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(54) **IMMUNOTHERAPEUTIC TARGETS AGAINST STAPHYLOCOCCUS AUREUS**

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

CPC ***A61K 39/085*** (2013.01); ***C07K 16/1271*** (2013.01); *A61K 2039/55505* (2013.01)

(57) **ABSTRACT**

The subject invention pertains to vaccine formulations and antibodies, and related methods, for the treatment and/or prevention of *S. aureus* infection. The present invention provides one or more *S. aureus* antigens for use in vaccine formulations, wherein two or more antigens act synergistically. Further, the present invention provides vaccines that can protect against hematic spread, pneumonia and skin infection.

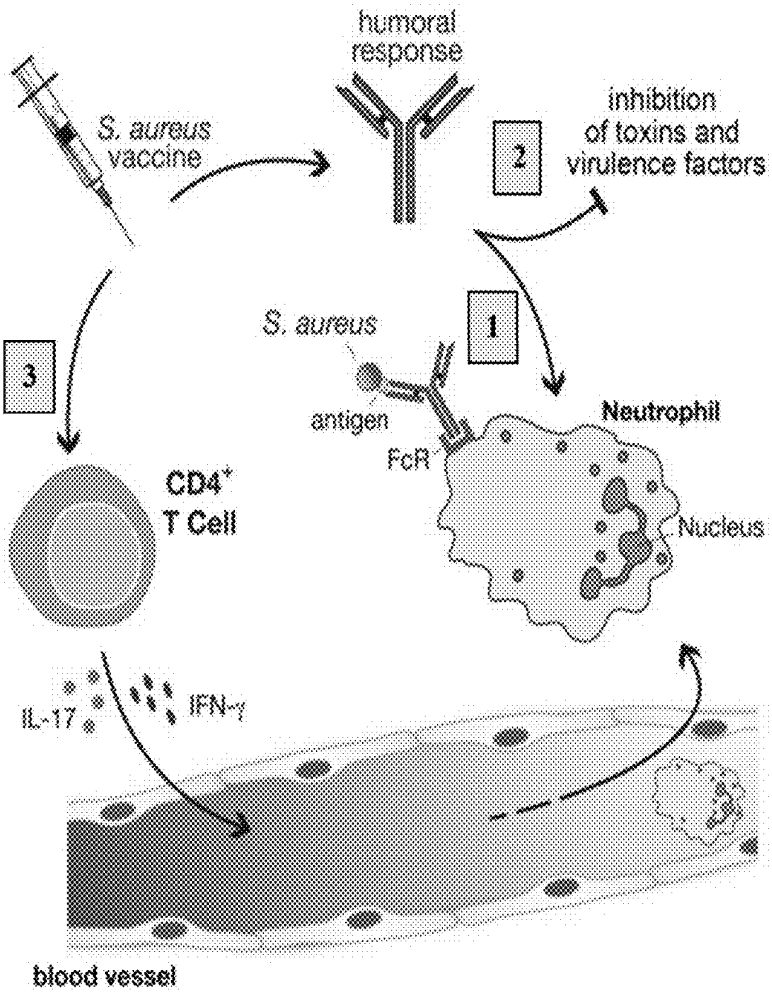
humoral response
S. aureus vaccine
2
inhibition of toxins and virulence factors
S. aureus
1
3
antigen
FcR
Neutrophil
CD4+ T Cell
Nucleus
IL-17
IFN-γ
blood vessel

FIGURE 1

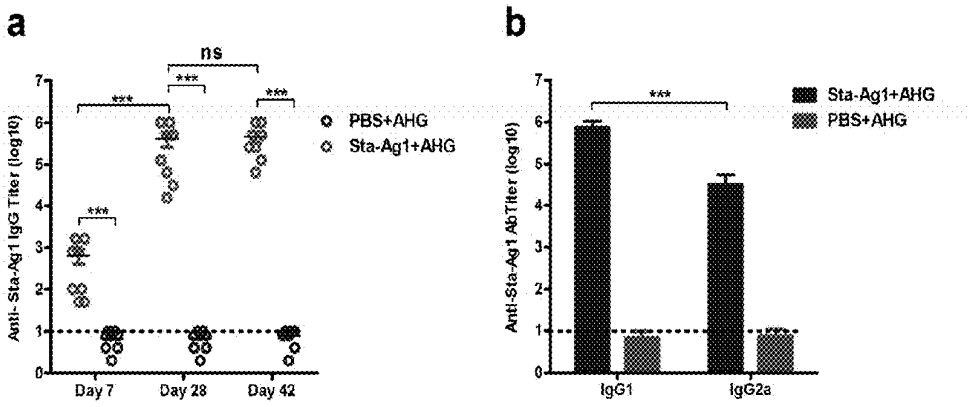

FIGURE 2

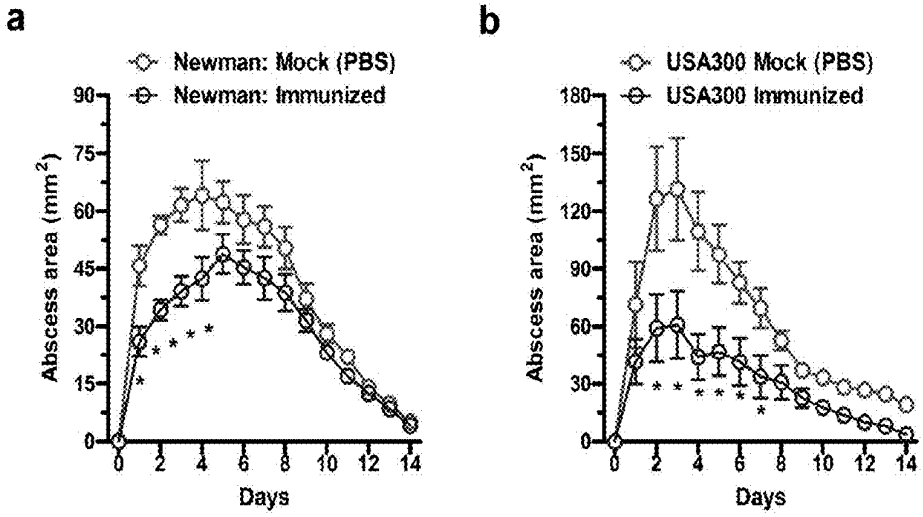

FIGURE 3

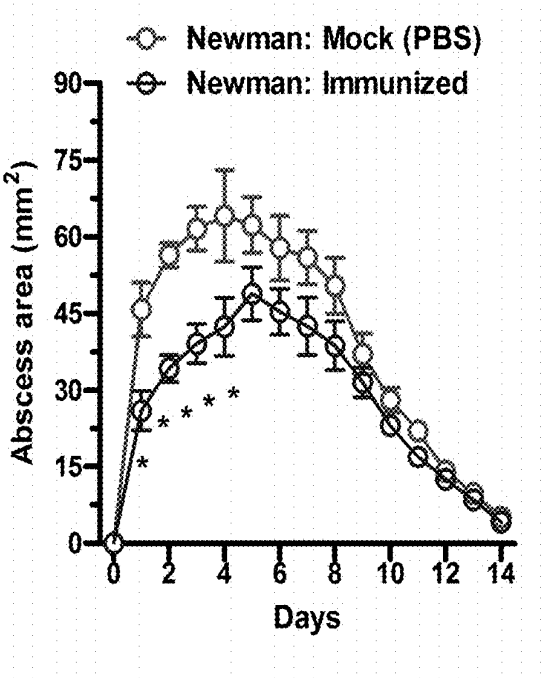

FIGURE 3A

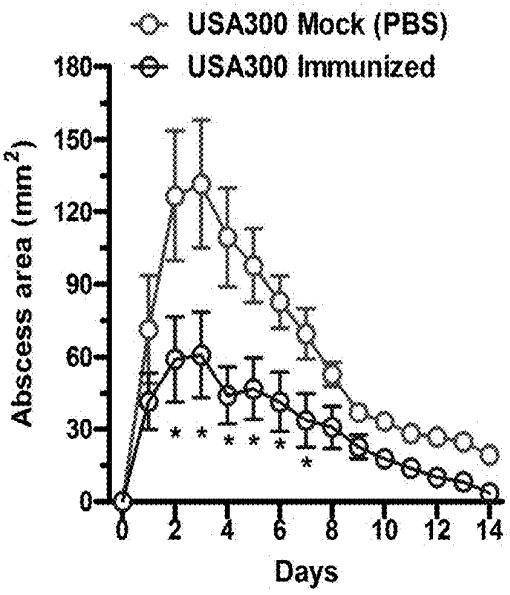

FIGURE 3B

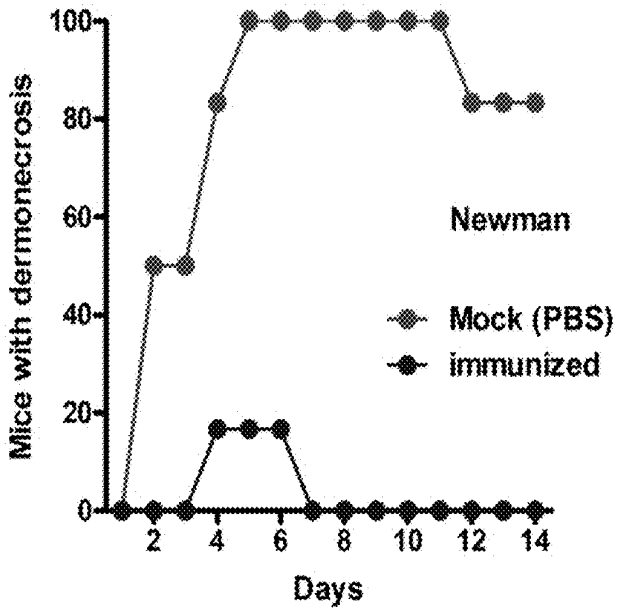

FIGURE 4A

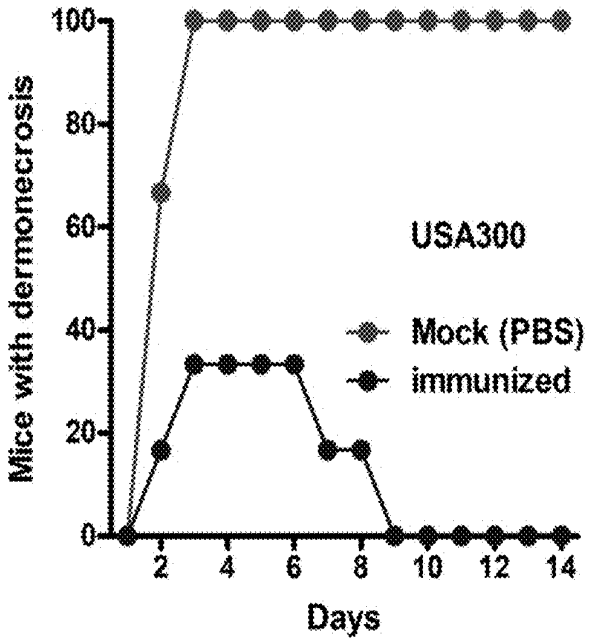

FIGURE 4B

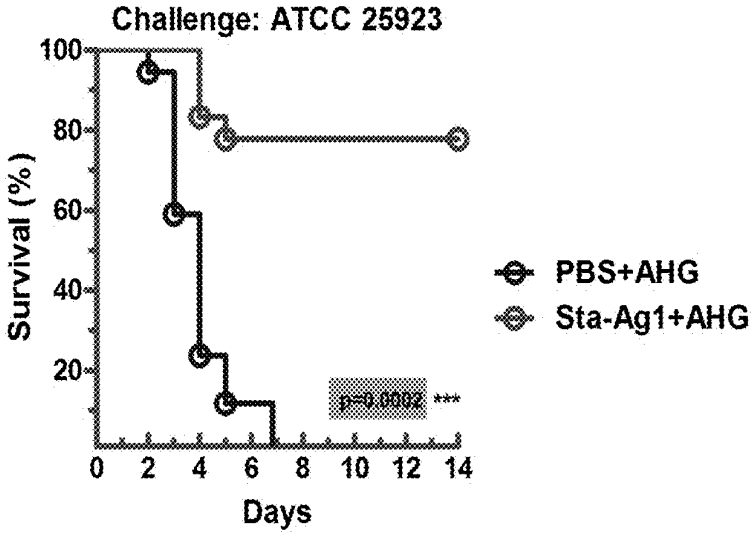

FIGURE 5

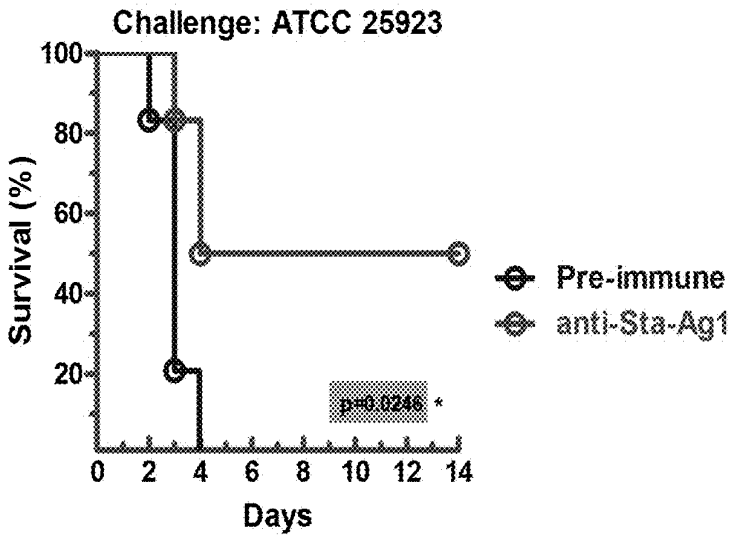

FIGURE 6

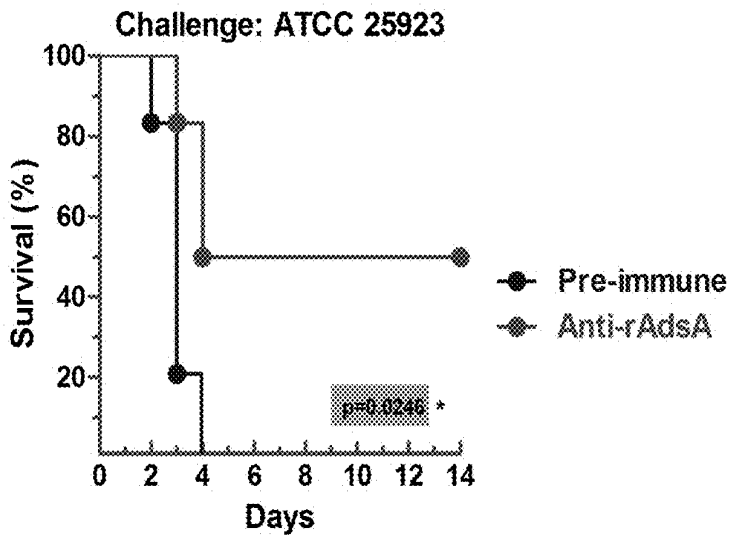
Challenge: ATCC 25923
Survival (%)
100
80
60
40
20
0
2
4
6
8
10
12
14
Days
Pre-immune
Anti-rAdsA
p=0.0246 *

FIGURE 7

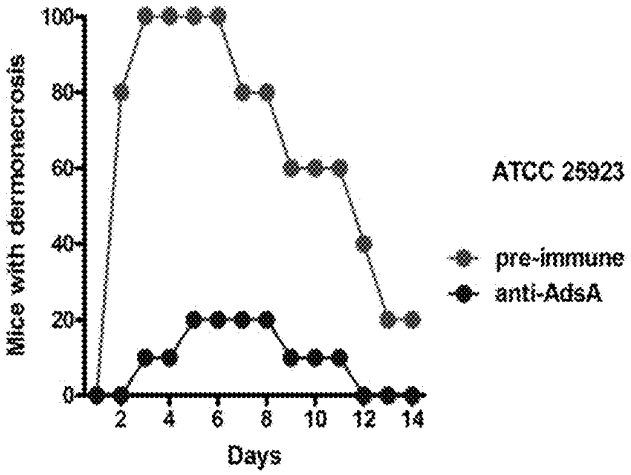

FIGURE 8A

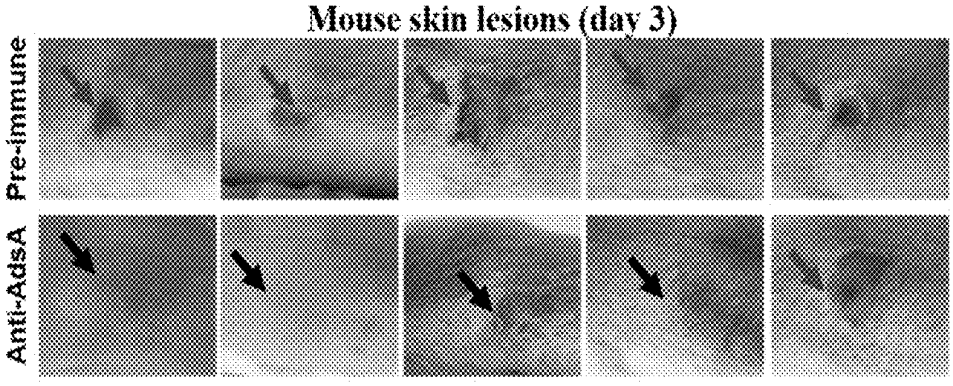

FIGURE 8B

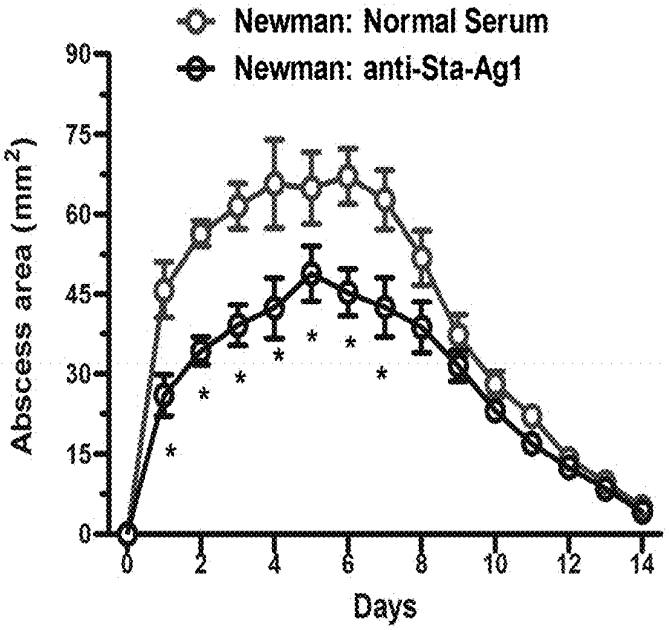

FIGURE 9A

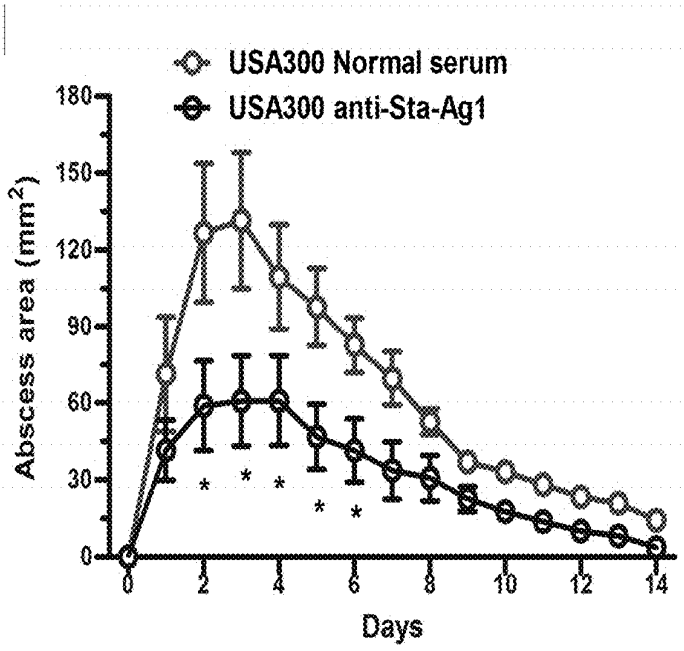

FIGURE 9B

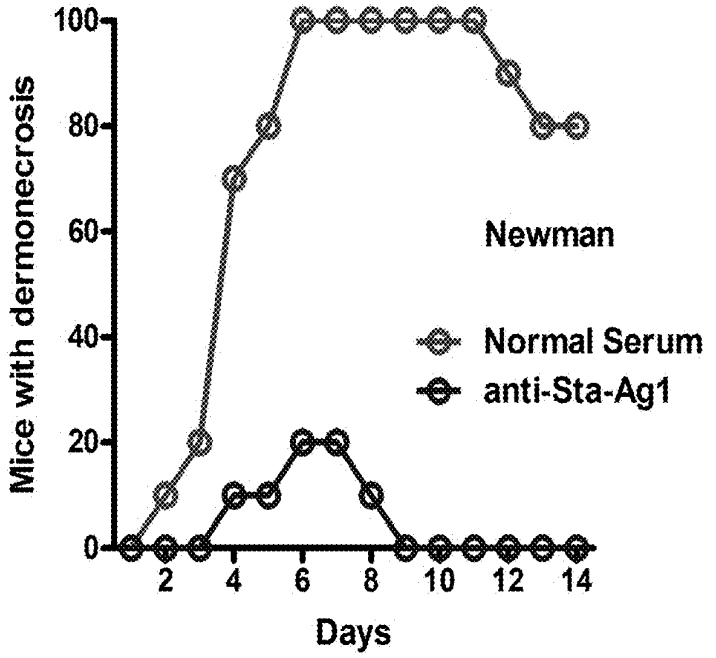

FIGURE 10A

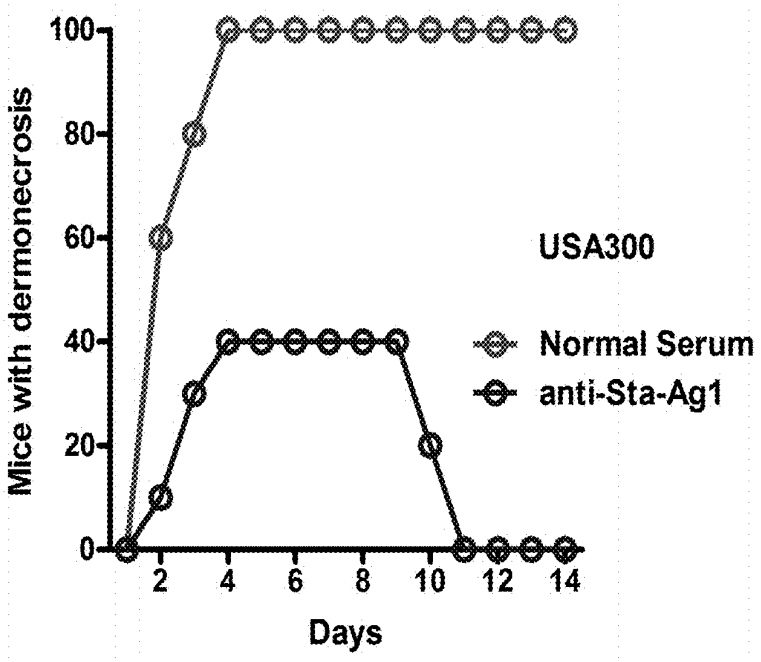

FIGURE 10B

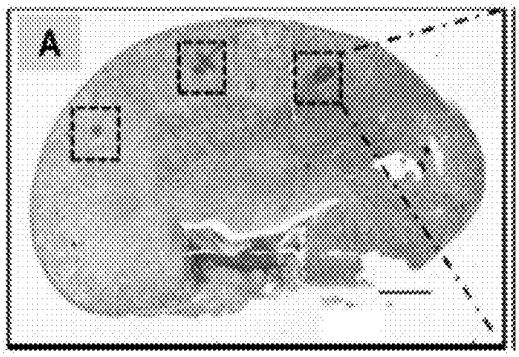

FIGURE 11A

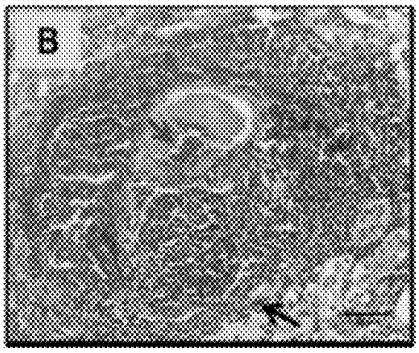

FIGURE 11B

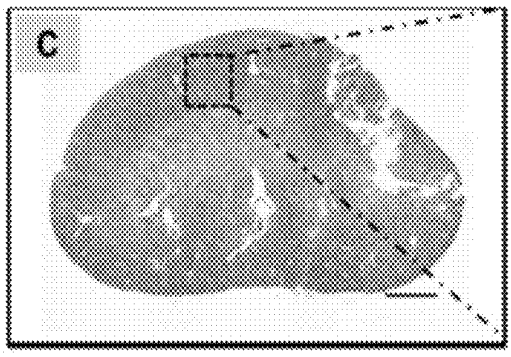

FIGURE 11C

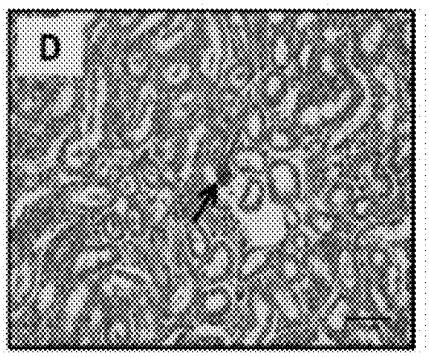

FIGURE 11D

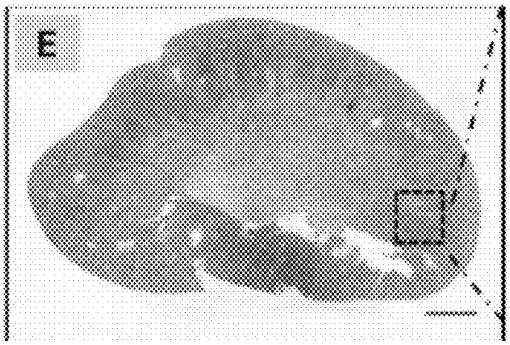

FIGURE 11E

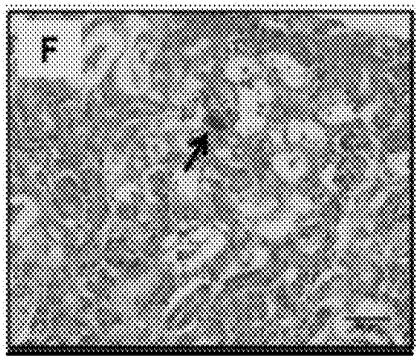

FIGURE 11F

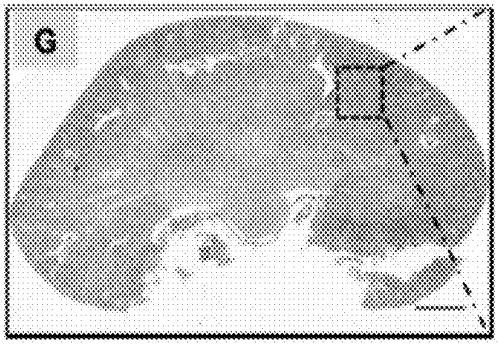

FIGURE 11G

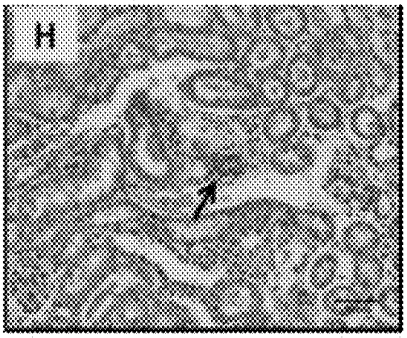

FIGURE 11H

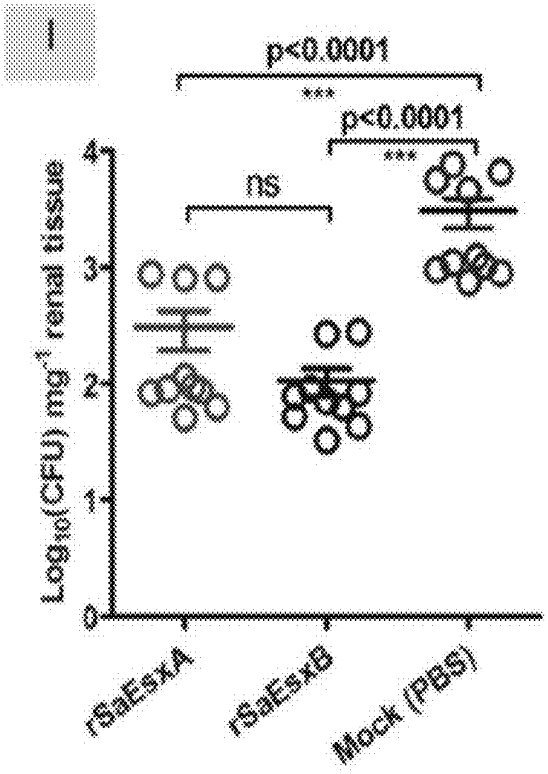

FIGURE 11I

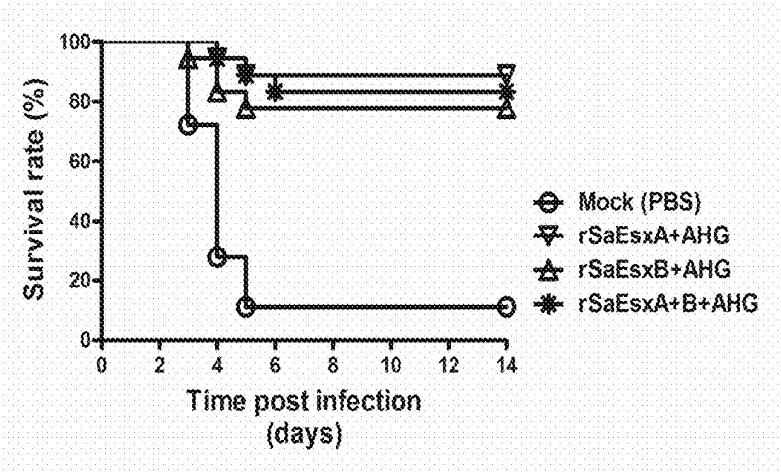

FIGURE 12

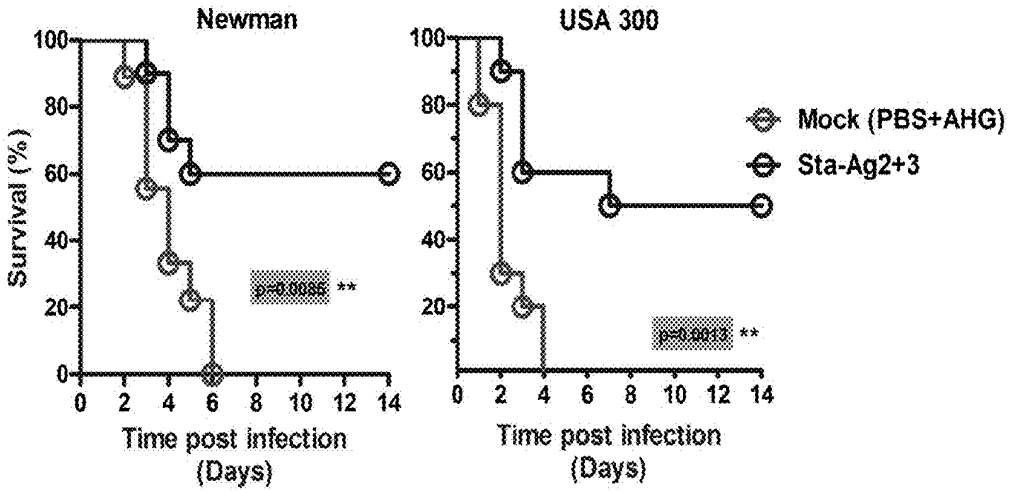

FIGURE 13

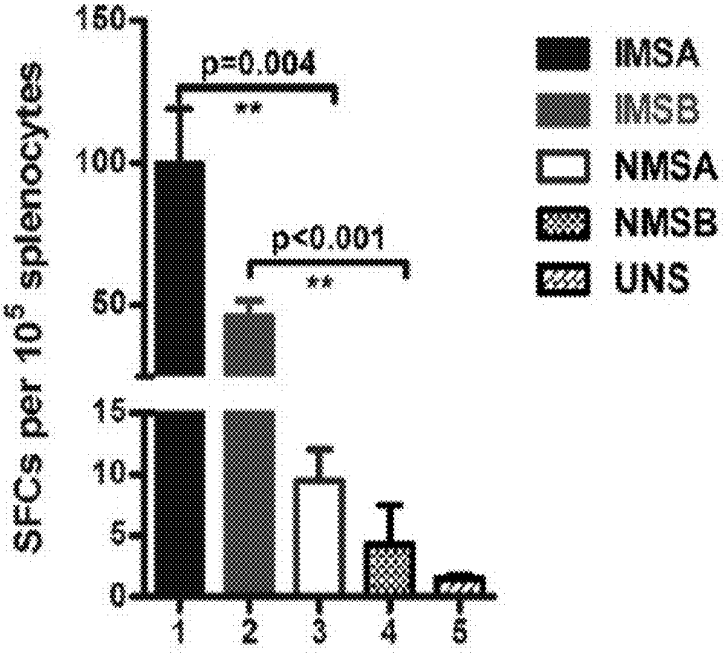

FIGURE 14A

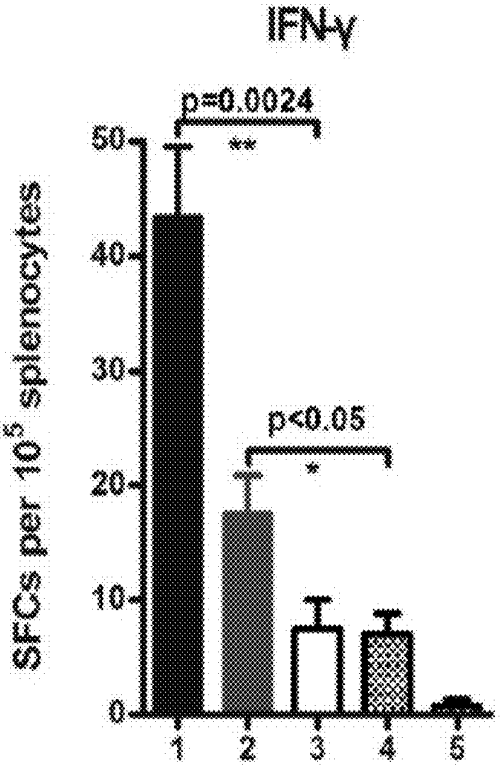

FIGURE 14B

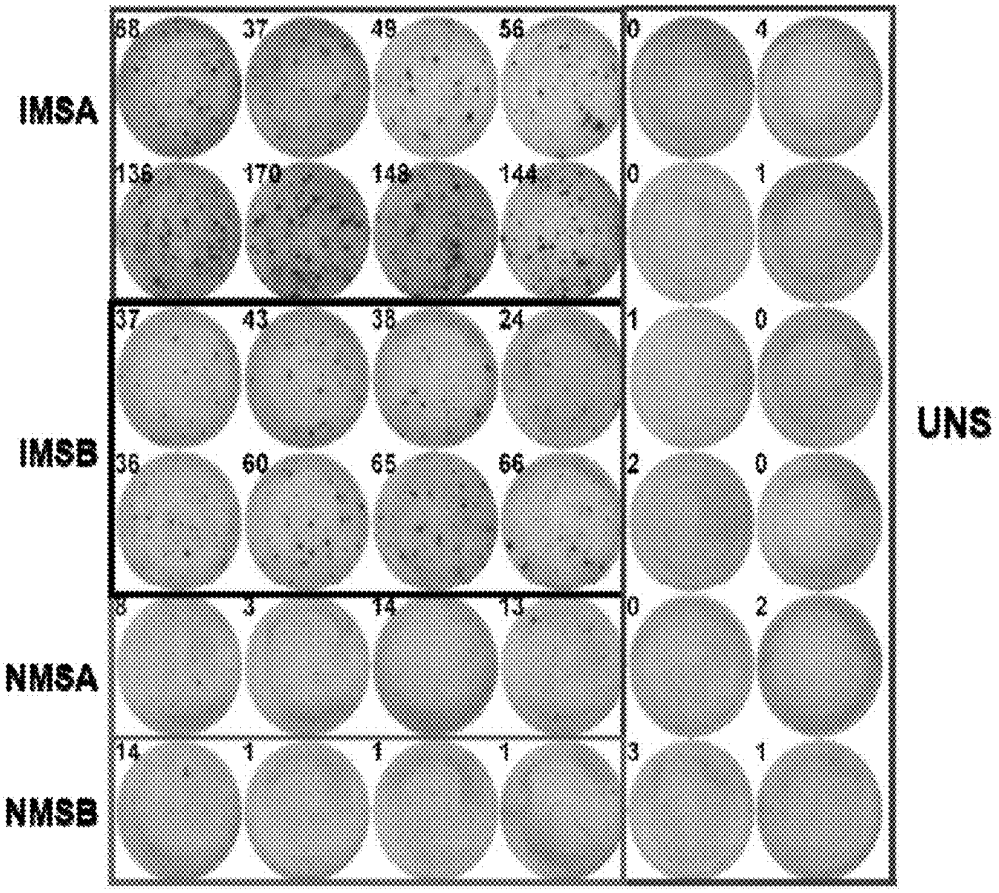
IMSA
IMSB
NMSA
NMSB
UNS
68
37
49
56
0
4
136
170
148
144
0
1
37
43
38
24
1
0
36
60
65
66
2
0
8
3
14
13
0
2
14
1
1
1
3
1

FIGURE 14C

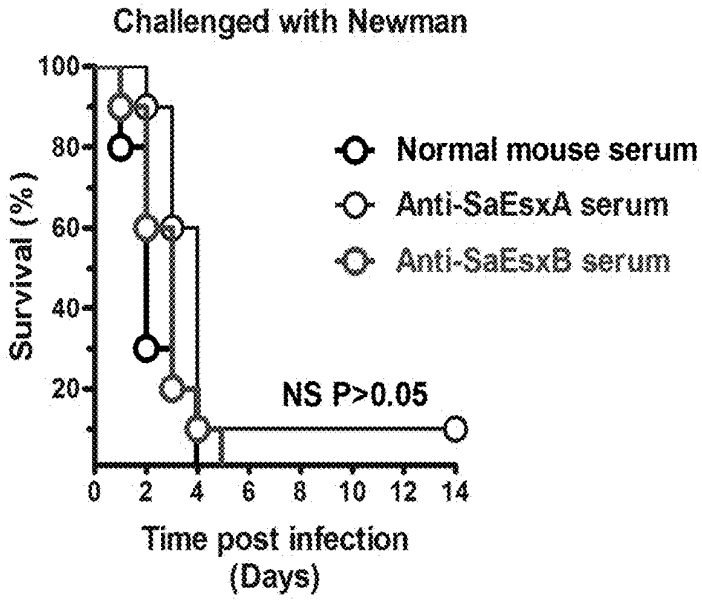
Challenged with Newman
100
80
60
40
20
Survival (%)
Normal mouse serum
Anti-SaEsxA serum
Anti-SaEsxB serum
NS P>0.05
0
2
4
6
8
10
12
14
Time post infection
(Days)

FIGURE 15

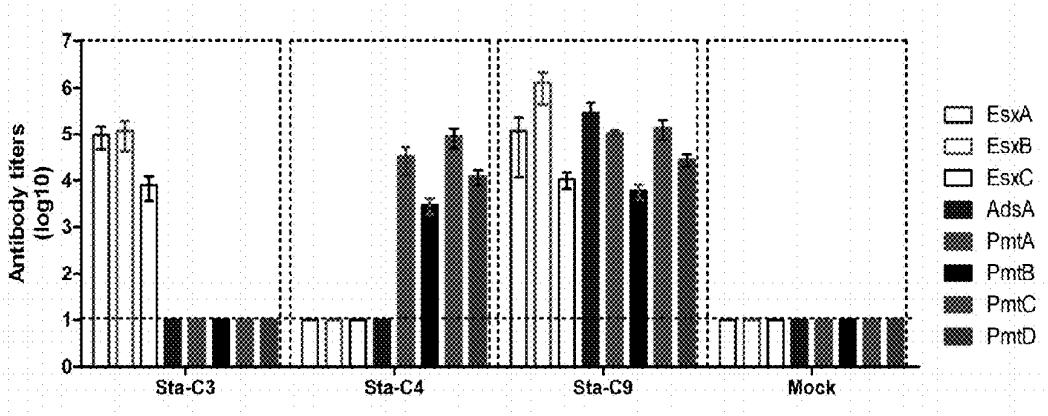
Antibody titers (log10)
0
1
2
3
4
5
6
7
Sta-C3
Sta-C4
Sta-C9
Mock
EsxA
EsxB
EsxC
AdsA
PmtA
PmtB
PmtC
PmtD

FIGURE 16

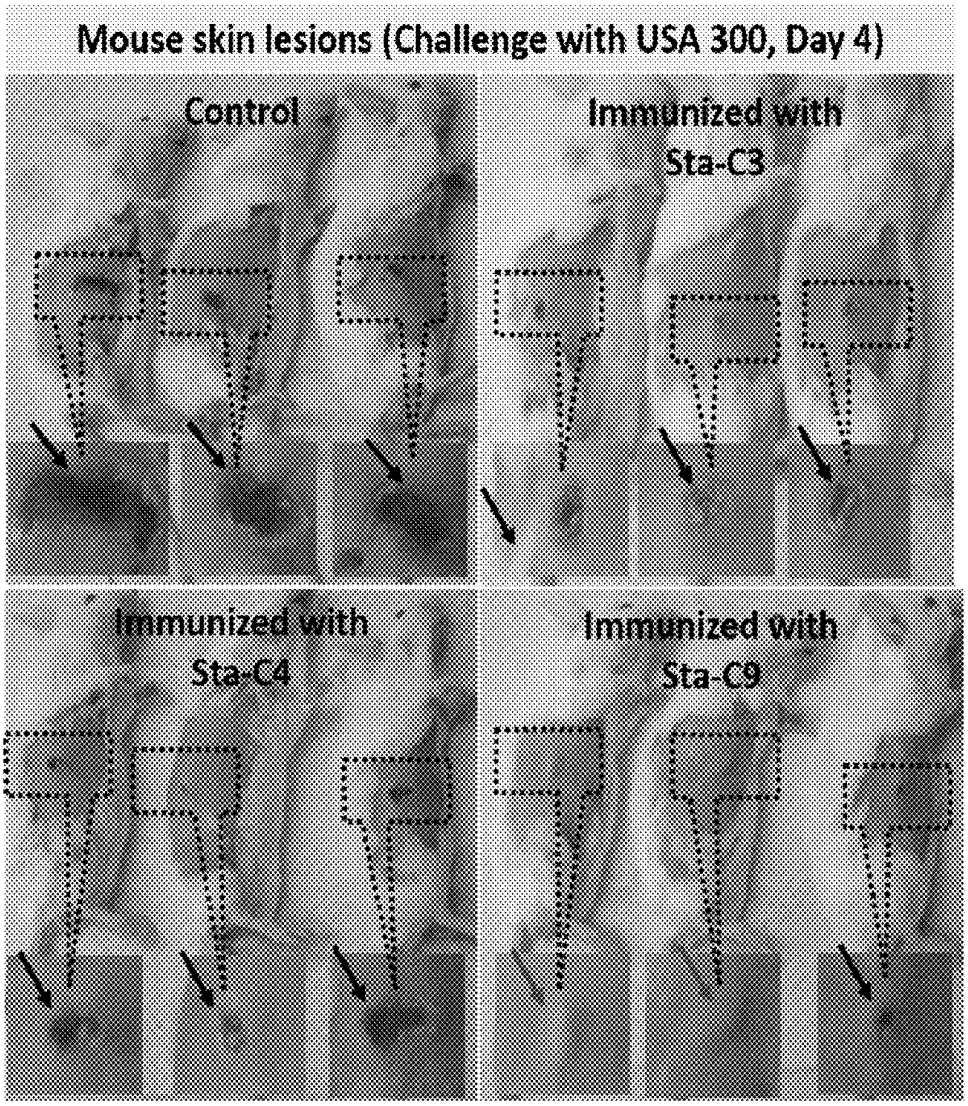
Mouse skin lesions (Challenge with USA 300, Day 4)
Control
Immunized with Sta-C3
Immunized with Sta-C4
Immunized with Sta-C9

FIGURE 17

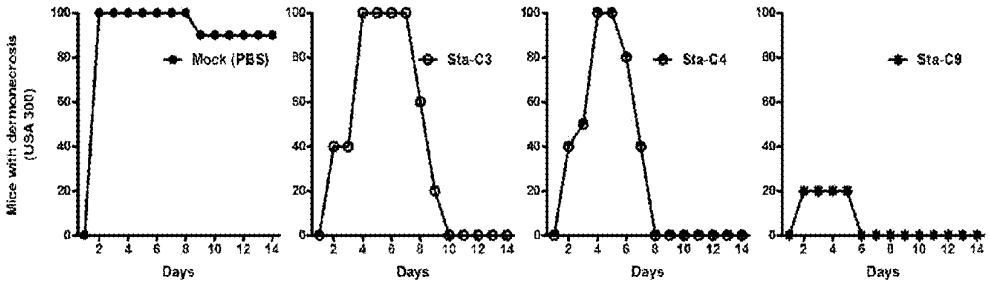

FIGURE 18

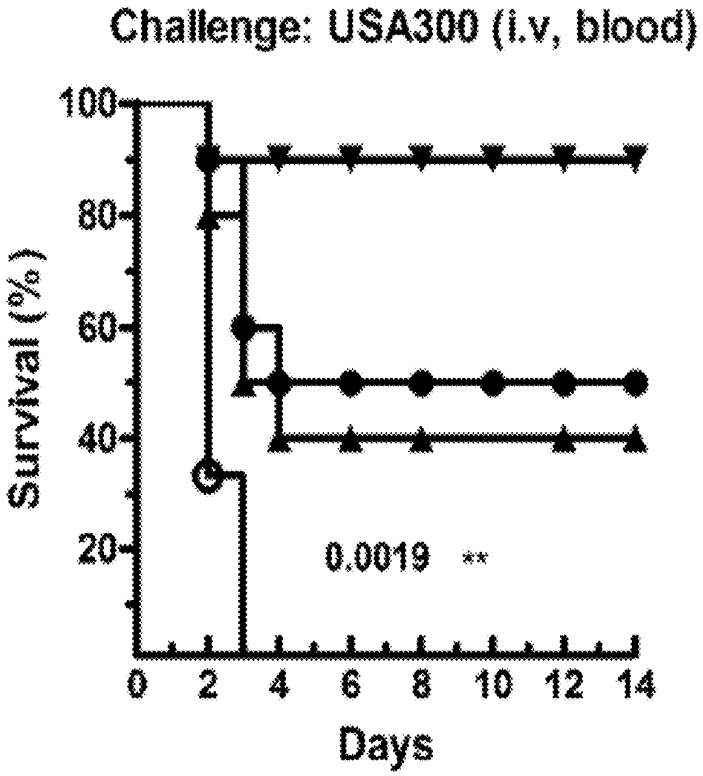

FIGURE 19A

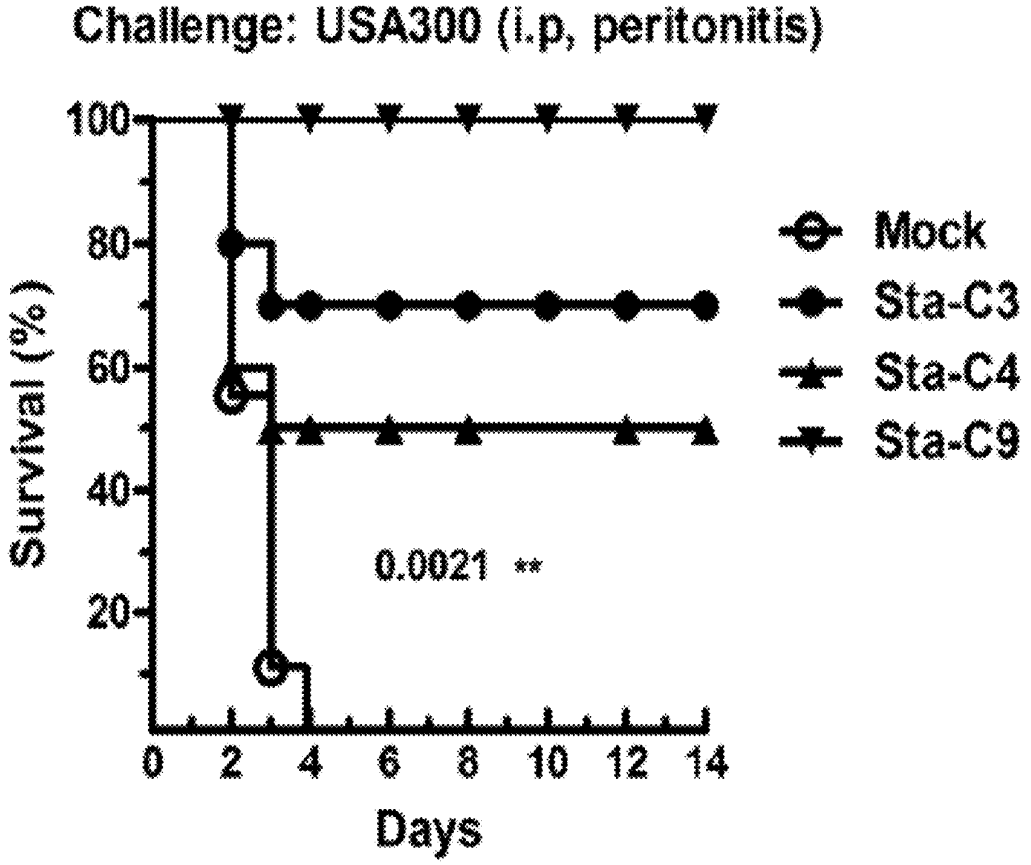
Challenge: USA300 (i.p, peritonitis)
Survival (%)
100
80
60
40
20
0
2
4
6
8
10
12
14
Days
Mock
Sta-C3
Sta-C4
Sta-C9
0.0021 **

FIGURE 19B

IMMUNOTHERAPEUTIC TARGETS AGAINST STAPHYLOCOCCUS AUREUS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. provisional application Ser. No. 62/093,752, filed Dec. 18, 2014, which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] *Staphylococcus aureus* (*S. aureus*) is a facultative anaerobic Gram-positive bacterium, frequently found as part of the normal flora on the skin and in the nasal passages [1, 2]. *S. aureus* can cause a range of illnesses ranging from minor skin infections (such as pimples, cellulitis folliculitis, carbuncles, scalded skin syndrome and abscesses) to life-threatening diseases (such as meningitis, pneumonia, toxic shock syndrome, bacteraemia and sepsis). Multidrug-resistant (MDR) pathogens are a global problem. Their ability to adapt enables emerging strains to develop resistance to new antibiotics. Vaccinations could be a better strategy to control MDR pathogen infections. Vaccination has been demonstrated to be effective in preventing many infectious diseases, including influenza, small pox and Hepatitis B Virus infections. However, for many MDR pathogens, a serotype-independent immune response may be required.

[0003] Similar ESAT-6-like proteins, esxA (Rv3875) and esxB (Rv3874), secreted by *M. tuberculosis* are known to play a vital role in its pathogenesis. These two proteins can trigger cell-mediated immune responses and IFN-γ production during tuberculosis [46, 39]. This study indicated that the ESAT-6 expressed by the virulent *M. tuberculosis* strain H37Rv, but not by BCG, promotes immunity by enhancing Th17 cell responses [40]. Activation of naïve T cells by pathogen antigens presented by antigen presenting cells in the presence of various cytokines leads to the generation of T helper cell-subsets such as Th1, Th2 and Th17. Universally, the Th1 cells regulate IFN-γ-dependent immunity against most intracellular pathogens. The Th1 subset could be inhibited by IL-4, subsequently inducing another T cell subset, Th2, which produced IL-4, IL-5, and IL-13 against helminth infection [41].

[0004] A study by Misstear et al. showed that mouse nasal vaccination with targeted nanoparticles loaded with *S. aureus* protein could protect against systemic *S. aureus* infection in the absence of any antigen-specific antibodies [42]. This study suggested that a cellular-only response could protect against *S. aureus* infection. Moreover, many reports show the importance of Th17/IL-17 in the protection against *S. aureus* infections [43, 44]. Many mucosal vaccination approaches can induce robust Th17 responses, suggesting Th17 cells are useful targets for vaccines that induce immunity [45]. Recently, several studies using mouse vaccine models showed that T helper cells, including Th17, were important for a $CD4^+$ T-cell-dependent immune response [45]. Th17 cells had a role in anti-microbial immunity at the epithelial/mucosal barrier [45]. Th17 cells produce cytokines, which stimulated epithelial cells to produce anti-microbial proteins to clear out certain types of opportunistic microbes.

[0005] Th17-mediated protective responses involve the release of anti-microbial peptides, recruitment of neutrophils, and IL-17-driven Th1 immunity. These signaling mechanisms could offer immunity against a range of MDR pathogens through the production and induction of inflammatory cytokines and other proteins. For staphylococcal vaccines to be effective, protection must be achieved against a wide variety of different clinical strains.

[0006] In the past, *S. aureus* infections were efficiently treated with antibiotics. However, the number of antibiotic resistant strains of *S. aureus* has been increasing in the past two decades. Most notably, Methicillin-resistant *S. aureus* (MRSA) is one of the most dangerous bacterial strains that have become resistant to many antibiotics. MRSA strains are very common in hospitals, but are also becoming increasingly prevalent in community-acquired infections [3, 4]. Hence, the development of immunotherapeutic approaches, either active or passive, has seen resurgence in recent years [5].

[0007] Previous studies show that *S. aureus* has many surface proteins and virulence factors, many of which have been evaluated as potential vaccine targets [6-15]. Past and present *S. aureus* vaccines or therapeutic antibody strategies mainly focus on the following targets: capsular polysaccharide, virulence factors, surface targets and iron-regulated proteins. The capsular polysaccharide is a putative protective antigen to develop as a *S. aureus* vaccine. The leading effort in this regard was StaphVAX, a bivalent polysaccharide and protein conjugated vaccine [16-19]. Some other strategies to develop a *S. aureus* vaccine have been based on virulence factors and surface proteins. Many virulence factors and surface proteins have been targeted by vaccination, including alpha-toxin (using non-toxic derivative H35L) [7, 20], clumping factor A (ClfA) [21], Fibronectin binding protein (FnBPA or FnBPB), Panton-Valentine leukocidin (PVL) [22] and protein A [11].

[0008] Another approach to develop a *S. aureus* vaccine has been based on iron-regulated proteins. Iron-regulated proteins are of fundamental importance to all bacterial pathogens (except *Borrelia burgdorferi*). The leading vaccine candidate in this regard was Merck V710, which is based on the *S. aureus* iron-regulated protein (IsdB) [6, 23]. The Merck V710 vaccine may be effective against hematic spread of the *S. aureus* infection, but may be ineffective against pneumonia and may not elicit any antibody opsonic activity.

[0009] The Sta-Ag1 protein is a cell wall-anchored enzyme, and acts as an immune evasion factor [29]. When both wild-type and AdsA mutant *Staphylococci* are mixed with fresh mouse or human blood, they are phagocytized by polymorphonuclear leukocytes (PMNs), particularly phagocytic neutrophils; however, wild-type *Staphylococci* survive within PMNs, but AdsA mutants are killed [29]. *S. aureus* can generate adenosine by converting AMP or ADP after infecting humans or mammals. In mammals, it is a two-step process to catalyze adenosine triphosphate to adenosine. First, ecto-nucleoside triphosphate diphosphohydrolases (ecto-NTDPases) hydrolyze ATP or ADP to produce AMP. AdsA contains two 5'-nucleotidase signature regions, which then catalyze the conversion of AMP to adenosine [30]. Extracellular adenosine is necessary for the regulation of inflammation, but excess production of adenosine is also harmful as in *S. aureus* infections [29]. *S. aureus* AdsA produces excessive adenosine, which disrupts the balance of the proinflammatory and anti-inflammatory response. *Staphylococci* survival in PMNs depends on adenosine receptor-mediated signaling. In addition, adenosine may also suppress adaptive immune responses by interfering with the antigen presenting cells (APCs) presenting *S. aureus* antigens [31].

[0010] Two typical *S. aureus* strains are Newman and USA 300. Newman is a methicillin-sensitive *S. aureus* strain and

USA 300 is a community-associated methicillin-resistant *S. aureus* strain. The 'Sta-Ag1' antigen is annotated as 'Adenosine synthase A (AdsA)', similar to 5'-nucleotidase family protein. In the Newman strain, Sta-Ag1 is designated NWMN_0022 and has an amino acid sequence of GI:150373034, GenBank: BAF66294.1.

[0011] The 'Sta-Ag2' antigen is annotated as 'Virulence factor SaEsxA' and belongs to the ESAT-6 (esx) family. In the Newman strain, Sta-Ag2 is NWMN_0219 and has the amino acid sequence of GI: 68565377, UniProtKB/Swiss-Prot: P0C046.1.

[0012] The 'Sta-Ag3' antigen is annotated as 'Virulence factor SaEsxB' and belongs to the ESAT-6 (esx) family. In the Newman strain, Sta-Ag3 is NWMN_0225 and has the amino acid sequence of GI: 166214927, UniProtKB/Swiss-Prot: P0C047.2. These two ESAT-6-like proteins, SaEsxA and SaEsxB of *S. aureus* are secreted by a specialized secretion system termed ESAT-6-like system and play an important role in virulence. Mutants that failed to secrete EsxA and EsxB are defective to cause *S. aureus*-induced murine abscesses [32].

[0013] The 'Sta-Ag4' antigen is annotated as 'Virulence factor SaEsxC' and belongs to the ESAT-6 (esx) family. In the Newman strain, Sta-Ag4 is NWMN_0224 and has the amino acid sequence of SEQ ID NO:4 (GI:446933033, UniProtKB/Swiss-Prot: P0C051).

[0014] The 'Sta-Ag5' antigen is annotated as 'phenol-soluble modulin alpha 1 (PSMal)'. In the Newman strain, Sta-Ag5 is NWMN_2619 and has an amino acid sequence of GI: 223670821, GenBank: AP009351.1. In the Newman strain, Sta-Ag5 is NWMN_2618 and has an amino acid sequence of.

[0015] The 'Sta-Ag6' antigen is annotated as 'phenol-soluble modulin alpha 2 (PSMα2)'. In the Newman strain, Sta-Ag6 is NWMN_2618 and has an amino acid sequence of GI: 223670820, GenBank: AP009351.1

[0016] The 'Sta-Ag7' antigen is annotated as 'phenol-soluble modulin alpha 3 (PSMα3)'. In the Newman strain, Sta-Ag7 is NWMN_2617 and has an amino acid sequence of GI: 223670819, GenBank: AP009351.1.

[0017] The 'Sta-Ag8' antigen is annotated as 'phenol-soluble modulin alpha 4 (PSMα4)'. In the Newman strain, Sta-Ag8 is NWMN_2616 and has an amino acid sequence of GI: 223670818, GenBank: AP009351.1.

[0018] The 'Sta-Ag9' antigen is annotated as 'Pmt A', which is an ABC transporter, ATP-binding protein. In the Newman strain, Sta-Ag9 is NWMN_1869 and has an amino acid sequence of GI: 150374881, GenBank: AP009351.1.

[0019] The 'Sta-Ag10' antigen is annotated as 'Pmt B', which is an ABC transporter, ATP-binding protein. In the Newman strain, Sta-Ag10 is NWMN_1868 and has an amino acid sequence of GI: 150374880, GenBank: AP009351.1.

[0020] The 'Sta-Ag11' antigen is annotated as 'Pmt C', which is an ABC transporter, ATP-binding protein. In the Newman strain, Sta-Ag11 is NWMN_1867 and has an amino acid sequence of GI: 150374879, GenBank: AP009351.1.

[0021] The 'Sta-Ag12' antigen is annotated as 'Pmt D', which is an ABC transporter, ATP-binding protein. In the Newman strain, Sta-Ag12 is NWMN_1866 and has an amino acid sequence of GI: 150374878, GenBank: AP009351.1.

[0022] Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11 and Sta-Ag12 represent desirable antigens for vaccine development.

[0023] In terms of passive immunization, most strategies are aimed to eliminate major *S. aureus* virulence determinants such as monoclonal alpha-toxin antibodies, polyclonal PVL antibodies and anti-ClfA monoclonal antibody (Aurexis). However, thus far, most of the clinical trials for *S. aureus* vaccines or passive immunization have ended in failure (Nabi, Types 5 and 8 CPS conjugated to pseudomonal exoprotein A [19]; Inhibitex, ClfA, SdrG (Veronate) [24, 25]; Nabi, Polyclonal anti-CPS 5 and 8 (Altastaph) [26, 27]). The reasons why the *S. aureus* vaccine clinical trials may have failed were analyzed by Protor [28].

BRIEF SUMMARY OF THE INVENTION

[0024] The present invention provides vaccine formulations and antibodies, and related methods, for the treatment and/or prevention of *S. aureus* infection. The present invention provides one or more *S. aureus* antigens for use in vaccine formulations, wherein two or more antigens act synergistically. Thus, the protection against *S. aureus* infection achieved by their combined administration exceeds that expected by mere addition of their individual protective efficacy. Further, the present invention provides vaccines that can protect against hematic spread, pneumonia and skin infection, and which may also elicit a protective antibody response. The invention also provides novel antibodies and antibody cocktails to treat *S. aureus* infection. Furthermore, the vaccine formulations and antibodies of the present invention can be utilized in the treatment of mastitis in lactating dairy cows caused by *S. aureus* infection.

[0025] In one aspect, the present invention provides a vaccine formulation comprising one or more *S. aureus* polypeptides, as well as fragments, variants or derivatives thereof. The *S. aureus* polypeptide is selected from Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag1, Sta-Ag12, and combinations thereof.

[0026] In some embodiments, the formulations may further include one or more adjuvants, such as, for example, a Th1 adjuvant, a Th2 adjuvant, a Th17 adjuvant, an aluminum hydroxide adjuvant, or combinations thereof. In additional embodiments, the formulation may include a histidine buffer.

[0027] In some embodiments, the *S. aureus* polypeptide is derived from a eukaryotic expression system. In other embodiments, the *S. aureus* polypeptide is derived from a prokaryotic expression system. In certain embodiments, the *S. aureus* polypeptide may be a synthetic polypeptide.

[0028] The Sta-Ag1 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 1, or a bioequivalent fragment, variant or derivative thereof. The Sta-Ag2 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 2, or a fragment, variant or derivative thereof. The Sta-Ag3 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 3, or a bioequivalent fragment, variant or derivative thereof. The Sta-Ag4 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 4, or a bioequivalent fragment, variant or derivative thereof. The Sta-Ag5 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 5, or a bioequivalent fragment, variant or derivative thereof. The Sta-Ag6 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 6, or a bioequivalent fragment, variant or derivative thereof. The

Sta-Ag7 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 7, or a bioequivalent fragment, variant or derivative thereof. The Sta-Ag8 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 8, or a bioequivalent fragment, variant or derivative thereof. The Sta-Ag9 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 9, or a bioequivalent fragment, variant or derivative thereof. The Sta-Ag10 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 10, or a fragment, variant or derivative thereof. The Sta-Ag11 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 11, or a fragment, variant or derivative thereof. The Sta-Ag12 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 12, or a fragment, variant or derivative thereof.

[0029] In preferred embodiments, the present invention provides combinations of Sta-Ags based on SEQ ID NOs: 1 to 12, which combinations exert synergistic effects in eliciting a *S. aureus*-specific immune response.

[0030] In another aspect, the present invention provides isolated antibodies that bind to at least one of Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11 and/or Sta-Ag12 *S. aureus* polypeptides, or bioequivalent fragments, variants, or derivatives thereof.

[0031] In another aspect, the present invention provides methods for preventing and/or treating *S. aureus* infection in a subject, comprising administering to the subject an effective amount of a vaccine formulation comprising one or more *S. aureus* polypeptides, or bioequivalent fragments, variants or derivatives thereof. The *S. aureus* polypeptide is selected from Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, Sta-Ag12, and combinations thereof.

[0032] In yet another aspect, the present invention provides methods for preventing and/or treating *S. aureus* infection in a subject comprising administering to the subject an effective amount of one or more antibodies selected from a Sta-Ag1 antibody, a Sta-Ag2 antibody, a Sta-Ag3 antibody, a Sta-Ag4 antibody, a Sta-Ag5 antibody, a Sta-Ag6 antibody, a Sta-Ag7 antibody, a Sta-Ag8 antibody, a Sta-Ag9 antibody, a Sta-Ag10 antibody, Sta-Ag11, and a Sta-Ag12 antibody.

[0033] The methods and compositions herein described can be used in connection with pharmaceutical, medical, and veterinary applications, as well as fundamental scientific research and methodologies, as would be identifiable by a skilled person upon reading of the present disclosure. These and other objects, features and advantages of the present invention will become clearer when the drawings as well as the detailed description are taken into consideration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] For a fuller understanding of the nature of the present invention, reference should be had to the following detailed description taken in connection with the accompanying figures described below.

[0035] FIG. **1** shows a model to generate protective immunity against *S. aureus* infections through vaccination (adapted from *Nature Medicine* 17, 168-169 (2011) doi: 10.1038/nm0211-168).

[0036] FIG. **2** shows evaluation of serum antibody responses in mice by ELISA. The results of two independent experiments are shown.

[0037] FIGS. **3**A and **3**B show graphs illustrating that active immunization with Sta-Ag1 decreases the size of abscesses caused by USA300 or Newman strains of *S. aureus*. Mice were injected intramuscularly with aluminium hydroxide gel (AHG) plus phosphate-buffered saline (PBS). A and B, Abscess formation was monitored once per day after subcutaneous infection with 1×10^7 of the indicated bacteria 49 days after primary immunization. Results are the mean value±standard error of the mean; n=12 mice per group. *$p<0.05$ versus wild-type USA300 or Newman strains using a 2-way analysis of variance and Bonferroni's post-test.

[0038] FIGS. **4**A, **4**B, and **4**C show the percentage of mice per group that had dermonecrosis on each day following active immunization with Sta-Ag1 and challenge with USA300 or Newman skin inoculation. A P value of $P<0.001$ was obtained for mock immunized mice after infection with either Newman (a) or USA300 (b) strains over the 14-day time course. C, shows representative mouse skin lesions on day 4. Red arrows indicate dermonecrosis, black arrows indicate abscess formation without dermonecrosis, and yellow boxes indicate scratches.

[0039] FIG. **5** shows a graph of the survival curve of vaccinated BALB/c mice challenged with *S. aureus*. Mice were challenged by intravenous injection of *S. aureus* ATCC 25923 (5×10^7 CFU). The results of two independent experiments (mice, n=12) are shown. The p value represents the likelihood of a significant difference between all groups by pair-wise log-rank analysis.

[0040] FIG. **6** shows a graph of the evaluation of the therapeutic effects of anti-Sta-Ag1 rabbit serum in the BALB/c mouse model. Mice were challenged by intravenous injection of *S. aureus* ATCC 25923 (5×10^7 CFU). After 2 hours, the experimental groups were treated with anti-Sta-Ag1 rabbit serum, whereas control mice were injected with normal rabbit serum. The p value represents the likelihood of a significant difference between all groups by pair-wise log-rank analysis.

[0041] FIG. **7** shows a graph of the evaluation of the therapeutic effects of anti-AdsA (anti-Sta-Ag1) mouse serum in the BALB/c mouse model. Mice were challenged by intravenous injection of *S. aureus* ATCC 25923 (5×10^7 CFU). After 2 hours, the experimental groups were treated with anti-AdsA mouse serum, whereas control mice were injected with normal mouse serum. The p value represents the likelihood of a significant difference between all groups by pair-wise log-rank analysis.

[0042] FIGS. **8**A and **8**B show that passive immunization with AdsA-specific (Sta-Ag1-specific) mouse anti-sera prevents dermonecrosis. FIG. **8**A, Percentage of mice per group that had dermonecrosis on each day. *$P<0.001$ for mice administered pre-immune versus anti-AdsA serum after infection with ATCC25923 strains over the 14-day time course. FIG. **8**B, Representative skin lesions of mice on day 3 for each of the treatment conditions anti-AdsA, AdsA-specific mouse anti-sera; Pre-immune, pre-immune mouse serum samples. Red arrows indicate dermonecrosis, and black arrows indicate abscess formation without dermonecrosis.

[0043] FIGS. **9**A and **9**B show graphs illustrating that passive immunization with Sta-Ag1-specific rabbit anti-sera (anti-Sta-Ag1) reduces size of lesions caused by USA300 or Newman strains of *S. aureus*. a and b, Mice received 100 mL

of pre-immune rabbit serum samples (pre-immune) or anti-Sta-Ag14 h before subcutaneous infection with 1×10^7 with USA300 or Newman strains and on day 2 after infection. Results are the mean value±standard error of the mean for all groups; n=10 mice per group. *p<0.05 versus wild-type USA300 or Newman strains using a 2-way analysis of variance and Bonferroni's post-test.

[0044] FIGS. **10**A and **10**B shows graphs illustrating that passive immunization with Sta-Ag1-specific rabbit anti-sera (anti-Sta-Ag1) prevents dermonecrosis. *p<0.001 for mice administered pre-immune versus anti-Sta-Ag1 serum after infection with Newman (a) or USA300 (b) strains over the 14-day time course.

[0045] FIGS. **11**A, **11**B, **11**C, **11**D, **11**E, **11**F, **11**G, **11**H, **11**I shows that immunization with the rSaEsxA, rSaEsxB and rSaEsxA+B generates protective immunity against *S. aureus* abscess formation in BALB/C mice. Animals were treated with PBS plus AHG (FIG. **11**A, FIG. **11**B) or immunized with rSaEsxA plus AHG (FIG. **11**C, FIG. **11**D), rSaEsxB (FIG. **11**E, FIG. **11**F) and rSaEsxA+B plus AHG (FIG. **11**G, FIG. **11**I); and challenged by intraperitoneal infection with *S. aureus* ATCC 25923. Four days after challenge, mice were killed, and the kidneys were collected for histopathology (A-H) or Staphylococcal load measurements (FIG. **11**I). Kidney was fixed with formalin, thin-sectioned, and stained with hematoxylin/eosin. Microscopic images of whole kidneys (FIG. **11**A, FIG. **11**C, FIG. **11**E and FIG. **11**G) or tissue at magnification (FIG. **11**B, FIG. **11**D, FIG. **11**F and FIG. **11**H) revealed abscess formation only in PBS control mice. Consistent results were obtained for six kidney tissues in each group. Staphylococcal abscess (black arrow) with a central concentration of *staphylococci* (red arrow) was marked in FIG. **11**B. Small infiltrates of PMNs (black arrow) were shown in FIG. **11**D, FIG. **11**F and FIG. **11**H. (Scale bars: A, C, E and G, 1000 μm; B, D, E and F, 50 μm.)

[0046] FIG. **12** shows the survival curve of vaccinated BALB/C mice following *S. aureus* challenge. Mice were challenged by intravenously injection of *S. aureus* ATCC2593 (5×10^7 CFU). P value represents the likelihood of a significant difference between all groups following pair-wise log-rank analysis between groups. "NS" indicates differences not significant; *p>0.05 following pair-wise log-rank analysis. The data are the results of three independent experiments.

[0047] FIG. **13** shows that immunization with Sta-Ag2+3 generates protective immunity against lethal challenge with two different clinical *S. aureus* strains. Mice (n=10) immunized with the Sta-Ag2+3 or treated with PBS+AHG as controls were challenged with *S. aureus* Newman or USA 300 strains by intravenous injection. The survival of mice was monitored for 14 days. Log-rank (Mantel-Cox) test was used to compare the protective immunity between control mice and the Sta-Ag2+3 immunized mice. Data from two replicate experiments is shown.

[0048] FIGS. **14**A, **14**B, and **14**C show the antigen-specific IL17A and IFN-γ responses elicited by Sta-Ag2 or Sta-Ag3 immunization. Immunized mice (n=8) were sacrificed 5 days after the third immunization and splenocytes were prepared and stimulated with Sta-Ag2 or Sta-Ag3 protein for 20 h. Detection of (FIG. **14**A) IL-17A and (FIG. **14**B) IFN-γ producing cells by ELISPOT. (FIG. **14**C) Representative images of splenic ELISPOT responses. Results for one of two representative experiments are shown. The Mann Whitney test was used for the statistical analysis. Data was expressed as mean+SEM. SFCs: spot-forming units; IMSA: immunized mice stimulated with Sta-Ag2; IMSB: immunized mice stimulated with Sta-Ag3; NMSA: naïve mice stimulated with Sta-Ag2; NMSB: naïve mice stimulated with Sta-Ag3; UNS: unstimulated wells (immunized mice).

[0049] FIG. **15** shows the survival curve of BALB/c mice passively treated with SaEsxA- or SaEsxB-specific mouse antisera and challenged with the Newman strain via the tail vein. BALB/c mice (n=10) received 100 μL of normal mouse serum or specific mouse antiserum (anti-SaEsxA or anti-SaEsxB) 4 hours before intravenous injection of *S. aureus* Newman strain (5×10^7 CFU) and on day 2 after infection. Log-rank (Mantel-Cox) test was used to compare between groups. NS: no significant differences (p>0.05). Data from two replicate experiments is shown.

[0050] FIG. **16** shows evaluation of serum antibody responses in mice by ELISA. Sta-C3 (Sta-Ag2, Sta-Ag3 and Sta-Ag4), Sta-C4 (Sta-Ag-9, Sta-Ag-10, Sta-Ag-11 and Sta-Ag-12) and Sta-C9 (Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag-9, Sta-Ag-10, Sta-Ag-11, Sta-Ag-12 and uric acid).

[0051] FIG. **17** shows representative mouse skin lesions (day 4). Black arrows indicate dermonecrosis, and red arrows indicate abscess formation without dermonecrosis. Sta-C3 (Sta-Ag2, Sta-Ag3 and Sta-Ag4), Sta-C4 (Sta-Ag-9, Sta-Ag-10, Sta-Ag-11 and Sta-Ag-12) and Sta-C9 (Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag-9, Sta-Ag-10, Sta-Ag-11, Sta-Ag-12 and uric acid).

[0052] FIG. **18** shows percentage of mice per group that had dermonecrosis on each day. Sta-C3 (Sta-Ag2, Sta-Ag3 and Sta-Ag4), Sta-C4 (Sta-Ag-9, Sta-Ag-10, Sta-Ag-11 and Sta-Ag-12) and Sta-C9 (Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag-9, Sta-Ag-10, Sta-Ag-11, Sta-Ag-12 and uric acid).

[0053] FIGS. **19**A and **19**B shows that immunization with combined vaccine generates protective immunity against lethal challenge with *S. aureus* USA300 strains. The survival of mice was monitored for 14 days. Log-rank (Mantel-Cox) test was used to compare the protective immunity between different groups. Sta-C3 (Sta-Ag2, Sta-Ag3 and Sta-Ag4), Sta-C4 (Sta-Ag-9, Sta-Ag-10, Sta-Ag-11 and Sta-Ag-12) and Sta-C9 (Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag-9, Sta-Ag-10, Sta-Ag-11, Sta-Ag-12 and uric acid).

BRIEF DESCRIPTION OF THE SEQUENCES

[0054] SEQ ID NO: 1 is an amino acid sequence of *S. aureus* Sta-Ag1.

[0055] SEQ ID NO: 2 is an amino acid sequence of *S. aureus* Sta-Ag2.

[0056] SEQ ID NO: 3 is an amino acid sequence of *S. aureus* Sta-Ag3.

[0057] SEQ ID NO: 4 is an amino acid sequence of *S. aureus* Sta-Ag4.

[0058] SEQ ID NO: 5 is an amino acid sequence of *S. aureus* Sta-Ag5.

[0059] SEQ ID NO: 6 is an amino acid sequence of *S. aureus* Sta-Ag6.

[0060] SEQ ID NO: 7 is an amino acid sequence of *S. aureus* Sta-Ag7.

[0061] SEQ ID NO: 8 is an amino acid sequence of *S. aureus* Sta-Ag8.

[0062] SEQ ID NO: 9 is an amino acid sequence of *S. aureus* Sta-Ag9.

[0063] SEQ ID NO: 10 is an amino acid sequence of *S. aureus* Sta-Ag10.

[0064] SEQ ID NO: 11 is an amino acid sequence of *S. aureus* Sta-Ag11.

[0065] SEQ ID NO: 12 is an amino acid sequence of *S. aureus* Sta-Ag12.

DETAILED DISCLOSURE OF THE INVENTION

[0066] Several aspects of the invention are described below, with reference to examples for illustrative purposes only. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or practiced with other methods, protocols, reagents, cell lines and animals. The present invention is not limited by the illustrated ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts, steps or events are required to implement a methodology in accordance with the present invention. Many of the techniques and procedures described, or referenced herein, are well understood and commonly employed using conventional methodology by those skilled in the art.

[0067] Generally, vaccines or antibodies to *S. aureus* are based on a single antigen and do not provide sufficient protection against hematic spread, pneumonia and skin infection. Therefore, the selection of potent antigenic targets which induce protective immunity is crucial in the development of therapeutics based on vaccines and/or antibodies.

[0068] The subject invention provides vaccine formulations and antibodies, and related methods, for the treatment and/or prevention of *S. aureus* infection. In some embodiments, the subject invention provides one or more *S. aureus* antigens for use in vaccine formulations, wherein two or more antigens act synergistically. Thus, the protection against *S. aureus* infection achieved by their combined administration exceeds that expected by mere addition of their individual protective efficacy.

[0069] In preferred embodiments, the subject invention provides vaccines which can protect against hematic spread, pneumonia and skin infection, and which may also elicit a protective antibody response. In further preferred embodiments, the subject invention provides novel antibodies and antibody cocktails to treat *S. aureus* infection.

[0070] As used herein, the term "vaccine" or "immunizing formulation" refers to any composition that stimulates an immune response to a particular antigen or antigens. Thus, a vaccine refers to any composition that is administered to a subject with the goal of establishing an immune response and/or immune memory to a particular antigen. In some embodiments of the subject invention, the vaccine compositions comprise other substances designed to increase the ability of the vaccine to generate an immune response.

[0071] In preferred embodiments, the vaccines of the subject invention can be therapeutic or prophylactic. Thus, for example, the vaccines disclosed herein can be used to prevent an infection, such as *S. aureus* infection. Alternatively, the vaccines disclosed herein can be used therapeutically to treat a subject with a *S. aureus* infection.

[0072] In some embodiments, the disclosed methods of the subject invention comprise the simultaneous or separate administration of multiple vaccines or vaccine components. Thus, in further embodiments, the subject invention provides the administration of a second, third, fourth, etc. *S. aureus* polypeptide, wherein the second, third, fourth, etc. *S. aureus* polypeptide is administered in a separate vaccine for administration at the same time as or 1, 2, 3, 4, 5, 6, 10, 14, 18, 21, 30, 60, 90, 120, 180, 360 days (or any number of days in between) after the first *S. aureus* polypeptide.

[0073] In some embodiments, the subject invention provides a vaccine formulation comprising one or more *S. aureus* polypeptides, or bioequivalent fragments, variants or derivatives thereof. In preferred embodiments, the *S. aureus* polypeptide is one of Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, Sta-Ag12, and/or combinations thereof. Fragments, variants, and derivatives are routinely prepared by those of ordinary skill in the art and their immunogenicity is readily and routinely determined. Immunogenic fragments, variants and derivatives are equivalents (also known as "bioequivalents") of these *S. aureus* polypeptides and are included in the scope of the subject invention.

[0074] In preferred embodiments, the subject invention provides vaccine formulations comprising the *S. aureus* polypeptides from Sta-Ag1, Sta-Ag2, and/or Sta-Ag3. In further preferred embodiments, the subject invention provides vaccine formulations comprising the *S. aureus* polypeptides from Sta-Ag2 combined with any of Sta-Ag1, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, and/or Sta-Ag12. In yet further preferred embodiments, the subject invention provides vaccine formulations comprising the *S. aureus* polypeptides from Sta-Ag3 combined with any of Sta-Ag1, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, and/or Sta-Ag12.

[0075] In further embodiments, the subject invention provides vaccine formulations comprising the *S. aureus* polypeptides from Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, and/or Sta-Ag12, and/or any combinations thereof.

[0076] In more preferred embodiments, the subject invention provides vaccine formulations comprising the *S. aureus* polypeptides from Sta-Ag2, Sta-Ag3, and Sta-Ag4, designated combination Sta-C3.

[0077] In other more preferred embodiments, the subject invention provides vaccine formulations comprising the *S. aureus* polypeptides from Sta-Ag9, Sta-Ag10, Sta-Ag11, and/or Sta-Ag12, designated combination Sta-C4.

[0078] In most preferred embodiments, the subject invention provides vaccine formulations comprising the *S. aureus* polypeptide from Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag9, Sta-Ag10, Sta-Ag1, and/or Sta-Ag12, designated combination Sta-C9.

[0079] In some embodiments, the formulations of the subject invention may further include one or more adjuvant, such as, for example, a Th1 adjuvant, a Th2 adjuvant, a Th17 adjuvant, an aluminum hydroxide adjuvant, or combinations thereof. In additional embodiments, the formulation may include a histidine buffer.

[0080] In some embodiments, the *S. aureus* polypeptide is derived from a eukaryotic expression system. In other embodiments, the *S. aureus* polypeptide is derived from a prokaryotic expression system. In certain embodiments, the *S. aureus* polypeptide may be a synthetic polypeptide or a recombinant polypeptide.

[0081] In preferred embodiments, the *S. aureus* polypeptides of the subject invention are derived from various strains of *S. aureus* bacteria, including, but not limited to, USA 300

and Newman strains. In further preferred embodiments, the polypeptides, fragments thereof, or antibodies are delivered to a subject by any means known in the art, including, but not limited to, *Salmonella* and virus-like particle (VLP) delivery systems.

[0082] In preferred embodiments of the subject invention, the Sta-Ag1 *S. aureus* polypeptides may comprise the amino acid sequence of SEQ ID NO: 1, or a fragment, variant or derivative thereof, which SEQ ID NO: 1 is derived from the Newman strain Sta-Ag1 designated NWMN_0022 and has an amino acid sequence of GI: 150373034, GenBank: BAF66294.1. In more preferred embodiments, fragments useful in the subject invention comprise amino acids from about amino acid 36 to about amino acid 430 of SEQ ID NO: 1, which fragments contain two 5'-nucleotidase motifs.

[0083] In other preferred embodiments of the subject invention, the Sta-Ag2 *S. aureus* polypeptides may comprise the amino acid sequence of SEQ ID NO: 2, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 2 is derived from Newman strain Sta-Ag2 designated NWMN_0219 and has an amino acid sequence of GI: 68565377, UniProtKB/Swiss-Prot: P0C046.1.

[0084] In other preferred embodiments of the subject invention, the Sta-Ag3 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 3, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 3 is derived from Newman strain Sta-Ag3 designated NWMN_0225 and has an amino acid sequence of GI: 166214927, UniProtKB/Swiss-Prot: P0C047.2.

[0085] In other preferred embodiments of the subject invention, the Sta-Ag4 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 4, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 4 is derived from Newman strain Sta-Ag4 designated NWMN_0224 and has an amino acid sequence of GI:446933033, GenBank: AP009351.1.

[0086] In other preferred embodiments of the subject invention, the Sta-Ag5 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 5, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 5 is derived from Newman strain Sta-Ag5 designated NWMN_2619 and has an amino acid sequence of GI: 223670821, GenBank: AP009351.1.

[0087] In other preferred embodiments of the subject invention, the Sta-Ag6 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 6, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 6 is derived from Newman strain Sta-Ag6 designated NWMN_2618 and has an amino acid sequence of GI: 223670820, GenBank: AP009351.1.

[0088] In other preferred embodiments of the subject invention, the Sta-Ag7 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 7, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 7 is derived from Newman strain Sta-Ag7 designated NWMN_2617 and has an amino acid sequence of GI: 223670819, GenBank: AP009351.1.

[0089] In other preferred embodiments of the subject invention, the Sta-Ag8 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 8, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 8 is derived from Newman strain Sta-Ag8 designated NWMN_2616 and has an amino acid sequence of GI:223670818, GenBank: AP009351.1.

[0090] In other preferred embodiments of the subject invention, the Sta-Ag9 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 9, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 9 is derived from Newman strain Sta-Ag9 designated NWMN_1869 and has an amino acid sequence of GI: 150374881, GenBank: AP009351.1.

[0091] In other preferred embodiments of the subject invention, the Sta-Ag10 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 10, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 10 is derived from Newman strain Sta-Ag10 designated NWMN_1868 and has an amino acid sequence of SEQ ID NO: 10 (GI: 150374880, GenBank: AP009351.1).

[0092] In other preferred embodiments of the subject invention, the Sta-Ag11 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 11, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 11 is derived from Newman strain Sta-Ag11 designated NWMN_1867 and has an amino acid sequence of GI: 150374879, GenBank: AP009351.1.

[0093] In other preferred embodiments of the subject invention, the Sta-Ag12 *S. aureus* polypeptides of the present invention may comprise the amino acid sequence of SEQ ID NO: 12, or a bioequivalent fragment, variant or derivative thereof, which SEQ ID NO: 12 is derived from Newman strain Sta-Ag12 designated NWMN_1866 and has an amino acid sequence of GI: 150374878, GenBank: AP009351.1.

[0094] In another preferred embodiment, the subject invention provides isolated antibodies or aptamers that bind to at least one of Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, and/or Sta-Ag12 *S. aureus* polypeptides, or bioequivalent fragments, variants, or derivatives thereof. Antibodies may include intact immunoglobulin molecules, as well as fragments thereof, which are capable of binding associated antigens of Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, and/or Sta-Ag12 and can include chimeric antibody molecules; $F(ab')_2$ and F (ab) fragments and Fv molecules; non-covalent heterodimers; single chain Fv molecules (scFv); dimeric and trimeric antibody fragment constructs; minibodies and humanized antibody molecules.

[0095] In further embodiments, the subject invention provides a cocktail of antibodies which are specific for PSM antigens and includes any of PSMα1 (Sta-Ag5), PSMα2 (Sta-Ag6), PSMα3 (Sta-Ag7), and PSMα4 (Sta-Ag8) Advantageously, PSM antigens are secreted and crucial to *S. aureus* virulence, therefore targeting PSMs with monoclonal antibodies (mAbs) can provide enhanced protection

[0096] In another embodiment, the subject invention provides methods for preventing and/or treating *S. aureus* infection in a subject, comprising administering to the subject an effective amount of a vaccine formulation comprising one or more *S. aureus* polypeptides, or bioequivalent fragments, variants or derivatives thereof, wherein the *S. aureus* polypeptide is one of Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag1, Sta-Ag12, and/or any combination thereof.

[0097] As used herein, the term "subject" refers to an animal. Typically, the terms "subject" and "patient" may be used

interchangeably herein in reference to a subject. As such, a "subject" includes an animal that is being treated for a disease, being immunized, or the recipient of a mixture of components as described herein, such as a vaccine formulation or antibody. The term "animal," includes, but is not limited to, mouse, rat, dog, guinea pig, cow, horse, chicken, cat, rabbit, pig, monkey, chimpanzee, and human.

[0098] In yet another embodiment, the present invention provides methods for preventing or treating *S. aureus* infection in a subject in need thereof comprising administering to the subject an effective amount of one or more antibody which antibody can be a Sta-Ag1 antibody, a Sta-Ag2 antibody, a Sta-Ag3 antibody, a Sta-Ag4 antibody, a Sta-Ag5 antibody, a Sta-Ag6 antibody, a Sta-Ag7 antibody, a Sta-Ag8 antibody, a Sta-Ag9 antibody, a Sta-Ag10 antibody, a Sta-Ag11 or a Sta-Ag12 antibody, and/or any combination thereof.

[0099] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

[0100] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLES

Example 1

[0101] Animal model to evaluate the immunogenicity of *S. aureus* antigens. The *S. aureus* ATCC 25923, ATCC 29213, Newman and USA 300 strains were stored at −80° C. until use. *E. coli* strain BL21 (DE3) was used for protein expression. Recombinant expression vector pETH was obtained from Prof. K Y Yuen. SPF BALB/c mice were supplied by the Laboratory Animal Unit of the University of Hong Kong. All animal experiments were approved by the Committee on the Use of Live Animals in Teaching & Research of the University of Hong Kong (CULATR 2596-11).

[0102] Animal immunization. Six-week-old female BALB/c mice (n=6 per group) were immunized with a range of doses (Table 1) of rSaEsxA or rSaEsxB proteins with Freund's adjuvant by intraperitoneal (i.p.) injection or with Aluminium hydroxide gel (Alhydrogel, AHG) by intramuscular (i.m.) injection. The two recombinant proteins were emulsified at a ratio of 1:1 with complete Freund's adjuvant (CFA) for priming and with incomplete Freund's adjuvant (IFA) for boosting. The two recombinant proteins were formulated at a ratio of 9:1 with AHG (100 μl of 2% AHG per 900 μl of antigen). These treatments were administered to mice on days 0, 14, and 28. Blood samples were drawn from the tail vein on days 0, 21, and 35.

[0103] Antibody detection. The rSaEsxA and rSaEsxB antibody titers were detected by enzyme-linked immunosorbent assay (ELISA). Briefly, rSaEsxA or rSaEsxB protein (1 μg/ml in 0.05 M carbonate/bicarbonate buffer, pH 9.6) were coated (200 μl/well) on ELISA plates (Nunc, Roskilde, Denmark) and incubated overnight at 4° C. The plates were blocked with PBS containing 5% (w/v) nonfat milk for 3 h at 37° C. and washed four times with PBS containing 0.05% Tween. Two-fold serially diluted mice sera were added into the wells and incubated for 1 h at 37° C. The plates were washed six times with PBS containing 0.05% Tween and incubated with HRP-conjugated goat anti-mouse IgG/IgG1/IgG2a for 1 h at 37° C. The color was developed using TMB solution (Sigma) and absorbance was measured using an ELISA reader at 450 nm. The antibody endpoint titer was defined as the serum dilution that produced an OD450 of 0.5 absorbance units in the ELISA assay.

[0104] ELISPOT assay. Mice were sacrificed 5 days after the third immunization. IFN-γ or IL17A producing splenocytes from vaccinated or naïve unvaccinated mice were analyzed using cytokine-specific enzyme-linked immunospot assay (ELISPOT) (BD PharMingen, United States). Briefly, plates were coated with capture antibodies (anti-IFN-γ or IL17A mAb) overnight at 4° C. and then blocked with a blocking solution (RPMI 1640 containing 10% fetal bovine serum and 1% L-glutamine-streptomycin-penicillin) for 1 h at 37° C. Splenocytes isolated from immunized mice were plated at a concentration of 1×10^5 cells/well and stimulated with rSaEsxA (IMSA, 0.2 μg/well) or rSaEsxB (IMSB, 0.2 μg/well) at a final concentration of 10 μg/ml in triplicate and incubated for 20 h at 37° C. Ionomycin (1 μg/ml) (Sigma, United States) and phorbol myristate acetate (PMA, 50 ng/ml) (Sigma) were used as positive controls. Splenocytes from naïve mice stimulated with rSaEsxA (NMSA) or rSaEsxB (NMSB), splenocytes from unstimulated mice (immunized mice, UNS) and RPMI 1640 treated splenocytes were used as negative controls. After washing, biotinylated anti-IFN-γ or IL17A mAb was added for 1 h at 37° C., followed by streptavidin-HRP conjugate for 1 h at 37° C. The color was developed with TMB solution and the spots were counted using an immunospot analyzer.

[0105] Renal Abscess. *S. aureus* strain ATCC 25923 was plated onto a TSA plate with 5% horse blood and cultured for 24 h at 37° C. The bacteria were harvested using endotoxin-free PBS, washed twice, and suspended in PBS at a concentration of 5×10^7 CFU/mL. On day 42 after the first vaccination, mice immunized with rSaEsxA (50 μg) or rSaEsxB (50 μg) were injected with 200 μl of the inoculums by i.p. at a total bacterial suspension concentration of 1×10^7 CFU. Four days after bacterial challenge, infected mice were euthanized by compressed CO_2 inhalation. The kidneys were removed and homogenized in 1% Triton X-100. Aliquots were diluted and plated on blood agar for CFU counting. Kidney tissue samples for histological analysis were incubated in 10% formalin for 24 h at room temperature. Tissues were embedded in paraffin and thin sections were obtained using a microtome. Sections were stained with hematoxylin-eosin and examined under a microscope.

[0106] Lethal Challenge. On day 42 after the first vaccination, immunized mice were injected intravenously in the tail vein with 5×10^7 CFU of *S. aureus* ATCC 25923, Newman (MSSA) or USA 300 (CA-MRSA) strains. Mice were monitored for mortality and clinical signs.

[0107] Passive immunization. Mouse polyclonal SaEsxA- or SaEsxB-specific antisera were generated and collected from the mice immunized with purified rSaEsxA or rSaEsxB. Female BALB/c mice (~8 weeks old) were administered 100 μL of mouse normal sera or polyclonal SaEsxA- or SaEsxB-specific antisera (~1:200000 antibody titers) by i.p. injection 4 h before *S. aureus* challenge and then 2 days after *S. aureus* challenge. Mice were monitored for mortality and clinical signs.

[0108] Statistical analysis. Student's t-test was used to analyze the statistical significance of ELISPOT assay results and

Staphylococcal load measurements. Log-rank (Mantel-Cox) analysis was used to analyze the statistical significance of the data from the lethal challenge experiments. Analyses were performed using GraphPad Prism 5 (GraphPad Software, United States) and a p value<0.05 was considered statistically significant.

EXAMPLE 2

[0109] Targeting Sta-Ag1 (AdsA) for immunotherapeutic drug development. A fragment of Sta-Ag1 (36-430 aa that contained two 5'-nucleotidase motifs; DNA sequence from 106 to 1290 bp) was expressed and purified in *E. coli*, and the immunogenicity of the Sta-Ag1 protein was tested in a BALB/c mouse model. Mice vaccinated with Sta-Ag1 produced antibodies specific for the protein, as determined by ELISA. Half-maximal anti-Sta-Ag1 antibody titers were about 1:500,000 for the vaccinated mouse group, whereas anti-Sta-Ag1 was undetectable in the mock group (FIG. **2***a*); furthermore, immunization with Sta-Ag1 in the presence of aluminium hydroxide gel (AHG) also elicited both Th1 and Th2-associated rAdsA-specific IgG2a and IgG1 antibody responses (FIG. **2***b*).

[0110] Active immunization. Immunization with Sta-Ag1 moderates severity of USA300 skin infections. Recently, research has indicated that adenosine synthase A (AdsA, Sta-Ag1), a *S. aureus* cell wall-anchored enzyme, acts as an immune evasion factor. When both wild-type and AdsA mutant *Staphylococci* are mixed with fresh mouse or human blood, they are phagocytized by polymorphonuclear leukocytes (PMNs), particularly phagocytic neutrophils; however, wild-type *Staphylococci* survive within PMNs, but AdsA mutants are killed.

[0111] To determine whether active immunization with Sta-Ag1 protects mice from severe *S. aureus* lethal or skin infections, mice were vaccinated intramuscularly with Sta-Ag1+AHG 35 days before infection with USA 300 or Newman strains. The results showed that *S. aureus* abscess size was reduced significant in mice vaccinated with Sta-Ag1 (FIG. **3**A and FIG. **3**B). Also, there was little or no dermonecrosis in infected mice that had been vaccinated (FIGS. **4**A, **4**B, **4**C), which demonstrates that active immunization with Sta-Ag1 moderates severity of *S. aureus* skin infections. Additionally, immunization of mice with Sta-Ag1 generated protective immunity against *S. aureus* lethal challenge in BalB/c mouse models (FIG. **5**).

[0112] Passive immunization. Since active immunization with Sta-Ag1 moderates severity of USA300 skin infections and increases survival rate in a lethal infection model, it was next determined whether passive immunization with rabbit and mouse sera directed against Sta-Ag1 would moderate the severity of skin disease and reduce mortality in lethal challenge of a mouse model. The studies demonstrate that intraperitoneal injection of Sta-Ag1-specific rabbit anti-sera protects mice from lethal *S. aureus* infection (FIG. **6**). The studies also demonstrate that intraperitoneal injection of Sta-Ag1-specific mouse anti-sera protects mice from lethal *S. aureus* infection (FIG. **7**).

[0113] Skin lesions of mice infected with ATCC 25923 strains were significantly smaller after passive immunization with AdsA-specific mouse antisera, compared with lesions of mice that received pre-immune serum samples. Mice that received AdsA-specific mouse antisera either failed to develop dermonecrotic lesions after infection with ATCC 25923 strains or the area of dermonecrosis was limited (FIGS. **8**A and **8**B).

[0114] Skin lesions of mice infected with USA300 or Newman strains were significantly smaller after passive immunization with Sta-Ag1-specific rabbit antisera, compared with lesions of mice that received normal serum samples (FIGS. **9**A and **9**B). In addition, mice that received Sta-Ag1-specific rabbit antisera either failed to develop dermonecrotic lesions after infection with USA300 or Newman strains or the area of dermonecrosis was limited (FIGS. **10**A and **10**B). EXAMPLE 2-Targeting Sta-Ag2-3 for immunotherapeutic drug development. Recombinant Sta-Ag2 or 3 protein was expressed in *E. coli* BL21 and purified using a three-step chromatography strategy. Results indicated that the majority of the Sta-Ag2 and Sta-Ag3 were expressed in soluble form at a high yield (>99%). To evaluate the immunogenicity of Sta-Ag2 or Sta-Ag3, mice were vaccinated i.p. with three doses of Sta-Ag2 or Sta-Ag3 protein. Serum samples obtained 7 days following each immunization were evaluated by ELISA to assess the development of the antibody response. The Sta-Ag2 or Sta-Ag3 specific IgG, IgG1 and IgG2a antibody titers were evaluated by ELISA. The data shows that immunization with the Sta-Ag2 or Sta-Ag3 results in the generation of specific antibodies. Table 1 shows that IgG antibody titers were increased with raising doses of protein. Therefore, mice immunized with 50 ptg Sta-Ag2 (rSaEsxA) or Sta-Ag3 (rSaEsxB) (+FA or AHG) produced the highest titers on day 35.

TABLE 1

Immunization for different dose and forms of Sta-Ag2 or Sta-Ag3 against *S. aureus* infection

			Titer[b]			
Treatment	Dose (μg)	Adjuvant[a]	Anti-Sta-Ag2 IgG	Anti-Sta-Ag2 IgG1	Anti-Sta-Ag2 IgG2a	Anti-Sta-Ag3 IgG
Sta-Ag2	50	N	$2.0 \times 10^4 \pm 3.0 \times 10^3$	$1.5 \times 10^4 \pm 3.9 \times 10^3$	$8.0 \times 10^3 \pm 1.5 \times 10^2$	<20
	50	AHG	$8.5 \times 10^4 \pm 1.1 \times 10^4$	$5.9 \times 10^4 \pm 1.2 \times 10^4$	$9.7 \times 10^4 \pm 2.5 \times 10^2$	<20
	50	FA	$9.6 \times 10^4 \pm 1.1 \times 10^4$	$6.4 \times 10^4 \pm 1.1 \times 10^4$	$1.1 \times 10^4 \pm 2.3 \times 10^2$	<20
	10	FA	$7.2 \times 10^4 \pm 2.5 \times 10^4$	ND	ND	<20
	3	FA	$5.4 \times 10^4 \pm 3.0 \times 10^4$	ND	ND	<20
Sta-Ag3	50	N	<50	ND	ND	$1.2 \times 10^3 = 3.1 \times 10^4$
	50	AHG	<50	ND	ND	$1.2 \times 10^5 = 1.8 \times 10^5$
	50	FA	<50	ND	ND	$2.6 \times 10^6 \pm 1.9 \times 10^5$
	10	FA	<10	ND	ND	$2.6 \times 10^6 \pm 8.0 \times 10^4$
	3	FA	<10	ND	ND	$1.9 \times 10^4 \pm 6.4 \times 10^4$

TABLE 1-continued

Immunization for different dose and forms of Sta-Ag2 or Sta-Ag3 against *S. aureus* infection

Sta-Ag2 + 3	50	AHG	$8.0 \times 10^4 \pm 1.1 \times 10^4$	$6.9 \times 10^4 \pm 1.0 \times 10^4$	$1.2 \times 10^4 \pm 2.1 \times 10^4$	$1.3 \times 10^6 \pm 1.9 \times 10^1$
PBS mock	0	FA	<10	<10	<10	<10
	0	AHG	<10	<10	<10	<10
	0	N	<10	<10	<10	<10

			Titer[b]			
Treatment	Dose (μg)	Adjuvant[a]	Anti-Sta-Ag3 IgG1	Anti-Sta-Ag3 IgG2a	Survival[c]	Significance[d]
Sta-Ag2	50	N	ND	ND	66.70%	$P < 0.05$
	50	AHG	ND	ND	88.90%	$P < 0.0001$
	50	FA	ND	ND	83.30%	$P < 0.0001$
	10	FA	ND	ND	75.00%	$P < 0.005$
	3	FA	ND	ND	75.00%	$P < 0.005$
Sta-Ag3	50	N	$9.6 \times 10^4 \pm 1.4 \times 10^2$	$4.3 \times 10^3 \pm 6.7 \times 10^4$	50.00%	$P < 0.05$
	50	AHG	$1.1 \times 10^4 \pm 1.7 \times 10^2$	$3.3 \times 10^5 \pm 4.5 \times 10^4$	77.78%	$P < 0.0001$
	50	FA	$1.8 \times 10^4 \pm 1.4 \times 10^5$	$3.8 \times 10^4 \pm 4.4 \times 10^4$	83.30%	$P < 0.0001$
	10	FA	ND	ND	62.50%	$P < 0.003$
	3	FA	ND	ND	50.00%	$P < 0.05$
Sta-Ag2 + 3	50	AHG	$1.0 \times 10^3 \pm 1.7 \times 10^3$	$6.0 \times 10^4 \pm 1.2 \times 10^4$	83.30%	$P < 0.0001$
PBS mock	0	FA	<10	<10	22.20%	$P = 0.7861$
	0	AHG	<10	<10	16.70%	$P = 0.7250$
	0	N	<10	<10	11.10%	—

[a]FA: Freund's adjuvant.

AHG: Aluminium hydroxide gel;

N: No adjuvant

[b]Antibody titers (titers ± SEM) were detected by ELISA with purified Sta-Ag2 or Sta-Ag3 (1 μg/ml); the antibody endpoint titer was defined as the serum dilution that produced an OD450 of 0.5 absorbance units in the ELISA assay.

[c]Vaccinated BALB/c mice were challenged with *S. aureus* ATCC 2593 (5×10^7 CFU) by intravenous injection and survival was monitored.

[d]Log-rank (Mantel-Cox) test was used to compare between PBS mock (No adjuvant) and treatment groups.

ND: Not determined

[0115] Renal Abscess. The potential protective effect of rSaEsxA and rSaEsxB in a mouse renal abscess model was evaluated. Mice were infected with 1×10^7 CFU of *S. aureus* strain ATCC 25923. Four days after challenge, mice were sacrificed and their kidneys were collected. Renal tissue of animals treated with PBS displayed a staphylococcal load of 3.50 (+0.29) log 10 CFU mg^{-1} of kidney tissues. Significant decreases in bacterial number were observed for animals treated with rSaEsxA [2.38 (+0.31) log 10 CFU mg^{-1}, p=0.0159] and rSaEsxB [1.97 (+0.05) log 10 CFU mg^{-1}, p=0.0079](FIG. **11**I). Histological analysis of kidney tissues failed to detect staphylococcal abscesses in animals that had been immunized with the rSaEsxA+B or rSaEsxA and rSaEsxB alone (F **11**C/FIG. **11**D, FIG. **11**E/FIG. **11**F and FIG. **11**G/FIG. **11**H). Conversely, kidneys collected from control mice harbored abscesses with central concentrations of *staphylococci* that were surrounded by large numbers of necrotic immune cells (FIG. **11**A/FIG. **11**B).

[0116] Lethal Challenge. The protective effect of the recombinant Sta-Ag2 or Sta-Ag3 proteins against lethal infections was investigated in mice immunized with purified rSaEsxA or rSaEsxB antigens, or a mixture of both. The immunized mice were challenged with 5×10' CFU of *S. aureus* ATCC 25923 by intravenous injection through the tail vein. Animals were monitored for more than 14 days. Survival rates between groups were compared using the pairwise, Log-rank (Mantel-Cox) test. The different survival rates of mice immunized with different treatments (protein+AHG or FA) and doses of Sta-Ag2 or Sta-Ag3 (3, 10 and 50 μg) against *S. aureus* ATCC 25923 are shown in Table 1. Survival rates increased with increasing doses of Sta-Ag2 or Sta-Ag3, with AHA or FA possibly playing non-specific roles against *S. aureus* infection. However, there were no significant differences between the adjuvant only groups (FA: 22.20%, P=0.7861; AHG: 16.70%, P=0.7250) and the PBS control group (11.10%). The results in FIG. **12** showed the vaccinated mice groups had significantly improved survival rates ($p<0.0001$). Specifically, mice vaccinated with Sta-Ag2 alone had the highest survival rate (16/18) compared to (14/18) for Sta-Ag3 alone and Sta-Ag2+3 vaccinated animals. In contrast, the majority of mice (16/18) in the control group died within 8 days after bacterial challenge. The survival rates between combined and individual antigens were not significantly different ($p>0.05$).

[0117] To test whether Sta-Ag2+3 could protect against a wide range of *S. aureus* clinical strains, two typical *S. aureus* strains, Newman and USA 300, were first tested. Newman is a methicillin-sensitive *S. aureus* strain and USA 300 is a community-associated methicillin-resistant *S. aureus* strain. Mice were treated with these strains in a similar manner to the above experiments and were monitored over 14 days. Compared with the control mice treated with PBS+AHG, mice vaccinated with combined Sta-Ag2+3 had significant protective immunity to Newman (60%; p=0.0085, Log-rank Mantel-Cox test) and USA 300 (50%; p=0.0013, Log-rank Mantel-Cox test) *S. aureus* strains (FIG. **13**).

[0118] Sta-Ag2 or Sta-Ag3-specific IFN-γ^+ and IL17A$^+$ T cell responses. IFN-γ and IL17A play essential roles in the protective immunity against *S. aureus* infection. The release of IFN-γ and IL17A are indicative of Th1- and Th17-biased immune responses [33]. Mice were sacrificed 5 days after the third immunization and splenocyte production of IFN-γ and IL17A cytokines was measured by ELISPOT. Splenocytes from mice immunized with Sta-Ag2 or Sta-Ag3 had more IFN-γ (FIG. **14**A) and IL17A (FIG. **14**B) producing cells compared to the control group. The numbers of IFN-γ-pro-

ducing splenocytes in these immunized mice were also significantly greater than in NMSA (naïve mice stimulated with Sta-Ag2) and NMSB (naïve mice stimulated with Sta-Ag3). Furthermore, immunization with Sta-Ag2 or Sta-Ag3 induced robust specific Th17 responses.

[0119] Finally, mice treated with SaEsxA- or SaEsxB-specific antisera did not exhibit any significant protective effects against *S. aureus* challenge ($p>0.05$, Log-rank Mantel-Cox test) (FIG. **15**). Treatment with SaEsxA- or SaEsxB-specific antisera alone could not provide effective immunity.

[0120] Taken together, these data indicate that SaEsxA or SaEsxB proteins promote the induction of Th1- and Th17-biased immune responses, but SaEsxA- or SaEsxB-specific antisera alone could not provide effective immunity.

EXAMPLE 3

[0121] Targeting Sta-Ag4-11 for drug development. Phenol-soluble modulins (PSMs) have recently emerged as a novel toxin family defining the virulence potential of highly aggressive *S. aureus* isolates. PSMs have multiple roles in staphylococcal pathogenesis, causing lysis of red and white blood cells, stimulating inflammatory responses and contributing to biofilm development and the dissemination of biofilm-associated infections [34, 35]. Moreover, the pronounced capacity of PSMs to kill human neutrophils after phagocytosis might explain failures in the development of anti-staphylococcal vaccines. In *S. aureus*, however, not all PSMs are cytolytic. The four PSMα peptides of *S. aureus*, PSMα1 (Sta-Ag5), PSMα2 (Sta-Ag6), PSMα3 (Sta-Ag7), and PSMα4 (Sta-Ag8), have a pronounced ability to lyse human leukocytes and erythrocytes, and PSMα3 has by far the strongest activity [35]. All PSMs are secreted without a signal peptide, carrying an amino terminal N-formyl methionine. The lack of a signal peptide indicates that PSM secretion takes place by a dedicated mechanism, which was recently identified to be a four-component ABC transporter, named PmtA (Sta-Ag9), PmtB (Sta-Ag10), PmtC (Sta-Ag111) and PmtD (Sta-Ag12). This transporter plays an essential part in *S. aureus* physiology because in its absence, PSM peptides accumulate in the cytosol, leading to cell death.

[0122] Targeting PSMs for vaccine development. Experiments led to the identification of *S. aureus* PSMs that kill human neutrophils and have a major impact on the ability of the recently emerged community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains to cause disease [34, 35]. PSMα1 (Sta-Ag5), PSMα2 (Sta-Ag6), PSMα3 (Sta-Ag7), and PSMα4 (Sta-Ag8) can be used as potential antigens in active vaccination approaches. In addition, Pmt [PmtA (Sta-Ag9), PmtB (Sta-Ag10), PmtC (Sta-Ag11) and PmtD (Sta-Ag12)] can also be potential targets for active vaccination, given surface location and essential role in growth and pathogenesis. Therefore, based on PSMs associated antigens, they can be utilized to develop a vaccine which elicits a potential antibody to prevent skin infection.

[0123] Targeting PSMs for immunotherapeutic antibodies development. MAb-dependent facilitation of opsonophagocytosis might not lead to enhanced killing of *S. aureus*, however, mAbs can eliminate PSM toxicity by sequestration. In addition, drugs blocking the Pmt [PmtA (Sta-Ag9), PmtB (Sta-Ag10), PmtC (Sta-Ag111) and PmtD (Sta-Ag12)]transport function should work on all PSM-producing species, as the Pmt system is well conserved.

EXAMPLE 4

[0124] Novel combined vaccines targeting Sta-Ag1, Sta-Ag2, Sta-Ag3, and Sta-Ag4-12 Th17 cell targets (Sta-Ag2, 3) and B cell targets (Sta-Ag1, 4-12) are combined to develop a novel vaccine, thereby acting synergistically to effectively prevent *S. aureus* hematic spread, pneumonia, skin infection and mastitis in dairy cows. Despite eliciting high levels of anti-SaEsxA IgG and anti-SaEsxB IgG after vaccination with the purified SaEsxA and SaEsxB proteins, these antibodies could not prevent *S. aureus* infection in a murine model. Studies showed that healthy individuals naturally have high levels of antibody titers to *S. aureus*, but those with defects in B cell immunity are not particularly prone to *S. aureus* infections (36, 7). The lack of humoral immunity protection against *S. aureus* must be compensated for by other immune mechanisms.

[0125] To study if mice immunized with rSaEsxA or rSaEsxB could prevent abscess formation, a murine model of staphylococcal load and abscess formation was chosen. The results indicate that SaEsxA or SaEsxA proteins could induce protective immunity against *S. aureus* renal abscess formation in this murine model. Furthermore, these results suggest that mice immunized with the rSaEsxA+B, rSaEsxA or rSaEsxB had significantly increased protection against lethal challenge by *S. aureus* ATCC 25923 and show that rSaEsxA and rSaEsxB proteins not only specifically trigger high levels of IL-17A but also high levels of IFN-γ. These data indicate that SaEsxA and SaEsxB promote the induction of Th1- and Th17-biased immune responses. The results also showed that mice immunized with rSaEsxA+B were protected against two typical *S. aureus* clinical strains, Newman (MSSA) and USA 300 (CA-MRSA).

[0126] At least 13 secreted proteins and 24 surface adhesion proteins from *S. aureus* have been implicated in the bacterial immune evasion (38). The secretion of SaEsxA and SaEsxB represents an important bacterial virulence strategy, which leads to bacterial replication and abscess formation (32). Therefore, instead of a vaccine targeting a single-antigen, multivalent antigens are a better alternative for inducing both B and T cell immune responses to achieve protection against *S. aureus*.

EXAMPLE 5

[0127] Mice were vaccinated with combinations of Sta-Ag1, Sta-Ag2, and Sta-Ag3 (Sta-C3), Sta-Ag9, Sta-Ag10, Sta-Ag11 and Sta-Ag12 (Sta-C4), or Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag9, Sta-Ag10, Sta-Ag11 and Sta-Ag12 (Sta-C9). Determination of antibody titers by ELISA revealed antibodies specific for Sta-Ag2, Sta-Ag3, and Sta-Ag4 in Sta-C3-treated animals, antibodies specific for Sta-Ag9, Sta-Ag10, Sta-Ag11 and Sta-Ag12 in Sta-C4-treated animals, and antibodies specific for Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag9, Sta-Ag10, Sta-Ag11 and Sta-Ag12 in Sta-C9-treated animals, respectively (FIG. **16**). No antibodies were detected in mock treated animals.

[0128] Active immunization. To determine whether active immunization with combinations of Sta-Ag2, Sta-Ag3, and Sta-Ag4 (Sta-C3), Sta-Ag9, Sta-Ag10, Sta-Ag11 and Sta-Ag12 (Sta-C4), or Sta-Ag1, Sta-Ag2, Sta-Ag3, Sta-Ag4, Sta-Ag9, Sta-Ag10, Sta-Ag1 and Sta-Ag12 (Sta-C9) protected mice from severe *S. aureus* skin infections, mice were vaccinated intramuscularly with Sta-C3, Sta-C4, or Sta-C9 before infection with the USA 300 strain. The results showed that *S.*

aureus abscess size was reduced significant in mice vaccinated with Sta-C3, Sta-C4, and Sta-C9 and vaccinated mice showed substantially reduced dermonecrosis (FIG. **17**). Remarkably, Sta-C9 prevented skin lesions in 80% of the mice (FIGS. **17** and **18**).

[0129] Lethal Challenge. The protective effect of Sta-C3, Sta-C4, and Sta-C9 against lethal infections was investigated in mice immunized with Sta-C3, Sta-C4, or Sta-C9. The immunized mice were challenged with USA300 either injected i.v. through the tail vein or i.p. Animals were monitored for more than 14 days. Survival rates between groups were compared using the pair-wise, Log-rank (Mantel-Cox) test. The results showed that vaccinated mice groups had significantly improved survival rates after i.v. challenge ($p<0.0019$) and i.p. challenge ($p<0.0021$). Specifically, mice vaccinated with Sta-C9 had the highest survival rate (90% after i.v. challenge and 100% after i.p. challenge) compared to Sta-C3 and Sta-C4. In contrast, the majority of mice in the control group died within 4 days after bacterial challenge. These results demonstrated that Sta-C3, Sta-C4, and Sta-C9 generated protective immunity against *S. aureus* lethal challenge in a mouse model (FIGS. **19**A and **19**B).

[0130] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

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

Lys Glu Thr Gly Lys Phe Glu Asn Ile Asp Leu Lys Arg Val Tyr His
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Val Thr Met Asn Asp Phe Thr Ala Ser Gly Gly Asp Gly Tyr Ser Met
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Phe Gly Gly Pro Arg Glu Glu Gly Ile Ser Leu Asp Gln Val Leu Ala
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Ser Tyr Leu Lys Thr Ala Asn Leu Ala Lys Tyr Asp Thr Thr Glu Pro
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Gln Arg Met Leu Leu Gly Lys Pro Ala Val Ser Glu Gln Pro Ala Lys
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Gly Gln Gln Gly Ser Lys Gly Ser Lys Ser Gly Lys Asp Thr Gln Pro
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Ile Gly Asp Asp Lys Val Met Asp Pro Ala Lys Lys Pro Ala Pro Gly
        675                 680                 685

Lys Val Val Leu Leu Leu Ala His Arg Gly Thr Val Ser Ser Gly Thr
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Glu Gly Ser Gly Arg Thr Ile Glu Gly Ala Thr Val Ser Ser Lys Ser
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Gly Lys Gln Leu Ala Arg Met Ser Val Pro Lys Gly Ser Ala His Glu
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Lys Gln Leu Pro Lys Thr Gly Thr Asn Gln Ser Ser Ser Pro Glu Ala
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Phe Ala Gln Leu Leu Glu Glu Ile Lys Gln Gln Leu Asn Ser Thr Ala
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35 40 45

Ser Phe Ala Leu Asp Asp Ile Glu Ser Thr Ala Lys Asp Val Gln Thr
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Ala Lys Ser Ser Ala Asn Lys Asp Ser Val Thr Val Lys Gly Lys Ala

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Tyr Phe Val Leu Ser Gln Ser Ser Phe Pro Asp Leu Tyr Ser Tyr Ile
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        35                  40                  45

Asn Ile Ile Lys Phe Asp Gly Lys Val Asp Ile Asp Asn Ala Asp Asn
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65                  70                  75                  80

Leu Tyr Asn Leu Lys Leu Phe Ala Gln Val Leu Gly Lys Gly Phe Asp
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Thr Glu Lys Tyr Leu Ala Glu His Phe Gln Leu Gln Asn Val Asp Lys
225                 230                 235                 240

Ala Asp Gly His Leu Met Ile Asn Ala Gln Lys Asn Tyr Gln Val Ile
                245                 250                 255

Leu Lys Ala Leu Ser Glu Leu Asp Ile Tyr Pro Lys Tyr Ile Glu Thr
            260                 265                 270

Arg Lys Ser Ser Leu Arg Asp Thr Tyr Phe Asn Ile Asn Gln Arg Gly
        275                 280                 285

Asp Lys
    290


<210> SEQ ID NO 12
<211> LENGTH: 246
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 12

Met Arg Ile Leu Asn Leu Val Lys Tyr Asp Phe Tyr Ser Ile Phe Lys
1               5                   10                  15

Ser Pro Leu Thr Tyr Leu Ala Ile Leu Val Val Ser Ser Leu Ile Ala
            20                  25                  30

Thr Gln Ser Ile Leu Met Ala Asn Ser Met Asp Asn Pro Lys His Ile
        35                  40                  45

Ile Val Tyr Gly Ser Val Phe Ala Ala Ala Lys Trp Leu Leu Leu Ile
    50                  55                  60

Ile Gly Leu Met Phe Val Val Lys Thr Ile Thr Arg Asp Phe Ser Gln
65                  70                  75                  80

Gly Thr Ile Gln Leu Tyr Met Ser Lys Val Lys Thr Arg Val Gly Tyr
                85                  90                  95

Ile Ile Ser Lys Thr Ile Ser Ile Ile Leu Ile Ser Ile Leu Phe Ala
            100                 105                 110

Leu Ile His Tyr Val Ile Leu Ile Val Val Gln Ala Ser Ser Asn Gly
        115                 120                 125

Lys Asn Leu Ala Phe Ser Lys Tyr Val Asp Asn Leu Trp Phe Phe Leu
    130                 135                 140

Ile Phe Leu Leu Phe Phe Gly Leu Phe Leu Phe Leu Ile Thr Leu Ala
145                 150                 155                 160

Ser Gln Lys Thr Ala Met Ile Phe Ser Leu Gly Val Phe Leu Val Leu
                165                 170                 175

Ile Val Pro Phe Ile Lys Pro Phe Ile Thr Phe Ile Pro Arg Tyr Gly
            180                 185                 190

Glu Lys Val Leu Asp Ala Phe Asp Tyr Ile Pro Phe Ala Tyr Leu Thr
        195                 200                 205

Asp Lys Met Ile Ser Ser Asn Phe Asp Phe Ser Asn Trp Gln Trp Val
    210                 215                 220

Ile Ser Leu Gly Ser Ile Val Ile Phe Phe Ile Leu Asn Ile Leu Tyr
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-continued

225 230 235 240

Val Ala Lys Lys Asp Ile
245

What is claimed is:

1. A vaccine formulation, comprising:

a *Staphylococcus aureus* polypeptide selected from Sta-Ag1, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, and Sta-Ag12; any combination thereof; a combination of Sta-Ag2 with any of Sta-Ag1, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, and Sta-Ag12; or a combination of Sta-Ag3 with any of Sta-Ag1, Sta-Ag4, Sta-Ag5, Sta-Ag6, Sta-Ag7, Sta-Ag8, Sta-Ag9, Sta-Ag10, Sta-Ag11, and Sta-Ag12.

2. The vaccine formulation of claim **1**, wherein the combination of two or more *Staphylococcus aureus* polypeptides achieves protection against hematic spread, pneumonia, or skin infection.

3. The vaccine formulation of claim **1** comprising Sta-Ag2, Sta-Ag3, and Sta-Ag4.

4. The vaccine formulation of claim **1** comprising Sta-Ag9, Sta-Ag10, Sta-Ag11, and Sta-Ag12.

5. The vaccine formulation of claim **1** comprising Sta-Ag1, Sta-Ag2, Sta-Ag-3, Sta-Ag4, Sta-Ag9, Sta-Ag100, Sta-Ag11, and Sta-Ag12.

6. The vaccine formulation of claim **1**, further comprising at least one adjuvant.

7. The vaccine formulation of claim **6**, wherein the adjuvant is selected from a Th1 adjuvant, a Th2 adjuvant, a Th17 adjuvant, and any combination thereof.

8. The vaccine formulation of claim **7**, further comprising an aluminum hydroxide adjuvant.

9. The vaccine formulation of claim **8**, further comprising a histidine buffer.

10. The vaccine formulation of claim **1**, wherein the Sta-Ag1 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO: 1.

11. The vaccine formulation of claim **1**, wherein the Sta-Ag2 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO:2.

12. The vaccine formulation of claim **1**, wherein the Sta-Ag3 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO:3.

13. The vaccine formulation of claim **1**, wherein the Sta-Ag4 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO:4.

14. The vaccine formulation of claim **1**, wherein the Sta-Ag5 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO:5.

15. The vaccine formulation of claim **1**, wherein the Sta-Ag6 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO:6.

16. The vaccine formulation of claim **1**, wherein the Sta-Ag7 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO:7.

17. The vaccine formulation of claim **1**, wherein the Sta-Ag8 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO:8.

18. The vaccine formulation of claim **1**, wherein the Sta-Ag9 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO:9.

19. The vaccine formulation of claim **1**, wherein the Sta-Ag10 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO: 10.

20. The vaccine formulation of claim **1**, wherein the Sta-Ag11 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO: 11.

21. The vaccine formulation of claim **1**, wherein the Sta-Ag12 *Staphylococcus aureus* polypeptide comprises the amino acid sequence of SEQ ID NO: 12.

22. An isolated aptamer or antibody that binds with specificity to a polypeptide selected from a Sta-Ag1 *Staphylococcus aureus* polypeptide, a Sta-Ag2 *Staphylococcus aureus* polypeptide, a Sta-Ag3 *Staphylococcus aureus* polypeptide, a Sta-Ag4 *Staphylococcus aureus* polypeptide, a Sta-Ag5 *Staphylococcus aureus* polypeptide, a Sta-Ag6 *Staphylococcus aureus* polypeptide, a Sta-Ag7 *Staphylococcus aureus* polypeptide, a Sta-Ag8 *Staphylococcus aureus* polypeptide, a Sta-Ag9 *Staphylococcus aureus* polypeptide, a Sta-Ag10 *Staphylococcus aureus* polypeptide, a Sta-Ag11 *Staphylococcus aureus* polypeptide, and a Sta-Ag12 *Staphylococcus aureus* polypeptide.

23. A method for inhibiting *Staphylococcus aureus* infection in a subject, comprising: administering to the subject an effective amount of a vaccine formulation of claim **1**.

24. A method for inhibiting *Staphylococcus aureus* infection in a subject, comprising: administering to the subject an effective amount of an antibody according to claim **22**.

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