certain embodiments, an immunoconjugate contains more than one compound against which an immune response is desired or beneficial or is associated with a protective or therapeutic immune response. Examples of these are described herein such as with an antigenic compound and a peptidoglycan found in the capsule of a capsular microbe. In some embodiments, however, the peptidoglycan component is connected to a polypeptide through a sortase binding site, but the polypeptide is not an antigenic polypeptide against which an immune response significantly contributes (i.e., is statistically significant and detectable) to the immunoconjugates' therapeutic effect. In certain embodiments, the second molecule generates an immune response that is the basis for a protective or therapeutic effect. In these embodiments, there is a peptide or polypeptide component that is attached to the second molecule, but the peptide or polypeptide is non-antigenic as described herein.

[0045] Antigenic or non-antigenic peptides or polypeptides may be 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990,

1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 or more amino acids in length, or any range derivable therein. In some embodiments, an antigenic polypeptide or peptide has a sequence that is at least or at most 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 percent identity or homology to the sequence of a particular antigenic polypeptide, such as can be found in GenBank. In particular embodiments, the antigenic polypeptide is a virulence factor from *Staphylococcus aureus* (e.g., alpha hemolysin, a non-toxigenic form of alpha hemolysin, coagulase, von Willebrand factor binding protein, protein A, a non-toxigenic form of protein A, clumping factor A, clumping factor B, IsdA, IsdB, IsdH, FhuD2, EsxA or EsxB); *Bordetella pertussis* (such as pertussis toxin); or is a protein from *Vibrio cholera* (such as cholera toxin subunit A or B); *Corynebacterium* diphtheria (such as diphtheria toxin), from *Clostridium tetani* (such as tetanus toxin); *Pseudomonas aeruginosa* (such as exotoxin A), from *Streptococcus pneumonia* (such as pneumolysin), from *Streptococcus pyogenes* (such as the C5a peptidase, M protein or T protein), from *Streptococcus agalactiae* (such as, Rib, alpha-C, beta-C or BipA), from *Neisseria meningitides* (such as a *N. meningitides* outer membrane protein), or from *H. influenza* (such as *H. influenzae*-derived protein D).

[0046] In specific embodiments, the antigenic polypeptide is protective antigen (PA) from anthrax. In some embodiments, the polypeptide comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760. 761, 762, 763, or 764 amino acids from SEQ ID NO:10, which is the full-length sequence of a protective antigen protein from anthrax (MKKRKVLIPLMALSTILVSSTGN-
LEVIQAEVKQENRLLNESESSSQGLLGYYFSDLNFQ
APMVVTSSTTGDLSIPSSELENIPSEN-
QYFQSAIWSGFIKVKKSDEYTFATSADNHVTMW
VDDQEVINKASNSNKIRLEKGRLY-
QIKIQYQRENPTEKGLDFKLYWTDSQNKKEVISSD
NLQLPELKQKSSNSRKKRSTSAGPTVP-
DRDNDGIPDSLEVEGYTVDVKNKRTFLSPWISN
IHEKKGLTKYKSSPEKWSTASDPYSD-
FEKVTGRIDKNVSPEARHPLVAAYPIVHVDMENI
ILSKNEDQSTQNTDSQTR-
TISKNTSTSRTHTSEVHGNAEVHASFF-
DIGGSVSAGFSNSNSS TVAIDHSLSLAGERTWAET-
MGLNTADTARLNANIRYVNTGTAPIYNVLPTTSLVL
GKNQ TLATIKAKENQLSQILAPNNYYPSKNLA-
PIALNAQDDFSSTPITMNYNQFLELEKTKQLR LDT-
DQVYGNIATYNFENGRVRVDTGSNWSEV-
LPQIQETTARIIFNGKDLNLVERRIAAV
NPSDPLETTKPDMTLKEALKIAFGFNEP-
NGNLQYQGKDITEFDFNFDQQTSQNIKNQLAE LNAT-
NIYTVLDKIKLNAKMNILIRDKRFHY-
DRNNIAVGADESVVKEAHREVINSSTEGLL
LNIDKDIRKILS-
GYIVEIEDTEGLKEVINDRYDMLNISSL-
RQDGKTFIDFKKYNDKLPLYIS NPNYKVNVYAVT-
KENTIINPSENGDTSTNGIKKILIFSKKGYEIG). The signal peptide is amino acids 1-29. The full length protective antigen (without signal peptide) is amino acids 30-764. Protective antigen 20 (PA-20) is amino acids 30-196. Protective antigen 63 (PA-63) is amino acids 197-764. Embodiments include these different polypeptides. In further embodiments, a polypeptide is any of the lengths above but is at least or at most 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical or homologous to the a polypeptide of that length from SEQ ID NO:10. In specific embodiments, a polypeptide that is included in an immunoconjugate is a truncated version of PA-63, which means it has fewer than amino acids 197-764 of SEQ ID NO:10. In additional embodiments, a polypeptide contains domain 4 but is lacking 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, or 700 residues (or any range derivable therein) from amino acids 197-764 of SEQ ID NO:10. In further embodiments, a polypeptide further is at most or at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical or homologous to amino acids 197-764 with respect to the truncated protein.

[0047] The D4 sequence in SEQ ID NO:10 is 149 aa in length and aligns to PA from position 625 to 764 which is the C-terminal part of the mature PA-63. Embodiment include a PA polypeptide that comprises an amino acid sequence that is at most or at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical or homologous to amino acids 625 to 764 of SEQ ID NO:10.

[0048] Embodiments include a second molecule against which an immune response can be generated. In some embodiments, a detectable immune response may not be generated against the second molecule but for the immunoconjugation described herein. In other embodiments the second molecule is made at least or at most about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 percent or at least about or at most about 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 fold more immunogenic than prior to the conjugation. In certain embodiments, the second molecule is one found in the capsule of a bacteria, such as a Gram negative bacteria. In particular cases, the bacteria includes but is not limited to *Escherichia coli, Klebsiella pneumonia; Haemophilus influenza; Bacillus megaterium; Pseudomonas aeruginosa*; or, *Salmonella*. Gram-positive bacteria that have a capsule are also included in compositions and methods described herein. In specific embodiments, the second molecule is a polysaccharide or a peptidoglycan or another entity discussed herein.

[0049] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil, or aqueous propylene glycol; and sterile powders for the extemporaneous prepara-

tion of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0050] The carrier also can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0051] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient, plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0052] As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem complications commensurate with a reasonable benefit/risk ratio. The term "pharmaceutically acceptable carrier," means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent.

[0053] Some variation in dosage will necessarily occur depending on the condition of the subject. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. An effective amount of therapeutic or prophylactic composition is determined based on the intended goal. The term "unit dose" or "dosage" refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the composition calculated to produce the desired responses discussed above in association with its administration, i.e., the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the effects desired. Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment (alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition.

[0054] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above.

[0055] Typically, for a human adult (weighing approximately 70 kilograms), from about 0.1 mg to about 3000 mg (including all values and ranges there between), or from about 5 mg to about 1000 mg (including all values and ranges there between), or from about 10 mg to about 100 mg (including all values and ranges there between), of a compound are administered. It is understood that these dosage ranges are by way of example only, and that administration can be adjusted depending on the factors known to the skilled artisan.

[0056] In certain embodiments, a subject is administered about, at least about, or at most about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7. 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0. 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 410, 420, 425, 430, 440, 441, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870, 875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 milligrams (mg) or micrograms (mcg) or μg/kg or micrograms/kg/minute or mg/kg/min or micrograms/kg/hour or mg/kg/hour, or any range derivable therein.

[0057] A dose may be administered on an as needed basis or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, or 24 hours (or any range derivable therein) or 1, 2, 3, 4, 5, 6, 7, 8, 9, or times per day (or any range derivable therein). A dose may be first administered before or after signs of an infection are exhibited or felt by a patient or after a clinician evaluates the patient for an infection. In some embodiments, the patient is administered a first dose of a regimen 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hours (or any range derivable therein) or 1, 2, 3, 4, or 5 days after the patient experiences or exhibits signs or symptoms of

an infection (or any range derivable therein). The patient may be treated for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more days (or any range derivable therein) or until symptoms of an infection have disappeared or been reduced or after 6, 12, 18, or 24 hours or 1, 2, 3, 4, or 5 days after symptoms of an infection have disappeared or been reduced.

EXAMPLES

[0058] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods described herein are presently representative of certain embodiments, are provided as an example, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1

Virulence of pagA Mutant *B. anthracis* Injected into the Peritoneal Cavity of Mice

[0059] Using allelic replacement and phage transduction, the entire open reading frame of the pagA gene was deleted from the pXO1 virulence plasmid of *B. anthracis* Ames (FIG. **1**A). The resulting pagA mutant strain formed spores and replicated in laboratory media at the same rate as wild-type bacilli (FIG. **1**B). Growth in the presence of 5% CO_2 gas led to formation of encapsulated vegetative forms for both wild-type and pagA mutant bacilli (FIG. **1**C). Cultures of vegetative bacilli grown in the presence of bicarbonate were centrifuged, thereby separating extracellular media and bacterial sediment. Proteins in the supernatant were precipitated with TCA and probed by immunoblotting (FIG. **1**D). As expected, *B. anthracis* Ames secreted PA into the culture medium whereas the pagA mutant strain did not (FIG. **1**D). As a control, the pagA mutant expressed the pXO1-encoded S-layer-associated protein BslA at a level similar to wild-type bacilli (FIG. **1**D).

[0060] Next, studies were undertaken to determine whether or not the pagA mutant displayed the same level of virulence as the variant with the omega-kanamycin cassette replacement of pagA reported by Chand and colleagues. To test this, 300 spores derived from *B. anthracis* Ames or its pagA mutant were inoculated into the peritoneal cavity of C57BL/6 mice (FIG. **1**E). Both wild-type and pagA mutant bacilli replicated in murine tissues and killed about half of the experimental animals. As a control, intraperitoneal injection of 300 spores derived from the *B. anthracis* Ames capD mutant did not cause lethal disease (FIG. **1**E). Thus, it was concluded that the pagA deletion mutant of *B. anthracis* Ames is virulent in mice similar to the pagA omega-kanamycin cassette replacement variant.

pagA Mutant Bacilli are Attenuated in the Guinea Pig Model of Anthrax Disease

[0061] Cohorts of guinea pigs (n=10, 6-week old females) were infected with *B. anthracis* spores via subcutaneous injection into the inguinal fold. The survival of animals was monitored over the next fourteen days (FIG. **2**A). All animals infected with spore preparations (20 or 200 CFU) of *B. anthracis* Ames succumbed to challenge within four days (FIG. **2**A). By comparison, animals infected with pagA mutant spores displayed delayed time-to-death and increased survival (FIG. **2**A). Thus, the pagA mutation attenuates *B. anthracis* Ames virulence in guinea pigs about 10 fold; by comparison, a capD mutation is known to reduce virulence 1,000 fold. To study the disease features of the pagA mutant, animals were subjected to necropsy and tissue from the site of infection (inguinal fold), liver, lung and spleen was homogenized, spread on agar plates and colony formation enumerated as a measure for bacterial load (FIG. **2**B). Tissue samples were also fixed with formalin, embedded, thin-sectioned and stained with hematoxylin-eosin (FIG. **2**C). Animals infected with wild-type *B. anthracis* Ames spores displayed massive replication of vegetative forms at the injection site (10^5 CFU) as well as pathogen dissemination to lung, liver and spleen (FIG. **2**B). Edema and hemorrhage were detected at the site of infection (FIG. **2**C). Histopathology revealed *B. anthracis* vegetative forms with massive invasion of polymorphonuclear leukocytes and macrophages in lung and spleen tissues (FIG. **2**C). By comparison, vegetative replication of the pagA mutant at the site of infection was reduced by about 3 $\log_{10}$ CFU ($P<0.0001$) and did not elicit edema or hemorrhage (FIG. **2**C). Dissemination of the pagA mutant to liver, lung and spleen tissues was also reduced by 2-3 $\log_{10}$ CFU (FIG. **2**B).

AVA or PA Immunized Guinea Pigs are Susceptible to Infection with pagA Bacilli

[0062] Cohorts of guinea-pigs (n=10) were immunized by intramuscular injection with three doses of 50 μg PA adsorbed to aluminum hydroxide or 250 μl AVA (Biothrax®) in fourteen day intervals. Two-weeks following the final immunization, animals were challenged with spores derived from *B. anthracis* Ames or its pagA mutant (FIG. **3**). PA as well as AVA immunization of guinea pigs afforded protection from *B. anthracis* Ames challenge, as both vaccine protocols caused similar increases in animal survival and time-to-death over a control cohort of mock (PBS) immunized guinea pigs exposed to the same challenge dose (FIG. **3**A). AVA immunization, and to a lesser degree vaccination with PA, reduced the bacterial load at the site of infection as well as in lung, liver and spleen tissues (FIG. **2**C). Nevertheless, neither of the two vaccines elicited full protection from anthrax disease (FIG. **3**AB). This is an expected result, as optimal protection is only achieved upon completion of a schedule of 5-6 AVA immunizations over a one-year period.

[0063] Surprisingly, AVA immunized animals succumbed at a faster rate to challenge with pagA mutant spores than animals of the mock-immunized control cohort (FIG. **3**B). This phenomenon must be caused by immune responses to the protective antigen as guinea pigs immunized with purified PA (FIG. **3**B) or the D4 domain of PA (FIG. **5**B) exhibited similar hyper-sensitivity to challenge with spores derived from pagA mutant *B. anthracis* Ames. To test whether immunization with AVA or PA affected the ability of pagA mutants to replicate in host tissues, the bacterial load at the injection site and distant organ sites was examined. Compared to mock immunized animals, AVA vaccinated guinea pigs harbored a dramatically increased load of the pagA mutant at the site of infection (FIG. **3**C).

Pathological Features of Anthrax Disease Caused by Wild-Type and pagA Mutant Bacilli

[0064] The studies shown in FIG. **3** suggest that AVA immunization enhanced the virulence attributes of the pagA mutant *B. anthracis* Ames strain. This conjecture is further supported by the histopathology of infected guinea pigs (FIG. **6**). AVA and PA immunization ameliorate the hemorrhagic lesions, immune cell necrosis and vegetative replication associated with *B. anthracis* Ames spore inoculation at the site of infection (FIG. **6**A). PA and AVA vaccinated animals established a granuloma at the site of infection, which appeared to restrict replication and dissemination of the germinated pathogen (FIG. **6**A). In mock immunized animals, vegetative replication of the pagA mutant occurred within a similar granuloma (FIG. **6**A). Site of infection lesions in AVA or PA immunized guinea pigs were marked by hemorrhagic zones and severe necrosis of immune cells (FIG. **6**). Mock immunized guinea pigs infected with *B. anthracis* Ames spores developed severe interstitial pneumonia with a large burden of vegetative bacilli (FIG. **6**B). Lung histopathology of the pagA mutant showed a large immune cell infiltrate and moderate replication of the pathogen (FIG. **6**B). AVA or PA immunization limited pathogen replication within the lung: only sporadic interstitial immune cell infiltrates and occasional vegetative forms could be detected (FIG. **6**B). When challenged with pagA mutant bacilli, lung interstitial space of AVA or PA-immunized guinea pigs was enlarged by infiltrates of healthy or necrotic immune cells and the vegetative forms of the pagA mutant strain (FIG. **6**B).

[0065] Virulent *B. anthracis* replicate to the highest numbers in the spleen of their infected hosts (FIG. **2**). As expected, large numbers of *B. anthracis* Ames vegetative forms established microcolonies in spleen tissues of mock immunized animals (FIG. **7**). Vegetative forms of pagA mutant were associated with moderate immune cell infiltrates without changing the overall architecture of the spleen (FIG. **7**). Splenic tissues of AVA immunized guinea pigs did not reveal anthrax pathology when infected with *B. anthracis* Ames spores (FIG. **7**). However, AVA-vaccinated animals challenged with pagA mutant spores displayed vegetative forms and immune cell infiltrates replacing the red and white pulp architecture of spleen tissues (FIG. **7**).

Sortase-Ligation Generates Conjugate Vaccines

[0066] Chemical cross-linking has been used to tether PDGA to PA carrier, thereby enabling MHC presentation and antibody responses to otherwise non-immunogenic capsular material. Nevertheless, the ability of such PDGA-PA conjugate vaccine to elicit protection against wild-type and pagA mutant *B. anthracis* Ames has not yet been explored. Sortase A, a transpeptidase that cleaves between the threonine (T) and glycine (G) of its LPXTG recognition motif, was therefore exploited for the development of conjugate vaccines (FIG. **4**A). A glutathione S-transferase hybrid with the D4 receptor binding domain of PA, which is the target of neutralizing PA antibodies, was engineered to harbor a C-terminal LPXTG motif Purified GST-D4 was incubated with sortase A and NH_2-Gly_5-γ-D-Glu_{10} nucleophile. The transpeptidation product, D4-LPXT-Gly_5-γ-D-Glu_{10} (designated PDGA-D4), was cleaved off GST and purified (FIG. **4**B). When injected into guinea pigs, Alhydrogel adsorbed PDGA-D4 elicited antibody responses against PDGA capsule and the D4 domain of PA (FIG. **4**C and FIG. **8**). As a control, non-conjugated D4 antigen did not elicit PDGA antibodies (FIG. **4**C).

PDGA-D4 Protects Guinea Pigs Against Wild-Type and pagA Mutant Bacilli

[0067] Guinea pigs were immunized with PDGA-D4 or D4 following the same schedule as AVA and challenged with *B. anthracis* Ames spores (FIG. **5**A). In contrast to D4 immunized animals, which exhibited partial protection against *B. anthracis* Ames spore challenge, guinea pigs that have received PDGA-D4 were completely protected (FIG. **5**A). At the end of the observation period, animals were euthanized and subjected to necropsy. Microbiological analysis of tissue homogenates from PDGA-D4 immunized guinea pigs failed to detect *B. anthracis*, suggesting that the conjugate vaccine induced sterilizing immunity (FIG. **5**C). Importantly, PDGA-D4 immunization protected guinea pigs against challenge with the pagA mutant strain (FIG. **5**BD). Compared to mock immunized animals, immunization with the D4 antigen reduced the survival of guinea pigs subsequently challenged with the pagA mutant strains (FIG. **5**B).

Example 2

Materials and Methods

[0068] *Bacillus anthracis* Growth and Spore Preparations

[0069] *B. anthracis* cultures were grown overnight in Luria broth with or without 0.85% sodium bicarbonate at 37° C. and diluted in fresh medium at 37° C. Antibiotics were added to cultures for plasmid selection: 100 μg/ml ampicillin and 50 μg/ml kanamycin for *Escherichia coli* strains and 20 μg/ml kanamycin for *B. anthracis* strains. For spore preparation, vegetative cultures of *B. anthracis* Ames wild-type, pagA or capD mutants were sporulated in modified G medium [0.2% yeast extract, 0.0025% $CaCl_2$ dihydrate, 0.05% KH_2PO_4, 0.00976% $MgSO_4$ anhydrous, 0.005% $MnCl_2.4H_2O$, 0.00073% $ZnSO_4.7H_2O$, 0.00005% $FeSO_4.7H_2O$, 0.2% $(NH_4)_2SO_4$] until >99% sporulation was observed by light microscopy. Endospores were heat-treated at 68° C. for 1 h to kill vegetative cell. Spores were washed with sterile ddH_2O three times, suspended in sterile H_2O and stored frozen at −80° C. Endospore preparations were plated on LB agar to determine CFUs. Endospore preparations were examined by microscopy and found to be >99% purity with no observable vegetative cells or debris. For capsule production *B. anthracis* strains were grown in a capsule inducing medium [0.8% nutrient broth (pH 6.8), 0.3% yeast extract, 0.7% $NaHCO_3$, 10% horse serum, 25 mM HEPES-KOH, pH 7.5, 1.5% agar] overnight at 37° C. in 5% CO_2.

Bacillus anthracis Mutants and Plasmids

[0070] *B. anthracis* Sterne 34F2 pXO1 was used as a template for PCR amplification of two 1 kb DNA fragments flanking the pagA gene using the primers pagA1 (5'-TTTGGATCCGAGATGAAAATGGTAATATAGCGAATA-3'; SEQ ID NO:1) and pagA2 (5'-TTTCCCGGGATACGTTCTCCTTTTTGTATAAAATTAAA-3'; SEQ ID NO:2) (PCR 1) as well as pagA3 (5'-TTTCCCGGG GGTAATTCTAGGTGATTTTTAAATTATCT-3'; SEQ ID NO:3) and pagA 4 (5'-TTTGAATTCATGTGCCATTGTTTTTAAAAGTTC-3'; SEQ ID NO:4) (PCR2). PCR products 1 and 2 were restricted with BamH1/XmaI and XmaI/EcoRI, respectively, and ligated into pTS1 cut with BamH1/EcoR1.

The recombinant plasmid, pJWK374A was cut with SmaI and ligated to the kanamycin resistance cassette flanked by SmaI1 sites to generate pJWK374B. Plasmid pJWK374B was transformed into *E. coli* strain K1077 (dam dcm), non-methylated DNA purified and electroporated into *B. anthracis* Sterne as previously described (Gaspar A H, et al. (2005)). Allelic replacement and selection for a kan resistant pagA mutant followed the protocol of Marraffini (Marraffini L A & Schneewind O (2006)). Nucleic acid sequences of wild-type and mutant allele were verified by DNA sequencing. The capD variant of *B. anthracis* Ames has been previously described in Richter G S, et al. (2009), which is incorporated herein by reference in its entirety). The *B. anthracis* Sterne pagA mutant allele was transduced into *B. anthracis* Ames strain using CP-51 phage. In brief, the *B. anthracis* pagA Sterne mutant was grown overnight at 30° C. in NBY supplemented with 0.5% glycerol broth and kanamycin 20 μg/ml, and then refreshed in NBY supplemented with 0.5% glycerol for 3-5 h at 37° C. Following infection of 100 μl of refreshed donor strain with 100 μl CP-51 WT phage stock, 4 ml of PA soft agar was added and the transduction mix was plated on NBY plates with 0.5% glycerol. Following 30-46 hours incubation at 30° C., the soft agar was scraped off into 5 ml PA broth. Following centrifugation, the supernatant was passed through 0.22 μm sterile filter.

Fractionation of *B. anthracis* Cultures

[0071] *B. anthracis* strains were grown overnight in LB with or without 0.8% sodium bicarbonate as indicated. Overnight cultures were diluted 1:100 in fresh medium and grown to an optical density of 3 at 600 nm (A600 3). Total proteins in the cell culture (Total) were obtained by precipitating 1 ml of the culture with 7.5% trichloroacetic acid (TCA). To assay for protein secretion in the medium, 3 ml of the culture was centrifuged for 5 min at 6,000×g. Proteins in 1 ml of supernatant (Sup) were precipitated with 7.5% trichloroacetic acid (TCA). All TCA precipitates were washed with ice-cold acetone, solubilized in 50 μl of 0.5 M Tris-HCl (pH 8.0)/4% SDS and heated at 90° C. for 10 min. Proteins were separated on SDS/PAGE and transferred to PVDF membrane for immunoblot analysis with appropriate rabbit polyclonal antibodies. Immunoreactive signals were revealed by using a secondary antibody coupled to horseradish peroxidase and chemiluminescence.

Sortase Conjugation

[0072] The D4 domain sequence was amplified with the primers D4-1 (5'-tttggatcctttcattatgatagaaataacatagcagttg-3'; SEQ ID NO:5) and D4-2 (5'-tttgaattcttattcacccgtagccg-gaagagcttgagctcctatctcatagcatttttagaaa-3'; SEQ ID NO:6) primers using pXO1 template DNA. The PCR product was digested with BamHI and EcoRI, inserted into pGEX-2T (GE healthcare, USA) to generate pYT10, which was transformed into *E. coli* and transformants selected on Luria agar with ampicillin (100 μg/ml). An N-terminal truncated recombinant sortase A ($SrtA_{\Delta N}$) was purified as described previously (Ton-That H, Liu G, Mazmanian S K, Faull K F, & Schneewind O (1999)). For GST-D4 purification, *E. coli* BL21 (DE3) (pYT10) was grown in LB broth at 37° C. to OD_{600} 0.6 and induced with 1 mM IPTG for 3 hrs. Cells were sedimented by centrifugation, suspended in 30 mL buffer A [50 mM Tris-HCl (pH 7.5), 150 mM NaCl] and lysed in a French pressure cell at 14,000 psi. The extract was centrifuged at 29,000×g for 30 min, and the supernatant was applied to 1 ml of glutathione agarose, pre-equilibrated with buffer A. The column was washed with 40 ml of buffer A, and GST-D4 protein was eluted in 5 ml of buffer B [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 20 mM glutathione]. Excess glutathione was removed by dialysis against buffer A.

PDGA Production and the Use of this PDGA in Synthesizing the Conjugate Vaccine

[0073] In *B. anthracis*, capsule biosynthesis and anchoring is encoded by the capBCADE operon on the virulence plasmid pXO2. The anthrax capsule filaments consist of poly-γ-D-glutamic acid (PDGA). CapBC catalyzes PDGA synthesis in the cytoplasm and the CapAE transmembrane complexes transport the polymers across the cell membrane. On the cell surface, CapD attaches the polymers to a peptidoglycan by covalently linking the γ-carboxyl groups of PDGA to the side chain amino groups of the meso-diaminopimelic acid (m-DAP) of the muropeptide.

[0074] To produce recombinant PDGA, a genetically altered version of the cap operon was utilized. To generate an altered version of the cap operon, a total DNA preparation of *B. anthracis* Ames was used as template in a PCR to amplify the intact capBCADE coding region (FIG. **9**A) flanked by an upstream KpnI site and a downstream EagI site for cloning into expression vector pLM5 with Kan^R gene for selection of transformants. The cap operon encoded by the complement strand of virulence plasmid pXO2 (GenBank accession number NC_007323, which is hereby incorporated by reference) was amplified by a forward primer incorporating a KpnI restriction site (SEQ ID NO 7) (5'-TGTCGA GGTACC TTGAGCCTTGATAGTGCGAG-3) (sequence in bold represents pXO2 complement strand positions 57,020 to 57,001; flanking KpnI site introduced for cloning is underlined) and reverse primer incorporating an EagI restriction site (SEQ ID NO 8) (5'-CTAACA CGGCCG TTAGGGGTTAGCCTGTA-GAT-3') (sequence in bold represents pXO2 positions 52,147 to 52,166; flanking EagI site introduced for cloning is underlined). The 4.9-kb PCR product (corresponding to positions 52,147 to 57,020 on pXO2) was digested with the restrictases and inserted into the corresponding cloning sites on pLM5, Marraffini et al. 2006), downstream of the IPTG-inducible spat promoter. The resulting construct pCAP1 was propagated in *E. coli* DH5a and then transformed into *E. coli* K1077 (dam^- dcm^-) to screen for PDGA-producing clones.

[0075] Minicultures of transformants were grown in selection medium (LB containing 50 μg×ml-1 kanamycin) supplemented with 1 mM IPTG for inducing the cap operon. After overnight incubation, culture supernatants were recovered by centrifugation and heat-treated for 20 min at 95° C. Cell pellets were immediately frozen at −80° C. For cell lysis, the pellets were thawed in 10 mM HEPES-KOH, pH 7.5, and treated with a sonicator until the lysates turned clear. Insoluble material was removed by centrifugation.

[0076] To precipitate PDGA from culture supernatants, 4 volumes of ethanol were added and samples were stored at −20° C. for 48 hours. Precipitates were recovered by centrifugation and dissolved in 10 mM HEPES-KOH, pH 7.5. Both supernatant and cell pellet samples were treated with 200 $\mu g \times ml^{-1}$ proteinase K for 2 hours at 37° C. The enzyme was heat-inactivated (20 min at 95° C.) and insoluble material was removed by centrifugation (FIG. **9**B). Partial digestion of high molecular weight PDGA with CapD is required to obtain a fragmented product with reactive amino groups that are needed for conjugation. Samples were tested for PDGA content by dot blot probed with an anti-capsule antibody. Plas-

mids from PDGA-positive clones were isolated and sequenced. Analysis revealed that the capsule-producing clone CAP1-5 (SEQ ID NO:9) carries a cap operon with a frame shift mutation in capD that inactivates the gene without a polar effect on the other genes. PDGA isolated from this strain has been analyzed for D-glutamic acid content and was used in immunoconjugation experiments.

[0077] To determine the D-glutamate content of recombinant PDGA, the purified product was hydrolyzed by incubation at 105° C. in pH 2 for 16 hours. This lyzed material was neutralized and incubated with Marfey's reagent (Sigma) that reacts with primary amines for UV detection (FIG. **9**C). Derivatized D- and L-glutamates were separated by reversed-phase HPLC with a linear gradient of acetonitrile in ammonium formate-methanol buffer on a ODS column (particle size 5 μm; column size 250×4.6 mm; Thermo Scientific). Glutamate enantiomers differ in their retention time with the D=form eluting after the L=form (FIG. **9**D). The HPLC protocol was optimized using commercial D- and L-glutamate preparations (Sigma) as standards. The analysis indicates that recombinant PDGA contains 85% D-form. Capsule isolated from fully virulent *B. anthracis* Ames has 92% D-glutamate (FIG. **9**E).

[0078] The transpeptidation reaction was carried out in 0.5 ml 100 mM HEPES, 150 mM NaCl, 5 mM $CaCl_2$ (pH 7.5) with 0.2 mM GST-D4, 0.1 mM sortase and 1 mM nucleophile (NH_2-Gly_5-$PDGA_{10}$) for 12 hours at 37° C. Reaction products were subjected to glutathione agarose affinity chromatography. After washing with buffer A, GST tag was cleaved off with thrombin. Ion-exchange chromatography through MonoQ column (GE-healthcare, USA) was used to purify PDGA-D4 from D4. The reaction products were analyzed by analytical RP-HPLC and characterized with ESI-MS.

Animal Models of Anthrax Infection

[0079] All animal experiments followed protocols that were reviewed, approved, and supervised by the Institutional Animal Care and Use Committee and the Select Agent Committee at the University of Chicago. Six week old, female C57BL/6 mice (Jackson Laboratory) were challenged by intraperitoneal injection of *B. anthracis* spore suspensions in 100 μl PBS. Aliquots of the spore inoculum were spread on agar plates to enumerate the challenge dose. Infected animals were monitored in 12 hour intervals for survival or a moribund state (inability to remain upright, weight loss or unresponsive to touch). Moribund animals were killed by inhalation of compressed CO_2 and removal of vital organs.

[0080] Six week old, female C57BL/6 mice (Jackson Laboratory) were challenged by intra-peritoneal injection of *B. anthracis* spore suspensions in 100 μl PBS. Aliquots of the spore inoculum were spread on agar plates to enumerate the challenge dose. Infected animals were monitored in 12 hour intervals for survival or a moribund state (inability to remain upright, weight loss, non-responsive to touch) for 14 days. Moribund animals were killed by inhalation of compressed CO_2 and cervical dislocation.

[0081] Female Hartley guinea pigs (250-350 g) were infected by subcutaneous injection of spores into the inguinal fold of the hind leg. Animals were observed for morbidity and mortality for 14 days. All animals were subjected to necropsy and their site of infection, spleen, liver and lungs removed. Organs were immediately fixed by submersion in 10% neutral-buffered formalin and embedded in paraffin. Samples were submitted to the University of Chicago Animal Pathology Core for serial 4-μm thin sections and staining with hematoxylin-eosin. Tissue samples were viewed by light microscopy. Organ samples isolated during necropsy were also homogenized in phosphate buffered saline, serially diluted, and plated on LB to enumerate bacterial load as CFU. Alternatively, samples were fixed with neutral buffered formalin and stained with India ink to visualize the capsule of bacilli.

[0082] Groups (n=10) of female Hartley guinea-pigs (250-280 g) were immunized by intramuscular injection into the hind leg with 0.2 ml of 50 μg of PA (List Biological Laboratories, Inc.), D4 or PDGA-D4 absorbed to 25% aluminum hydroxide (Alhydrogel) on days 1, 14, 28. As a control, 250 μl AVA (Biothrax®) was administered at the same time. Blood was collected on day 35 to measure serum antibody titers before challenge. Levels of guinea pig serum immunoglobulin G (IgG) reactive with specific antigens were determined by a custom enzyme-linked immunosorbent assay (ELISA). Briefly, serum samples representative of the immunization groups were aliquoted on microtiter plates pre-coated with purified PA, D4 (5 μg/ml) or PDGA-D4 (20 μg/ml). Binding of serum antibody was detected with secondary antibodies against specific immunoglobulin (anti-guinea pig).

Statistical Analysis

[0083] Data were processed using GraphPad PRISM® 5.0 software to generate graphs and for statistical analyses. Statistical analysis of serum antibody levels was performed in pairwise comparison using the unpaired two-tailed Student's t-test. Bacterial load data were analyzed for statistical significance with the unpaired two-tailed Student's t-test. Comparisons of animal survival between two groups were evaluated with the log-rank test.

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[0084] The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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<210> SEQ ID NO 10
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<212> TYPE: PRT
<213> ORGANISM: Bacillus anthracis

<400> SEQUENCE: 10

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Gln Glu Asn Arg Leu Leu Asn Glu Ser Glu Ser Ser Ser Gln Gly Leu
        35                  40                  45

Leu Gly Tyr Tyr Phe Ser Asp Leu Asn Phe Gln Ala Pro Met Val Val
    50                  55                  60

Thr Ser Ser Thr Thr Gly Asp Leu Ser Ile Pro Ser Ser Glu Leu Glu
65                  70                  75                  80

Asn Ile Pro Ser Glu Asn Gln Tyr Phe Gln Ser Ala Ile Trp Ser Gly
                85                  90                  95

Phe Ile Lys Val Lys Lys Ser Asp Glu Tyr Thr Phe Ala Thr Ser Ala
            100                 105                 110

Asp Asn His Val Thr Met Trp Val Asp Asp Gln Glu Val Ile Asn Lys
        115                 120                 125

Ala Ser Asn Ser Asn Lys Ile Arg Leu Glu Lys Gly Arg Leu Tyr Gln
    130                 135                 140

Ile Lys Ile Gln Tyr Gln Arg Glu Asn Pro Thr Glu Lys Gly Leu Asp
145                 150                 155                 160

Phe Lys Leu Tyr Trp Thr Asp Ser Gln Asn Lys Lys Glu Val Ile Ser
                165                 170                 175

Ser Asp Asn Leu Gln Leu Pro Glu Leu Lys Gln Lys Ser Ser Asn Ser
            180                 185                 190

Arg Lys Lys Arg Ser Thr Ser Ala Gly Pro Thr Val Pro Asp Arg Asp
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Asn Asp Gly Ile Pro Asp Ser Leu Glu Val Glu Gly Tyr Thr Val Asp
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Glu Lys Lys Gly Leu Thr Lys Tyr Lys Ser Ser Pro Glu Lys Trp Ser
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Thr Ala Ser Asp Pro Tyr Ser Asp Phe Glu Lys Val Thr Gly Arg Ile
            260                 265                 270

Asp Lys Asn Val Ser Pro Glu Ala Arg His Pro Leu Val Ala Ala Tyr
        275                 280                 285

Pro Ile Val His Val Asp Met Glu Asn Ile Ile Leu Ser Lys Asn Glu
    290                 295                 300

Asp Gln Ser Thr Gln Asn Thr Asp Ser Gln Thr Arg Thr Ile Ser Lys
305                 310                 315                 320

Asn Thr Ser Thr Ser Arg Thr His Thr Ser Glu Val His Gly Asn Ala
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Glu Val His Ala Ser Phe Phe Asp Ile Gly Gly Ser Val Ser Ala Gly
            340                 345                 350

Phe Ser Asn Ser Asn Ser Ser Thr Val Ala Ile Asp His Ser Leu Ser
        355                 360                 365

Leu Ala Gly Glu Arg Thr Trp Ala Glu Thr Met Gly Leu Asn Thr Ala
    370                 375                 380

Asp Thr Ala Arg Leu Asn Ala Asn Ile Arg Tyr Val Asn Thr Gly Thr
385                 390                 395                 400

Ala Pro Ile Tyr Asn Val Leu Pro Thr Thr Ser Leu Val Leu Gly Lys
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Asn Gln Thr Leu Ala Thr Ile Lys Ala Lys Glu Asn Gln Leu Ser Gln
            420                 425                 430

Ile Leu Ala Pro Asn Asn Tyr Tyr Pro Ser Lys Asn Leu Ala Pro Ile
        435                 440                 445

Ala Leu Asn Ala Gln Asp Asp Phe Ser Ser Thr Pro Ile Thr Met Asn
    450                 455                 460

Tyr Asn Gln Phe Leu Glu Leu Glu Lys Thr Lys Gln Leu Arg Leu Asp
465                 470                 475                 480

Thr Asp Gln Val Tyr Gly Asn Ile Ala Thr Tyr Asn Phe Glu Asn Gly
                485                 490                 495

Arg Val Arg Val Asp Thr Gly Ser Asn Trp Ser Glu Val Leu Pro Gln
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Ile Gln Glu Thr Thr Ala Arg Ile Ile Phe Asn Gly Lys Asp Leu Asn
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Leu Val Glu Arg Arg Ile Ala Ala Val Asn Pro Ser Asp Pro Leu Glu
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Thr Thr Lys Pro Asp Met Thr Leu Lys Glu Ala Leu Lys Ile Ala Phe
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Gly Phe Asn Glu Pro Asn Gly Asn Leu Gln Tyr Gln Gly Lys Asp Ile
                565                 570                 575

Thr Glu Phe Asp Phe Asn Phe Asp Gln Gln Thr Ser Gln Asn Ile Lys
            580                 585                 590

Asn Gln Leu Ala Glu Leu Asn Ala Thr Asn Ile Tyr Thr Val Leu Asp
        595                 600                 605

Lys Ile Lys Leu Asn Ala Lys Met Asn Ile Leu Ile Arg Asp Lys Arg
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Phe His Tyr Asp Arg Asn Asn Ile Ala Val Gly Ala Asp Glu Ser Val
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Val Lys Glu Ala His Arg Glu Val Ile Asn Ser Ser Thr Glu Gly Leu
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Leu Leu Asn Ile Asp Lys Asp Ile Arg Lys Ile Leu Ser Gly Tyr Ile
            660                 665                 670

Val Glu Ile Glu Asp Thr Glu Gly Leu Lys Glu Val Ile Asn Asp Arg
        675                 680                 685

Tyr Asp Met Leu Asn Ile Ser Ser Leu Arg Gln Asp Gly Lys Thr Phe
    690                 695                 700

Ile Asp Phe Lys Lys Tyr Asn Asp Lys Leu Pro Leu Tyr Ile Ser Asn
705                 710                 715                 720

Pro Asn Tyr Lys Val Asn Val Tyr Ala Val Thr Lys Glu Asn Thr Ile
                725                 730                 735

Ile Asn Pro Ser Glu Asn Gly Asp Thr Ser Thr Asn Gly Ile Lys Lys
            740                 745                 750

Ile Leu Ile Phe Ser Lys Lys Gly Tyr Glu Ile Gly
        755                 760
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1. A method for producing an antigenic composition comprising contacting an antigenic polypeptide and a second molecule comprising a reactive amino group with an isolated sortase enzyme to produce an immunoconjugate, wherein administration of the immunoconjugate to a subject produces an immune response to the second molecule.

2. The method of claim **1**, wherein the second molecule is a peptide, a hapten, a peptidoglycan or a carbohydrate with a reactive amino group.

3. The method of claim **1**, wherein the second molecule is a bacterial capsule component.

4. The method of claim **1**, wherein the antigenic polypeptide and the second molecule are from the same organism.

5. The method of claim **4**, wherein the antigenic polypeptide and the second molecule are both from *B. anthracis*.

6.-**11**. (canceled)

12. The method of claim **1**, wherein the antigenic polypeptide is the protective antigen (PA) from *B. anthracis* or an antigenic fragment thereof.

13. The method of claim **12**, wherein antigenic polypeptide is a fragment of PA comprising the D4 domain.

14. The method of claim **3**, wherein the bacterial capsule component is a capsular polysaccharide from *Streptococcus pneumoniae, Neisseria meningitides, Staphylococcus aureus, Hemophilus influenza* or *Streptococcus agalactiae*.

15. The method of claim **3**, wherein the bacterial capsule component comprises poly-γ-glutamic acid.

16. The method of claim **15**, wherein the bacterial capsule component is the poly-D-γ-glutamic acid capsule of *B. anthracis*.

17.-**22**. (canceled)

23. An antigenic composition comprising an isolated and essentially homogenous population of antigenic polypeptide covalently linked to a bacterial capsule component wherein the covalent linkage comprises a peptide bond at the carboxyl-terminus of the antigenic polypeptide.

24. The antigenic composition of claim **23**, wherein the antigenic polypeptide and the covalently linked bacterial capsule component are present in a 1:1 ratio.

25.-**28**. (canceled)

29. The antigenic composition of claim **23**, wherein the covalent linkage between the antigenic polypeptide and the bacterial capsule component consists of a peptide bond at the carboxyl-terminus of the antigenic polypeptide.

30. The antigenic composition of claim **23**, wherein the antigenic polypeptide and the bacterial capsule component are from the same organism.

31. The antigenic composition of claim **30**, wherein the antigenic polypeptide and the bacterial capsule component are both from *B. anthracis*.

32. The antigenic composition of claim **23**, wherein the antigenic polypeptide is the protective antigen (PA) from *B. anthracis* or an antigenic fragment thereof.

33. The antigenic composition of claim **32**, wherein antigenic polypeptide is a fragment of PA comprising the D4 domain.

34. The antigenic composition of claim **23**, wherein the bacterial capsule component comprises poly-γ-glutamic acid.

35. The antigenic composition of claim **34**, wherein the bacterial capsule component is the poly-D-γ-glutamic acid capsule of *B. anthracis*.

36. (canceled)

37. A method of inducing a protective immune response in a subject comprising administering a composition comprising an antigenic portion of protective antigen (PA) from *B. anthracis* conjugated to a poly-D-γ-glutamic acid capsule component, wherein administration of the composition provides resistance to *B. anthracis*-induced mortality in the subject.

38.-**60**. (canceled)

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