[0018] The compositions and methods for their use can "comprise," "consist essentially of," or "consist of" any of the ingredients or steps disclosed throughout the specification. The phrase "consisting of" excludes any element, step, or ingredient not specified. The phrase "consisting essentially of" limits the scope of described subject matter to the specified materials or steps and those that do not materially affect its basic and novel characteristics. It is contemplated that embodiments described in the context of the term "comprising" may also be implemented in the context of the term "consisting of" or "consisting essentially of."

[0019] It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention. Aspects of an embodiment set forth in the Examples are also embodiments that may be implemented in the context of embodiments discussed elsewhere in a different Example or elsewhere in the application, such as in the Summary of Invention, Detailed Description of the Embodiments, Claims, and description of Figure Legends.

[0020] 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.

SUMMARY

[0021] Compositions and methods are provided for improved Protein A variants with reduced binding to VH3 of immunoglobulins to reduce their toxigenicty yet stimulate humoral immune responses that protect against staphylococcal disease. In certain examples the improved Protein A variant stimulates humoral immune responses that can (i) prevent colonization and (ii) lead to decolonization of actively colonized individuals.

[0021a] In one aspect, the present disclosure refers to a composition comprising the isolated polypeptide of any of the preceding claims, wherein the composition contains less than 1% by weight of the total protein amount a protein other than the isolated polypeptide, optionally wherein the composition further comprises:

(A) an adjuvant, optionally wherein the isolated polypeptide is coupled to an adjuvant and/or wherein the adjuvant comprises alum; and/or

(B) a pharmaceutically acceptable excipient.

[0021b] In another aspect, the present disclosure refers to a use of a composition in the manufacture of a medicament for eliciting a safe immune response against a Staphylococcus bacteria in a human patient, wherein the composition is to be administered to the human patient, wherein the composition is the composition as described herein.

[0021c] In another aspect, the present disclosure refers to an isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues corresponding to positions 9 and 10 in each of domains A-E and (ii) at least one other amino acid substitution corresponding to position 33 and/or 29 in SEQ ID NO:2 in each of domains A-E, wherein the SpA variant has a K_D binding affinity for VH3 from human IgG that is greater than 1.0×10^{-4} M and/or a K_D binding affinity for VH3 from human IgE that is greater than 1.0×10^{-6} M.

[0021d] In another aspect, the present disclosure refers to a composition as described herein, wherein the composition is capable of stimulating an immune response in a subject in need thereof, optionally wherein:

(a) the composition contains less than 1% by weight of the total protein amount a protein other than the isolated polypeptide; and/or

(b) the composition further comprises an adjuvant, optionally wherein the isolated polypeptide is coupled to an adjuvant, optionally wherein the adjuvant comprises alum; and/or

(c) the composition further comprises a pharmaceutically acceptable excipient.

[0021e] In another aspect, the present disclosure refers to a use of a composition in the manufacture of a medicament for preventing or treating staphylococcal infection, wherein the composition is to be administered to a patient in need thereof, wherein the composition is the isolated polypeptide as described herein or the composition as described herein.

[0021f] In another aspect, the present disclosure refers to a use of a composition in the manufacture of a medicament for eliciting an immune response against a staphylococcus bacterium in a subject, wherein the composition comprises the isolated polypeptide as described herein or the composition as described herein, wherein an effective amount of the isolated polypeptide or the composition is to be provided to the subject.

[0021g] In yet another aspect, the present disclosure refers to a use of a composition in the manufacture of a medicament for treating a staphylococcal infection in a subject, wherein the

composition comprises an isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues corresponding to positions 9 and 10 in each of domains A-E and (ii) at least one other amino acid substitution corresponding to position 33 and/or 29 in SEQ ID NO:2 in each of domains A-E, wherein the SpA variant has a K_D binding affinity for IgG that is greater than 1.0×10^{-5} M, and wherein the composition is to be administered to a subject having, suspected of having or at risk of developing a staphylococcal infection an effective amount.

[0021h] In yet another aspect, the present disclosure refers to a use of of a composition in the manufacture of a medicament for decolonization or for preventing colonization or recolonization of Staphylococcus bacteria in a subject, wherein the composition comprises the isolated polypeptide as described herein or the composition as described herein, wherein the composition is to be administered to the subject in an effective amount.

[0021i] In yet another aspect, the present disclosure refers to an isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues in each of domains A-E corresponding to positions 9 and 10 in domain D and (ii) glutamate substitutions in each of domains A-E corresponding to position 33 in domain D, wherein the polypeptide does not, relative to a negative control, detectably crosslink IgG and IgE in blood or activate basophils or mast cells; wherein the SpA variant does not have substitutions in any of the domains A-E corresponding to positions 36 and 37 in domain D.

[0021j] In yet another aspect, the present disclosure refers to the isolated polypeptide as described herein or the composition as described herein for use in therapy.

[0022] In some aspects, compositions and methods invove an isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues in each of domains A-E corresponding to positions 9 and 10 in domain D and (ii) glutamate substitutions in each of domains A-E corresponding to position 33 in domain D, wherein the

polypeptide does not, relative to a negative control, detectably crosslink IgG and IgE in blood or activate basophils or mast cells. The variant SpA region, in some examples, is contemplated as not crosslinking IgG and/or IgE in blood and/or activate basophils so as to pose a significant safety or toxicity issue to human patients or to pose a significant risk of anaphylactic shock in a human patient. In some examples, the K_A binding affinity for VH3 from human IgG is reduced as compared to a SpA variant consisting of lysine substitutions for glutamine residues in each of domains A-E corresponding to position 9 and 10 in domain D and alanine substitutions for aspartic acid in domains A-E corresponding to position 36 and 37 of domain D (SpA_{KKAA}). In some examples, a SpA variant has a K_A binding affinity for VH3 from human IgG that is reduced by at least 2-fold as compared to $SpA_{KKAA.}$ In some examples, KA binding affinity may be measured by any method disclosed herein. In some examples, the SpA variant has a K_A binding affinity for VH3 from human IgG that is reduced by at least 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3-fold or more (or any range derivable therein) as compared to $SpA_{KKAA.}$ In some examples, the SpA variant has a K_A binding affinity for VH3 from human IgG that is reduced by at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300% or more (or any range derivable therein) as compared to $SpA_{KKAA.}$ Of course it will be understand that the comparison is done using the same or comparable assays. In some examples, the SpA variant has a K_A binding affinity for VH3 from human IgG that is less than about 1×10^5 M^{-1}. In some examples, the SpA variant has a K_A binding affinity for human IgG VH3 that is less than about 3, 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.70.6, 0.5, 0.4, 0.3, 0.2, 0.1×10^5 M^{-1} (or any arrange derivable therein). In some examples, the isolated polypeptide comprising the SpA variant does not have substitutions in any of the domains A-E corresponding to positions 36 and 37 in domain D. In some examples, the only substititions in the SpA variant are (i) lysine substitutions for glutamine residues in each of domains A-E corresponding to positions 9 and 10 in domain D and (ii) glutamate substitutions in each of domains A-E corresponding to position 33 in domain D (referred to as $SpA_{Q9,10K/S33E}$). In some examples, the isolated polypeptide consists of the SpA variant. In some examples, the isolated polypeptide consists of $SpA_{Q9,10K/S33E.}$

[0023] In some aspects, there is an isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues in each of domains A-E

corresponding to positions 9 and 10 in domain D and (ii) threonine substitutions in each of domains A-E corresponding to position 33 in domain D, wherein the polypeptide does not, relative to a negative control, detectably crosslink IgG and IgE in blood or activate basophils or mast cells. The variant SpA region, in some examples, is contemplated as not crosslinking IgG and/or IgE in blood and/or activate basophils so as to pose a significant safety or toxicity issue to human patients or to pose a significant risk of anaphylactic shock in a human patient. In some examples, the KA binding affinity for VH3 from human IgG is reduced as compared to a SpA variant consisting of lysine substitutions for glutamine residues in each of domains A-E corresponding to position 9 and 10 in domain D and alanine substitutions for aspartic acid in domains A-E corresponding to position 36 and 37 of domain D (SpA_{KKAA}). In some examples, a SpA variant has a K_A binding affinity for VH3 from human IgG that is reduced by at least 2-fold as compared to $SpA_{KKAA.}$ In some examples, KA binding affinity may be measured by any method disclosed herein. In some examples, the SpA variant has a K_A binding affinity for VH3 from human IgG that is reduced by at least 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3-fold or more (or any range derivable therein) as compared to $SpA_{KKAA.}$ In some examples, the SpA variant has a K_A binding affinity for VH3 from human IgG that is reduced by at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300% or more (or any range derivable therein) as compared to $SpA_{KKAA.}$ Of course it will be understand that the comparison is done using the same or comparable assays. In some examples, the SpA variant has a K_A binding affinity for VH3 from human IgG that is less than about 1 x 10^5 M^{-1}. In some examples, the SpA variant has a K_A binding affinity for human IgG VH3 that is less than about 3, 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.70.6, 0.5, 0.4, 0.3, 0.2, 0.1 x 10^5 M^{-1} (or any arrange derivable therein). In some examples, the isolated polypeptide comprising the SpA variant does not have substitutions in any of the domains A-E corresponding to positions 36 and 37 in domain D. In some examples, the only substititions in the SpA variant are (i) lysine substitutions for glutamine residues in each of domains A-E corresponding to positions 9 and 10 in domain D and (ii) threonine substitutions in each of domains A-E corresponding to position 33 in domain D (referred to as $SpA_{Q9,10K/S33T}$). In some examples, the isolated polypeptide consists of the SpA variant. In some examples, the isolated polypeptide consists of $SpA_{Q9,10K/S33T.}$

[0024] In some examples, compositions and methods involve $SpA_{Q9,10K/S33E}$. Any example discussed below in the context of a SpA variant can be implemented specifically with $SpA_{Q9,10K/S33E}$, which has the critical advantage of reduced activity that is associated with toxicity and/or anaphylactic shock. In some examples, there are methods for eliciting a safe immune response against a Staphylococcus bacteria in a human patient comprising administering to the human patient an effective amount of a composition comprising $SpA_{Q9,10K/S33E}$. In some examples, there are methods for eliciting a safe immune response against a Staphylococcus bacteria in a subject comprising administering to the subject an effective amount of a composition comprising $SpA_{Q9,10K/S33E}$. The subject may be a laboratory animal, or industrial animal such as cow, pig, sheep goat or any other animal grown for human consumption and that may suffer Staph infections such as mastitis.

[0025] In some examples, compositions and methods involve $SpA_{Q9,10K/S33E}$. Any examplesdiscussed below in the context of a SpA variant can be implemented specifically with $SpA_{Q9,10K/S33T}$, which has the critical advantage of reduced activity that is associated with toxicity and/or anaphylactic shock. In some examples, there are methods for eliciting a safe immune response against a Staphylococcus bacteria in a human patient comprising administering to the human patient an effective amount of a composition comprising $SpA_{Q9,10K/S33T}$.

[0026] In some examples, compositions and methods involve an isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues corresponding to positions 9 and 10 in each of domains A-E and (ii) at least one other amino acid substitution corresponding to position 29 and/or 33 in SEQ ID NO:2 in each of domains A-E, wherein the SpA variant has a K_D binding affinity for VH3 from human IgG that is greater than 1.0×10^{-4} M. In the same or additional examples, the SpA variant has K_D binding affinity for VH3 from human IgE that is greater than 1.0×10^{-6} M.

[0027] Examples include the generation of protein A variants that are safe for use in humans, specifically variants that cannot bind to crosslink the variant heavy chains of VH3 idiotypic IgG and IgE molecules bound to Fc receptors on the surface of immune cells, which are capable of releasing immune mediators such as histamine that trigger vascular leakage and anaphylactic reactions.

[0028] Amino acids substitutions are made in each of domains A, B, C, D. and E (Domains A-E) of a variant SpA meaning the amino acids corresponding to the glutamine residues at

positions 9 and 10 as they are numbered in Domain D, which is SEQ ID NO:2, are substituted; the same amino acid substitutions are made to the corresponding amino acids in Domains A, B, C, and E. Examples concern a SpA in which lysine residues have been substituted in each of Domains A-E for the glutaminic acid residues corresponding to positions 9 and 10 in Domain D.

[0029] In certain examples, a SpA variant has a K_D binding affinity for VH3 from human IgG that is greater than about 1.0×10^{-4} M, 5.0×10^{-4} M, 1.0×10^{-3} M, 5.0×10^{-3} M, 1.0×10^{-2} M, 5.0×10^{-2} M, 1.0×10^{-1} M, 5.0×10^{-1} M (or any range derivable therein). In some examples, the binding affinity for VH3 from human IgG is greater than about 1.0×10^{-2} M or 5.0×10^{-2} M. In the same or additional examples, the SpA variant has K_D binding affinity for VH3 from human IgE that is greater than about about 1.0×10^{-6} M, 5.0×10^{-6} M, 1.0×10^{-5} M, 5.0×10^{-5} M, 1.0×10^{-4} M, 5.0×10^{-4} M, 1.0×10^{-3} M, 5.0×10^{-3} M, 1.0×10^{-2} M, 5.0×10^{-2} M,1.0×10^{-1} M, or 5.0×10^{-1} M (or any range derivable therein). Affinity of SpA and its variant to IgG may be measured with purified human IgG (from pooled serum) and with purified human monoclonal antibodies (trastuzumab human IgG1 VH3 clonal antibody and IgE trastuzumab). The assays used may be ELISA and/or surface plasmon resonance.

[0030] In specific examples, the variant SpA comprises an amino acid substitution corresponding to position 29 in SEQ ID NO:2 in each of domains A-E. In some cases, the substitution of the amino acid corresponding to position 29 is alanine, leucine, proline, phenylalanine, glutamic acid, arginine, lysine, serine, threonine or glutamine. In some examples, the variant SpA comprises a substitution of the amino acid corresponding to position 29 that is alanine, phenylalanine, or arginine. In other examples, the substitution is not with one of the following: leucine, proline, glutamic acid, lysine, serine, threonine, or glutamine. In some examples, a variant SpA comprises an amino acid substitution corresponding to position 33 in SEQ ID NO:2 in each of domains A-E. In specific examples, the substitution of the amino acid corresponding to position 33 is with alanine, phenylalanine, glutamic acid, lysine, or glutamine. In particular cases the substitution is with phenylalanine, glutamic acid, or glutamine. In other examples, the substitution is not with alanine or lysine. In some SpA variants, there are substitutions described herein corresponding to positions 29 and 33 in SEQ ID NO:2 in each of domains A-E. These may or may not be combined with an amino acid substitution corresponding to one or both of positions 36 and 37 in SEQ ID NO:2 in each of domains A-E. In some cases, there are substitutions at both positions

36 and 37. In some cases, the amino acid substitutions corresponding to positions 36 and 37 are alanine residues for aspartic acid residues.

[0031] In some examples, a variant SpA polypeptide comprises variant A-E domains (meaning a polypeptide with a variant Domain A, a variant Domain B, a variant Domain C, a variant Domain D, and a variant Domain E) and the variant Domains are each at least 70%, 75%, 80%, 85%, 90%, 95%, or 96%, identical to a Protein A domain D polypeptide (SEQ ID NO:2), domain E (SEQ ID NO:3), domain A (SEQ ID NO:4), domain C (SEQ ID NO:5), domain B (SEQ ID NO:6). Alternatively, a variant SpA polypeptide comprises variant A-E domains (meaning a polypeptide with a variant Domain A, a variant Domain B, a variant Domain C, a variant Domain D, and a variant Domain E) and the variant Domains are each at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a Protein A domain D polypeptide (SEQ ID NO:2), domain E (SEQ ID NO:3), domain A (SEQ ID NO:4), domain C (SEQ ID NO:5), domain B (SEQ ID NO:6) with respect to amino acids other than those specifically articulated as having an amino acid substitution. In other words, a SpA variant with substitutions at positions 9, 10 and 29 may have Domains A-E otherwise 100% identical to the non-variant sequences. In some examples, for example, a variant SpaA comprises variant A-E domains that do not comprise any amino acid substitutions in SEQ ID NO:2 except at corresponding positions 9, 10, 29, 33, 36, and/or 37.

[0032] In particular examples, the variant SpaA comprises variant A-E domains consisting only of amino acid substitutions corresponding to positions 9, 10, and 29 in SEQ ID NO:2. In certain other examples, the variant SpaA comprises variant A-E domains consisting only of amino acid substitutions corresponding to positions 9, 10, and 33 in SEQ ID NO:2. In additional examples, the variant SpaA comprises variant A-E domains consisting only of amino acid substitutions corresponding to positions 9, 10, 29, and 33 in SEQ ID NO:2. Additionally, other examplesconcern a variant SpaA that comprises variant A-E domains consisting only of amino acid substitutions corresponding to positions 9, 10, 29, 36, and 37 in SEQ ID NO:2. In further examples, a variant SpaA comprises variant A-E domains consisting only of amino acid substitutions corresponding to positions 9, 10, 33, 36, and 37 in SEQ ID NO:2. In other examples, a variant SpaA comprises variant A-E domains consisting only of amino acid substitutions corresponding to positions 9, 10, 29, 33, 36, and 37 in SEQ ID NO:2.

[0033] In additional examples, a variant SpA is part of an isolated polypeptide. The isolated polypeptide may or may not include a region that is not from Protein A. In some cases,

the non-Protein A segment is a second antigen segment, which may or may not be a staphylococcal antigen segment. In some examples, the segment may comprise an Emp, EsxA, EsxB, EsaC, Eap, Ebh, EsaB, Coa, vWbp, vWh, Hla, SdrC, SdrD, SdrE, IsdA, IsdB, IsdC, ClfA, ClfB, and/or SasF segment.

[0034] Examples include the use of Protein A variants in methods and compositions for the treatment of bacterial and/or staphylococcal infection. This application also provides an immunogenic composition comprising a Protein A variant or immunogenic fragment thereof. In certain examples, the immunogenic fragment is a Protein A domain D segment. Furthermore, the present disclosure provides methods and compositions that can be used to treat (*e.g.*, limiting staphylococcal abscess formation and/or persistence in a subject) or prevent bacterial infection. In some cases, methods for stimulating an immune response involve administering to the subject an effective amount of a composition including or encoding all or part of a Protein A variant polypeptide or antigen, and in certain examples other bacterial proteins. Other bacterial proteins include, but are not limited to (i) a secreted virulence factor, and/or a cell surface protein or peptide, or (ii) a recombinant nucleic acid molecule encoding a secreted virulence factor, and/or a cell surface protein or peptide.

[0035] In some examples, there are methods involving a SpA variant. Methods include methods for treating a staphylococcal infection in a subject, eliciting an immune response against a staphylococcus bacterium in a subject, preventing a staphylococcal infection in a subject, preventing colonization or re-colonization of staphylococcus bacterium in a subject, reducing colonization or re-colonization of staphylococcus bacterium in a subject, treating a staphylococcal infection in a subject without significant toxicity, and/or inducing killing of staphylococcal bacteria in a subject. Steps may include administering an effective amount of a SpA variant or a composition comprising a SpA variant to a subject. The subject may be in need to the treatment or prevention because of an existing infection or a risk of infection.

[0036] In some examples, the subject does not exhibit any signs of toxicity from the isolated polypeptide or composition. In further examples, the subject does not exhibit any signs of anaphylactic shock from the isolated polypeptide or composition. In some examples, the subject is evaluated for signs of toxicity, which may include signs of anaphylactic shock.

[0037] The polypeptide can be formulated in a pharmaceutically acceptable composition. The composition can further comprise one or more of at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9,

10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 additional staphylococcal antigen or immunogenic fragment thereof (*e.g.*, Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla (*e.g.*, H35 mutants), IsdC, SasF, vWbp, or vWh). Additional staphylococcal antigens that can be used in combination with a Protein A variant include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa (GenBank CAC80837), Aap (GenBank accession AJ249487), Ant (GenBank accession NP_372518), autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein (see PCT publications WO2007/113222, WO2007/113223, WO2006/032472, WO2006/032475, WO2006/032500, each of which is incorporated herein by reference in their entirety). The staphylococcal antigen or immunogenic fragment can be administered concurrently with the Protein A variant. The staphylococcal antigen or immunogenic fragment and the Protein A variant can be administered in the same composition. The Protein A variant can also be a recombinant nucleic acid molecule encoding a Protein A variant. A recombinant nucleic acid molecule can encode the Protein A variant and at least one staphylococcal antigen or immunogenic fragment thereof. As used herein, the term "modulate" or "modulation" encompasses the meanings of the words "enhance," or "inhibit." "Modulation" of activity may be either an increase or a decrease in activity. As used herein, the term “modulator” refers to compounds that effect the function of a moiety, including up-regulation, induction, stimulation, potentiation, inhibition, down-regulation, or suppression of a protein, nucleic acid, gene, organism or the like.

[0038] In certain examples the methods and compositions use or include or encode all or part of the Protein A variant or antigen. In other examples, the Protein A variant may be used in combination with secreted factors or surface antigens including, but not limited to one or more of an isolated Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh polypeptide or immunogenic segment thereof. Additional staphylococcal antigens that can be used in combination with a Protein A

variant include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. In certain examples, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. can be specifically excluded from a formulation.

[0039] In still further examples, the isolated Protein A variant is multimerized, *e.g.*, dimerized or a linear fusion of two or more polypeptides or peptide segments. In certain examples of the disclosure, a composition comprises multimers or concatamers of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more isolated cell surface proteins or segments thereof. Concatamers are linear polypeptides having one or more repeating peptide units. SpA polypeptides or fragments can be consecutive or separated by a spacer or other peptide sequences, *e.g.*, one or more additional bacterial peptide. In a further example, the other polypeptides or peptides contained in the multimer or concatamer can include, but are not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 of Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh or immunogenic fragments thereof. Additional staphylococcal antigens that can be used

in combination with a Protein A variant include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein.

[0040] The term "Protein A variant" or "SpA variant" refers to polypeptides that include a SpA IgG domain having two or more amino acid substitutions that disrupt binding to Fc and V_H3. In certain example, a SpA variant includes a variant domain D peptide, as well as variants of SpA polypeptides and segments thereof that are non-toxigenic and stimulate an immune response against staphylococcus bacteria Protein A and/or bacteria expressing such.

[0041] Examples also include methods for eliciting an immune response against a staphylococcus bacterium or staphylococci in a subject comprising providing to the subject an effective amount of a Protein A variant or a segment thereof. In certain examples, the methods for eliciting an immune response against a staphylococcus bacterium or staphylococci in a subject comprising providing to the subject an effective amount of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or more secreted proteins and/or cell surface proteins or segments/fragments thereof. A secreted protein or cell surface protein includes, but is not limited to Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, and/or vWh proteins and immunogenic fragments thereof. Additional staphylococcal antigens that can be used in combination with a Protein A variant include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG

/ Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein.

[0042] Examples including method, composition, and polypeptide examples, include specific examples in which the Staphylococcus bacteria comprises the WU1 or JSNZ strain of *Staphylococcus aureus*. In some examples, the Staphylococcus bacteria comprises type ST88 isolate.

[0043] In some examples, the subject or patient described herein, such as the human patient is a pediatric patient. A pediatric patient is one that is defined as less than 18 years old. In some examples, the patient is at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 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, 85, or 90 years old (or any range derivable therein). In some examples, the pediatric patient is 2 years old or less. In some examples, the pediatric patient is lest than 1 year old. In some examples, the pediatric patient is less than 6 months old. In some examples, the pediatric patient is 2 months old or less. In some examples, the human patient is 65 years old or older. In some examples, the human patient is a health care worker. In some examples, the patient is one that will receive a surgical procedure.

[0044] In some examples, the patient the isolated polypeptide of composition is administered in four doses and wherein the interval between doses is at least four weeks. In some examples, the isolated polypeptide is given in 4 doses or in exactly 4 doses. In some examples, the isolated polypeptide or composition is given in at least, at most, or exactly 1, 2, 3, 4, 5, 6, 7, or 8 doses. In some examples, the first dose is administered at 6-8 weeks of age. In some examples, all four doses are administered at or before 2 years of age. In some examples, the polypeptide or composition is to be administered as a four-dose series at 2, 4, 6, and 12–15 months of age. Dose 1 may be given as early as 6 weeks of age. The interval between dosing may be about 4 to 8 weeks. In some examples, the fourth dose is administered at approximately 12–15 months of age, and at least 2 months after the third dose.

[0045] Wxamples include compositions that include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to Protein A, or a second protein or peptide that is a secreted bacterial protein or a bacterial cell surface protein. In a further example a composition may include a

polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a Protein A domain D polypeptide (SEQ ID NO:2), domain E (SEQ ID NO:3), domain A (SEQ ID NO:4), domain C (SEQ ID NO:5), domain B (SEQ ID NO:6), or a nucleic acid sequence encoding a Protein A domain D, domain E, domain A, domain C, or domain B polypeptide. In certain examples a Protein A polypeptide segment will have an amino acid sequence of SEQ ID NO:8. Similarity or identity, with identity being preferred, is known in the art and a number of different programs can be used to identify whether a protein (or nucleic acid) has sequence identity or similarity to a known sequence. Sequence identity and/or similarity is determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman (1981), by the sequence identity alignment algorithm of Needleman & Wunsch (1970), by the search for similarity method of Pearson & Lipman (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.), the Best Fit sequence program described by Devereux *et al.* (1984), preferably using the default settings, or by inspection. Preferably, percent identity is calculated by using alignment tools known to and readily ascertainable to those of skill in the art. Percent identity is essentially the number of identical amino acids divided by the total number of amino acids compared times one hundred.

[0046] Still further examples include methods for stimulating in a subject a protective or therapeutic immune response against a staphylococcus bacterium comprising administering to the subject an effective amount of a composition including (i) a SpA variant, *e.g.*, a variant SpA domains A-E polypeptide or peptide thereof; or, (ii) a nucleic acid molecule encoding such a SpA variant polypeptide or peptide thereof, or (iii) administering a SpA variant domain D polypeptide with any combination or permutation of bacterial proteins described herein. In a preferred examples the composition is not a staphylococcus bacterium. In certain examples the subject is a human or a cow or pig or sheep or goat or any other animal grown for human consumption and that may suffer Staph infections such as mastitis. In a further example the composition is formulated in a pharmaceutically acceptable formulation. The staphylococci may be *Staphylococcus aureus*.

[0047] Yet still further examples include vaccines comprising a pharmaceutically acceptable composition having an isolated SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described herein, wherein the composition is capable

of stimulating an immune response against a staphylococcus bacterium. The vaccine may comprise an isolated SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described. In certain examples of the disclosure the isolated SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described are multimerized, *e.g.*, dimerized or concatamerized. In a further example, the vaccine composition is contaminated by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5, 0.25, 0.05% (or any range derivable therein) of other Staphylococcal proteins. A composition may further comprise an isolated non-SpA polypeptide. Typically the vaccine comprises an adjuvant. In certain examples a protein or peptide of the disclosure is linked (covalently or non-covalently) to the adjuvant, preferably the adjuvant is chemically conjugated to the protein. In some examples, the adjuvant comprises alum. In some examples, the adjuvant comprises an adjuvant described herein.

[0048] In still yet further examples, a vaccine composition is a pharmaceutically acceptable composition having a recombinant nucleic acid encoding all or part of a SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described herein, wherein the composition is capable of stimulating an immune response against a staphylococcus bacteria. The vaccine composition may comprise a recombinant nucleic acid encoding all or part of a SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described herein. In certain examplesthe recombinant nucleic acid contains a heterologous promoter. Preferably the recombinant nucleic acid is a vector. More preferably the vector is a plasmid or a viral vector. In some examples the vaccine includes a recombinant, non-staphylococcus bacterium containing the nucleic acid. The recombinant non-staphylococci may be Salmonella or another gram-positive bacteria. The vaccine may comprise a pharmaceutically acceptable excipient, more preferably an adjuvant. In some examples, the adjufvant comprises alum or an adjuvant described herein.

[0049] Still further examples include methods for stimulating in a subject a protective or therapeutic immune response against a staphylococcus bacterium comprising administering to the subject an effective amount of a composition of a SpA variant polypeptide or segment/fragment thereof and further comprising one or more of a Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh protein or peptide thereof. In a preferred example the composition comprises a non-staphylococcus bacterium. In a further example the composition is formulated in a pharmaceutically acceptable formulation. The staphylococci for which a subject is being

treated may be *Staphylococcus aureus*. Methods of the disclosure also include SpA variant compositions that contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or more secreted virulence factors and/or cell surface proteins, such as Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh in various combinations. In certain examples a vaccine formulation includes Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, and vWh. In certain examples an antigen combination can include (1) a SpA variant and IsdA; (2) SpA variant and ClfB; (3) SpA variant and SdrD; (4) SpA variant and Hla or Hla variant; (5) SpA variant and ClfB, SdrD, and Hla or Hla variant; (6) SpA variant, IsdA, SdrD, and Hla or Hla variant; (7) SpA variant, IsdA, ClfB, and Hla or Hla variant; (8) SpA variant, IsdA, ClfB, and SdrD; (9) SpA variant, IsdA, ClfB, SdrD and Hla or Hla variant; (10) SpA variant, IsdA, ClfB, and SdrD; (11) SpA variant, IsdA, SdrD, and Hla or Hla variant; (12) SpA variant, IsdA, and Hla or Hla variant; (13) SpA variant, IsdA, ClfB, and Hla or Hla variant; (14) SpA variant, ClfB, and SdrD; (15) SpA variant, ClfB, and Hla or Hla variant; or (16) SpA variant, SdrD, and Hla or Hla variant.

[0050] In certain examples, a bacterium delivering a composition of the disclosure will be limited or attenuated with respect to prolonged or persistent growth or abscess formation. In yet a further example, SpA variant(s) can be overexpressed in an attenuated bacterium to further enhance or supplement an immune response or vaccine formulation.

[0051] The term "EsxA protein" refers to a protein that includes isolated wild-type EsxA polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria EsxA proteins.

[0052] The term "EsxB protein" refers to a protein that includes isolated wild-type EsxB polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria EsxB proteins.

[0053] The term "SdrD protein" refers to a protein that includes isolated wild-type SdrD polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria SdrD proteins.

[0054] The term "SdrE protein" refers to a protein that includes isolated wild-type SdrE polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria SdrE proteins.

[0065] The term "Coa protein" refers to a protein that includes isolated wild-type Coa polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria Coa proteins.

[0066] The term "Hla protein" refers to a protein that includes isolated wild-type Hla polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria Hla proteins.

[0067] The term "IsdC protein" refers to a protein that includes isolated wild-type IsdC polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria IsdC proteins.

[0068] The term "SasF protein" refers to a protein that includes isolated wild-type SasF polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria SasF proteins.

[0069] The term "vWbp protein" refers to a protein that includes isolated wild-type vWbp (von Willebrand factor binding protein) polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria vWbp proteins.

[0070] The term "vWh protein" refers to a protein that includes isolated wild-type vWh (von Willebrand factor binding protein homolog) polypeptides from staphylococcus bacteria and segments thereof, as well as variants that stimulate an immune response against staphylococcus bacteria vWh proteins.

[0071] An immune response refers to a humoral response, a cellular response, or both a humoral and cellular response in an organism. An immune response can be measured by assays that include, but are not limited to, assays measuring the presence or amount of antibodies that specifically recognize a protein or cell surface protein, assays measuring T-cell activation or proliferation, and/or assays that measure modulation in terms of activity or expression of one or more cytokines.

[0072] In certain examples, a polypeptide or segment/fragment can have a sequence that is at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% or more identical to the amino acid sequence of the reference polypeptide. The term "similarity" refers to a polypeptide that has a sequence that has a certain percentage of amino acids that are either identical with the reference polypeptide or constitute conservative substitutions with the reference polypeptides.

[0073] The polypeptides described herein may include or exclude amino acids at positions 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, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids, or any range derivable therein, of SEQ ID NOs:1-6.

[0074] A polypeptide segment as described herein may include 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, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids, or any range derivable therein, of SEQ ID NOs:1-6.

[0075] The compositions may be formulated in a pharmaceutically acceptable composition. In certain examples of the disclosure the staphylococcus bacterium is an *S. aureus* bacterium.

[0076] In further examples, a composition may be administered more than one time to the subject, and may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more times. The

administration of the compositions include, but is not limited to oral, parenteral, subcutaneous, intramuscular, intravenous, or various combinations thereof, including inhalation or aspiration.

[0077] In still further examples, a composition comprises a recombinant nucleic acid molecule encoding a polypeptide described herein or segments/fragments thereof. Typically a recombinant nucleic acid molecule encoding a polypeptide described herein contains a heterologous promoter. In certain examples, a recombinant nucleic acid molecule of the disclosure is a vector, in still other examples the vector is a plasmid. In certain examples the vector is a viral vector. In certain examples a composition includes a recombinant, non-staphylococcus bacterium containing or expressing a polypeptide described herein. In particular examples the recombinant non-staphylococcus bacteria is *Salmonella* or another gram-positive bacteria. A composition is typically administered to mammals, such as human subjects, but administration to other animals that are capable of eliciting an immune response is contemplated. In further examples the staphylococcus bacterium containing or expressing the polypeptide is *Staphylococcus aureus*. In further examples the immune response is a protective immune response.

[0078] In further examplesa composition comprises a recombinant nucleic acid molecule encoding all or part of one or more of a Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, SpA, vWbp, or vWh protein or peptide or variant thereof. Additional staphylococcal antigens that can be used in combination with the polypeptides described herein include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. In particular examples, a bacteria is a recombinant non-staphylococcus bacteria, such as a *Salmonella* or other gram-positive bacteria.

[0079] Compositions of the disclosure are typically administered to human subjects, but administration to other animals that are capable of eliciting an immune response to a

staphylococcus bacterium is contemplated, particularly cattle, horses, goats, sheep and other domestic animals, *i.e.*, mammals.

[0080] In certain examples the staphylococcus bacterium is a *Staphylococcus aureus*. In further examples the immune response is a protective immune response. In still further examples, the methods and compositions of the disclosure can be used to prevent, ameliorate, reduce, or treat infection of tissues or glands, *e.g.*, mammary glands, particularly mastitis and other infections. Other methods include, but are not limited to prophylactically reducing bacterial burden in a subject not exhibiting signs of infection, particularly those subjects suspected of or at risk of being colonized by a target bacteria, *e.g.*, patients that are or will be at risk or susceptible to infection during a hospital stay, treatment, and/or recovery.

[0081] Any example discussed with respect to one example of the disclosure applies to other examples of the disclosure as well. In particular, any example discussed in the context of a SpA variant polypeptide or peptide or nucleic acid may be implemented with respect to other antigens, such as Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein (or nucleic acids), and vice versa. It is also understood that any one or more of Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689),

SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein can be specifically excluded from a claimed composition.

[0082] Examples of the disclosure include compositions that contain or do not contain a bacterium. A composition may or may not include an attenuated or viable or intact staphylococcal bacterium. In certain examples, the composition comprises a bacterium that is not a staphylococcal bacterium or does not contain staphylococcal bacteria. In certain examples a bacterial composition comprises an isolated or recombinantly expressed staphylococcal Protein A variant or a nucleotide encoding the same. The composition may be or include a recombinantly engineered staphylococcus bacterium that has been altered in a way that comprises specifically altering the bacterium with respect to a secreted virulence factor or cell surface protein. For example, the bacteria may be recombinantly modified to express more of the virulence factor or cell surface protein than it would express if unmodified.

[0083] The term "isolated" can refer to a nucleic acid or polypeptide that is substantially free of cellular material, bacterial material, viral material, or culture medium (when produced by recombinant DNA techniques) of their source of origin, or chemical precursors or other chemicals (when chemically synthesized). Moreover, an isolated compound refers to one that can be administered to a subject as an isolated compound; in other words, the compound may not simply be considered "isolated" if it is adhered to a column or embedded in an agarose gel. Moreover, an "isolated nucleic acid fragment" or "isolated peptide" is a nucleic acid or protein fragment that is not naturally occurring as a fragment and/or is not typically in the functional state.

[0084] Moieties of the disclosure, such as polypeptides, peptides, antigens, or immunogens, may be conjugated or linked covalently or noncovalently to other moieties such as adjuvants, proteins, peptides, supports, fluorescence moieties, or labels. The term "conjugate" or "immunoconjugate" is broadly used to define the operative association of one moiety with another agent and is not intended to refer solely to any type of operative association, and is particularly not limited to chemical "conjugation." Recombinant fusion proteins are particularly contemplated. Compositions of the disclosure may further comprise an adjuvant or a pharmaceutically acceptable excipient. In some examples, the adjuvant comprises alum or an adjuvant described herein. An adjuvant may be covalently or non-covalently coupled to a polypeptide or peptide of the disclosure. In certain examples, the adjuvant is chemically conjugated to a protein, polypeptide, or peptide.

[0085] The term "providing" is used according to its ordinary meaning to indicate "to supply or furnish for use." In some examples, the protein is provided directly by administering the protein, while in other examples, the protein is effectively provided by administering a nucleic acid that encodes the protein. In certain examples the disclosure contemplates compositions comprising various combinations of nucleic acid, antigens, peptides, and/or epitopes.

[0086] The subject will have (*e.g.*, are diagnosed with a staphylococcal infection), will be suspected of having, or will be at risk of developing a staphylococcal infection. Compositions of the present disclosure include immunogenic compositions wherein the antigen(s) or epitope(s) are contained in an amount effective to achieve the intended purpose. More specifically, an effective amount means an amount of active ingredients necessary to stimulate or elicit an immune response, or provide resistance to, amelioration of, or mitigation of infection. In more specific examples, an effective amount prevents, alleviates or ameliorates symptoms of disease or infection, or prolongs the survival of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any preparation used in the methods of the disclosure, an effective amount or dose can be estimated initially from *in vitro* studies, cell culture, and/or animal model assays. For example, a dose can be formulated in animal models to achieve a desired immune response or circulating antibody concentration or titer. Such information can be used to more accurately determine useful doses in humans.

[0087] In some examples, the polypeptide of the disclosure (SpA variant) provides a histamine release of less than or equal to 20, 19.5, 19, 18.5, 18, 17.5, 17, 16.5, 16, 15.5, 15, 14.5, 14, 13.5, 13, 12.5, 12, 11.5, 11, 10.5, 10, 9.5, 9, 8.5, 8, 7.5, 7, 6.5, 6, 5.5, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5, 1, 0.5, 0.4, 0.3, 0.2, 0.1, or 0% (or any derivable range therein) in a basophil histamine release assay.

[0088] The examples in the Example section are understood to be examples of the disclosure that are applicable to all examples of the disclosure.

[0089] 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.

[0090] 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.

[0091] Following long-standing patent law, the words "a" and "an," when used in conjunction with the word "comprising" in the claims or specification, denotes one or more, unless specifically noted.

[0092] Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific examples of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE DRAWINGS

[0093] So that the matter in which the above-recited features, advantages and objects of the invention as well as others which will become clear are attained and can be understood in detail, more particular descriptions and certain embodiments of the invention briefly summarized above are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate certain embodiments of the invention and therefore are not to be considered limiting in their scope.

[0094] **FIGs. 1A-E.** ***Staphylococcus aureus*** **ST88 isolate WU1, a mouse pathogen.** (**A**) Domain structure and sequence homology of the *vwb* gene products from *S. aureus* WU1 and *S. aureus* Newman, a human clinical isolate. The percent amino acid (a.a.) identity of vWbp for its signal peptide (S), D1 and D2 domains (responsible for binding and activation of host prothrombin), linker (white box) and C-terminal fibrinogen binding domain (C) is displayed. (**B**) Immunoblot of *S. aureus* whole culture samples of strains Newman (WT, wild-type) as well as its Δ*coa*, Δ*vwb*, Δ*coa-vwb*, and Δ*clfA* variants, strains WU1, JSNZ, USA300 LAC and its Δ*vwb* variant were analyzed for the production of vWbp (αvWbp), Coa (αCoa), Hla (αHla), and ClfA (αClfA) using polyclonal rabbit antibodies. (**C**) Polyclonal antibodies against the vWbp-C domain identify the vWbp allelic variant from strains JSNZ and WU1 as well as vWbp from strain USA300 LAC. (**D-E**) Agglutination of Syto-9 stained *S. aureus* strains in human (**D**) or mouse (**E**) plasma was measured as average size and standard error of the means of clumped bacteria in 12 fields of microscopic view and statistical significance was assessed in

CLAIMS

1. An isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues in each of domains A-E corresponding to positions 9 and 10 in domain D and (ii) glutamate substitutions in each of domains A-E corresponding to position 33 in domain D, wherein the polypeptide does not, relative to a negative control, detectably crosslink IgG and IgE in blood or activate basophils or mast cells.

2. An isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues in each of domains A-E corresponding to positions 9 and 10 in domain D and (ii) threonine substitutions in each of domains A-E corresponding to position 33 in domain D, wherein the polypeptide does not, relative to a negative control, detectably crosslink IgG and IgE in blood or activate basophils or mast cells.

3. The isolated polypeptide of claim 1 or 2, wherein:

(A) the K_A binding affinity for VH3 from human IgG is reduced as compared to a SpA variant consisting of lysine substitutions for glutamine residues in each of domains A-E corresponding to position 9 and 10 in domain D and alanine substitutions for aspartic acid in domains A-E corresponding to position 36 and 37 of domain D (SpA_{KKAA}); and/or

(B) the isolated polypeptide has a K_A binding affinity for VH3 from human IgG that is reduced by at least 2-fold as compared to SpA_{KKAA}; and/or

(C) the isolated polypeptide has a K_A binding affinity for VH3 from human IgG is less than 1×10^5 M^{-1}; and/or

(D) the SpA variant does not have substitutions in any of the domains A-E corresponding to positions 36 and 37 in domain D; and/or

(E) the only substitititions in the SpA variant are (i) and (ii); and/or

(F) the isolated polypeptide consists of the SpA variant.

4. A composition comprising the isolated polypeptide of any of the preceding claims, wherein the composition contains less than 1% by weight of the total protein amount a protein other than the isolated polypeptide, optionally wherein the composition further comprises:

(A) an adjuvant, optionally wherein the isolated polypeptide is coupled to an adjuvant and/or wherein the adjuvant comprises alum; and/or

(B) a pharmaceutically acceptable excipient.

5. Use of a composition in the manufacture of a medicament for eliciting a safe immune response against a Staphylococcus bacteria in a human patient, wherein the composition is to be administered to the human patient, wherein the composition is the composition of claim 4.

6. The use according to claim 5, wherein:

(A) the Staphylococcus bacteria comprises type ST88 isolate or any other human Staphylococcus bacterium now adapted to an animal; and/or

(B) the Staphylococcus bacteria comprises the WU1 or JSNZ strain of *Staphylococcus aureus*; and/or

(C) the human patient is:

(a) a pediatric patient, optionally wherein the pediatric patient is 2 years old or less; or

(b) 65 years old or older; and/or

(c) a health care worker; and/or

(D) the isolated polypeptide of the composition is to be administered in four doses and wherein the interval between doses is at least four weeks.

7. An isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues corresponding to positions 9 and 10 in each of domains A-E and (ii) at least one other amino acid substitution corresponding to position 33 and/or 29 in SEQ ID NO:2 in each of domains A-E, wherein the SpA variant has a K_D binding affinity for VH3 from human IgG that is greater than 1.0×10^{-4} M and/or a K_D binding affinity for VH3 from human IgE that is greater than 1.0×10^{-6} M.

8. The isolated polypeptide of claim 7, wherein:

(A) the variant SpA comprises:

(a) an amino acid substitution corresponding to position 33 in SEQ ID NO:2 in each of domains A-E; or

(b) an amino acid substitution corresponding to position 29 in SEQ ID NO:2 in each of domains A-E; or

(c) an amino acid substitution corresponding to positions 29 and 33 in SEQ ID NO:2 in each of domains A-E; and/or

(B) the variant SpA comprises an amino acid substitution corresponding to one or both of positions 36 and 37 in SEQ ID NO:2 in each of domains A-E, optionally wherein the variant SpA comprises amino acid substitutions corresponding to both positions 36 and 37 of SEQ ID NO:2 in each of domains A-E, further optionally wherein the amino acid substitutions corresponding to positions 36 and 37 are alanine residues for aspartic acid residues; and/or

(C) the variant SpA comprises variant A-E domains that are at least 70%, at least 80%, or at least 90% identical to the amino acid sequence of SEQ ID NO:2, optionally wherein:

(a) the variant SpA comprises variant A-E domains that do not comprise any amino acid substitutions in SEQ ID NO:2 except at corresponding positions 33, 9, 10, 29, 36, and/or 37; and/or

(b) the variant SpA comprises variant A-E domains consisting only of amino acid substitutions corresponding to:

(I) positions 9, 10, and 33 in SEQ ID NO:2; or

(II) positions 9, 10, and 29 in SEQ ID NO:2; or

(III) positions 9, 10, 29, and 33 in SEQ ID NO:2; or

(IV) positions 9, 10, 29, 36, and 37 in SEQ ID NO:2; or

(V) positions 9, 10, 33, 36, and 37 in SEQ ID NO:2; or

(VI) positions 9, 10, 29, 33, 36, and 37 in SEQ ID NO:2 and/or

(D) the substitution of the amino acid corresponding to:

(a) position 33 is phenylalanine, glutamic acid, or glutamine; or

(b) position 29 is alanine, leucine, proline, phenylalanine, glutamic acid, arginine, lysine, serine, threonine or glutamine, optionally wherein the substitution of the amino acid corresponding to position 29 is alanine, phenylalanine, or arginine; or

(c) position 33 is alanine, phenylalanine, glutamic acid, lysine, or glutamine; and/or

and/or

(E) the SpA variant has a K_D binding affinity for VH3 that is greater than 1.0 x 10^{-2} M; and/or

(F) the isolated polypeptide further comprises a non-Protein A segment, optionally wherein:

the non-Protein A segment is a second antigen segment, further optionally wherein the second antigen segment is a staphylococcal antigen segment;

further optionally wherein the staphylococcal antigen segment is an Emp, EsxA, EsxB, EsaC, Eap, Ebh, EsaB, Coa, vWbp, vWh, Hla, SdrC, SdrD, SdrE, IsdA, IsdB, IsdC, ClfA, ClfB, and/or SasF segment.

9. A composition comprising the isolated polypeptide of claim 7 or 8, wherein the composition is capable of stimulating an immune response in a subject in need thereof, optionally wherein:

(a) the composition contains less than 1% by weight of the total protein amount a protein other than the isolated polypeptide; and/or

(b) the composition further comprises an adjuvant, optionally wherein the isolated polypeptide is coupled to an adjuvant, optionally wherein the adjuvant comprises alum; and/or

(c) the composition further comprises a pharmaceutically acceptable excipient.

10. Use of a composition in the manufacture of a medicament for preventing or treating staphylococcal infection, wherein the composition is to be administered to a patient in need thereof, wherein the composition is the isolated polypeptide of claim 7 or 8 or the composition of claim 9.

11. The use according to claim 10, wherein:

(A) the Staphylococcus bacteria comprises type ST88 isolate or any other human Staphylococcus bacterium now adapted to an animal; and/or

(B) the Staphylococcus bacteria comprises the WU1 or JSNZ strain of *Staphylococcus aureus*; and/or

(C) the human patient is:

(a) a pediatric patient, optionally wherein the pediatric patient is 2 years old or less; or

(b) 65 years old or older; and/or

(c) a health care worker; and/or

(D) the isolated polypeptide or composition is to be administered in four doses and wherein the interval between doses is at least four weeks.

12. Use of a composition in the manufacture of a medicament for eliciting an immune response against a staphylococcus bacterium in a subject, wherein the composition comprises the isolated polypeptide of claim 7 or 8 or the composition of claim 9, wherein an effective amount of the isolated polypeptide or the composition is to be provided to the subject.

13. The use of claim 12, wherein:

(A) the subject is also to be administered an adjuvant; and/or

(B) the composition comprises an adjuvant, optionally wherein the SpA variant is coupled to an adjuvant, further optionally wherein the adjuvant comprises alum; and/or

(C) the composition is formulated in a pharmaceutically acceptable composition; and/or

(D) the staphylococcus bacterium is a *S. aureus* bacterium, optionally wherein the staphyococcus bacterium is resistant to one or more treatments, further optionally wherein the bacterium is methicillin resistant; and/or

(E) the isolated polypeptide or composition is to be further administered more than one time to the subject; and/or

(F) the composition is to be administered orally, parenterally, subcutaneously, intramuscularly, or intravenously; and/or

(G) a composition comprising a second staphylococcal antigen is to be further administered to the subject, optionally wherein the second staphylococcal antigen is one or more of Emp, EsxA, EsxB, EsaC, Eap, Ebh, EsaB, Coa, vWbp, vWh, Hla, SdrC, SdrD, SdrE, IsdA, IsdB, IsdC, ClfA, ClfB, and SasF; and/or

(H) the subject is a mammal, optionally the subject is human; and/or

(I) the immune response is a protective immune response; or

(J) the subject does not exhibit any signs of toxicity from the isolated polypeptide or composition, optionally wherein the subject does not exhibit any signs of anaphylactic shock from the isolated polypeptide or composition;

and/or

(K) the Staphylococcus bacteria comprises type ST88 isolate or any other human Staphylococcus bacterium now adapted to an animal; and/or

(L) the Staphylococcus bacteria comprises the WU1 or JSN4Z strain of *Staphylococcus aureus*; and/or

(M) the subject is a human patient and is:

(a) a pediatric patient, optionally wherein the pediatric patient is 2 years old or less; or

(b) 65 years old or older; and/or

(c) a health care worker; and/or

(N) the isolated polypeptide or composition is to be administered in four doses and wherein the interval between doses is at least four weeks.

14. Use of a composition in the manufacture of a medicament for treating a staphylococcal infection in a subject, wherein the composition comprises an isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues corresponding to positions 9 and 10 in each of domains A-E and (ii) at least one other amino acid substitution corresponding to position 33 and/or 29 in SEQ ID NO:2 in each of domains A-E, wherein the SpA variant has a K_D binding affinity for IgG that is greater than 1.0×10^{-5} M, and wherein the composition is to be administered to a subject having, suspected of having or at risk of developing a staphylococcal infection an effective amount.

15. The use of claim 14, wherein:

(A) the variant SpA comprises an amino acid substitution corresponding to position 33 in SEQ ID NO:2 in each of domains A-E; or

(B) the variant SpA comprises an amino acid substitution corresponding to position 29 in SEQ ID NO:2 in each of domains A-E; or

(C) the variant SpA comprises an amino acid substitution corresponding to positions 29 and 33 in SEQ ID NO:2 in each of domains A-E; and/or

(D) the variant SpA comprises an amino acid substitution corresponding to one or both of positions 36 and 37 in SEQ ID NO:2 in each of domains A-E, optionally wherein the variant SpA comprises amino acid substitutions corresponding to both positions 36 and 37 of SEQ ID NO:2 in each of domains A-E and/or wherein the

amino acid substitutions corresponding to positions 36 and 37 are alanine residues for aspartic acid residues; and/or

(E) the variant SpA comprises variant A-E domains that are at least 70%, at least 80%, or at least 90% identical to the amino acid sequence of SEQ ID NO:2, optionally wherein

(a) the variant SpA comprises variant A-E domains that do not comprise any amino acid substitutions in SEQ ID NO:2 except at corresponding positions 33, 9, 10, 29, 36, and/or 37; and/or

(b) the variant SpA comprises variant A-E domains consisting only of amino acid substitutions corresponding to:

(I) positions 9, 10, and 33 in SEQ ID NO:2; or

(II) positions 9, 10, and 29 in SEQ ID NO:2; or

(III) positions 9, 10, 29, and 33 in SEQ ID NO:2; or

(IV) positions 9, 10, 29, 36, and 37 in SEQ ID NO:2; or

(V) positions 9, 10, 33, 36, and 37 in SEQ ID NO:2; or

(VI) positions 9, 10, 29, 33, 36, and 37 in SEQ ID NO:2; and/or

(F) the substitution of the amino acid corresponding to:

(a) position 33 is phenylalanine, glutamic acid, or glutamine; and/or

(b) position 29 is alanine, leucine, proline, phenylalanine, glutamic acid, arginine, lysine, serine, threonine or glutamine, optionally wherein the substitution of the amino acid corresponding to position 29 is alanine, phenylalanine, or arginine; or

(c) position 33 is alanine, phenylalanine, glutamic acid, lysine, or glutamine; and/or

(G) the SpA variant has a K_D binding affinity for VH3 that is greater than 1.0 x 10^{-2} M; and/or(H) a non-Protein A segment, optionally wherein the non-Protein A segment is a second antigen segment;

further optionally wherein the second antigen segment is a staphylococcal antigen segment;

further optionally wherein the staphylococcal antigen segment is an Emp, EsxA, EsxB, EsaC, Eap, Ebh, EsaB, Coa, vWbp, vWh, Hla, SdrC, SdrD, SdrE, IsdA, IsdB, IsdC, ClfA, ClfB, and/or SasF segment; and/or

(I) the subject is diagnosed with a persistent staphylococcal infection; and/or

(J) the SpA variant elicits production of an antibody that binds Protein A in the subject; and/or

(K) the isolated polypeptide is to be administered with an adjuvant, optionally wherein the isolated polypeptide is coupled to an adjuvant; and/or

(L) the isolated polypeptide is formulated in a pharmaceutically acceptable composition; and/or

(M) a second staphylococcal antigen is to be administered, optionally wherein

(a) the second staphylococcal antigen is to be administered concurrently with the isolated polypeptide; optionally wherein the second staphylococcal antigen and the isolated polypeptide are to be administered in the same composition; further optionally wherein the second staphylococcal antigen is fused with the SpA variant; and/or

(b) the second staphylococcal antigen is one or more of Emp, EsxA, EsxB, EsaC, Eap, Ebh, EsaB, Coa, vWbp, vWh, Hla, SdrC, SdrD, SdrE, IsdA, IsdB, IsdC, ClfA, ClfB, and SasF peptide; and/or

(N) the staphylococcal infection is a *Staphylococcus aureus* infection; and/or

(O) the peptide is to be administered orally, parenterally, transdermally, transmucosally, subcutaneously, intramuscularly, or by inhalation; and/or

(P) the isolated polypeptide is to be further administered more than one time to the subject; and/or

(Q) the subject:

(a) is a mammal, optionally wherein the subject is human; or

(b) does not exhibit any signs of toxicity from the isolated polypeptide or composition, optionally wherein the subject does not exhibit any signs of anaphylactic shock from the isolated polypeptide or composition; and/or

(R) the Staphylococcus bacteria comprises type ST88 isolate or any other human Staphylococcus bacterium now adapted to an animal; and/or

(S) the Staphylococcus bacteria comprises the WU1 or JSN4Z strain of *Staphylococcus aureus*; and/or

(T) the subject is a human patient, wherein the human patient is:

(a) a pediatric patient, optionally wherein the pediatric patient is 2 years old or less; or

(b) 65 years old or older; and/or

(c) a health care worker; and/or

(U) the isolated polypeptide or composition is to be administered in four doses and wherein the interval between doses is at least four weeks.

16. Use of a composition in the manufacture of a medicament for decolonization or for preventing colonization or recolonization of Staphylococcus bacteria in a subject, wherein the composition comprises the isolated polypeptide of claim 7 or 8 or the composition of claim 9, wherein the composition is to be administered to the subject in an effective amount.

17. An isolated polypeptide comprising a variant Protein A (SpA) having (i) lysine substitutions for glutamine residues in each of domains A-E corresponding to positions 9 and 10 in domain D and (ii) glutamate substitutions in each of domains A-E corresponding to position 33 in domain D, wherein the polypeptide does not, relative to a negative control, detectably crosslink IgG and IgE in blood or activate basophils or mast cells; wherein the SpA variant does not have substitutions in any of the domains A-E corresponding to positions 36 and 37 in domain D.

18. The isolated polypeptide of claim 7 or 8 or the composition of claim 4 or 9 for use in therapy.