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(54) **COMPOSITIONS AND METHODS RELATED TO PROTEIN A (SPA) VARIANTS**

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(58) **Field of Classification Search**

None
See application file for complete search history.

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(57) **ABSTRACT**

The present invention concerns methods and compositions for treating or preventing a bacterial infection, particularly infection by a *Staphylococcus* bacterium. The invention provides methods and compositions for stimulating an immune response against the bacteria. In certain embodiments, the methods and compositions involve a non-toxicogenic Protein A (SpA) variant.

9 Claims, 15 Drawing Sheets

Specification includes a Sequence Listing.

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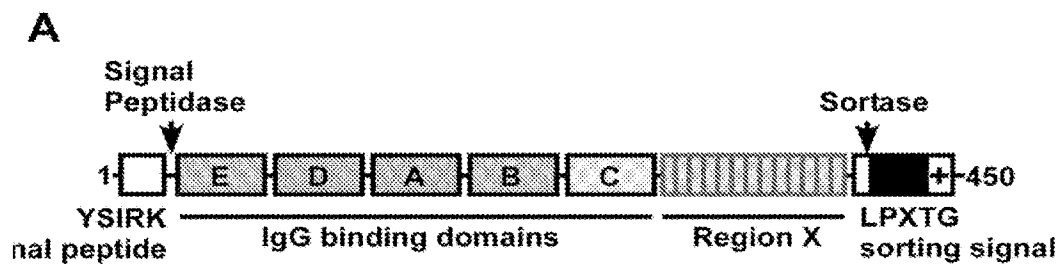


FIG. 1A

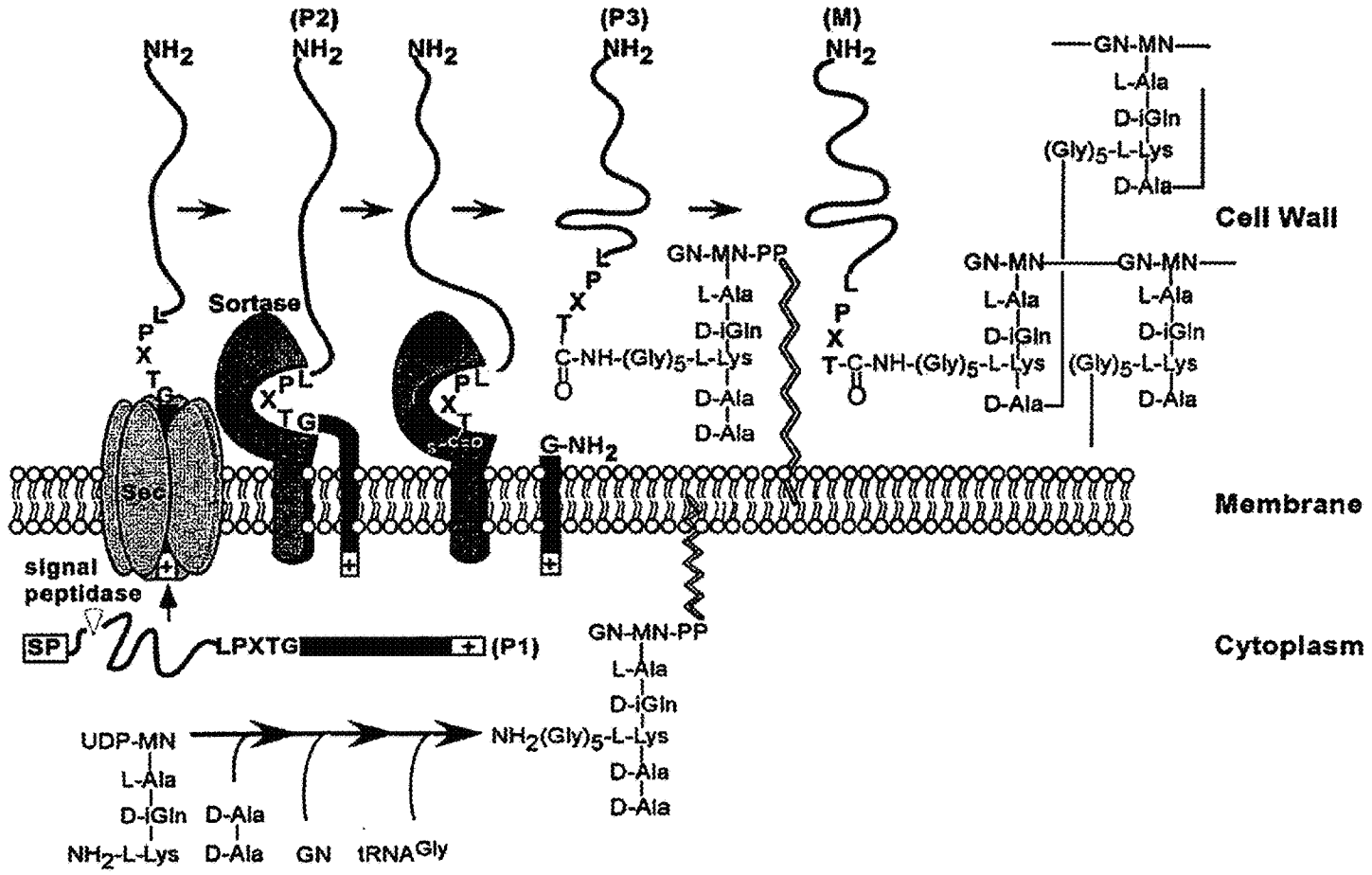
B

FIG. 1B

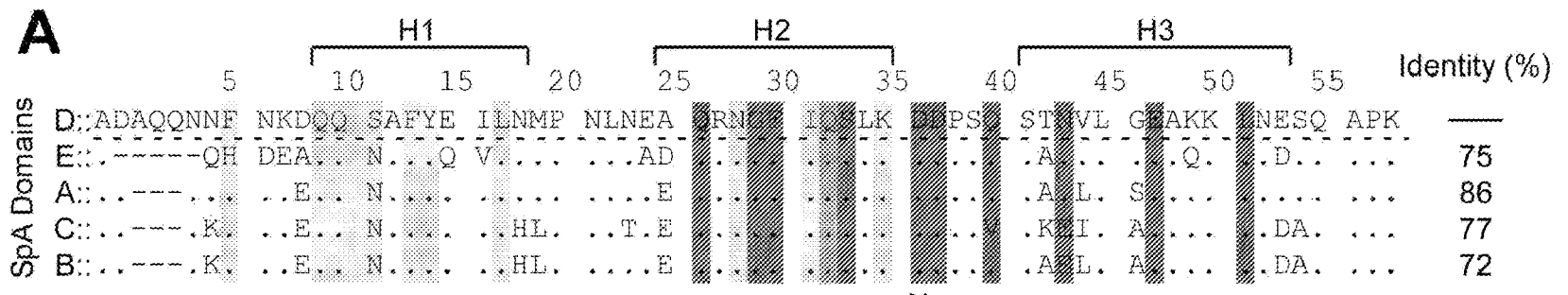


FIG. 2A

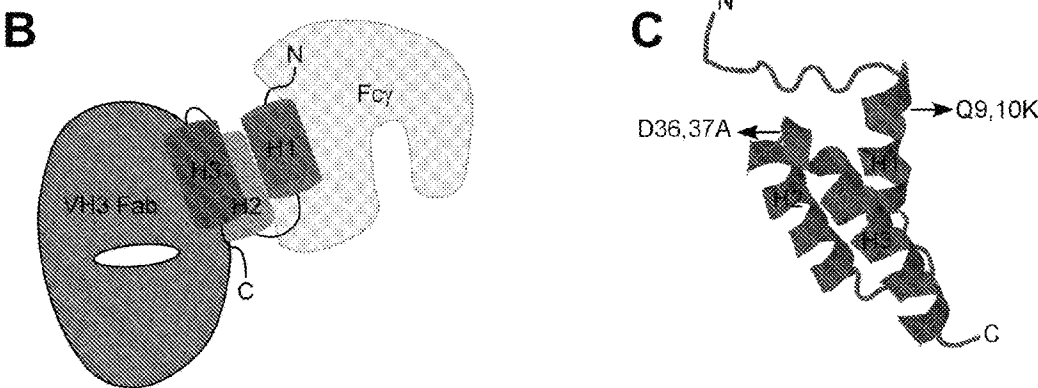


FIG. 2B-2C

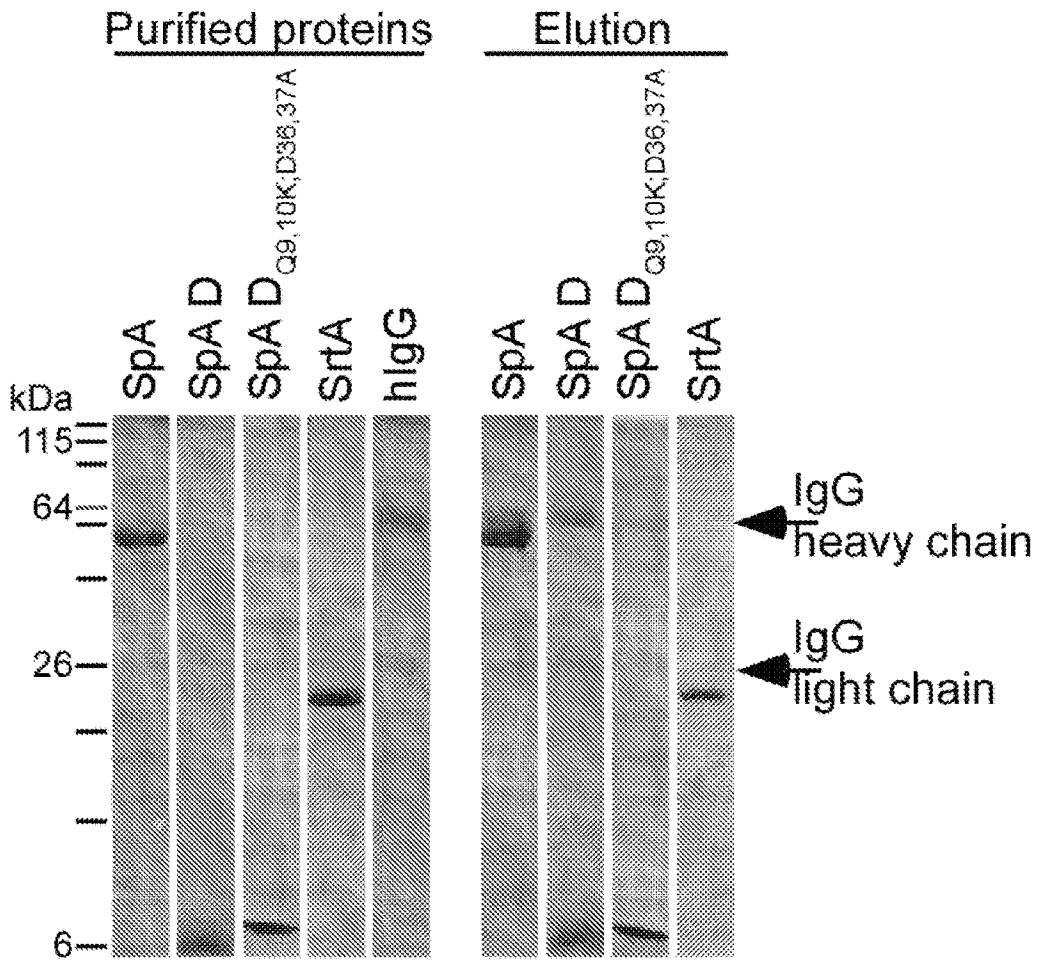


FIG. 3

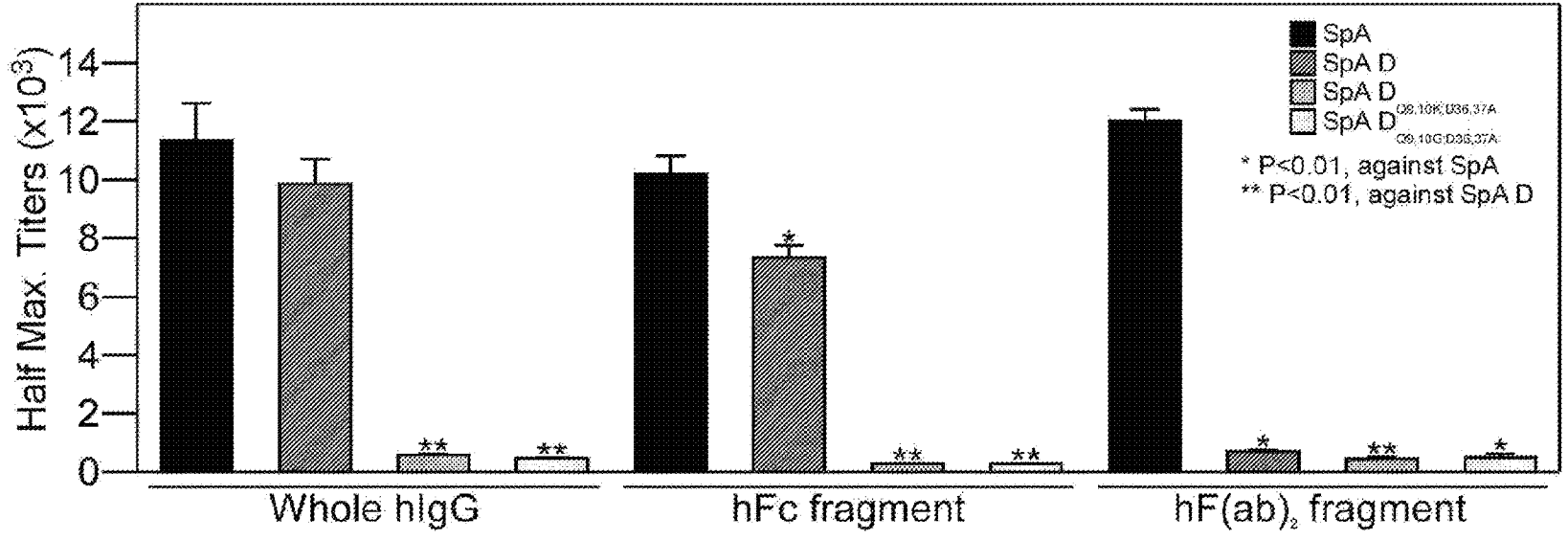


FIG. 4

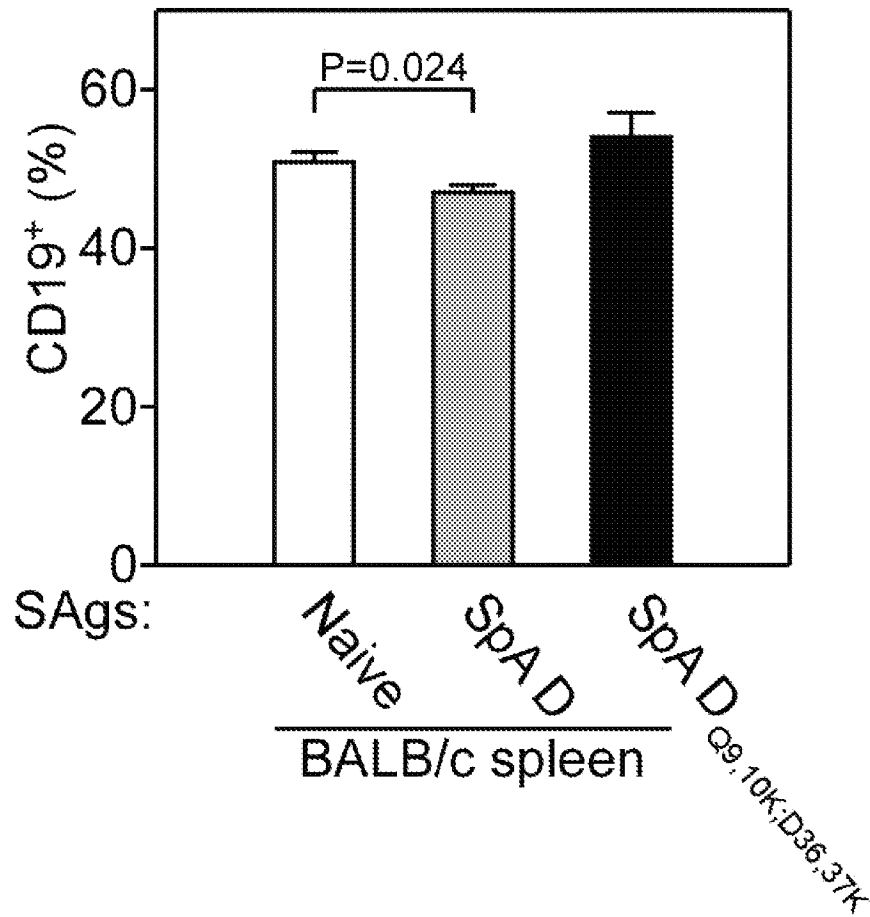


FIG. 5

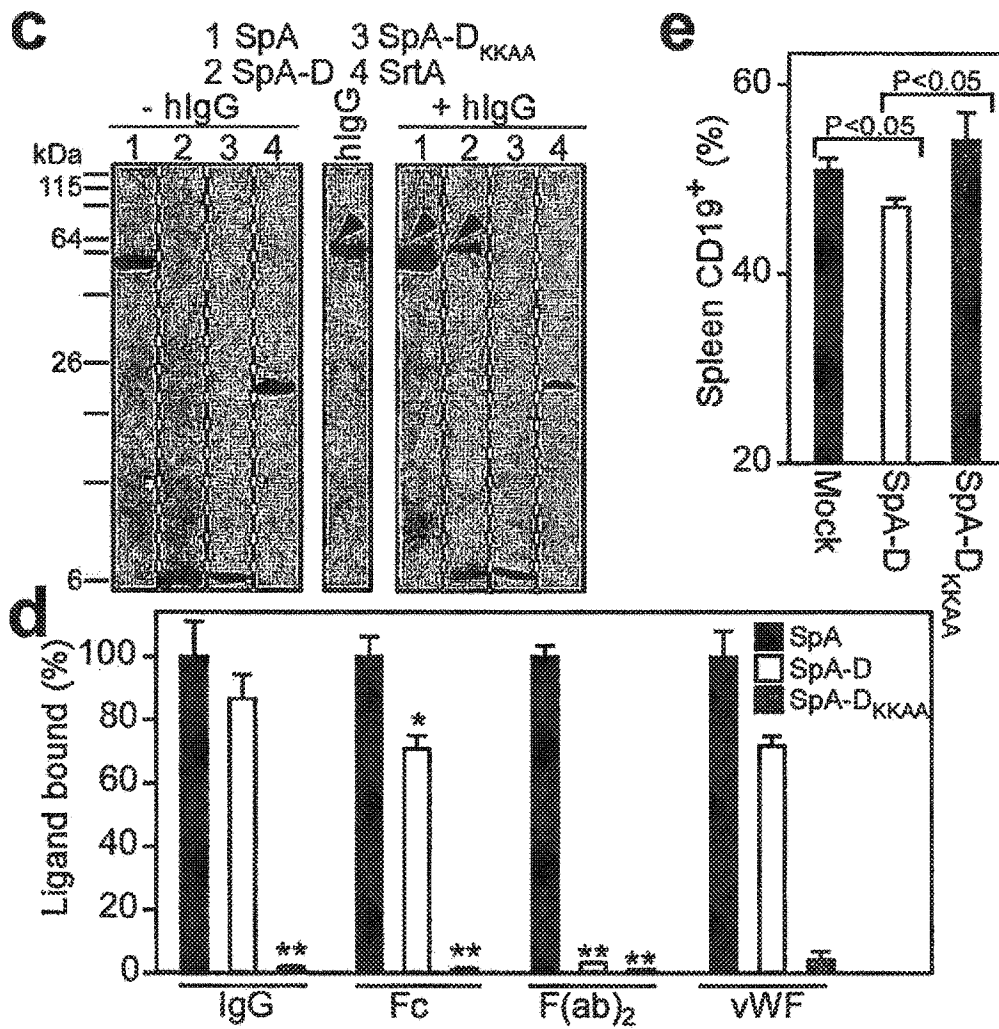


FIG. 6C-6E

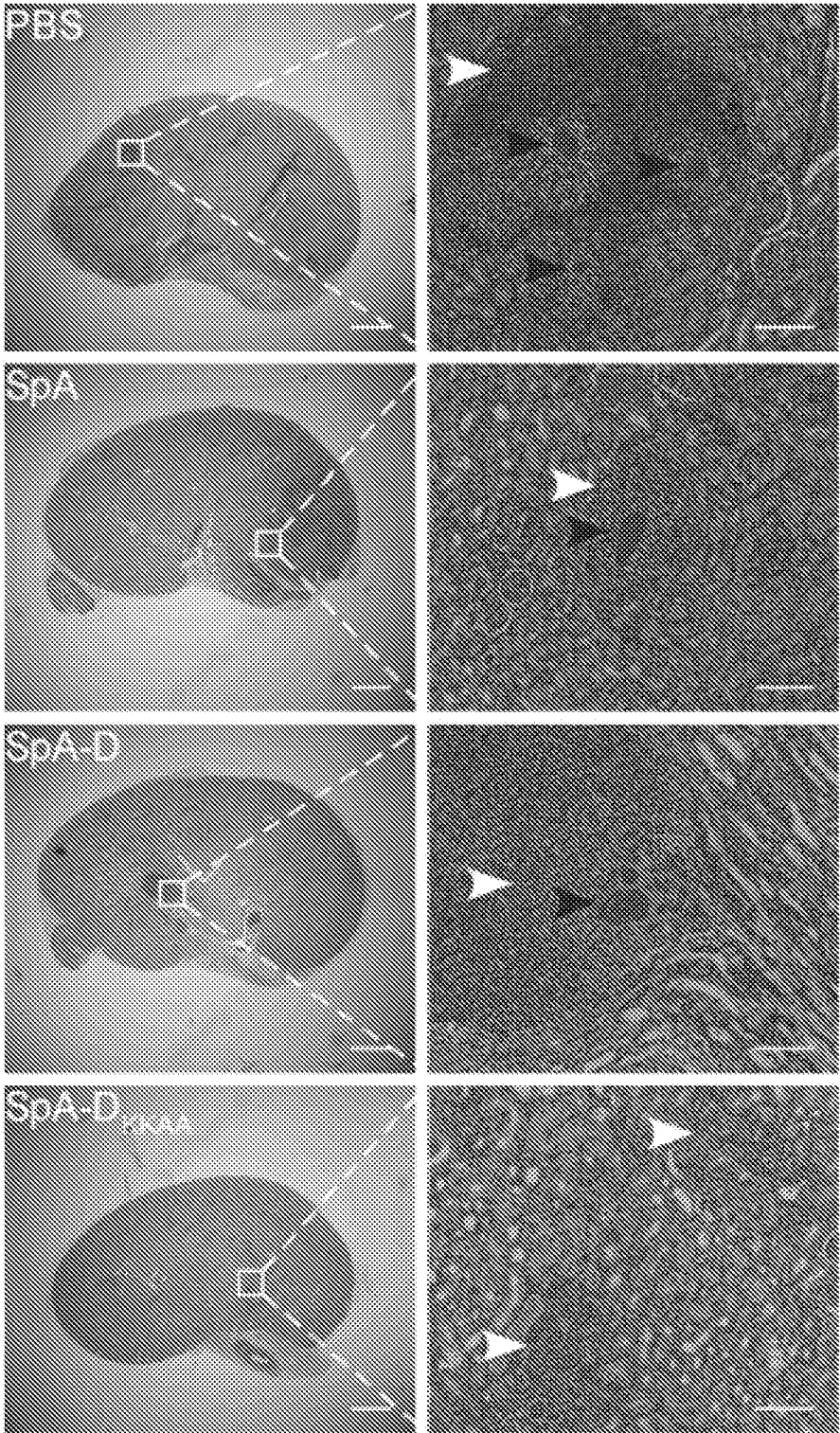


FIG. 7

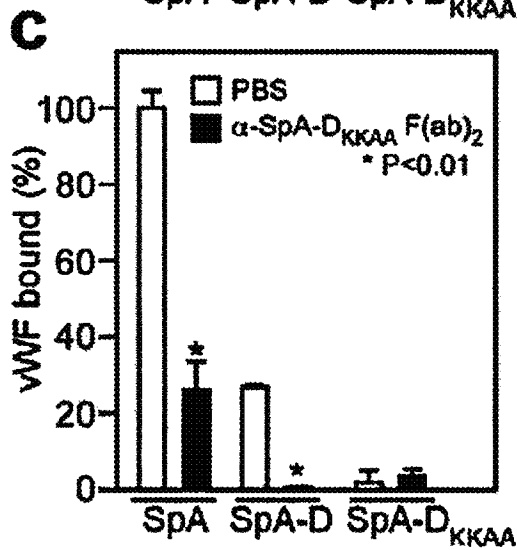
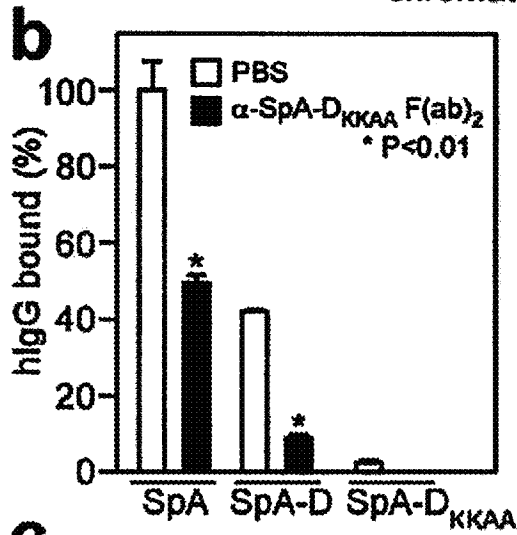
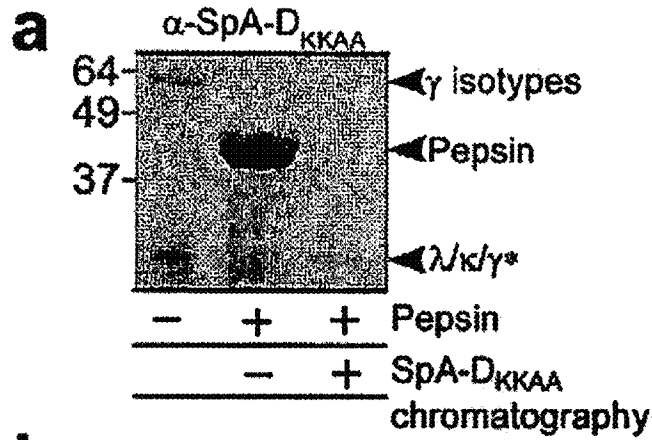


FIG. 8A-8C

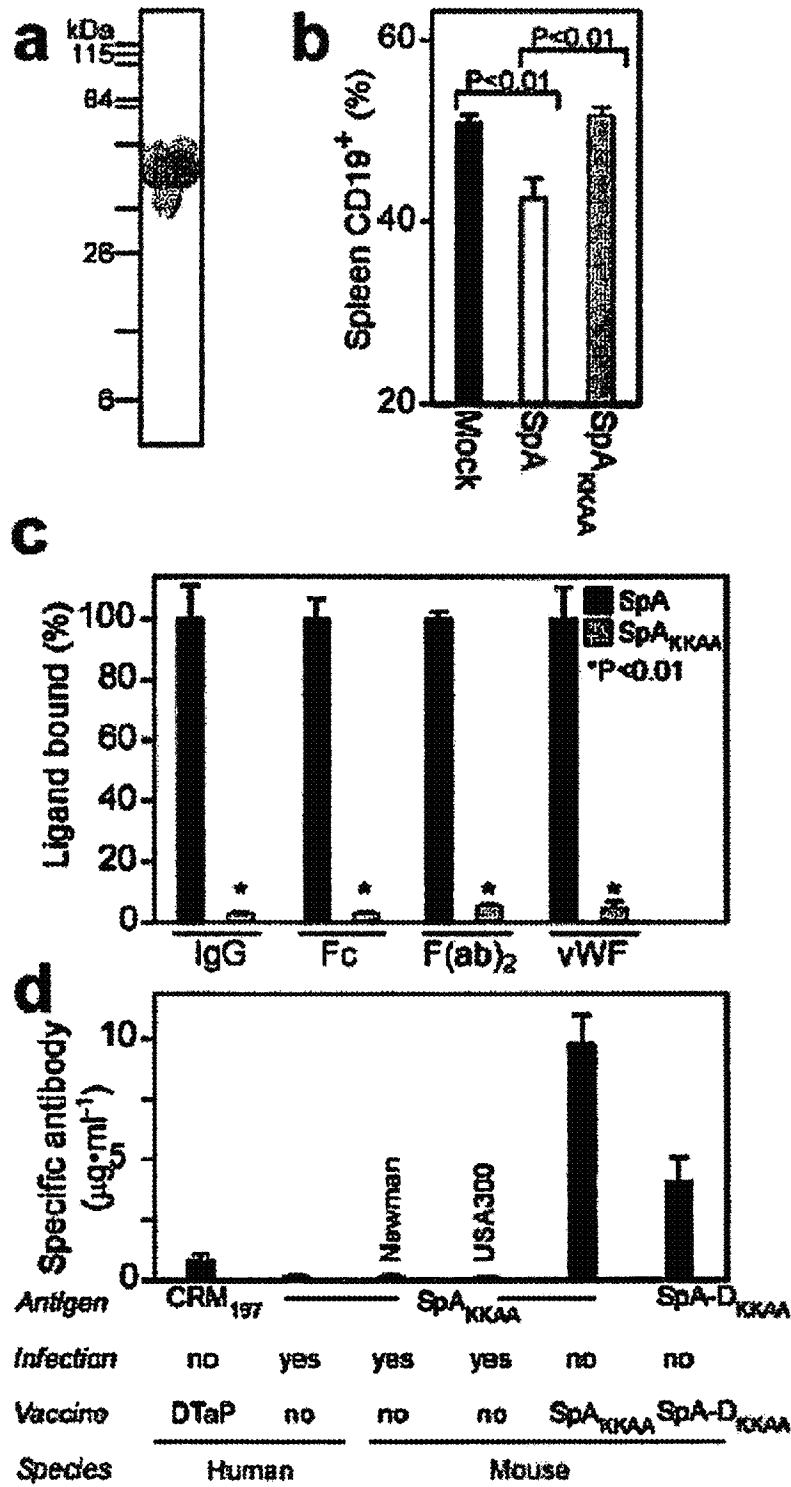


FIG. 9A-9D

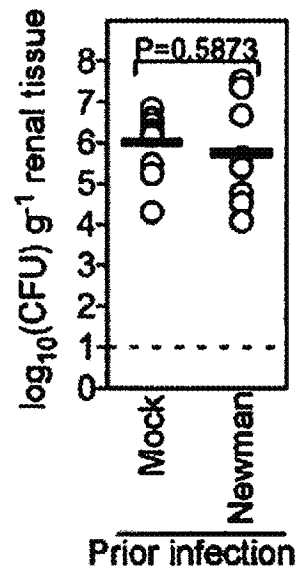


FIG. 10

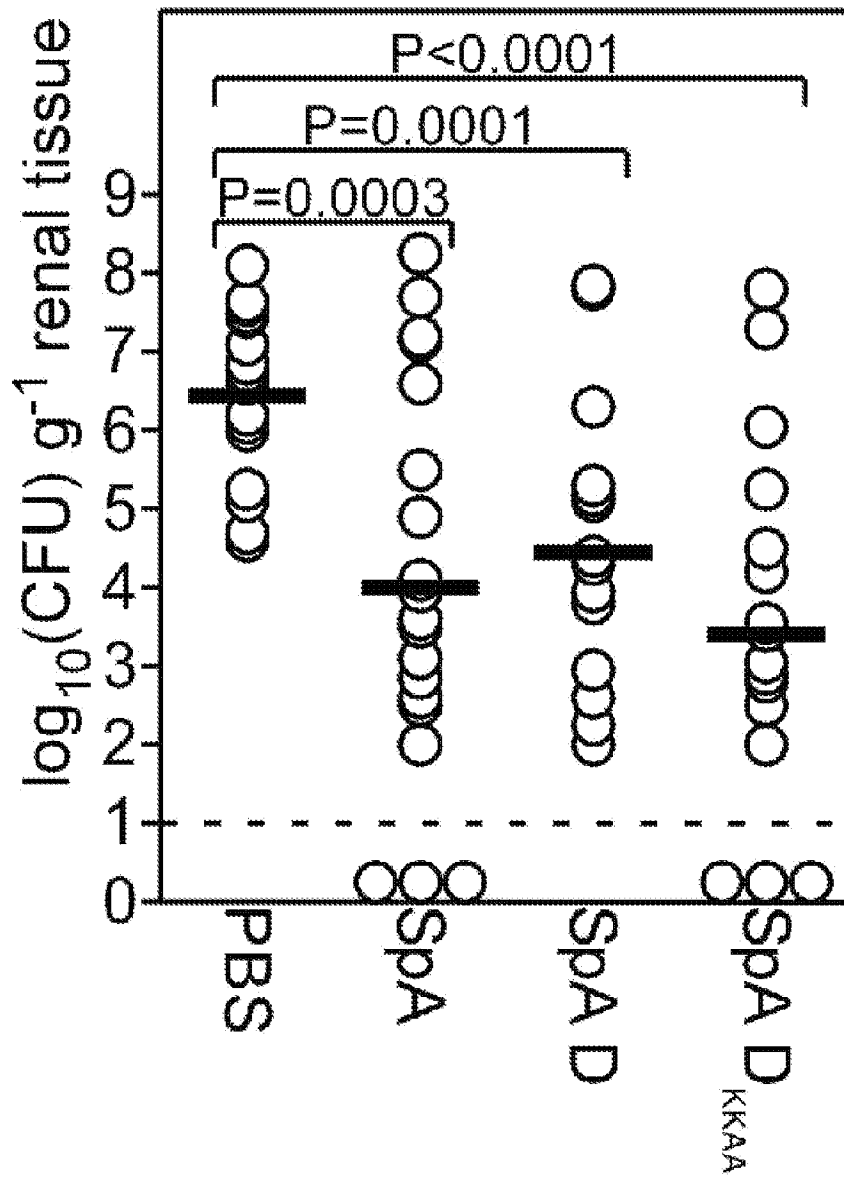


FIG. 11

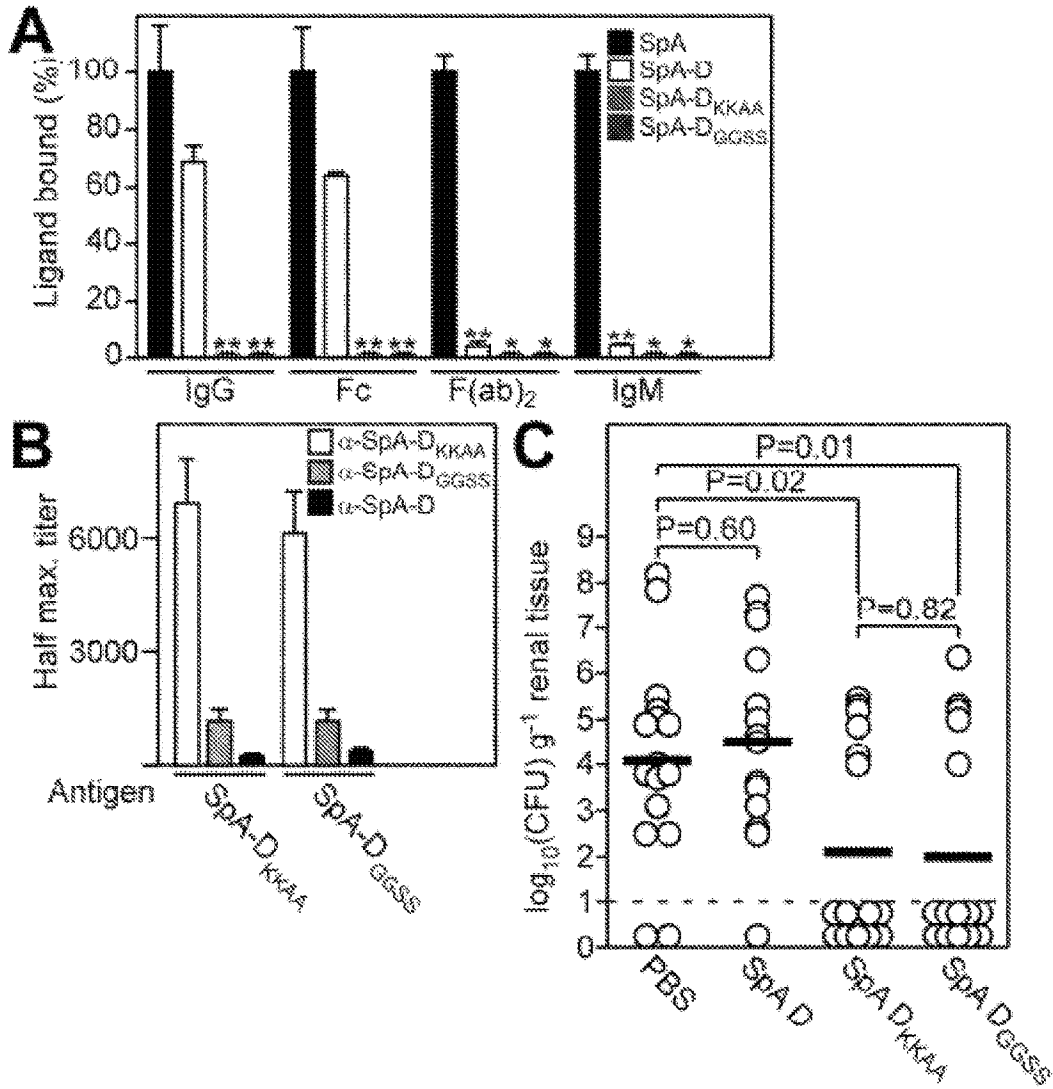


FIG. 12A-12C

COMPOSITIONS AND METHODS RELATED TO PROTEIN A (SPA) VARIANTS

This application is a continuation of U.S. patent application Ser. No. 15/060,861, filed Mar. 4, 2016, which is a continuation of U.S. patent application Ser. No. 14/466,514, filed Aug. 22, 2014, now U.S. Pat. No. 9,315,554, which is a continuation of U.S. patent application Ser. No. 13/807,598, filed Mar. 19, 2013, now U.S. Pat. No. 8,821,894, which is a national phase application under 35 U.S.C. § 371 of International Application No. PCT/US2011/042845, filed Jul. 1, 2011, which claims the benefit of priority to U.S. Provisional Patent Application Ser. No. 61/361,218 filed Jul. 2, 2010, and 61/370,725 filed Aug. 4, 2010. The entire contents of each of the above-referenced disclosures are specifically incorporated herein by reference without disclaimer.

This invention was made with government support under AI057153, AI052474, and GM007281 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

I. Field of the Invention

The present invention relates generally to the fields of immunology, microbiology, and pathology. More particularly, it concerns methods and compositions involving bacterial Protein A variants, which can be used to invoke an immune response against the bacteria.

II. Background

The number of both community acquired and hospital acquired infections have increased over recent years with the increased use of intravascular devices. Hospital acquired (nosocomial) infections are a major cause of morbidity and mortality, more particularly in the United States, where it affects more than 2 million patients annually. The most frequent infections are urinary tract infections (33% of the infections), followed by pneumonia (15.5%), surgical site infections (14.8%) and primary bloodstream infections (13%) (Emorl and Gaynes, 1993).

The major nosocomial pathogens include *Staphylococcus aureus*, coagulase-negative Staphylococci (mostly *Staphylococcus epidermidis*), *enterococcus* spp., *Escherichia coli* and *Pseudomonas aeruginosa*. Although these pathogens cause approximately the same number of infections, the severity of the disorders they can produce combined with the frequency of antibiotic resistant isolates balance this ranking towards *S. aureus* and *S. epidermidis* as being the most significant nosocomial pathogens.

Staphylococci can cause a wide variety of diseases in humans and other animals through either toxin production or invasion. Staphylococcal toxins are also a common cause of food poisoning, as the bacteria can grow in improperly-stored food.

Staphylococcus epidermidis is a normal skin commensal which is also an important opportunistic pathogen responsible for infections of impaired medical devices and infections at sites of surgery. Medical devices infected by *S. epidermidis* include cardiac pacemakers, cerebrospinal fluid shunts, continuous ambulatory peritoneal dialysis catheters, orthopedic devices and prosthetic heart valves.

Staphylococcus aureus is the most common cause of nosocomial infections with a significant morbidity and mor-

ality. It is the cause of some cases of osteomyelitis, endocarditis, septic arthritis, pneumonia, abscesses, and toxic shock syndrome. *S. aureus* can survive on dry surfaces, increasing the chance of transmission. Any *S. aureus* infection can cause the staphylococcal scalded skin syndrome, a cutaneous reaction to exotoxin absorbed into the bloodstream. It can also cause a type of septicemia called pyaemia that can be life-threatening. Problematically, Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major cause of hospital-acquired infections.

S. aureus and *S. epidermidis* infections are typically treated with antibiotics, with penicillin being the drug of choice, whereas vancomycin is used for methicillin resistant isolates. The percentage of staphylococcal strains exhibiting wide-spectrum resistance to antibiotics has become increasingly prevalent, posing a threat for effective antimicrobial therapy. In addition, the recent emergence of vancomycin resistant *S. aureus* strain has aroused fear that MRSA strains are emerging and spreading for which no effective therapy is available.

An alternative to antibiotic treatment for staphylococcal infections is under investigation that uses antibodies directed against staphylococcal antigens. This therapy involves administration of polyclonal antisera (WO00/15238, WO00/12132) or treatment with monoclonal antibodies against lipoteichoic acid (WO98/57994).

An alternative approach would be the use of active vaccination to generate an immune response against staphylococci. The *S. aureus* genome has been sequenced and many of the coding sequences have been identified (WO02/094868, EP0786519), which can lead to the identification of potential antigens. The same is true for *S. epidermidis* (WO01/34809). As a refinement of this approach, others have identified proteins that are recognized by hyperimmune sera from patients who have suffered staphylococcal infection (WO01/98499, WO02/059148).

S. aureus secretes a plethora of virulence factors into the extracellular milieu (Archer, 1998; Dinges et al., 2000; Foster, 2005; Shaw et al., 2004; Sibbald et al., 2006). Like most secreted proteins, these virulence factors are translocated by the Sec machinery across the plasma membrane. Proteins secreted by the Sec machinery bear an N-terminal leader peptide that is removed by leader peptidase once the pre-protein is engaged in the Sec translocon (Dalbey and Wickner, 1985; van Wely et al., 2001). Recent genome analysis suggests that Actinobacteria and members of the Firmicutes encode an additional secretion system that recognizes a subset of proteins in a Sec-independent manner (Pallen, 2002). ESAT-6 (early secreted antigen target 6 kDa) and CFP-10 (culture filtrate antigen 10 kDa) of *Mycobacterium tuberculosis* represent the first substrates of this novel secretion system termed ESX-1 or Snm in *M. tuberculosis* (Andersen et al., 1995; Hsu et al., 2003; Pym et al., 2003; Stanley et al., 2003). In *S. aureus*, two ESAT-6 like factors designated EsxA and EsxB are secreted by the Ess pathway (ESAT-6 secretion system) (Burts et al., 2005).

The first generation of vaccines targeted against *S. aureus* or against the exoproteins it produces have met with limited success (Lee, 1996). There remains a need to develop effective vaccines against staphylococcal infections. Additional compositions for treating staphylococcal infections are also needed.

SUMMARY OF THE INVENTION

Protein A (SpA)(SEQ ID NO:33), a cell wall anchored surface protein of *Staphylococcus aureus*, provides for bac-

terial evasion from innate and adaptive immune responses. Protein A binds immunoglobulins at their Fc portion, interacts with the VH3 domain of B cell receptors inappropriately stimulating B cell proliferation and apoptosis, binds to von Willebrand factor A1 domains to activate intracellular clotting, and also binds to the TNF Receptor-1 to contribute to the pathogenesis of staphylococcal pneumonia. Due to the fact that Protein A captures immunoglobulin and displays toxic attributes, the possibility that this surface molecule may function as a vaccine in humans has not been rigorously pursued. Here the inventors demonstrate that Protein A variants no longer able to bind to immunoglobulins, which are thereby removed of their toxigenic potential, i.e., are non-toxigenic, stimulate humoral immune responses that protect against staphylococcal disease.

In certain embodiments the SpA variant is a full length SpA variant comprising a variant A, B, C, D, and/or E domain. In certain aspects, the SpA variant comprises or consists of the amino acid sequence that is 80, 90, 95, 98, 99, or 100% identical to the amino acid sequence of SEQ ID NO:34. In other embodiments the SpA variant comprises a segment of SpA. The SpA segment can comprise at least or at most 1, 2, 3, 4, 5 or more IgG binding domains. The IgG domains can be at least or at most 1, 2, 3, 4, 5 or more variant A, B, C, D, or E domains. In certain aspects the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant A domains. In a further aspect the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant B domains. In still a further aspect the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant C domains. In yet a further aspect the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant D domains. In certain aspects the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant E domains. In a further aspect the SpA variant comprises a combination of A, B, C, D, and E domains in various combinations and permutations. The combinations can include all or part of a SpA signal peptide segment, a SpA region X segment, and/or a SpA sorting signal segment. In other aspects the SpA variant does not include a SpA signal peptide segment, a SpA region X segment, and/or a SpA sorting signal segment. In certain aspects a variant A domain comprises a substitution at position(s) 7, 8, 34, and/or 35 of SEQ ID NO:4. In another aspect a variant B domain comprises a substitution at position(s) 7, 8, 34, and/or 35 of SEQ ID NO:6. In still another aspect a variant C domain comprises a substitution at position(s) 7, 8, 34, and/or 35 of SEQ ID NO:5. In certain aspects a variant D domain comprises a substitution at position(s) 9, 10, 36, and/or 37 of SEQ ID NO:2. In a further aspect a variant E domain comprises a substitution at position(s) 6, 7, 33, and/or 34 of SEQ ID NO:3.

In certain aspects, an SpA domain D variant or its equivalent can comprise a mutation at position 9 and 36; 9 and 37; 9 and 10; 36 and 37; 10 and 36; 10 and 37; 9, 36, and 37; 10, 36, and 37, 9, 10 and 36; or 9, 10 and 37 of SEQ ID NO:2. In a further aspect, analogous mutations can be included in one or more of domains A, B, C, or E.

In further aspects, the amino acid glutamine (Q) at position 9 of SEQ ID NO:2 (or its analogous amino acid in other SpA domains) can be replaced with an alanine (A), an asparagine (N), an aspartic acid (D), a cysteine (C), a glutamic acid (E), a phenylalanine (F), a glycine (G), a histidine (H), an isoleucine (I), a lysine (K), a leucine (L), a methionine (M), a proline (P), a serine (S), a threonine (T), a valine (V), a tryptophane (W), or a tyrosine (Y). In some aspects the glutamine at position 9 can be substituted with an arginine (R). In a further aspect, the glutamine at position 9

of SEQ ID NO:2, or its equivalent, can be substituted with a lysine or a glycine. Any 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more of the substitutions can be explicitly excluded.

In another aspect, the amino acid glutamine (Q) at position 10 of SEQ ID NO:2 (or its analogous amino acid in other SpA domains) can be replaced with an alanine (A), an asparagine (N), an aspartic acid (D), a cysteine (C), a glutamic acid (E), a phenylalanine (F), a glycine (G), a histidine (H), an isoleucine (I), a lysine (K), a leucine (L), a methionine (M), a proline (P), a serine (S), a threonine (T), a valine (V), a tryptophane (W), or a tyrosine (Y). In some aspects the glutamine at position 10 can be substituted with an arginine (R). In a further aspect, the glutamine at position 10 of SEQ ID NO:2, or its equivalent, can be substituted with a lysine or a glycine. Any 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more of the substitutions can be explicitly excluded.

In certain aspects, the aspartic acid (D) at position 36 of SEQ ID NO:2 (or its analogous amino acid in other SpA domains) can be replaced with an alanine (A), an asparagine (N), an arginine (R), a cysteine (C), a phenylalanine (F), a glycine (G), a histidine (H), an isoleucine (I), a lysine (K), a leucine (L), a methionine (M), a proline (P), a glutamine (Q), a serine (S), a threonine (T), a valine (V), a tryptophane (W), or a tyrosine (Y). In some aspects the aspartic acid at position 36 can be substituted with a glutamic acid (E). In certain aspects, an aspartic acid at position 36 of SEQ ID NO:2, or its equivalent, can be substituted with an alanine or a serine. Any 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more of the substitutions can be explicitly excluded.

In another aspect, the aspartic acid (D) at position 37 of SEQ ID NO:2 (or its analogous amino acid in other SpA domains) can be replaced with an alanine (A), an asparagine (N), an arginine (R), a cysteine (C), a phenylalanine (F), a glycine (G), a histidine (H), an isoleucine (I), a lysine (K), a leucine (L), a methionine (M), a proline (P), a glutamine (Q), a serine (S), a threonine (T), a valine (V), a tryptophane (W), or a tyrosine (Y). In some aspects the aspartic acid at position 37 can be substituted with a glutamic acid (E). In certain aspects, an aspartic acid at position 37 of SEQ ID NO:2, or its equivalent, can be substituted with an alanine or a serine. Any 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more of the substitutions can be explicitly excluded.

In a particular embodiment the amino at position 9 of SEQ ID NO:2 (or an analogous amino acid in another SpA domain) is replaced by an alanine (A), a glycine (G), an isoleucine (I), a leucine (L), a proline (P), a serine (S), or a valine (V). In certain aspects the amino acid at position 9 of SEQ ID NO:2 is replaced by a glycine. In a further aspect the amino acid at position 9 of SEQ ID NO:2 is replaced by a lysine.

In a particular embodiment the amino at position 10 of SEQ ID NO:2 (or an analogous amino acid in another SpA domain) is replaced by an alanine (A), a glycine (G), an isoleucine (I), a leucine (L), a proline (P), a serine (S), or a valine (V). In certain aspects the amino acid at position 10 of SEQ ID NO:2 is replaced by a glycine. In a further aspect the amino acid at position 10 of SEQ ID NO:2 is replaced by a lysine.

In a particular embodiment the amino at position 36 of SEQ ID NO:2 (or an analogous amino acid in another SpA domain) is replaced by an alanine (A), a glycine (G), an isoleucine (I), a leucine (L), a proline (P), a serine (S), or a valine (V). In certain aspects the amino acid at position 36 of SEQ ID NO:2 is replaced by a serine. In a further aspect the amino acid at position 36 of SEQ ID NO:2 is replaced by an alanine.

In a particular embodiment the amino acid at position 37 of SEQ ID NO:2 (or an analogous amino acid in another SpA domain) is replaced by an alanine (A), a glycine (G), an isoleucine (I), a leucine (L), a proline (P), a serine (S), or a valine (V). In certain aspects the amino acid at position 37 of SEQ ID NO:2 is replaced by a serine. In a further aspect the amino acid at position 37 of SEQ ID NO:2 is replaced by an alanine.

In certain aspects the SpA variant includes a substitution of (a) one or more amino acid substitution in an IgG Fc binding sub-domain of SpA domain A, B, C, D, and/or E that disrupts or decreases binding to IgG Fc, and (b) one or more amino acid substitution in a V_H3 binding sub-domain of SpA domain A, B, C, D, and/or E that disrupts or decreases binding to V_H3 . In still further aspects the amino acid sequence of a SpA variant comprises an amino acid sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, or 100% identical, including all values and ranges there between, to the amino acid sequence of SEQ ID NOs:2-6.

In a further aspect the SpA variant includes (a) one or more amino acid substitution in an IgG Fc binding sub-domain of SpA domain D, or at a corresponding amino acid position in other IgG domains, that disrupts or decreases binding to IgG Fc, and (b) one or more amino acid substitution in a V_H3 binding sub-domain of SpA domain D, or at a corresponding amino acid position in other IgG domains, that disrupts or decreases binding to V_H3 . In certain aspects amino acid residue F5, Q9, Q10, S11, F13, Y14, L17, N28, I31, and/or K35 (SEQ ID NO:2, QQNNFNKDDQQA-FYEILNMPNLNEAQRNGFIQSLKDDPSQSTNV-LGEAKKLNES) of the IgG Fc binding sub-domain of domain D are modified or substituted. In certain aspects amino acid residue Q26, G29, F30, S33, D36, D37, Q40, N43, and/or E47 (SEQ ID NO:2) of the V_H3 binding sub-domain of domain D are modified or substituted such that binding to Fc or V_H3 is attenuated. In further aspects corresponding modifications or substitutions can be engineered in corresponding positions of the domain A, B, C, and/or E. Corresponding positions are defined by alignment of the domain D amino acid sequence with one or more of the amino acid sequences from other IgG binding domains of SpA, for example see FIG. 2A. In certain aspects the amino acid substitution can be any of the other 20 amino acids. In a further aspect conservative amino acid substitutions can be specifically excluded from possible amino acid substitutions. In other aspects only non-conservative substitutions are included. In any event, any substitution or combination of substitutions that reduces the binding of the domain such that SpA toxicity is significantly reduced is contemplated. The significance of the reduction in binding refers to a variant that produces minimal to no toxicity when introduced into a subject and can be assessed using in vitro methods described herein.

In certain embodiments, a variant SpA comprises at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more variant SpA domain D peptides. In certain aspects 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 or more amino acid residues of the variant SpA are substituted or modified—including but not limited to amino acids F5, Q9, Q10, S11, F13, Y14, L17, N28, I31, and/or K35 (SEQ ID NO:2) of the IgG Fc binding sub-domain of domain D and amino acid residue Q26, G29, F30, S33, D36, D37, Q40, N43, and/or E47 (SEQ ID NO:2) of the V_H3 binding sub-domain of domain D. In one aspect of the invention glutamine residues at position 9 and/or 10 of SEQ ID NO:2 (or corresponding positions in other domains) are mutated. In another aspect, aspartic acid residues 36 and/or 37 of SEQ ID NO:2 (or

corresponding positions in other domains) are mutated. In a further aspect, glutamine 9 and 10, and aspartic acid residues 36 and 37 are mutated. Purified non-toxicogenic SpA or SpA-D mutants/variants described herein are no longer able to significantly bind (i.e., demonstrate attenuated or disrupted binding affinity) Fcγ or F(ab)₂ V_H3 and also do not stimulate B cell apoptosis. These non-toxicogenic Protein A variants can be used as subunit vaccines and raise humoral immune responses and confer protective immunity against *S. aureus* challenge. Compared to wild-type full-length Protein A or the wild-type SpA-domain D, immunization with SpA-D variants resulted in an increase in Protein A specific antibody. Using a mouse model of staphylococcal challenge and abscess formation, it was observed that immunization with the non-toxicogenic Protein A variants generated significant protection from staphylococcal infection and abscess formation. As virtually all *S. aureus* strains express Protein A, immunization of humans with the non-toxicogenic Protein A variants can neutralize this virulence factor and thereby establish protective immunity. In certain aspects the protective immunity protects or ameliorates infection by drug resistant strains of *Staphylococcus*, such as USA300 and other MRSA strains.

Embodiments 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 aspects, the immunogenic fragment is a Protein A domain D segment. Furthermore, the present invention 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 aspects 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.

In other aspects, the subject can be administered all or part of a Protein A variant, such as a variant Protein A domain D segment. The polypeptide of the invention 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, CHB, 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 52 kDa 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 (U.S. Pat. No. 6,288,214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (U.S. Pat. No. 6,008,341), Fibronectin binding protein (U.S. Pat. No. 5,840,846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (U.S. Pat. No. 5,648,240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK, SBI, SdrF (WO 00/12689), SdrG/

TABLE 1-continued

SpA and staphylococcal antigen combinations.							
Coa	+	+	+	+	+	+	+
Hla		+	+	+	+	+	+
Hla _{FF35A}			+	+	+	+	+
IsdC				+	+	+	+
SasF					+	+	+
vWbp						+	+
vWh							+
ClfB	+	+	+	+	+	+	+
Coa		+	+	+	+	+	+
Hla			+	+	+	+	+
Hla _{FF35A}				+	+	+	+
IsdC					+	+	+
SasF						+	+
vWbp							+
vWh							+
Coa	+	+	+	+	+	+	+
Hla			+	+	+	+	+
Hla _{FF35A}				+	+	+	+
IsdC					+	+	+
SasF						+	+
vWbp							+
vWh							+
Hla		+	+	+	+	+	+
Hla _{FF35A}			+	+	+	+	+
IsdC				+	+	+	+
SasF					+	+	+
vWbp						+	+
vWh							+
Hla		+	+	+	+	+	+
Hla _{FF35A}			+	+	+	+	+
IsdC				+	+	+	+
SasF					+	+	+
vWbp						+	+
vWh							+
Hla		+	+	+	+	+	+
Hla _{FF35A}			+	+	+	+	+
IsdC				+	+	+	+
SasF					+	+	+
vWbp						+	+
vWh							+
IsdC				+	+	+	+
SasF					+	+	+
vWbp						+	+
vWh							+
SasF					+	+	+
vWbp						+	+
vWh							+
vWbp						+	+
vWh							+
vWh							+

40

In still further aspects, the isolated Protein A variant is multimerized, e.g., dimerized or a linear fusion of two or more polypeptides or peptide segments. In certain aspects of the invention, 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 aspect, 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 52 kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (U.S. Pat. No. 6,288,214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (U.S. Pat. No. 6,008,341), Fibronectin binding protein (U.S. Pat. No. 5,840,846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin

receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (U.S. Pat. No. 5,648,240), 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 (U.S. Pat. No. 5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein.

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 aspect, a SpA variant includes a variant domain D peptide, as well as variants of SpA polypeptides and segments thereof that are non-toxicogenic and stimulate an immune response against *staphylococcus* bacteria Protein A and/or bacteria expressing such.

Embodiments of the present invention 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 aspects, 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.

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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 52 kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (U.S. Pat. No. 6,288,214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (U.S. Pat. No. 6,008,341), Fibronectin binding protein (U.S. Pat. No. 5,840,846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (U.S. Pat. No. 5,648,240), 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 (U.S. Pat. No. 5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein.

Embodiments of the invention 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 embodiment of the invention 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 aspects 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.

Still further embodiments 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 domain D 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 bacte-

rial proteins described herein. In a preferred embodiment the composition is not a *staphylococcus* bacterium. In certain aspects the subject is a human or a cow. In a further aspect the composition is formulated in a pharmaceutically acceptable formulation. The staphylococci may be *Staphylococcus aureus*.

Yet still further embodiments 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 aspects of the invention 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 aspect, 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 aspects a protein or peptide of the invention is linked (covalently or non-covalently) to the adjuvant, preferably the adjuvant is chemically conjugated to the protein.

In still yet further embodiments, 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 embodiments the 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 aspects 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.

Still further embodiments 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 embodiment the composition comprises a non-*staphylococcus* bacterium. In a further aspect 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 invention 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 aspects 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 aspects an antigen combination can include (1) a SpA variant and IsdA; (2) SpA variant and CHB; (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, CHB, 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.

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

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.

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.

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.

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.

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

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

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

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

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

The term "EsaB protein" refers to a protein that includes isolated wild-type EsaB polypeptides from *staphylococcus*

bacteria and segments thereof, as well as variants that stimulate an immune response against *staphylococcus* bacteria EsaB proteins.

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

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

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

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

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.

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.

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.

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.

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.

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.

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.

In still further embodiments of the invention 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 an EsxA protein. In

certain aspects the EsxA protein will have all or part of the amino acid sequence of SEQ ID NO:11.

In still further embodiments of the invention 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 an EsxB protein. In certain aspects the EsxB protein will have all or part of the amino acid sequence of SEQ ID NO:12.

In yet still further embodiments of the invention 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 an SdrD protein. In certain aspects the SdrD protein will have all or part of the amino acid sequence of SEQ ID NO:13.

In further embodiments of the invention 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 an SdrE protein. In certain aspects the SdrE protein will have all or part of the amino acid sequence of SEQ ID NO:14.

In still further embodiments of the invention 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 an IsdA protein. In certain aspects the IsdA protein will have all or part of the amino acid sequence of SEQ ID NO:15.

In yet still further embodiments of the invention 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 an IsdB protein. In certain aspects the IsdB protein will have all or part of the amino acid sequence of SEQ ID NO:16.

Embodiments of the invention 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 a EsaB protein. In certain aspects the EsaB protein will have all or part of the amino acid sequence of SEQ ID NO:17.

In a further embodiments of the invention 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 ClfB protein. In certain aspects the ClfB protein will have all or part of the amino acid sequence of SEQ ID NO:18.

In still further embodiments of the invention 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 an IsdC protein. In certain aspects the IsdC protein will have all or part of the amino acid sequence of SEQ ID NO:19.

In yet further embodiments of the invention 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 SasF protein. In certain aspects the SasF protein will have all or part of the amino acid sequence of SEQ ID NO:20.

In yet still further embodiments of the invention 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 SdrC protein. In certain aspects the SdrC protein will have all or part of the amino acid sequence of SEQ ID NO:21.

In yet still further embodiments of the invention 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 ClfA protein. In certain

aspects the ClfA protein will have all or part of the amino acid sequence of SEQ ID NO:22.

In yet still further embodiments of the invention 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 an Eap protein. In certain aspects the Eap protein will have all or part of the amino acid sequence of SEQ ID NO:23.

In yet still further embodiments of the invention 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 an Ebh protein. In certain aspects the Ebh protein will have all or part of the amino acid sequence of SEQ ID NO:24.

In yet still further embodiments of the invention 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 an Emp protein. In certain aspects the Emp protein will have all or part of the amino acid sequence of SEQ ID NO:25.

In yet still further embodiments of the invention 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 an EsaC protein. In certain aspects the EsaC protein will have all or part of the amino acid sequence of SEQ ID NO:26. Sequence of EsaC polypeptides can be found in the protein databases and include, but are not limited to accession numbers ZP_02760162 (GI:168727885), NP_645081.1 (GI:21281993), and NP_370813.1 (GI:15923279), each of which is incorporated herein by reference as of the priority date of this application.

In yet still further embodiments of the invention 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 Coa protein. In certain aspects the Coa protein will have all or part of the amino acid sequence of SEQ ID NO:27.

In yet still further embodiments of the invention 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 Hla protein. In certain aspects the Hla protein will have all or part of the amino acid sequence of SEQ ID NO:28.

In yet still further embodiments of the invention 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 vWa protein. In certain aspects the vWa protein will have all or part of the amino acid sequence of SEQ ID NO:29.

In yet still further embodiments of the invention 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 vWbp protein. In certain aspects the vWbp protein will have all or part of the amino acid sequence of SEQ ID NO:32.

In certain aspects, 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.

The polypeptides described herein may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more variant amino acids within at least, or at most 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 NO:2-30, or SEQ ID NO:32-34.

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 NO:2-30, or SEQ ID NO:33-34.

The compositions may be formulated in a pharmaceutically acceptable composition. In certain aspects of the invention the *staphylococcus* bacterium is an *S. aureus* bacterium.

In further aspects, 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.

In still further embodiments, 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 aspects, a recombinant nucleic acid molecule of the invention is a vector, in still other aspects the vector is a plasmid. In certain embodiments the vector is a viral vector. In certain aspects a composition includes a recombinant, non-*staphylococcus* bacterium containing or expressing a polypeptide described herein. In particular aspects 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 aspects the *staphylococcus* bacterium containing or expressing the polypeptide is *Staphylococcus aureus*. In further embodiments the immune response is a protective immune response.

In further embodiments a composition comprises a recombinant nucleic acid molecule encoding all or part of one or more of a Eap, Ehb, 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 52 kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (U.S. Pat. No. 6,288,214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (U.S. Pat. No. 6,008,341), Fibronectin binding protein (U.S. Pat. No. 5,840,846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (U.S. Pat. No. 5,648,240), 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 (U.S. Pat. No. 5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. In particular aspects, a bacteria is a recombinant non-*staphylococcus* bacteria, such as a *Salmonella* or other gram-positive bacteria.

Compositions of the invention 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.

In certain aspects the *staphylococcus* bacterium is a *Staphylococcus aureus*. In further embodiments the immune response is a protective immune response. In still further aspects, the methods and compositions of the invention 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.

Any embodiment discussed with respect to one aspect of the invention applies to other aspects of the invention as well. In particular, any embodiment 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, Ehb, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52 kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (U.S. Pat. No. 6,288,214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (U.S. Pat. No. 6,008,341), Fibronectin binding protein (U.S. Pat. No. 5,840,846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (U.S. Pat. No.

5,648,240), 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 (U.S. Pat. No. 5,801,234), 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, Ehb, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52 kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (U.S. Pat. No. 6,288,214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (U.S. Pat. No. 6,008,341), Fibronectin binding protein (U.S. Pat. No. 5,840,846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg²⁺ transporter, MHC II analogue (U.S. Pat. No. 5,648,240), 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 (U.S. Pat. No. 5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein can be specifically excluded from a claimed composition.

Embodiments of the invention 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 aspects, the composition comprises a bacterium that is not a staphylococcal bacterium or does not contain staphylococcal bacteria. In certain embodiments 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.

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.

Moieties of the invention, 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 invention may further comprise an adjuvant or a pharma-

ceutically acceptable excipient. An adjuvant may be covalently or non-covalently coupled to a polypeptide or peptide of the invention. In certain aspects, the adjuvant is chemically conjugated to a protein, polypeptide, or peptide.

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

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 invention 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 aspects, 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 invention, 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.

The embodiments in the Example section are understood to be embodiments of the invention that are applicable to all aspects of the invention.

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.

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.

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.

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

DESCRIPTION OF THE DRAWINGS

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 embodi-

ments 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.

FIGS. 1A-1B. (FIG. 1A) Primary structure of the Protein A precursor with an N-terminal YSIRK motif signal peptide, five immunoglobulin binding domains as tandem repeats designated E, D, A, B, C, region X, and the LPXTG sorting signal. (FIG. 1B) Following synthesis of the Protein A precursor, staphylococci secrete this product via the Sec pathway, and sortase A cleaves the LPXTG sorting signal between the T and G residues. Nucleophilic attack of the amino group within lipid II at the sortase-Protein A thioester-linked intermediate forms the amide bond that links Protein A to the cell wall envelope and enables its display on the bacterial surface.

FIG. 2A-2C. Three dimensional model of the molecular interactions between the SpA-domain D of Protein A, the VH3 Fab domain of the B cell receptor, and of the Fc γ domain of immunoglobulin. The model is derived from two crystal structures (Graille et al., 2000 and Gouda et al., 1992) that revealed side chain residues involved in the formation of ionic bonds that enable these complexes. Gln-9 and Gln-10 of SpA-D promote binding to Fc γ , whereas Asp-36 and Asp-37 enable complex formation with VH3 Fab.

FIG. 3. Left panel—Coomassie Blue stained SDS-PAGE reveals the migrational position of purified His-tagged SpA, SpA-D, SpA-D_{Q9,10K;D36,37A}, human IgG, and sortase A (SrtA), a control protein. Right panel—Coomassie Blue stained SDS-PAGE to reveal the elution of Protein A immunoglobulin complexes eluted following affinity chromatography of human IgG on Ni-NTA columns pre-charged with His-tagged SpA, SpA-D, SpA-D_{Q9,10K;D36,37A} or SrtA.

FIG. 4. ELISA assays to quantify human immunoglobulin (hIgG), human F(ab)₂ IgG fragments and human Fc fragments of immunoglobulin (hFc). Plates were coated with equal amounts of His-tagged SpA, SpA-D, SpA-D_{Q9,10K;D36,37A} or SrtA. hIgG-HRP, F(ab)₂-HRP and hFc-HRP were added onto the plates and incubated for an hour. Absorbance at 450 nm was recorded and plotted to determine the half maximal titers.

FIG. 5. Purified SpA-D, SpA-D_{Q9,10K;D36,37A} or a PBS mock control were injected into the peritoneum of mice and analyzed for their ability to reduce the B cell population in the spleen of experimental BALB/c mice. Animals were killed 4 hours following injection, their spleen removed, tissue homogenized and stained with CD19 antibodies directed against B cells. The number of B cells was quantified by FACS sorting.

FIG. 6A-6E. Generation of a non-toxicigenic protein A vaccine. a, Translational protein A (SpA) product of *S. aureus* Newman and USA300 LAC with an N-terminal signal peptide (white box), five immunoglobulin binding domains (IgBDs designated E, D, A, B and C), variable region X and C-terminal sorting signal (black box). b, Amino acid sequence of the five IgBDs as well as nontoxicigenic SpA-D_{KKAA}, with the positions of triple α -helical bundles (H1, H2 and H3) as well as glutamine (Q) 9, 10 and aspartate (D) 36, 37 indicated. c, Coomassie Blue-stained SDS-PAGE of SpA, SpA-D, SpA-D_{KKAA} or SrtA purified on Ni-NTA sepharose in the presence or absence of human immunoglobulin (hIgG). d, ELISA examining the association of immobilized SpA, SpA-D or SpA-D_{KKAA} with human IgG as well as its Fc or F(ab)₂ fragments and von Willebrand factor (vWF). e, CD19+ B lymphocytes in

splenic tissue of BALB/c mice that had been mock immunized or treated with SpA-D or SpA-D_{KKAA} were quantified by FACS.

FIG. 7. Non-toxicigenic protein A vaccine prevents abscess formation. Histopathology of renal tissue isolated during necropsy of BALB/c mice that had been mock immunized (PBS) or vaccinated with SpA, SpA-D as well as SpA-D_{KKAA} and challenged with *S. aureus* Newman. Thin sectioned tissues were stained with hematoxylin-eosin. White arrows identify polymorphonuclear leukocyte (PMN) infiltrates. Dark arrows identify staphylococcal abscess communities.

FIG. 8A-8C. Antibodies raised by the non-toxicigenic protein A vaccine block the B cell superantigen function of SpA. a, Rabbit antibodies raised against SpA-D_{KKAA} were purified on a matrix with immobilized antigen and analyzed by Coomassie Blue-stained SDS-PAGE. Antibodies were cleaved with pepsin and F(ab)₂ fragments were purified by a second round of affinity chromatography on SpA-D_{KKAA} matrix. b, SpA-D_{KKAA} specific F(ab)₂ interfere with the binding of SpA or SpA-D to human immunoglobulin (hIgG) or, c, to von Willebrand Factor (vWF).

FIG. 9A-9D. Full-length non-toxicigenic protein A generates improved immune responses. a, Full-length SpA_{KKAA} was purified on Ni-NTA sepharose and analyzed by Coomassie-Blue stained SDS-PAGE. b, CD19+ B lymphocytes in splenic tissue of BALB/c mice that had been mock immunized or treated with SpA or SpA_{KKAA} were quantified by FACS. c, ELISA examining the association of immobilized SpA or SpA_{KKAA} with human IgG as well as its Fc or F(ab)₂ fragments or von Willebrand factor (vWF). d, Human or mouse serum antibody titers to diphtheria toxoid (CRM197) and non-toxicigenic SpA_{KKAA} or SpA-D_{KKAA}. Human volunteers with a history of DTaP immunization and staphylococcal infection (n=16) as well as mice (n=20) that had been infected with *S. aureus* Newman or USA 300 LAC or immunized with SpA_{KKAA} or SpA-D_{KKAA} were examined by quantitative dot blot.

FIG. 10. Staphylococcal infection does not generate protective immunity. BALB/c mice (n=20) were infected with *S. aureus* Newman or mock challenged (PBS) for thirty days and infection cleared with chloramphenicol treatment. Both cohorts of animals were then challenged with *S. aureus* Newman and bacterial load (CFU) in kidney tissue homogenate analyzed following necropsy on day 4.

FIG. 11. Comparison of abscess formation in mice treated with PBS, SpA, SpA-D, and SpA-D_{KKAA}.

FIGS. 12A-12C. (A) ELISA examining the association of immobilized SpA, SpA-D, SpA-D_{KKAA} or SpA-DGGSS with human IgG as well as its Fc or F(ab)₂ fragments and IgM. Statistical significance of SpA-D_{KKAA} and SpA-DGGSS binding to each ligand was compared against SpA-D; SpA-D binding was compared against SpA (n=3); * signifies P<0.05; ** signifies P<0.01. (B) ELISA examining the level of cross-reactive antibodies of hyperimmune sera samples collected from actively immunized mice (n=5) with SpA-D, SpA-D_{KKAA} and SpA-DGGSS. (C) Abscess formation in mice treated with PBS, SpA-D, SpA-D_{KKAA} and SpA-DGGSS.

DETAILED DESCRIPTION

Staphylococcus aureus is a commensal of the human skin and nares, and the leading cause of bloodstream, skin and soft tissue infections (Klebens et al., 2007). Recent dramatic increases in the mortality of staphylococcal diseases are attributed to the spread of methicillin-resistant *S. aureus*

(MRSA) strains often not susceptible to antibiotics (Kennedy et al., 2008). In a large retrospective study, the incidence of MRSA infections was 4.6% of all hospital admissions in the United States (Klevens et al., 2007). The annual health care costs for 94,300 MRSA infected individuals in the United States exceed \$2.4 billion (Klevens et al., 2007). The current MRSA epidemic has precipitated a public health crisis that needs to be addressed by development of a preventive vaccine (Boucher and Corey, 2008). To date, an FDA licensed vaccine that prevents *S. aureus* diseases is not available.

The inventors describe here the use of Protein A, a cell wall anchored surface protein of staphylococci, for the generation of variants that can serve as subunit vaccines. The pathogenesis of staphylococcal infections is initiated as bacteria invade the skin or blood stream via trauma, surgical wounds, or medical devices (Lowy, 1998). Although the invading pathogen may be phagocytosed and killed, staphylococci can also escape innate immune defenses and seed infections in organ tissues, inducing inflammatory responses that attract macrophages, neutrophils, and other phagocytes (Lowy, 1998). The responsive invasion of immune cells to the site of infection is accompanied by liquefaction necrosis as the host seeks to prevent staphylococcal spread and allow for removal of necrotic tissue debris (Lam et al., 1963). Such lesions can be observed by microscopy as hypercellular areas containing necrotic tissue, leukocytes, and a central nidus of bacteria (Lam et al., 1963). Unless staphylococcal abscesses are surgically drained and treated with antibiotics, disseminated infection and septicemia produce a lethal outcome (Sheagren, 1984).

II. Staphylococcal Antigens

A. Staphylococcal Protein A (SpA)

All *Staphylococcus aureus* strains express the structural gene for Protein A (*spa*) (Jensen, 1958; Said-Salim et al., 2003), a well characterized virulence factor whose cell wall anchored surface protein product (SpA) encompasses five highly homologous immunoglobulin binding domains designated E, D, A, B, and C (Sjodahl, 1977). These domains display ~80% identity at the amino acid level, are 56 to 61 residues in length, and are organized as tandem repeats (Uhlen et al., 1984). SpA is synthesized as a precursor protein with an N-terminal YSIRK/GS signal peptide and a C-terminal LPXTG motif sorting signal (DeDent et al., 2008; Schneewind et al., 1992). Cell wall anchored Protein A is displayed in great abundance on the staphylococcal surface (DeDent et al., 2007; Sjoquist et al., 1972). Each of its immunoglobulin binding domains is composed of anti-parallel α -helices that assemble into a three helix bundle and bind the Fc domain of immunoglobulin G (IgG) (Deisenhofer, 1981; Deisenhofer et al., 1978), the VH3 heavy chain (Fab) of IgM (i.e., the B cell receptor) (Graillet et al., 2000), the von Willebrand factor at its A1 domain [vWF A1 is a ligand for platelets] (O'Seaghda et al., 2006) and the tumor necrosis factor α (TNF- α) receptor I (TNFRI) (Gomez et al., 2006), which is displayed on surfaces of airway epithelia (Gomez et al., 2004; Gomez et al., 2007).

SpA impedes neutrophil phagocytosis of staphylococci through its attribute of binding the Fc component of IgG (Jensen, 1958; Uhlen et al., 1984). Moreover, SpA is able to activate intravascular clotting via its binding to von Willebrand factor A1 domains (Hartleib et al., 2000). Plasma proteins such as fibrinogen and fibronectin act as bridges between staphylococci (C1FA and C1FB) and the platelet integrin GPIIb/IIIa (O'Brien et al., 2002), an activity that is

supplemented through Protein A association with vWF A1, which allows staphylococci to capture platelets via the GPIIb- α platelet receptor (Foster, 2005; O'Seaghda et al., 2006). SpA also binds TNFRI and this interaction contributes to the pathogenesis of staphylococcal pneumonia (Gomez et al., 2004). SpA activates proinflammatory signaling through TNFR1 mediated activation of TRAF2, the p38/c-Jun kinase, mitogen activate protein kinase (MAPK) and the Rel-transcription factor NF-KB. SpA binding further induces TNFR1 shedding, an activity that appears to require the TNF-converting enzyme (TACE)(Gomez et al., 2007). All of the aforementioned SpA activities are mediated through its five IgG binding domains and can be perturbed by the same amino acid substitutions, initially defined by their requirement for the interaction between Protein A and human IgG1 (Cedergren et al., 1993).

SpA also functions as a B cell superantigen by capturing the Fab region of VH3 bearing IgM, the B cell receptor (Gomez et al., 2007; Goodyear et al., 2003; Goodyear and Silverman, 2004; Roben et al., 1995). Following intravenous challenge, staphylococcal Protein A (SpA) mutations show a reduction in staphylococcal load in organ tissues and dramatically diminished ability to form abscesses (described herein). During infection with wildtype *S. aureus*, abscesses are formed within forty-eight hours and are detectable by light microscopy of hematoxylin-eosin stained, thin-sectioned kidney tissue, initially marked by an influx of polymorphonuclear leukocytes (PMNs). On day 5 of infection, abscesses increase in size and enclosed a central population of staphylococci, surrounded by a layer of eosinophilic, amorphous material and a large cuff of PMNs. Histopathology revealed massive necrosis of PMNs in proximity to the staphylococcal nidus at the center of abscess lesions as well as a mantle of healthy phagocytes. The inventors also observed a rim of necrotic PMNs at the periphery of abscess lesions, bordering the eosinophilic pseudocapsule that separated healthy renal tissue from the infectious lesion. Staphylococcal variants lacking Protein A are unable to establish the histopathology features of abscesses and are cleared during infection.

In previous studies, Cedergren et al. (1993) engineered five individual substitutions in the Fc fragment binding sub-domain of the B domain of SpA, L17D, N28A, I31A and K35A. These authors created these proteins to test data gathered from a three dimensional structure of a complex between one domain of SpA and Fc₁. Cedergren et al. determined the effects of these mutations on stability and binding, but did not contemplate use of such substitutions for the production of a vaccine antigen.

Brown et al. (1998) describe studies designed to engineer new proteins based on SpA that allow the use of more favorable elution conditions when used as affinity ligands. The mutations studied included single mutations of Q13A, Q14H, N15A, N15H, F17H, Y18F, L21H, N32H, or K39H. Brown et al. report that Q13A, N15A, N15H, and N32H substitutions made little difference to the dissociation constant values and that the Y18F substitution resulted in a 2 fold decrease in binding affinity as compared to wild type SpA. Brown et al. also report that L21H and F17H substitutions decrease the binding affinity by five-fold and a hundred-fold respectively. The authors also studied analogous substitutions in two tandem domains. Thus, the Brown et al. studies were directed to generating a SpA with a more favorable elution profile, hence the use of His substitutions to provide a pH sensitive alteration in the binding affinity. Brown et al. is silent on the use of SpA as a vaccine antigen.

Graille et al. (2000) describe a crystal structure of domain D of SpA and the Fab fragment of a human IgM antibody. Graille et al. define by analysis of a crystal structure the D domain amino acid residues that interact with the Fab fragment as residues Q26, G29, F30, Q32, S33, D36, D37, Q40, N43, E47, or L51, as well as the amino acid residues that form the interface between the domain D sub-domains. Graille et al. define the molecular interactions of these two proteins, but is silent in regard to any use of substitutions in the interacting residues in producing a vaccine antigen.

O'Seaghda et al. (2006) describe studies directed at elucidating which sub-domain of domain D binds vWF. The authors generated single mutations in either the Fc or VH3 binding sub-domains, i.e., amino acid residues F5A, Q9A, Q10A, F13A, Y14A, L17A, N28A, I31A, K35A, G29A, F30A, S33A, D36A, D37A, Q40A, E47A, or Q32A. The authors discovered that vWF binds the same sub-domain that binds Fc. O'Seaghda et al. define the sub-domain of domain D responsible for binding vWF, but is silent in regard to any use of substitutions in the interacting residues in producing a vaccine antigen.

Gomez et al. (2006) describe the identification of residues responsible for activation of the TNFR1 by using single mutations of F5A, F13A, Y14A, L17A, N21A, I31A, Q32A, and K35A. Gomez et al. is silent in regard to any use of substitutions in the interacting residues in producing a vaccine antigen.

Recombinant affinity tagged Protein A, a polypeptide encompassing the five IgG domains (EDCAB) (Sjodahl, 1977) but lacking the C-terminal Region X (Guss et al., 1984), was purified from recombinant *E. coli* and used as a vaccine antigen (Stranger-Jones et al., 2006). Because of the attributes of SpA in binding the Fc portion of IgG, a specific humoral immune response to Protein A could not be measured (Stranger-Jones et al., 2006). The inventors have overcome this obstacle through the generation of SpA-DQ9, 10K;D36,37A. BALB/c mice immunized with recombinant Protein A (SpA) displayed significant protection against intravenous challenge with *S. aureus* strains: a 2.951 log reduction in staphylococcal load as compared to the wild-type ($P > 0.005$; Student's t-test) (Stranger-Jones et al., 2006). SpA specific antibodies may cause phagocytic clearance prior to abscess formation and/or impact the formation of the aforementioned eosinophilic barrier in abscesses that separate staphylococcal communities from immune cells since these do not form during infection with Protein A mutant strains. Each of the five SpA domains (i.e., domains formed from three helix bundles designated E, D, A, B, and C) exerts similar binding properties (Jansson et al., 1998). The solution and crystal structure of the domain D has been solved both with and without the Fc and VH3 (Fab) ligands, which bind Protein A in a non-competitive manner at distinct sites (Graille et al., 2000). Mutations in residues known to be involved in IgG binding (FS, Q9, Q10, S11, F13, Y14, L17, N28, 131 and K35) are also required for vWF AI and TNFR1 binding (Cedergren et al., 1993; Gomez et al., 2006; O'Seaghda et al., 2006), whereas residues important for the VH3 interaction (Q26, G29, F30, S33, D36, D37, Q40, N43, E47) appear to have no impact on the other binding activities (Graille et al., 2000; Jansson et al., 1998). SpA specifically targets a subset of B cells that express VH3 family related IgM on their surface, i.e., VH3 type B cell receptors (Roben et al., 1995). Upon interaction with SpA, these B cells proliferate and commit to apoptosis, leading to preferential and prolonged deletion of innate-like B lymphocytes (i.e., marginal zone B cells and follicular B2 cells) (Goodyear et al., 2003; Goodyear et al., 2004).

Molecular Basis of Protein a Surface Display and Function.

Protein A is synthesized as a precursor in the bacterial cytoplasm and secreted via its YSIRK signal peptide at the cross wall, i.e. the cell division septum of staphylococci (FIG. 1) (DeDent et al., 2007; DeDent et al., 2008). Following cleavage of the C-terminal LPXTG sorting signal, Protein A is anchored to bacterial peptidoglycan cross-bridges by sortase A (Mazmanian et al., 1999; Schneewind et al., 1995; Mazmanian et al., 2000). Protein A is the most abundant surface protein of staphylococci; the molecule is expressed by virtually all *S. aureus* strains (Cespedes et al., 2005; Kennedy et al., 2008; Said-Salim et al., 2003). Staphylococci turn over 15-20% of their cell wall per division cycle (Navarre and Schneewind, 1999). Murine hydrolases cleave the glycan strands and wall peptides of peptidoglycan, thereby releasing Protein A with its attached C-terminal cell wall disaccharide tetrapeptide into the extracellular medium (Ton-That et al., 1999). Thus, by physiological design, Protein A is both anchored to the cell wall and displayed on the bacterial surface but also released into surrounding tissues during host infection (Marraffini et al., 2006).

Protein A captures immunoglobulins on the bacterial surface and this biochemical activity enables staphylococcal escape from host innate and acquired immune responses (Jensen, 1958; Goodyear et al., 2004). Interestingly, region X of Protein A (Guss et al., 1984), a repeat domain that tethers the IgG binding domains to the LPXTG sorting signal/cell wall anchor, is perhaps the most variable portion of the staphylococcal genome (Said-Salim, 2003; Schneewind et al., 1992). Each of the five immunoglobulin binding domains of Protein A (SpA), formed from three helix bundles and designated E, D, A, B, and C, exerts similar structural and functional properties (Sjodahl, 1977; Jansson et al., 1998). The solution and crystal structure of the domain D has been solved both with and without the Fc and V_{H3} (Fab) ligands, which bind Protein A in a non-competitive manner at distinct sites (Graille 2000).

In the crystal structure complex, the Fab interacts with helix II and helix III of domain D via a surface composed of four VH region β -strands (Graille 2000). The major axis of helix II of domain D is approximately 50° to the orientation of the strands, and the interhelical portion of domain D is most proximal to the C0 strand. The site of interaction on Fab is remote from the Ig light chain and the heavy chain constant region. The interaction involves the following domain D residues: Asp-36 of helix II, Asp-37 and Gln-40 in the loop between helix II and helix III and several other residues (Graille 2000). Both interacting surfaces are composed predominantly of polar side chains, with three negatively charged residues on domain D and two positively charged residues on the 2A2 Fab buried by the interaction, providing an overall electrostatic attraction between the two molecules. Of the five polar interactions identified between Fab and domain D, three are between side chains. A salt bridge is formed between Arg-H19 and Asp-36 and two hydrogen bonds are made between Tyr-H59 and Asp-37 and between Asn-H82a and Ser-33. Because of the conservation of Asp-36 and Asp-37 in all five IgG binding domains of Protein A, the inventors mutated these residues.

The SpA-D sites responsible for Fab binding are structurally separate from the domain surface that mediates Fc γ binding. The interaction of Fc γ with domain D primarily involves residues in helix I with lesser involvement of helix II (Gouda et al., 1992; Deisenhofer, 1981). With the exception of the Gln-32, a minor contact in both complexes, none of the residues that mediate the Fc γ interaction are involved

in Fab binding. To examine the spatial relationship between these different Ig-binding sites, the SpA domains in these complexes have been superimposed to construct a model of a complex between Fab, the SpA-domain D, and the Fc γ molecule. In this ternary model, Fab and Fc γ form a sandwich about opposite faces of the helix II without evidence of steric hindrance of either interaction. These findings illustrate how, despite its small size (i.e., 56-61 aa), an SpA domain can simultaneously display both activities, explaining experimental evidence that the interactions of Fab with an individual domain are noncompetitive. Residues for the interaction between SpA-D and Fc γ are Gln-9 and Gln-10.

In contrast, occupancy of the Fc portion of IgG on the domain D blocks its interaction with vWF A1 and probably also TNFR1 (O'Seaghda et al., 2006). Mutations in residues essential for IgG Fc binding (F5, Q9, Q10, S11, F13, Y14, L17, N28, 131 and K35) are also required for vWF A1 and TNFR1 binding (O'Seaghda et al., 2006; Cedergren et al., 1993; Gomez et al., 2006), whereas residues critical for the VH3 interaction (Q26, G29, F30, S33, D36, D37, Q40, N43, E47) have no impact on the binding activities of IgG Fc, vWF A1 or TNFR1 (Jansson et al., 1998; Graille et al., 2000). The Protein A immunoglobulin Fab binding activity targets a subset of B cells that express V_H3 family related IgM on their surface, i.e., these molecules function as VH3type B cell receptors (Roben et al., 1995). Upon interaction with SpA, these B cells rapidly proliferate and then commit to apoptosis, leading to preferential and prolonged deletion of innate-like B lymphocytes (i.e., marginal zone B cells and follicular B2 cells) (Goodyear and Silverman, 2004; Goodyear and Silverman, 2003). More than 40% of circulating B cells are targeted by the Protein A interaction and the V_H3 family represents the largest family of human B cell receptors to impart protective humoral responses against pathogens (Goodyear and Silverman, 2004; Goodyear and Silverman, 2003). Thus, Protein A functions analogously to staphylococcal superantigens (Roben et al., 1995), albeit that the latter class of molecules, for example SEB, TSST-1, TSST-2, form complexes with the T cell receptor to inappropriately stimulate host immune responses and thereby precipitating characteristic disease features of staphylococcal infections (Roben et al., 1995; Tiedemann et al., 1995). Together these findings document the contributions of Protein A in establishing staphylococcal infections and in modulating host immune responses.

In sum, Protein A domains can be viewed as displaying two different interfaces for binding with host molecules and any development of Protein A based vaccines must consider the generation of variants that do not perturb host cell signaling, platelet aggregation, sequestration of immunoglobulins or the induction of B cell proliferation and apoptosis. Such Protein A variants should also be useful in analyzing vaccines for the ability of raising antibodies that block the aforementioned SpA activities and occupy the five repeat domains at their dual binding interfaces. This goal is articulated and pursued here for the first time and methods are described in detail for the generation of Protein A variants that can be used as a safe vaccine for humans. To perturb IgG Fc γ , vWF AI and TNFR1 binding, glutamine (Q) 9 and 10 [numbering derived from the SpA domain D as described in Uhlen et al., 1984] were mutated, and generated lysine substitutions for both glutamines with the expectation that these abolish the ligand attributes at the first binding interface. To perturb IgM Fab VH3 binding, aspartate (D) 36 and 37 were mutated, each of which is required for the association with the B cell receptor. D36 and D37 were both substituted with alanine. Q9,10K and D36,37A mutations

are here combined in the recombinant molecule SpA-DQ9, 10K;D36,37A and tested for the binding attributes of Protein A. Further, SpA-D and SpA-DQ9,10K;D36,37A are subjected to immunization studies in mice and rabbits and analyzed for [1] the production of specific antibodies (SpA-D Ab); [2] the ability of SpA-D Ab to block the association between Protein A and its four different ligands; and, [3] the attributes of SpA-D Ab to generate protective immunity against staphylococcal infections. (See Examples section below).

B. Staphylococcal Coagulases

Coagulases are enzymes produced by *Staphylococcus* bacteria that convert fibrinogen to fibrin. Coa and vW_h activate prothrombin without proteolysis (Friedrich et al., 2003). The coagulase-prothrombin complex recognizes fibrinogen as a specific substrate, converting it directly into fibrin. The crystal structure of the active complex revealed binding of the D1 and D2 domains to prothrombin and insertion of its Ile1-Val² N-terminus into the Ile¹⁶ pocket, inducing a functional active site in the zymogen through conformational change (Friedrich et al., 2003). Exosite I of α -thrombin, the fibrinogen recognition site, and proexosite I on prothrombin are blocked by the D2 of Coa (Friedrich et al., 2003). Nevertheless, association of the tetrameric (Coa-prothrombin)₂ complex binds fibrinogen at a new site with high affinity (Panizzi et al., 2006). This model explains the coagulant properties and efficient fibrinogen conversion by coagulase (Panizzi et al., 2006).

Fibrinogen is a large glycoprotein (Mr~340,000), formed by three pairs of A α -, B β -, and γ -chains covalently linked to form a "dimer of trimers," where A and B designate the fibrinopeptides released by thrombin cleavage (Panizzi et al., 2006). The elongated molecule folds into three separate domains, a central fragment E that contains the N-termini of all six chains and two flanking fragments D formed mainly by the C-termini of the B β - and γ -chains. These globular domains are connected by long triple-helical structures. Coagulase-prothrombin complexes, which convert human fibrinogen to the self-polymerizing fibrin, are not targeted by circulating thrombin inhibitors (Panizzi et al., 2006). Thus, staphylococcal coagulases bypass the physiological blood coagulation pathway.

All *S. aureus* strains secrete coagulase and vWbp (Bjerketorp et al., 2004; Field and Smith, 1945). Although early work reported important contributions of coagulase to the pathogenesis of staphylococcal infections (Ekstedt and Yotis, 1960; Smith et al., 1947), more recent investigations with molecular genetics tools challenged this view by observing no virulence phenotypes with endocarditis, skin abscess and mastitis models in mice (Moreillon et al., 1995; Phonimdaeng et al., 1990). Generating isogenic variants of *S. aureus* Newman, a fully virulent clinical isolate (Duthie et al., 1952), it is described herein that coa mutants indeed display virulence defects in a lethal bacteremia and renal abscess model in mice. In the inventors experience, *S. aureus* 8325-4 is not fully virulent and it is presumed that mutational lesions in this strain may not be able to reveal virulence defects in vivo. Moreover, antibodies raised against Coa or vWbp perturb the pathogenesis of *S. aureus* Newman infections to a degree mirroring the impact of gene deletions. Coa and vWbp contribute to staphylococcal abscess formation and lethal bacteremia and may also function as protective antigens in subunit vaccines.

Biochemical studies document the biological value of antibodies against Coa and vWbp. By binding to antigen and blocking its association with clotting factors, the antibodies prevent the formation of Coa-prothrombin and vWbp-pro-

thrombin complexes. Passive transfer studies revealed protection of experimental animals against staphylococcal abscess formation and lethal challenge by Coa and vWbp antibodies. Thus, Coa and vWbp neutralizing antibodies generate immune protection against staphylococcal disease.

Earlier studies revealed a requirement of coagulase for resisting phagocytosis in blood (Smith et al., 1947) and the inventors observed a similar phenotype for Δ coa mutants in lepirudin-treated mouse blood (see Example 3 below). As vWbp displays higher affinity for human prothrombin than the mouse counterpart, it is suspected the same may be true for Δ vWbp variants in human blood. Further, expression of Coa and vWbp in abscess lesions as well as their striking distribution in the eosinophilic pseudocapsule surrounding (staphylococcal abscess communities (SACs) or the peripheral fibrin wall, suggest that secreted coagulases contribute to the establishment of these lesions. This hypothesis was tested and, indeed, Δ coa mutants were defective in the establishment of abscesses. A corresponding test, blocking Coa function with specific antibodies, produced the same effect. Consequently, it is proposed that the clotting of fibrin is a critical event in the establishment of staphylococcal abscesses that can be targeted for the development of protective vaccines. Due to their overlapping function on human prothrombin, both Coa and vWbp are considered excellent candidates for vaccine development.

C. Other Staphylococcal Antigens

Research over the past several decades identified *S. aureus* exotoxins, surface proteins and regulatory molecules as important virulence factors (Foster, 2005; Mazmanian et al., 2001; Novick, 2003). Much progress has been achieved regarding the regulation of these genes. For example, staphylococci perform a bacterial census via the secretion of auto-inducing peptides that bind to a cognate receptor at threshold concentration, thereby activating phospho-relay reactions and transcriptional activation of many of the exotoxin genes (Novick, 2003). The pathogenesis of staphylococcal infections relies on these virulence factors (secreted exotoxins, exopolysaccharides, and surface adhesins). The development of staphylococcal vaccines is hindered by the multifaceted nature of staphylococcal invasion mechanisms. It is well established that live attenuated micro-organisms are highly effective vaccines; immune responses elicited by such vaccines are often of greater magnitude and of longer duration than those produced by non-replicating immunogens. One explanation for this may be that live attenuated strains establish limited infections in the host and mimic the early stages of natural infection. Embodiments of the invention are directed to compositions and methods including variant SpA polypeptides and peptides, as well as other immunogenic extracellular proteins, polypeptides, and peptides (including both secreted and cell surface proteins or peptides) of gram positive bacteria for the use in mitigating or immunizing against infection. In particular embodiments the bacteria is a *staphylococcus* bacteria. Extracellular proteins, polypeptides, or peptides include, but are not limited to secreted and cell surface proteins of the targeted bacteria.

The human pathogen *S. aureus* secretes EsxA and EsxB, two ESAT-6 like proteins, across the bacterial envelope (Burts et al., 2005, which is incorporated herein by reference). Staphylococcal esxA and esxB are clustered with six other genes in the order of transcription: esxA esaA esaA esaB essB essC esaC esxB. The acronyms esa, ess, and esx stand for ESAT-6 secretion accessory, system, and extracellular, respectively, depending whether the encoded proteins play an accessory (esa) or direct (ess) role for secretion, or are secreted (esx) in the extracellular milieu. The entire

cluster of eight genes is herein referred to as the Ess cluster. EsxA, esxB, essA, essB, and essC are all required for synthesis or secretion of EsxA and EsxB. Mutants that fail to produce EsxA, EsxB, and EssC display defects in the pathogenesis of *S. aureus* murine abscesses, suggesting that this specialized secretion system may be a general strategy of human bacterial pathogenesis. Secretion of non-WXG100 substrates by the ESX-1 pathway has been reported for several antigens including EspA, EspB, Rv3483c, and Rv3615c (Fortune et al., 2005; MacGurn et al., 2005; McLaughlin et al., 2007; Xu et al., 2007). The alternate ESX-5 pathway has also been shown to secrete both WXG100 and non-WXG100 proteins in pathogenic mycobacteria (Abdallah et al., 2007; Abdallah et al., 2006).

The *Staphylococcus aureus* Ess pathway can be viewed as a secretion module equipped with specialized transport components (Ess), accessory factors (Esa) and cognate secretion substrates (Esx). EssA, EssB and EssC are required for EsxA and EsxB secretion. Because EssA, EssB and EssC are predicted to be transmembrane proteins, it is contemplated that these proteins form a secretion apparatus. Some of the proteins in the ess gene cluster may actively transport secreted substrates (acting as motor) while others may regulate transport (regulator). Regulation may be achieved, but need not be limited to, transcriptional or post-translational mechanisms for secreted polypeptides, sorting of specific substrates to defined locations (e.g., extracellular medium or host cells), or timing of secretion events during infection. At this point, it is unclear whether all secreted Esx proteins function as toxins or contribute indirectly to pathogenesis.

Staphylococci rely on surface protein mediated-adhesion to host cells or invasion of tissues as a strategy for escape from immune defenses. Furthermore, *S. aureus* utilize surface proteins to sequester iron from the host during infection. The majority of surface proteins involved in staphylococcal pathogenesis carry C-terminal sorting signals, i.e., they are covalently linked to the cell wall envelope by sortase. Further, staphylococcal strains lacking the genes required for surface protein anchoring, i.e., sortase A and B, display a dramatic defect in the virulence in several different mouse models of disease. Thus, surface protein antigens represent a validated vaccine target as the corresponding genes are essential for the development of staphylococcal disease and can be exploited in various embodiments of the invention. The sortase enzyme superfamily are Gram-positive transpeptidases responsible for anchoring surface protein virulence factors to the peptidoglycan cell wall layer. Two sortase isoforms have been identified in *Staphylococcus aureus*, SrtA and SrtB. These enzymes have been shown to recognize a LPXTG motif in substrate proteins. The SrtB isoform appears to be important in heme iron acquisition and iron homeostasis, whereas the SrtA isoform plays a critical role in the pathogenesis of Gram-positive bacteria by modulating the ability of the bacterium to adhere to host tissue via the covalent anchoring of adhesins and other proteins to the cell wall peptidoglycan. In certain embodiments the SpA variants described herein can be used in combination with other staphylococcal proteins such as Coa, Eap, Ebh, Emp, EsaC, EsaB, EsxA, EsxB, Hla, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, IsdC, SasF, vWbp, and/or vWh proteins.

Certain aspects of the invention include methods and compositions concerning proteinaceous compositions including polypeptides, peptides, or nucleic acid encoding SpA variant(s) and other staphylococcal antigens such as

other proteins transported by the Ess pathway, or sortase substrates. These proteins may be modified by deletion, insertion, and/or substitution.

The Exs polypeptides include the amino acid sequence of Exs proteins from bacteria in the *Staphylococcus* genus. The Exs sequence may be from a particular *staphylococcus* species, such as *Staphylococcus aureus*, and may be from a particular strain, such as Newman. In certain embodiments, the ExsA sequence is SAV0282 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number Q99WU4 (gil68565539), which is hereby incorporated by reference. In other embodiments, the ExsB sequence is SAV0290 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number Q99WT7 (gil68565532), which is hereby incorporated by reference. In further embodiments, other polypeptides transported by the Ess pathway may be used, the sequences of which may be identified by one of skill in the art using databases and internet accessible resources.

The sortase substrate polypeptides include, but are not limited to the amino acid sequence of SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, IsdC or SasF proteins from bacteria in the *Staphylococcus* genus. The sortase substrate polypeptide sequence may be from a particular *staphylococcus* species, such as *Staphylococcus aureus*, and may be from a particular strain, such as Newman. In certain embodiments, the SdrD sequence is from strain N315 and can be accessed using Genbank Accession Number NP_373773.1 (gil15926240), which is incorporated by reference. In other embodiments, the SdrE sequence is from strain N315 and can be accessed using Genbank Accession Number NP_373774.1 (gil15926241), which is incorporated by reference. In other embodiments, the IsdA sequence is SAV1130 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number NP_371654.1 (gil15924120), which is incorporated by reference. In other embodiments, the IsdB sequence is SAV1129 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number NP_371653.1 (gil15924119), which is incorporated by reference. In further embodiments, other polypeptides transported by the Ess pathway or processed by sortase may be used, the sequences of which may be identified by one of skill in the art using databases and internet accessible resources.

Examples of various proteins that can be used in the context of the present invention can be identified by analysis of database submissions of bacterial genomes, including but not limited to accession numbers NC_002951 (GI:57650036 and GenBank CP000046), NC_002758 (GI:57634611 and GenBank BA000017), NC_002745 (GI:29165615 and GenBank BA000018), NC_003923 (GI:21281729 and GenBank BA000033), NC_002952 (GI:49482253 and GenBank BX571856), NC_002953 (GI:49484912 and GenBank BX571857), NC_007793 (GI:87125858 and GenBank CP000255), NC_007795 (GI:87201381 and GenBank CP000253) each of which are incorporated by reference.

As used herein, a "protein" or "polypeptide" refers to a molecule comprising at least ten amino acid residues. In some embodiments, a wild-type version of a protein or polypeptide are employed, however, in many embodiments of the invention, a modified protein or polypeptide is employed to generate an immune response. The terms described above may be used interchangeably. A "modified protein" or "modified polypeptide" or a "variant" refers to a protein or polypeptide whose chemical structure, particu-

larly its amino acid sequence, is altered with respect to the wild-type protein or polypeptide. In some embodiments, a modified/variant protein or polypeptide has at least one modified activity or function (recognizing that proteins or polypeptides may have multiple activities or functions). It is specifically contemplated that a modified/variant protein or polypeptide may be altered with respect to one activity or function yet retain a wild-type activity or function in other respects, such as immunogenicity.

In certain embodiments the size of a protein or polypeptide (wild-type or modified) may comprise, but is not limited to, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000, 1100, 1200, 1300, 1400, 1500, 1750, 2000, 2250, 2500 amino molecules or greater, and any range derivable therein, or derivative of a corresponding amino sequence described or referenced herein. It is contemplated that polypeptides may be mutated by truncation, rendering them shorter than their corresponding wild-type form, but also they might be altered by fusing or conjugating a heterologous protein sequence with a particular function (e.g., for targeting or localization, for enhanced immunogenicity, for purification purposes, etc.).

As used herein, an "amino molecule" refers to any amino acid, amino acid derivative, or amino acid mimic known in the art. In certain embodiments, the residues of the proteinaceous molecule are sequential, without any non-amino molecule interrupting the sequence of amino molecule residues. In other embodiments, the sequence may comprise one or more non-amino molecule moieties. In particular embodiments, the sequence of residues of the proteinaceous molecule may be interrupted by one or more non-amino molecule moieties.

Accordingly, the term "proteinaceous composition" encompasses amino molecule sequences comprising at least one of the 20 common amino acids in naturally synthesized proteins, or at least one modified or unusual amino acid.

Proteinaceous compositions may be made by any technique known to those of skill in the art, including (i) the expression of proteins, polypeptides, or peptides through standard molecular biological techniques, (ii) the isolation of proteinaceous compounds from natural sources, or (iii) the chemical synthesis of proteinaceous materials. The nucleotide as well as the protein, polypeptide, and peptide sequences for various genes have been previously disclosed, and may be found in the recognized computerized databases. One such database is the National Center for Biotechnology Information's Genbank and GenPept databases (on the World Wide Web at ncbi.nlm.nih.gov/). The coding regions for these genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art.

Amino acid sequence variants of SpA, coagulases and other polypeptides of the invention can be substitutional, insertional, or deletion variants. A variation in a polypeptide of the invention may affect 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, or more non-contiguous or contiguous amino acids of the polypeptide, as compared to wild-type. A

variant can comprise an amino acid sequence that is at least 50%, 60%, 70%, 80%, or 90%, including all values and ranges there between, identical to any sequence provided or referenced herein, e.g., SEQ ID NO:2-8 or SEQ ID NO:11-30, A variant can include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more substitute amino acids. A

leucine. Alternatively, substitutions may be non-conservative such that a function or activity of the polypeptide is affected. Non-conservative changes typically involve substituting a residue with one that is chemically dissimilar, such as a polar or charged amino acid for a nonpolar or uncharged amino acid, and vice versa.

TABLE 2

Exemplary surface proteins of <i>S. aureus</i> strains.								
SAV #	SA#	Surface	MW2	Mu50	N315	Newman	MRSA252*	MSSA476*
SAV0111	SA0107	Spa	492	450	450	520	516	492
SAV2503	SA2291	FnBPA	1015	1038	1038	741	—	1015
SAV2502	SA2290	FnBPB	943	961	961	677	965	957
SAV0811	SA0742	ClfA	946	935	989	933	1029	928
SAV2630	SA2423	ClfB	907	877	877	913	873	905
Np	Np	Cna	1183	—	—	—	1183	1183
SAV0561	SA0519	SdrC	955	953	953	947	906	957
SAV0562	SA0520	SdrD	1347	1385	1385	1315	—	1365
SAV0563	SA0521	SdrE	1141	1141	1141	1166	1137	1141
Np	Np	Pls	—	—	—	—	—	—
SAV2654	SA2447	SasA	2275	2271	2271	2271	1351	2275
SAV2160	SA1964	SasB	686	2481	2481	2481	2222	685
	SA1577	SasC	2186	213	2186	2186	2189	2186
SAV0134	SA0129	SasD	241	241	241	241	221	241
SAV1130	SA0977	SasE/IsdA	350	350	350	350	354	350
SAV2646	SA2439	SasF	635	635	635	635	627	635
SAV2496		SasG	1371	525	927	—	—	1371
SAV0023	SA0022	SasH	772	—	772	772	786	786
SAV1731	SA1552	SasI	895	891	891	891	534	895
SAV1129	SA0976	SasJ/IsdB	645	645	645	645	652	645
	SA2381	SasK	198	211	211	—	—	197
	Np	SasL	—	232	—	—	—	—
SAV1131	SA0978	IsdC	227	227	227	227	227	227

polypeptide processed or secreted by the Ess pathway or other surface proteins (see Table 1) or sortase substrates from any *staphylococcus* species and strain are contemplated for use in compositions and methods described herein.

Deletion variants typically lack one or more residues of the native or wild-type protein. Individual residues can be deleted or a number of contiguous amino acids can be deleted. A stop codon may be introduced (by substitution or insertion) into an encoding nucleic acid sequence to generate a truncated protein. Insertional mutants typically involve the addition of material at a non-terminal point in the polypeptide. This may include the insertion of one or more residues. Terminal additions, called fusion proteins, may also be generated. These fusion proteins include multimers or concatamers of one or more peptide or polypeptide described or referenced herein.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, with or without the loss of other functions or properties. Substitutions may be conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or

Proteins of the invention may be recombinant, or synthesized in vitro. Alternatively, a non-recombinant or recombinant protein may be isolated from bacteria. It is also contemplated that a bacteria containing such a variant may be implemented in compositions and methods of the invention. Consequently, a protein need not be isolated.

The term "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids (see Table 2, below).

TABLE 3

Codon Table			
Amino Acids			Codons
Alanine	Ala	A	GCA GCC GCG GCU
Cysteine	Cys	C	UGC UGU
Aspartic acid	Asp	D	GAC GAU
Glutamic acid	Glu	E	GAA GAG
Phenylalanine	Phe	F	UUC UUU
Glycine	Gly	G	GGA GGC GGG GGU
Histidine	His	H	CAC CAU
Isoleucine	Ile	I	AUA AUC AUU
Lysine	Lys	K	AAA AAG
Leucine	Leu	L	UUA UUG CUA CUC CUG CUU

TABLE 3-continued

Codon Table			
Amino Acids		Codons	
Methionine	Met M	AUG	
Asparagine	Asn N	AAC AAU	
Proline	Pro P	CCA CCC CCG CCU	
Glutamine	Gln Q	CAA CAG	
Arginine	Arg R	AGA AGG CGA CGC CGG CGU	
Serine	Ser S	AGC AGU UCA UCC UCG UCU	
Threonine	Thr T	ACA ACC ACG ACU	
Valine	Val V	GUA GUC GUG GUU	
Tryptophan	Trp W	UGG	
Tyrosine	Tyr Y	UAC UAU	

It also will be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids, or 5' or 3' sequences, respectively, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity (e.g., immunogenicity) where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region.

The following is a discussion based upon changing of the amino acids of a protein to create a variant polypeptide or peptide. For example, certain amino acids may be substituted for other amino acids in a protein structure with or without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's functional activity, certain amino acid substitutions can be made in a protein sequence, and in its underlying DNA coding sequence, and nevertheless produce a protein with a desirable property. It is thus contemplated by the inventors that various changes may be made in the DNA sequences of genes.

It is contemplated that in compositions of the invention, there is between about 0.001 mg and about 10 mg of total polypeptide, peptide, and/or protein per ml. The concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg/ml or more (or any range derivable therein). Of this, about, at least about, or at most about 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% may be an SpA variant or a coagulase, and may be used in combination with other peptides or polypeptides, such as other bacterial peptides and/or antigens.

The present invention contemplates the administration of variant SpA polypeptides or peptides to effect a preventative

therapy or therapeutic effect against the development of a disease or condition associated with infection by a *staphylococcus* pathogen.

In certain aspects, combinations of staphylococcal antigens are used in the production of an immunogenic composition that is effective at treating or preventing staphylococcal infection. Staphylococcal infections progress through several different stages. For example, the staphylococcal life cycle involves commensal colonization, initiation of infection by accessing adjoining tissues or the bloodstream, and/or anaerobic multiplication in the blood. The interplay between *S. aureus* virulence determinants and the host defense mechanisms can induce complications such as endocarditis, metastatic abscess formation, and sepsis syndrome. Different molecules on the surface of the bacterium are involved in different steps of the infection cycle. Combinations of certain antigens can elicit an immune response which protects against multiple stages of staphylococcal infection. The effectiveness of the immune response can be measured either in animal model assays and/or using an opsonophagocytic assay.

D. Polypeptides and Polypeptide Production

The present invention describes polypeptides, peptides, and proteins and immunogenic fragments thereof for use in various embodiments of the present invention. For example, specific polypeptides are assayed for or used to elicit an immune response. In specific embodiments, all or part of the proteins of the invention can also be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, (1984); Tam et al., (1983); Merrifield, (1986); and Barany and Merrifield (1979), each incorporated herein by reference.

Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of the invention is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression.

One embodiment of the invention includes the use of gene transfer to cells, including microorganisms, for the production and/or presentation of polypeptides or peptides. The gene for the polypeptide or peptide of interest may be transferred into appropriate host cells followed by culture of cells under the appropriate conditions. The generation of recombinant expression vectors, and the elements included therein, are well known in the art and briefly discussed herein. Alternatively, the protein to be produced may be an endogenous protein normally synthesized by the cell that is isolated and purified.

Another embodiment of the present invention uses autologous B lymphocyte cell lines, which are transfected with a viral vector that expresses an immunogen product, and more specifically, a protein having immunogenic activity. Other examples of mammalian host cell lines include, but are not limited to Vero and HeLa cells, other B- and T-cell lines, such as CEM, 721.221, H9, Jurkat, Raji, as well as cell lines of Chinese hamster ovary, W138, BHK, COS-7, 293, HepG2, 3T3, RIN and MDCK cells. In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or that modifies and processes the gene product in the manner desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be

chosen to ensure the correct modification and processing of the foreign protein expressed.

A number of selection systems may be used including, but not limited to HSV thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes, in tk-, hgp^rt- or apr^t-cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection: for dhfr, which confers resistance to trimethoprim and methotrexate; gpt, which confers resistance to mycophenolic acid; neo, which confers resistance to the aminoglycoside G418; and hygro, which confers resistance to hygromycin.

Animal cells can be propagated in vitro in two modes: as non-anchorage-dependent cells growing in suspension throughout the bulk of the culture or as anchorage-dependent cells requiring attachment to a solid substrate for their propagation (i.e., a monolayer type of cell growth).

Non-anchorage dependent or suspension cultures from continuous established cell lines are the most widely used means of large scale production of cells and cell products. However, suspension cultured cells have limitations, such as tumorigenic potential and lower protein production than adherent cells.

Where a protein is specifically mentioned herein, it is preferably a reference to a native or recombinant protein or optionally a protein in which any signal sequence has been removed. The protein may be isolated directly from the staphylococcal strain or produced by recombinant DNA techniques. Immunogenic fragments of the protein may be incorporated into the immunogenic composition of the invention. These are fragments comprising at least 10 amino acids, 20 amino acids, 30 amino acids, 40 amino acids, 50 amino acids, or 100 amino acids, including all values and ranges there between, taken contiguously from the amino acid sequence of the protein. In addition, such immunogenic fragments are immunologically reactive with antibodies generated against the Staphylococcal proteins or with antibodies generated by infection of a mammalian host with Staphylococci. Immunogenic fragments also include fragments that when administered at an effective dose, (either alone or as a hapten bound to a carrier), elicit a protective or therapeutic immune response against Staphylococcal infection, in certain aspects it is protective against *S. aureus* and/or *S. epidermidis* infection. Such an immunogenic fragment may include, for example, the protein lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment according to the invention comprises substantially all of the extracellular domain of a protein which has at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, or at least 97-99% identity, including all values and ranges there between, to a sequence selected segment of a polypeptide described or referenced herein.

Also included in immunogenic compositions of the invention are fusion proteins composed of one or more Staphylococcal proteins, or immunogenic fragments of staphylococcal proteins. Such fusion proteins may be made recombinantly and may comprise one portion of at least 1, 2, 3, 4, 5, or 6 staphylococcal proteins or segments. Alternatively, a fusion protein may comprise multiple portions of at least 1, 2, 3, 4 or 5 staphylococcal proteins. These may combine different Staphylococcal proteins and/or multiples of the same protein or protein fragment, or immunogenic fragments in the same protein (forming a multimer or a concatamer).

Alternatively, the invention also includes individual fusion proteins of Staphylococcal proteins or immunogenic fragments thereof, as a fusion protein with heterologous sequences such as a provider of T-cell epitopes or purification tags, for example: β -galactosidase, glutathione-S-transferase, green fluorescent proteins (GFP), epitope tags such as FLAG, myc tag, poly histidine, or viral surface proteins such as influenza virus haemagglutinin, or bacterial proteins such as tetanus toxoid, diphtheria toxoid, or CRM197.

II. Nucleic Acids

In certain embodiments, the present invention concerns recombinant polynucleotides encoding the proteins, polypeptides, peptides of the invention. The nucleic acid sequences for SpA, coagulases and other bacterial proteins are included, all of which are incorporated by reference, and can be used to prepare peptides or polypeptides.

As used in this application, the term "polynucleotide" refers to a nucleic acid molecule that either is recombinant or has been isolated free of total genomic nucleic acid. Included within the term "polynucleotide" are oligonucleotides (nucleic acids of 100 residues or less in length), recombinant vectors, including, for example, plasmids, cosmids, phage, viruses, and the like. Polynucleotides include, in certain aspects, regulatory sequences, isolated substantially away from their naturally occurring genes or protein encoding sequences. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be RNA, DNA (genomic, cDNA or synthetic), analogs thereof, or a combination thereof. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide.

In this respect, the term "gene," "polynucleotide," or "nucleic acid" is used to refer to a nucleic acid that encodes a protein, polypeptide, or peptide (including any sequences required for proper transcription, post-translational modification, or localization). As will be understood by those in the art, this term encompasses genomic sequences, expression cassettes, cDNA sequences, and smaller engineered nucleic acid segments that express, or may be adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. A nucleic acid encoding all or part of a polypeptide may contain a contiguous nucleic acid sequence of: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1095, 1100, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 9000, 10000, or more nucleotides, nucleosides, or base pairs, including all values and ranges therebetween, of a polynucleotide encoding one or more amino acid sequence described or referenced herein. It also is contemplated that a particular polypeptide may be encoded by nucleic acids containing variations having slightly different nucleic acid sequences but, nonetheless, encode the same or substantially similar protein (see Table 3 above).

In particular embodiments, the invention concerns isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode a variant SpA or coagulase. The term "recombinant" may be used in con-

junction with a polynucleotide or polypeptide and generally refers to a polypeptide or polynucleotide produced and/or manipulated in vitro or that is a replication product of such a molecule.

In other embodiments, the invention concerns isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode a variant SpA or coagulase polypeptide or peptide to generate an immune response in a subject. In various embodiments the nucleic acids of the invention may be used in genetic vaccines.

The nucleic acid segments used in the present invention can be combined with other nucleic acid sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant nucleic acid protocol. In some cases, a nucleic acid sequence may encode a polypeptide sequence with additional heterologous coding sequences, for example to allow for purification of the polypeptide, transport, secretion, post-translational modification, or for therapeutic benefits such as targeting or efficacy. As discussed above, a tag or other heterologous polypeptide may be added to the modified polypeptide-encoding sequence, wherein "heterologous" refers to a polypeptide that is not the same as the modified polypeptide.

In certain other embodiments, the invention concerns isolated nucleic acid segments and recombinant vectors that include within their sequence a contiguous nucleic acid sequence from SEQ ID NO:1 (SpA domain D) or SEQ ID NO:3 (SpA) or any other nucleic acid sequences encoding coagulases or other secreted virulence factors and/or surface proteins including proteins transported by the Ess pathway, processed by sortase, or proteins incorporated herein by reference.

In certain embodiments, the present invention provides polynucleotide variants having substantial identity to the sequences disclosed herein; those comprising at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher sequence identity, including all values and ranges there between, compared to a polynucleotide sequence of this invention using the methods described herein (e.g., BLAST analysis using standard parameters).

The invention also contemplates the use of polynucleotides which are complementary to all the above described polynucleotides.

A. Vectors

Polypeptides of the invention may be encoded by a nucleic acid molecule comprised in a vector. The term "vector" is used to refer to a carrier nucleic acid molecule into which a heterologous nucleic acid sequence can be inserted for introduction into a cell where it can be replicated and expressed. A nucleic acid sequence can be "heterologous," which means that it is in a context foreign to the cell in which the vector is being introduced or to the nucleic acid in which is incorporated, which includes a sequence homologous to a sequence in the cell or nucleic acid but in a position within the host cell or nucleic acid where it is ordinarily not found. Vectors include DNAs, RNAs, plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g., YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques (for example Sambrook et al., 2001; Ausubel et al., 1996, both incorporated herein by reference). In addition to encoding a

variant SpA polypeptide the vector can encode other polypeptide sequences such as a one or more other bacterial peptide, a tag, or an immunogenicity enhancing peptide. Useful vectors encoding such fusion proteins include pIN vectors (Inouye et al., 1985), vectors encoding a stretch of histidines, and pGEX vectors, for use in generating glutathione S-transferase (GST) soluble fusion proteins for later purification and separation or cleavage.

The term "expression vector" refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described herein.

1. Promoters and Enhancers

A "promoter" is a control sequence. The promoter is typically a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. The phrases "operatively positioned," "operatively linked," "under control," and "under transcriptional control" mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and expression of that sequence. A promoter may or may not be used in conjunction with an "enhancer," which refers to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence.

Naturally, it may be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the cell type or organism chosen for expression. Those of skill in the art of molecular biology generally know the use of promoters, enhancers, and cell type combinations for protein expression (see Sambrook et al., 2001, incorporated herein by reference). The promoters employed may be constitutive, tissue-specific, or inducible and in certain embodiments may direct high level expression of the introduced DNA segment under specified conditions, such as large-scale production of recombinant proteins or peptides.

Various elements/promoters may be employed in the context of the present invention to regulate the expression of a gene. Examples of such inducible elements, which are regions of a nucleic acid sequence that can be activated in response to a specific stimulus, include but are not limited to Immunoglobulin Heavy Chain (Banerji et al., 1983; Gilles et al., 1983; Grosschedl et al., 1985; Atchinson et al., 1986, 1987; Imler et al., 1987; Weinberger et al., 1984; Kiledjian et al., 1988; Porton et al.; 1990), Immunoglobulin Light Chain (Queen et al., 1983; Picard et al., 1984), T Cell Receptor (Luria et al., 1987; Winoto et al., 1989; Redondo et al.; 1990), HLA DQ α and/or DQ β (Sullivan et al., 1987), β Interferon (Goodbourn et al., 1986; Fujita et al., 1987; Goodbourn et al., 1988), Interleukin-2 (Greene et al., 1989), Interleukin-2 Receptor (Greene et al., 1989; Lin et al., 1990), MHC Class II 5 (Koch et al., 1989), MHC Class II HLA-DR α (Sherman et al., 1989), β -Actin (Kawamoto et al., 1988; Ng et al.; 1989), Muscle Creatine Kinase (MCK) (Jaynes et al., 1988; Horlick et al., 1989; Johnson et al., 1989), Prealbumin (Transthyretin) (Costa et al., 1988),

Elastase I (Ornitz et al., 1987), Metallothionein (MTII) (Karin et al., 1987; Culotta et al., 1989), Collagenase (Pinkert et al., 1987; Angel et al., 1987), Albumin (Pinkert et al., 1987; Tronche et al., 1989, 1990), α -Fetoprotein (Godbout et al., 1988; Campere et al., 1989), γ -Globin (Bodine et al., 1987; Perez-Stable et al., 1990), β -Globin (Trudel et al., 1987), c-fos (Cohen et al., 1987), c-Ha-Ras (Triesman, 1986; Deschamps et al., 1985), Insulin (Edlund et al., 1985), Neural Cell Adhesion Molecule (NCAM) (Hirsh et al., 1990), α 1-Antitrypsin (Latimer et al., 1990), H2B (TH2B) Histone (Hwang et al., 1990), Mouse and/or Type I Collagen (Ripe et al., 1989), Glucose-Regulated Proteins (GRP94 and GRP78) (Chang et al., 1989), Rat Growth Hormone (Larsen et al., 1986), Human Serum Amyloid A (SAA) (Edbrooke et al., 1989), Troponin I (TN I) (Yutzey et al., 1989), Platelet-Derived Growth Factor (PDGF) (Pech et al., 1989), Duchenne Muscular Dystrophy (Klamut et al., 1990), SV40 (Banerji et al., 1981; Moreau et al., 1981; Sleight et al., 1985; Firak et al., 1986; Herr et al., 1986; Imbra et al., 1986; Kadesch et al., 1986; Wang et al., 1986; Ondek et al., 1987; Kuhl et al., 1987; Schaffner et al., 1988), Polyoma (Swartzendruber et al., 1975; Vasseur et al., 1980; Katinka et al., 1980, 1981; Tyndell et al., 1981; Dandolo et al., 1983; de Villiers et al., 1984; Hen et al., 1986; Satake et al., 1988; Campbell et al., 1988), Retroviruses (Kriegler et al., 1982, 1983; Levinson et al., 1982; Kriegler et al., 1983, 1984a, b, 1988; Bosze et al., 1986; Miksicek et al., 1986; Celander et al., 1987; Thiesen et al., 1988; Celander et al., 1988; Choi et al., 1988; Reisman et al., 1989), Papilloma Virus (Campo et al., 1983; Lusky et al., 1983; Spandidos and Wilkie, 1983; Spalholz et al., 1985; Lusky et al., 1986; Cripe et al., 1987; Gloss et al., 1987; Hirochika et al., 1987; Stephens et al., 1987), Hepatitis B Virus (Bulla et al., 1986; Jameel et al., 1986; Shaul et al., 1987; Spandau et al., 1988; Vannice et al., 1988), Human Immunodeficiency Virus (Muesing et al., 1987; Hauber et al., 1988; Jakobovits et al., 1988; Feng et al., 1988; Takebe et al., 1988; Rosen et al., 1988; Berkhout et al., 1989; Laspia et al., 1989; Sharp et al., 1989; Braddock et al., 1989), Cytomegalovirus (CMV) IE (Weber et al., 1984; Boshart et al., 1985; Foecking et al., 1986), Gibbon Ape Leukemia Virus (Holbrook et al., 1987; Quinn et al., 1989).

Inducible elements include, but are not limited to MT II-Phorbol Ester (TFA)/Heavy metals (Palmiter et al., 1982; Haslinger et al., 1985; Searle et al., 1985; Stuart et al., 1985; Imagawa et al., 1987; Karin et al., 1987; Angel et al., 1987b; McNeall et al., 1989); MMTV (mouse mammary tumor virus)—Glucocorticoids (Huang et al., 1981; Lee et al., 1981; Majors et al., 1983; Chandler et al., 1983; Lee et al., 1984; Ponta et al., 1985; Sakai et al., 1988); β -Interferon-poly(rI)x/poly(rc) (Tavernier et al., 1983); Adenovirus 5 E2-E1A (Imperiale et al., 1984); Collagenase—Phorbol Ester (TPA) (Angel et al., 1987a); Stromelysin—Phorbol Ester (TPA) (Angel et al., 1987b); SV40—Phorbol Ester (TPA) (Angel et al., 1987b); Murine MX Gene—Interferon, Newcastle Disease Virus (Hug et al., 1988); GRP78 Gene—A23187 (Resendez et al., 1988); α -2-Macroglobulin—IL-6 (Kunz et al., 1989); Vimentin—Serum (Rittling et al., 1989); MHC Class I Gene H-2kb—Interferon (Blonar et al., 1989); HSP70—E1A/SV40 Large T Antigen (Taylor et al., 1989, 1990a, 1990b); Proliferin—Phorbol Ester/TPA (Mordacq et al., 1989); Tumor Necrosis Factor—PMA (Hensel et al., 1989); and Thyroid Stimulating Hormone a Gene—Thyroid Hormone (Chatterjee et al., 1989).

The particular promoter that is employed to control the expression of peptide or protein encoding polynucleotide of the invention is not believed to be critical, so long as it is

capable of expressing the polynucleotide in a targeted cell, preferably a bacterial cell. Where a human cell is targeted, it is preferable to position the polynucleotide coding region adjacent to and under the control of a promoter that is capable of being expressed in a human cell. Generally speaking, such a promoter might include either a bacterial, human or viral promoter.

In embodiments in which a vector is administered to a subject for expression of the protein, it is contemplated that a desirable promoter for use with the vector is one that is not down-regulated by cytokines or one that is strong enough that even if down-regulated, it produces an effective amount of a variant SpA for eliciting an immune response. Non-limiting examples of these are CMV IE and RSV LTR. Tissue specific promoters can be used, particularly if expression is in cells in which expression of an antigen is desirable, such as dendritic cells or macrophages. The mammalian MHC I and MHC II promoters are examples of such tissue-specific promoters.

2. Initiation Signals and Internal Ribosome Binding Sites (IRES)

A specific initiation signal also may be required for efficient translation of coding sequences. These signals include the ATG initiation codon or adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals.

In certain embodiments of the invention, the use of internal ribosome entry sites (IRES) elements are used to create multigene, or polycistronic, messages. IRES elements are able to bypass the ribosome scanning model of 5' \square methylated Cap dependent translation and begin translation at internal sites (Pelletier and Sonenberg, 1988; Macejak and Sarnow, 1991). IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message (see U.S. Pat. Nos. 5,925,565 and 5,935,819, herein incorporated by reference).

3. Selectable and Screenable Markers

In certain embodiments of the invention, cells containing a nucleic acid construct of the present invention may be identified *in vitro* or *in vivo* by encoding a screenable or selectable marker in the expression vector. When transcribed and translated, a marker confers an identifiable change to the cell permitting easy identification of cells containing the expression vector. Generally, a selectable marker is one that confers a property that allows for selection. A positive selectable marker is one in which the presence of the marker allows for its selection, while a negative selectable marker is one in which its presence prevents its selection. An example of a positive selectable marker is a drug resistance marker.

B. Host Cells

As used herein, the terms "cell," "cell line," and "cell culture" may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, "host cell" refers to a prokaryotic or eukaryotic cell, and it includes any transformable organism that is capable of replicating a vector or expressing a heterologous gene encoded by a vector. A host cell can, and has been, used as a recipient for vectors or viruses. A host cell may be

“transfected” or “transformed,” which refers to a process by which exogenous nucleic acid, such as a recombinant protein-encoding sequence, is transferred or introduced into the host cell. A transformed cell includes the primary subject cell and its progeny.

Host cells may be derived from prokaryotes or eukaryotes, including bacteria, yeast cells, insect cells, and mammalian cells for replication of the vector or expression of part or all of the nucleic acid sequence(s). Numerous cell lines and cultures are available for use as a host cell, and they can be obtained through the American Type Culture Collection (ATCC), which is an organization that serves as an archive for living cultures and genetic materials (www.atcc.org).

C. Expression Systems

Numerous expression systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with the present invention to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. Many such systems are commercially and widely available.

The insect cell/baculovirus system can produce a high level of protein expression of a heterologous nucleic acid segment, such as described in U.S. Pat. Nos. 5,871,986, 4,879,236, both herein incorporated by reference, and which can be bought, for example, under the name MAXBAC® 2.0 from INVITROGEN® and BACPACK™ BACULOVIRUS EXPRESSION SYSTEM FROM CLONTECH®.

In addition to the disclosed expression systems of the invention, other examples of expression systems include STRATAGENE®'s COMPLETE CONTROL™ Inducible Mammalian Expression System, which involves a synthetic ecdysone-inducible receptor, or its pET Expression System, an *E. coli* expression system. Another example of an inducible expression system is available from INVITROGEN®, which carries the T-REX™ (tetracycline-regulated expression) System, an inducible mammalian expression system that uses the full-length CMV promoter. INVITROGEN® also provides a yeast expression system called the *Pichia methanolica* Expression System, which is designed for high-level production of recombinant proteins in the methylotrophic yeast *Pichia methanolica*. One of skill in the art would know how to express a vector, such as an expression construct, to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide.

III. Polysaccharides

The immunogenic compositions of the invention may further comprise capsular polysaccharides including one or more of PIA (also known as PNAG) and/or *S. aureus* Type V and/or type VIII capsular polysaccharide and/or *S. epidermidis* Type I, and/or Type II and/or Type III capsular polysaccharide.

A. PIA (PNAG)

It is now clear that the various forms of staphylococcal surface polysaccharides identified as PS/A, PIA and SAA are the same chemical entity—PNAG (Maira-Litran et al., 2004). Therefore the term PIA or PNAG encompasses all these polysaccharides or oligosaccharides derived from them.

PIA is a polysaccharide intercellular adhesion and is composed of a polymer of β -(1 \rightarrow 6)-linked glucosamine substituted with N-acetyl and O-succinyl constituents. This polysaccharide is present in both *S. aureus* and *S. epidermidis* and can be isolated from either source (Joyce et al., 2003;

Maira-Litran et al., 2002). For example, PNAG may be isolated from *S. aureus* strain MN8m (WO04/43407). PIA isolated from *S. epidermidis* is an integral constituent of biofilm. It is responsible for mediating cell-cell adhesion and probably also functions to shield the growing colony from the host's immune response. The polysaccharide previously known as poly-N-succinyl- β -(1 \rightarrow 6)-glucosamine (PNSG) was recently shown not to have the expected structure since the identification of N-succinylation was incorrect (Maira-Litran et al., 2002). Therefore the polysaccharide formally known as PNSG and now found to be PNAG is also encompassed by the term PIA.

PIA (or PNAG) may be of different sizes varying from over 400 kDa to between 75 and 400 kDa to between 10 and 75 kDa to oligosaccharides composed of up to 30 repeat units (of β -(1 \rightarrow 6)-linked glucosamine substituted with N-acetyl and O-succinyl constituents). Any size of PIA polysaccharide or oligosaccharide may be used in an immunogenic composition of the invention, in one aspect the polysaccharide is over 40 kDa. Sizing may be achieved by any method known in the art, for instance by microfluidization, ultrasonic irradiation or by chemical cleavage (WO 03/53462, EP497524, EP497525). In certain aspects PIA (PNAG) is at least or at most 40-400 kDa, 40-300 kDa, 50-350 kDa, 60-300 kDa, 50-250 kDa and 60-200 kDa.

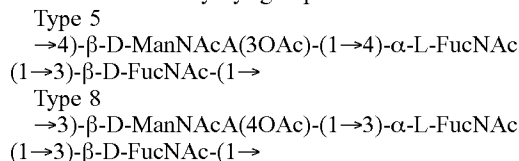
PIA (PNAG) can have different degree of acetylation due to substitution on the amino groups by acetate. PIA produced in vitro is almost fully substituted on amino groups (95-100%). Alternatively, a deacetylated PIA (PNAG) can be used having less than 60%, 50%, 40%, 30%, 20%, 10% acetylation. Use of a deacetylated PIA (PNAG) is preferred since non-acetylated epitopes of PNAG are efficient at mediating opsonic killing of Gram positive bacteria, preferably *S. aureus* and/or *S. epidermidis*. In certain aspects, the PIA (PNAG) has a size between 40 kDa and 300 kDa and is deacetylated so that less than 60%, 50%, 40%, 30% or 20% of amino groups are acetylated.

The term deacetylated PNAG (dPNAG) refers to a PNAG polysaccharide or oligosaccharide in which less than 60%, 50%, 40%, 30%, 20% or 10% of the amino groups are acetylated. In certain aspects, PNAG is deacetylated to form dPNAG by chemically treating the native polysaccharide. For example, the native PNAG is treated with a basic solution such that the pH rises to above 10. For instance the PNAG is treated with 0.1-5 M, 0.2-4 M, 0.3-3 M, 0.5-2 M, 0.75-1.5 M or 1 M NaOH, KOH or NH₄OH. Treatment is for at least 10 to 30 minutes, or 1, 2, 3, 4, 5, 10, 15 or 20 hours at a temperature of 20-100, 25-80, 30-60 or 30-50 or 35-45° C. dPNAG may be prepared as described in WO 04/43405.

The polysaccharide(s) can be conjugated or unconjugated to a carrier protein.

B. Type 5 and Type 8 Polysaccharides from *S. aureus*

Most strains of *S. aureus* that cause infection in man contain either Type 5 or Type 8 polysaccharides. Approximately 60% of human strains are Type 8 and approximately 30% are Type 5. The structures of Type 5 and Type 8 capsular polysaccharide antigens are described in Moreau et al., (1990) and Fournier et al., (1984). Both have FucNAcp in their repeat unit as well as ManNAcA which can be used to introduce a sulfhydryl group. The structures are:



Recently (Jones, 2005) NMR spectroscopy revised the structures to:

Type 5
 $\rightarrow 4$)- β -D-ManNAcA-(1 \rightarrow 4)- α -L-FucNAc(3OAc)-
 (1 \rightarrow 3)- β -D-FucNAc-(1 \rightarrow
 Type 8
 $\rightarrow 3$)- β -D-ManNAcA(4OAc)-(1 \rightarrow 3)- α -L-FucNAc
 (1 \rightarrow 3)- α -D-FucNAc-(1 \rightarrow

Polysaccharides may be extracted from the appropriate strain of *S. aureus* using method well known to of skill in the art, See U.S. Pat. No. 6,294,177. For example, ATCC 12902 is a Type 5 *S. aureus* strain and ATCC 12605 is a Type 8 *S. aureus* strain.

Polysaccharides are of native size or alternatively may be sized, for instance by microfluidisation, ultrasonic irradiation, or by chemical treatment. The invention also covers oligosaccharides derived from the type 5 and 8 polysaccharides from *S. aureus*. The type 5 and 8 polysaccharides included in the immunogenic composition of the invention are preferably conjugated to a carrier protein as described below or are alternatively unconjugated. The immunogenic compositions of the invention alternatively contains either type 5 or type 8 polysaccharide.

C. *S. aureus* 336 Antigen

In an embodiment, the immunogenic composition of the invention comprises the *S. aureus* 336 antigen described in U.S. Pat. No. 6,294,177. The 336 antigen comprises β -linked hexosamine, contains no O-acetyl groups, and specifically binds to antibodies to *S. aureus* Type 336 deposited under ATCC 55804. In an embodiment, the 336 antigen is a polysaccharide which is of native size or alternatively may be sized, for instance by microfluidisation, ultrasonic irradiation, or by chemical treatment. The invention also covers oligosaccharides derived from the 336 antigen. The 336 antigen can be unconjugated or conjugated to a carrier protein.

D. Type I, II and III Polysaccharides from *S. epidermidis*

Amongst the problems associated with the use of polysaccharides in vaccination, is the fact that polysaccharides per se are poor immunogens. It is preferred that the polysaccharides utilized in the invention are linked to a protein carrier which provide bystander T-cell help to improve immunogenicity. Examples of such carriers which may be conjugated to polysaccharide immunogens include the Diphtheria and Tetanus toxoids (DT, DT CRM197 and TT respectively), Keyhole Limpet Haemocyanin (KLH), and the purified protein derivative of Tuberculin (PPD), *Pseudomonas aeruginosa* exoprotein A (rEPA), protein D from *Haemophilus influenzae*, pneumolysin or fragments of any of the above. Fragments suitable for use include fragments encompassing T-helper epitopes. In particular the protein D fragment from *H. influenzae* will preferably contain the N-terminal $\frac{1}{3}$ of the protein. Protein D is an IgD-binding protein from *Haemophilus influenzae* (EP 0 594 610 B1) and is a potential immunogen. In addition, staphylococcal proteins may be used as a carrier protein in the polysaccharide conjugates of the invention.

A carrier protein that would be particularly advantageous to use in the context of a staphylococcal vaccine is staphylococcal alpha toxoid. The native form may be conjugated to a polysaccharide since the process of conjugation reduces toxicity. Preferably genetically detoxified alpha toxins such as the His35Leu or His35Arg variants are used as carriers since residual toxicity is lower. Alternatively the alpha toxin is chemically detoxified by treatment with a cross-linking reagent, formaldehyde or glutaraldehyde. A genetically detoxified alpha toxin is optionally chemically detoxified,

preferably by treatment with a cross-linking reagent, formaldehyde or glutaraldehyde to further reduce toxicity.

The polysaccharides may be linked to the carrier protein(s) by any known method (for example those methods described in U.S. Pat. Nos. 4,372,945, 4,474,757, and 4,356,170). Preferably, CDAP conjugation chemistry is carried out (see WO95/08348). In CDAP, the cyanylating reagent 1-cyano-dimethylaminopyridinium tetrafluoroborate (CD-AP) is preferably used for the synthesis of polysaccharide-protein conjugates. The cyanilation reaction can be performed under relatively mild conditions, which avoids hydrolysis of the alkaline sensitive polysaccharides. This synthesis allows direct coupling to a carrier protein.

Conjugation preferably involves producing a direct linkage between the carrier protein and polysaccharide. Optionally a spacer (such as adipic dihydride (ADH)) may be introduced between the carrier protein and the polysaccharide.

IV. Immune Response and Assays

As discussed above, the invention concerns evoking or inducing an immune response in a subject against a variant SpA or coagulase peptide. In one embodiment, the immune response can protect against or treat a subject having, suspected of having, or at risk of developing an infection or related disease, particularly those related to staphylococci. One use of the immunogenic compositions of the invention is to prevent nosocomial infections by inoculating a subject prior to undergoing procedures in a hospital or other environment having an increased risk of infection.

A. Immunoassays

The present invention includes the implementation of serological assays to evaluate whether and to what extent an immune response is induced or evoked by compositions of the invention. There are many types of immunoassays that can be implemented. Immunoassays encompassed by the present invention include, but are not limited to, those described in U.S. Pat. No. 4,367,110 (double monoclonal antibody sandwich assay) and U.S. Pat. No. 4,452,901 (western blot). Other assays include immunoprecipitation of labeled ligands and immunocytochemistry, both in vitro and in vivo.

Immunoassays generally are binding assays. Certain preferred immunoassays are the various types of enzyme linked immunosorbent assays (ELISAs) and radioimmunoassays (RIA) known in the art. Immunohistochemical detection using tissue sections is also particularly useful. In one example, antibodies or antigens are immobilized on a selected surface, such as a well in a polystyrene microtiter plate, dipstick, or column support. Then, a test composition suspected of containing the desired antigen or antibody, such as a clinical sample, is added to the wells. After binding and washing to remove non specifically bound immune complexes, the bound antigen or antibody may be detected. Detection is generally achieved by the addition of another antibody, specific for the desired antigen or antibody, that is linked to a detectable label. This type of ELISA is known as a "sandwich ELISA." Detection also may be achieved by the addition of a second antibody specific for the desired antigen, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label.

Competition ELISAs are also possible implementations which test samples compete for binding with known amounts of labeled antigens or antibodies. The amount of reactive species in the unknown sample is determined by

mixing the sample with the known labeled species before or during incubation with coated wells. The presence of reactive species in the sample acts to reduce the amount of labeled species available for binding to the well and thus reduces the ultimate signal. Irrespective of the format employed, ELISAs have certain features in common, such as coating, incubating or binding, washing to remove non specifically bound species, and detecting the bound immune complexes.

Antigen or antibodies may also be linked to a solid support, such as in the form of plate, beads, dipstick, membrane, or column matrix, and the sample to be analyzed is applied to the immobilized antigen or antibody. In coating a plate with either antigen or antibody, one will generally incubate the wells of the plate with a solution of the antigen or antibody, either overnight or for a specified period. The wells of the plate will then be washed to remove incompletely-adsorbed material. Any remaining available surfaces of the wells are then "coated" with a nonspecific protein that is antigenically neutral with regard to the test antisera. These include bovine serum albumin (BSA), casein, and solutions of milk powder. The coating allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

B. Diagnosis of Bacterial Infection

In addition to the use of proteins, polypeptides, and/or peptides, as well as antibodies binding these polypeptides, proteins, and/or peptides, to treat or prevent infection as described above, the present invention contemplates the use of these polypeptides, proteins, peptides, and/or antibodies in a variety of ways, including the detection of the presence of Staphylococci to diagnose an infection, whether in a patient or on medical equipment which may also become infected. In accordance with the invention, a preferred method of detecting the presence of infections involves the steps of obtaining a sample suspected of being infected by one or more staphylococcal bacteria species or strains, such as a sample taken from an individual, for example, from one's blood, saliva, tissues, bone, muscle, cartilage, or skin. Following isolation of the sample, diagnostic assays utilizing the polypeptides, proteins, peptides, and/or antibodies of the present invention may be carried out to detect the presence of staphylococci, and such assay techniques for determining such presence in a sample are well known to those skilled in the art and include methods such as radioimmunoassay, western blot analysis and ELISA assays. In general, in accordance with the invention, a method of diagnosing an infection is contemplated wherein a sample suspected of being infected with staphylococci has added to it the polypeptide, protein, peptide, antibody, or monoclonal antibody in accordance with the present invention, and staphylococci are indicated by antibody binding to the polypeptides, proteins, and/or peptides, or polypeptides, proteins, and/or peptides binding to the antibodies in the sample.

Accordingly, antibodies in accordance with the invention may be used for the prevention of infection from staphylococcal bacteria (i.e., passive immunization), for the treatment of an ongoing infection, or for use as research tools. The term "antibodies" as used herein includes monoclonal, polyclonal, chimeric, single chain, bispecific, simianized, and humanized or primate antibodies as well as Fab fragments, such as those fragments which maintain the binding specificity of the antibodies, including the products of an Fab immunoglobulin expression library. Accordingly, the invention contemplates the use of single chains such as

the variable heavy and light chains of the antibodies. Generation of any of these types of antibodies or antibody fragments is well known to those skilled in the art. Specific examples of the generation of an antibody to a bacterial protein can be found in U.S. Patent Application Pub. No. 20030153022, which is incorporated herein by reference in its entirety.

Any of the above described polypeptides, proteins, peptides, and/or antibodies may be labeled directly with a detectable label for identification and quantification of staphylococcal bacteria. Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances, including colored particles such as colloidal gold or latex beads. Suitable immunoassays include enzyme-linked immunosorbent assays (ELISA).

C. Protective Immunity

In some embodiments of the invention, proteinaceous compositions confer protective immunity to a subject. Protective immunity refers to a body's ability to mount a specific immune response that protects the subject from developing a particular disease or condition that involves the agent against which there is an immune response. An immunogenically effective amount is capable of conferring protective immunity to the subject.

As used herein in the specification and in the claims section that follows, the term polypeptide or peptide refer to a stretch of amino acids covalently linked there amongst via peptide bonds. Different polypeptides have different functionalities according to the present invention. While according to one aspect, a polypeptide is derived from an immunogen designed to induce an active immune response in a recipient, according to another aspect of the invention, a polypeptide is derived from an antibody which results following the elicitation of an active immune response in, for example, an animal, and which can serve to induce a passive immune response in the recipient. In both cases, however, the polypeptide is encoded by a polynucleotide according to any possible codon usage.

As used herein the phrase "immune response" or its equivalent "immunological response" refers to the development of a humoral (antibody mediated), cellular (mediated by antigen-specific T cells or their secretion products) or both humoral and cellular response directed against a protein, peptide, carbohydrate, or polypeptide of the invention in a recipient patient. Such a response can be an active response induced by administration of immunogen or a passive response induced by administration of antibody, antibody containing material, or primed T-cells. A cellular immune response is elicited by the presentation of polypeptide epitopes in association with Class I or Class II MHC molecules, to activate antigen-specific CD4 (+) T helper cells and/or CD8 (+) cytotoxic T cells. The response may also involve activation of monocytes, macrophages, NK cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils or other components of innate immunity. As used herein "active immunity" refers to any immunity conferred upon a subject by administration of an antigen.

As used herein "passive immunity" refers to any immunity conferred upon a subject without administration of an antigen to the subject. "Passive immunity" therefore includes, but is not limited to, administration of activated immune effectors including cellular mediators or protein mediators (e.g., monoclonal and/or polyclonal antibodies) of an immune response. A monoclonal or polyclonal antibody composition may be used in passive immunization for the prevention or treatment of infection by organisms that carry

the antigen recognized by the antibody. An antibody composition may include antibodies that bind to a variety of antigens that may in turn be associated with various organisms. The antibody component can be a polyclonal antiserum. In certain aspects the antibody or antibodies are affinity purified from an animal or second subject that has been challenged with an antigen(s). Alternatively, an antibody mixture may be used, which is a mixture of monoclonal and/or polyclonal antibodies to antigens present in the same, related, or different microbes or organisms, such as gram-positive bacteria, gram-negative bacteria, including but not limited to *staphylococcus* bacteria.

Passive immunity may be imparted to a patient or subject by administering to the patient immunoglobulins (Ig) and/or other immune factors obtained from a donor or other non-patient source having a known immunoreactivity. In other aspects, an antigenic composition of the present invention can be administered to a subject who then acts as a source or donor for globulin, produced in response to challenge with the antigenic composition ("hyperimmune globulin"), that contains antibodies directed against *Staphylococcus* or other organism. A subject thus treated would donate plasma from which hyperimmune globulin would then be obtained, via conventional plasma-fractionation methodology, and administered to another subject in order to impart resistance against or to treat *staphylococcus* infection. Hyperimmune globulins according to the invention are particularly useful for immune-compromised individuals, for individuals undergoing invasive procedures or where time does not permit the individual to produce their own antibodies in response to vaccination. See U.S. Pat. Nos. 6,936,258, 6,770,278, 6,756,361, 5,548,066, 5,512,282, 4,338,298, and 4,748,018, each of which is incorporated herein by reference in its entirety, for exemplary methods and compositions related to passive immunity.

For purposes of this specification and the accompanying claims the terms "epitope" and "antigenic determinant" are used interchangeably to refer to a site on an antigen to which B and/or T cells respond or recognize. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols (1996). Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen. T-cells recognize continuous epitopes of about nine amino acids for CD8 cells or about 13-15 amino acids for CD4 cells. T cells that recognize the epitope can be identified by in vitro assays that measure antigen-dependent proliferation, as determined by ³H-thymidine incorporation by primed T cells in response to an epitope (Burke et al., 1994), by antigen-dependent killing (cytotoxic T lymphocyte assay, Tigges et al., 1996) or by cytokine secretion.

The presence of a cell-mediated immunological response can be determined by proliferation assays (CD4 (+) T cells) or CTL (cytotoxic T lymphocyte) assays. The relative contributions of humoral and cellular responses to the protective or therapeutic effect of an immunogen can be distinguished

by separately isolating IgG and T-cells from an immunized syngeneic animal and measuring protective or therapeutic effect in a second subject.

As used herein and in the claims, the terms "antibody" or "immunoglobulin" are used interchangeably and refer to any of several classes of structurally related proteins that function as part of the immune response of an animal or recipient, which proteins include IgG, IgD, IgE, IgA, IgM and related proteins.

Under normal physiological conditions antibodies are found in plasma and other body fluids and in the membrane of certain cells and are produced by lymphocytes of the type denoted B cells or their functional equivalent. Antibodies of the IgG class are made up of four polypeptide chains linked together by disulfide bonds. The four chains of intact IgG molecules are two identical heavy chains referred to as H-chains and two identical light chains referred to as L-chains.

In order to produce polyclonal antibodies, a host, such as a rabbit or goat, is immunized with the antigen or antigen fragment, generally with an adjuvant and, if necessary, coupled to a carrier. Antibodies to the antigen are subsequently collected from the sera of the host. The polyclonal antibody can be affinity purified against the antigen rendering it monospecific.

Monoclonal antibodies can be produced by hyperimmunization of an appropriate donor with the antigen or ex-vivo by use of primary cultures of splenic cells or cell lines derived from spleen (Anavi, 1998; Huston et al., 1991; Johnson et al., 1991; Merzhaug et al., 1995).

As used herein and in the claims, the phrase "an immunological portion of an antibody" includes a Fab fragment of an antibody, a Fv fragment of an antibody, a heavy chain of an antibody, a light chain of an antibody, a heterodimer consisting of a heavy chain and a light chain of an antibody, a variable fragment of a light chain of an antibody, a variable fragment of a heavy chain of an antibody, and a single chain variant of an antibody, which is also known as scFv. In addition, the term includes chimeric immunoglobulins which are the expression products of fused genes derived from different species, one of the species can be a human, in which case a chimeric immunoglobulin is said to be humanized. Typically, an immunological portion of an antibody competes with the intact antibody from which it was derived for specific binding to an antigen.

Optionally, an antibody or preferably an immunological portion of an antibody, can be chemically conjugated to, or expressed as, a fusion protein with other proteins. For purposes of this specification and the accompanying claims, all such fused proteins are included in the definition of antibodies or an immunological portion of an antibody.

As used herein the terms "immunogenic agent" or "immunogen" or "antigen" are used interchangeably to describe a molecule capable of inducing an immunological response against itself on administration to a recipient, either alone, in conjunction with an adjuvant, or presented on a display vehicle.

D. Treatment Methods

A method of the present invention includes treatment for a disease or condition caused by a *staphylococcus* pathogen. An immunogenic polypeptide of the invention can be given to induce an immune response in a person infected with *staphylococcus* or suspected of having been exposed to *staphylococcus*. Methods may be employed with respect to individuals who have tested positive for exposure to *staphylococcus* or who are deemed to be at risk for infection based on possible exposure.

In particular, the invention encompasses a method of treatment for staphylococcal infection, particularly hospital acquired nosocomial infections. The immunogenic compositions and vaccines of the invention are particularly advantageous to use in cases of elective surgery. Such patients will know the date of surgery in advance and could be inoculated in advance. The immunogenic compositions and vaccines of the invention are also advantageous to use to inoculate health care workers.

In some embodiments, the treatment is administered in the presence of adjuvants or carriers or other staphylococcal antigens. Furthermore, in some examples, treatment comprises administration of other agents commonly used against bacterial infection, such as one or more antibiotics.

The use of peptides for vaccination can require, but not necessarily, conjugation of the peptide to an immunogenic carrier protein, such as hepatitis B surface antigen, keyhole limpet hemocyanin, or bovine serum albumin. Methods for performing this conjugation are well known in the art.

V. Vaccine and Other Pharmaceutical Compositions and Administration

A. Vaccines

The present invention includes methods for preventing or ameliorating staphylococcal infections, particularly hospital acquired nosocomial infections. As such, the invention contemplates vaccines for use in both active and passive immunization embodiments. Immunogenic compositions, proposed to be suitable for use as a vaccine, may be prepared from immunogenic SpA polypeptide(s), such as a SpA domain D variant, or immunogenic coagulases. In other embodiments SpA or coagulases can be used in combination with other secreted virulence proteins, surface proteins or immunogenic fragments thereof. In certain aspects, antigenic material is extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready formulation into a desired vehicle.

Other options for a protein/peptide-based vaccine involve introducing nucleic acids encoding the antigen(s) as DNA vaccines. In this regard, recent reports described construction of recombinant vaccinia viruses expressing either 10 contiguous minimal CTL epitopes (Thomson, 1996) or a combination of B cell, cytotoxic T-lymphocyte (CTL), and T-helper (Th) epitopes from several microbes (An, 1997), and successful use of such constructs to immunize mice for priming protective immune responses. Thus, there is ample evidence in the literature for successful utilization of peptides, peptide-pulsed antigen presenting cells (APCs), and peptide-encoding constructs for efficient *in vivo* priming of protective immune responses. The use of nucleic acid sequences as vaccines is exemplified in U.S. Pat. Nos. 5,958,895 and 5,620,896.

The preparation of vaccines that contain polypeptide or peptide sequence(s) as active ingredients is generally well understood in the art, as exemplified by U.S. Pat. Nos. 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and 4,578,770, all of which are incorporated herein by reference. Typically, such vaccines are prepared as injectables either as liquid solutions or suspensions: solid forms suitable for solution in or suspension in liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredient is often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the

vaccine may contain amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants that enhance the effectiveness of the vaccines. In specific embodiments, vaccines are formulated with a combination of substances, as described in U.S. Pat. Nos. 6,793,923 and 6,733,754, which are incorporated herein by reference.

Vaccines may be conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkalene glycols or triglycerides: such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10%, preferably about 1% to about 2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 10% to about 95% of active ingredient, preferably about 25% to about 70%.

The polypeptides and polypeptide-encoding DNA constructs may be formulated into a vaccine as neutral or salt forms. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the peptide) and those that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like.

Typically, vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including the capacity of the individual's immune system to synthesize antibodies and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are of the order of several hundred micrograms of active ingredient per vaccination. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by subsequent inoculations or other administrations.

The manner of application may be varied widely. Any of the conventional methods for administration of a vaccine are applicable. These are believed to include oral application within a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection and the like. The dosage of the vaccine will depend on the route of administration and will vary according to the size and health of the subject.

In certain instances, it will be desirable to have multiple administrations of the vaccine, e.g., 2, 3, 4, 5, 6 or more administrations. The vaccinations can be at 1, 2, 3, 4, 5, 6, 7, 8, to 5, 6, 7, 8, 9,10, 11, 12 twelve week intervals, including all ranges there between. Periodic boosters at intervals of 1-5 years will be desirable to maintain protective levels of the antibodies. The course of the immunization may be followed by assays for antibodies against the antigens, as described in U.S. Pat. Nos. 3,791,932; 4,174,384 and 3,949,064.

1. Carriers

A given composition may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or

polypeptide to a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin, or rabbit serum albumin can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimide, and bis-biazotized benzidine.

2. Adjuvants

The immunogenicity of polypeptide or peptide compositions can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Suitable adjuvants include all acceptable immunostimulatory compounds, such as cytokines, toxins, or synthetic compositions. A number of adjuvants can be used to enhance an antibody response against a variant SpA polypeptide or coagulase, or any other bacterial protein or combination contemplated herein. Adjuvants can (1) trap the antigen in the body to cause a slow release; (2) attract cells involved in the immune response to the site of administration; (3) induce proliferation or activation of immune system cells; or (4) improve the spread of the antigen throughout the subject's body.

Adjuvants include, but are not limited to, oil-in-water emulsions, water-in-oil emulsions, mineral salts, polynucleotides, and natural substances. Specific adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, γ -interferon, GMCSF, BCG, aluminum salts, such as aluminum hydroxide or other aluminum compound, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM), and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion. MEW antigens may even be used. Others adjuvants or methods are exemplified in U.S. Pat. Nos. 6,814,971, 5,084,269, 6,656,462, each of which is incorporated herein by reference).

Various methods of achieving adjuvant affect for the vaccine includes use of agents such as aluminum hydroxide or phosphate (alum), commonly used as about 0.05 to about 0.1% solution in phosphate buffered saline, admixture with synthetic polymers of sugars (Carbopol®) used as an about 0.25% solution, aggregation of the protein in the vaccine by heat treatment with temperatures ranging between about 70° to about 101° C. for a 30-second to 2-minute period, respectively. Aggregation by reactivating with pepsin-treated (Fab) antibodies to albumin; mixture with bacterial cells (e.g., *C. parvum*), endotoxins or lipopolysaccharide components of Gram-negative bacteria; emulsion in physiologically acceptable oil vehicles (e.g., mannide monooleate (Aracel A)); or emulsion with a 20% solution of a perfluorocarbon (Fluosol-DA®) used as a block substitute may also be employed to produce an adjuvant effect.

Examples of and often preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants, and aluminum hydroxide.

In some aspects, it is preferred that the adjuvant be selected to be a preferential inducer of either a Th1 or a Th2 type of response. High levels of Th1-type cytokines tend to favor the induction of cell mediated immune responses to a given antigen, while high levels of Th2-type cytokines tend to favor the induction of humoral immune responses to the antigen.

The distinction of Th1 and Th2-type immune response is not absolute. In reality an individual will support an immune

response which is described as being predominantly Th1 or predominantly Th2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4+ T cell clones by Mosmann and Coffman (Mosmann, and Coffman, 1989). Traditionally, Th1-type responses are associated with the production of the INF- γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of Th1-type immune responses are not produced by T-cells, such as IL-12. In contrast, Th2-type responses are associated with the secretion of IL-4, IL-5, IL-6, IL-10.

In addition to adjuvants, it may be desirable to co-administer biologic response modifiers (BRM) to enhance immune responses. BRMs have been shown to upregulate T cell immunity or downregulate suppresser cell activity. Such BRMs include, but are not limited to, Cimetidine (CIM; 1200 mg/d) (Smith/Kline, P A); or low-dose Cyclophosphamide (CYP; 300 mg/m²) (Johnson/Mead, N J) and cytokines such as γ -interferon, IL-2, or IL-12 or genes encoding proteins involved in immune helper functions, such as B-7.

B. Lipid Components and Moieties

In certain embodiments, the present invention concerns compositions comprising one or more lipids associated with a nucleic acid or a polypeptide/peptide. A lipid is a substance that is insoluble in water and extractable with an organic solvent. Compounds other than those specifically described herein are understood by one of skill in the art as lipids, and are encompassed by the compositions and methods of the present invention. A lipid component and a non-lipid may be attached to one another, either covalently or non-covalently.

A lipid may be a naturally occurring lipid or a synthetic lipid. However, a lipid is usually a biological substance. Biological lipids are well known in the art, and include for example, neutral fats, phospholipids, phosphoglycerides, steroids, terpenes, lysolipids, glycosphingolipids, glucolipids, sulphatides, lipids with ether and ester-linked fatty acids and polymerizable lipids, and combinations thereof.

A nucleic acid molecule or a polypeptide/peptide, associated with a lipid may be dispersed in a solution containing a lipid, dissolved with a lipid, emulsified with a lipid, mixed with a lipid, combined with a lipid, covalently bonded to a lipid, contained as a suspension in a lipid or otherwise associated with a lipid. A lipid or lipid-poxvirus-associated composition of the present invention is not limited to any particular structure. For example, they may also simply be interspersed in a solution, possibly forming aggregates which are not uniform in either size or shape. In another example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. In another non-limiting example, a lipofectamine(Gibco BRL)-poxvirus or Superfect (Qiagen)-poxvirus complex is also contemplated.

In certain embodiments, a composition may comprise about 1%, about 2%, about 3%, about 4% about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%,

about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or any range therebetween, of a particular lipid, lipid type, or non-lipid component such as an adjuvant, antigen, peptide, polypeptide, sugar, nucleic acid or other material disclosed herein or as would be known to one of skill in the art. In a non-limiting example, a composition may comprise about 10% to about 20% neutral lipids, and about 33% to about 34% of a cerebroside, and about 1% cholesterol. In another non-limiting example, a liposome may comprise about 4% to about 12% terpenes, wherein about 1% of the micelle is specifically lycopene, leaving about 3% to about 11% of the liposome as comprising other terpenes; and about 10% to about 35% phosphatidyl choline, and about 1% of a non-lipid component. Thus, it is contemplated that compositions of the present invention may comprise any of the lipids, lipid types or other components in any combination or percentage range.

C. Combination Therapy

The compositions and related methods of the present invention, particularly administration of a secreted virulence factor or surface protein, including a variant SpA polypeptide or peptide, and/or other bacterial peptides or proteins to a patient/subject, may also be used in combination with the administration of traditional therapies. These include, but are not limited to, the administration of antibiotics such as streptomycin, ciprofloxacin, doxycycline, gentamycin, chloramphenicol, trimethoprim, sulfamethoxazole, ampicillin, tetracycline or various combinations of antibiotics.

In one aspect, it is contemplated that a polypeptide vaccine and/or therapy is used in conjunction with antibacterial treatment. Alternatively, the therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agents and/or a proteins or polynucleotides are administered separately, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agent and antigenic composition would still be able to exert an advantageously combined effect on the subject. In such instances, it is contemplated that one may administer both modalities within about 12-24 h of each other or within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for administration significantly, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

Various combinations may be employed, for example antibiotic therapy is "A" and the immunogenic molecule given as part of an immune therapy regime, such as an antigen, is

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B
 B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A
 B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

Administration of the immunogenic compositions of the present invention to a patient/subject will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the SpA composition, or other compositions described herein. It is expected that the treatment cycles would be repeated as necessary. It also is

contemplated that various standard therapies, such as hydration, may be applied in combination with the described therapy.

D. General Pharmaceutical Compositions

In some embodiments, pharmaceutical compositions are administered to a subject. Different aspects of the present invention involve administering an effective amount of a composition to a subject. In some embodiments of the present invention, staphylococcal antigens, members of the Ess pathway, including polypeptides or peptides of the Esa or Esx class, and/or members of sortase substrates may be administered to the patient to protect against infection by one or more *staphylococcus* pathogens. Alternatively, an expression vector encoding one or more such polypeptides or peptides may be given to a patient as a preventative treatment. Additionally, such compounds can be administered in combination with an antibiotic or an antibacterial. Such compositions will generally be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

In addition to the compounds formulated for parenteral administration, such as those for intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g., tablets or other solids for oral administration; time release capsules; and any other form currently used, including creams, lotions, mouthwashes, inhalants and the like.

The active compounds of the present invention can be formulated for parenteral administration, e.g., formulated for injection via the intravenous, intramuscular, sub-cutaneous, or even intraperitoneal routes. The preparation of an aqueous composition that contains a compound or compounds that increase the expression of an WIC class I molecule will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified.

Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil, or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

The proteinaceous compositions may be formulated into a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric

hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

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

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

Administration of the compositions according to the present invention will typically be via any common route. This includes, but is not limited to oral, nasal, or buccal administration. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, intranasal, or intravenous injection. In certain embodiments, a vaccine composition may be inhaled (e.g., U.S. Pat. No. 6,651,655, which is specifically incorporated by reference). Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients. As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem complications commensurate with a reasonable benefit/risk ratio. The term "pharmaceutically acceptable carrier," means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in isotonic NaCl solution and either added to hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, Remington's Pharmaceutical Sciences, 1990). Some variation in dosage will necessarily occur

depending on the condition of the subject. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

An effective amount of therapeutic or prophylactic composition is determined based on the intended goal. The term "unit dose" or "dosage" refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the composition calculated to produce the desired responses discussed above in association with its administration, i.e., the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the protection desired.

Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment (alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition.

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

E. In Vitro, Ex Vivo, or In Vivo Administration

As used herein, the term in vitro administration refers to manipulations performed on cells removed from or outside of a subject, including, but not limited to cells in culture. The term ex vivo administration refers to cells which have been manipulated in vitro, and are subsequently administered to a subject. The term in vivo administration includes all manipulations performed within a subject.

In certain aspects of the present invention, the compositions may be administered either in vitro, ex vivo, or in vivo. In certain in vitro embodiments, autologous B-lymphocyte cell lines are incubated with a virus vector of the instant invention for 24 to 48 hours or with a variant SpA and/or cogulase and/or any other composition described herein for two hours. The transduced cells can then be used for in vitro analysis, or alternatively for ex vivo administration. U.S. Pat. Nos. 4,690,915 and 5,199,942, both incorporated herein by reference, disclose methods for ex vivo manipulation of blood mononuclear cells and bone marrow cells for use in therapeutic applications.

F. Antibodies and Passive Immunization

Another aspect of the invention is a method of preparing an immunoglobulin for use in prevention or treatment of staphylococcal infection comprising the steps of immunizing a recipient or donor with the vaccine of the invention and isolating immunoglobulin from the recipient or donor. An immunoglobulin prepared by this method is a further aspect of the invention. A pharmaceutical composition comprising the immunoglobulin of the invention and a pharmaceutically acceptable carrier is a further aspect of the invention which could be used in the manufacture of a medicament for the treatment or prevention of staphylococcal disease. A method for treatment or prevention of staphylococcal infection comprising a step of administering to a patient an effective amount of the pharmaceutical preparation of the invention is a further aspect of the invention.

Inocula for polyclonal antibody production are typically prepared by dispersing the antigenic composition in a physiologically tolerable diluent such as saline or other adjuvants suitable for human use to form an aqueous composition. An immunostimulatory amount of inoculum is administered to

a mammal and the inoculated mammal is then maintained for a time sufficient for the antigenic composition to induce protective antibodies.

The antibodies can be isolated to the extent desired by well known techniques such as affinity chromatography (Harlow and Lane, 1988). Antibodies can include antiserum preparations from a variety of commonly used animals, e.g. goats, primates, donkeys, swine, horses, guinea pigs, rats or man.

An immunoglobulin produced in accordance with the present invention can include whole antibodies, antibody fragments or subfragments. Antibodies can be whole immunoglobulins of any class (e.g., IgG, IgM, IgA, IgD or IgE), chimeric antibodies or hybrid antibodies with dual specificity to two or more antigens of the invention. They may also be fragments (e.g., F(ab')₂, Fab', Fab, Fv and the like) including hybrid fragments. An immunoglobulin also includes natural, synthetic, or genetically engineered proteins that act like an antibody by binding to specific antigens to form a complex.

A vaccine of the present invention can be administered to a recipient who then acts as a source of immunoglobulin, produced in response to challenge from the specific vaccine. A subject thus treated would donate plasma from which hyperimmune globulin would be obtained via conventional plasma fractionation methodology. The hyperimmune globulin would be administered to another subject in order to impart resistance against or treat staphylococcal infection. Hyperimmune globulins of the invention are particularly useful for treatment or prevention of staphylococcal disease in infants, immune compromised individuals, or where treatment is required and there is no time for the individual to produce antibodies in response to vaccination.

An additional aspect of the invention is a pharmaceutical composition comprising two or more monoclonal antibodies (or fragments thereof; preferably human or humanised) reactive against at least two constituents of the immunogenic composition of the invention, which could be used to treat or prevent infection by Gram positive bacteria, preferably staphylococci, more preferably *S. aureus* or *S. epidermidis*. Such pharmaceutical compositions comprise monoclonal antibodies that can be whole immunoglobulins of any class, chimeric antibodies, or hybrid antibodies with specificity to two or more antigens of the invention. They may also be fragments (e.g., F(ab')₂, Fab', Fab, Fv and the like) including hybrid fragments.

Methods of making monoclonal antibodies are well known in the art and can include the fusion of splenocytes with myeloma cells (Kohler and Milstein, 1975; Harlow and Lane, 1988). Alternatively, monoclonal Fv fragments can be obtained by screening a suitable phage display library (Vaughan et al., 1998). Monoclonal antibodies may be humanized or part humanized by known methods.

VI. Examples

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encom-

passed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1

Non-Toxicogenic Protein a Variants as Subunit Vaccines to Prevent *Staphylococcus Aureus* Infections

A. Results

An animal model for *S. aureus* infection BALB/c mice were infected by intravenous injection with 1×10^7 CFU of the human clinical isolate *S. aureus* Newman (Baba et al., 2007). Within 6 hours following infection, 99.999% of staphylococci disappeared from the blood stream and were distributed via the vasculature. Staphylococcal dissemination to peripheral tissues occurred rapidly, as the bacterial load in kidney and other peripheral organ tissues reached 1×10^5 CFU g^{-1} within the first three hours. The staphylococcal load in kidney tissues increased by 1.5 log CFU within twenty-four hours. Forty-eight hours following infection, mice developed disseminated abscesses in multiple organs, detectable by light microscopy of hematoxylin-eosin stained, thin-sectioned kidney tissue. The initial abscess diameter was $524 \mu m (\pm 65 \mu m)$; lesions were initially marked by an influx of polymorphonuclear leukocytes (PMNs) and harbored no discernable organization of staphylococci, most of which appeared to reside within PMNs. On day 5 of infection, abscesses increased in size and enclosed a central population of staphylococci, surrounded by a layer of eosinophilic, amorphous material and a large cuff of PMNs. Histopathology revealed massive necrosis of PMNs in proximity to the staphylococcal nidus at the center of abscess lesions as well as a mantle of healthy phagocytes. A rim of necrotic PMNs were observed at the periphery of abscess lesions, bordering eosinophilic, amorphous material that separates healthy renal tissue from lesions. Abscesses eventually reached a diameter of $\geq 1,524 \mu m$ on day 15 or 36. At later time intervals, the staphylococcal load was increased to 10^4 - 10^6 CFU g^{-1} and growing abscess lesions migrated towards the organ capsule. Peripheral lesions were prone to rupture, thereby releasing necrotic material and staphylococci into the peritoneal cavity or the retroperitoneal space. These events resulted in bacteremia as well as a secondary wave of abscesses, eventually precipitating a lethal outcome.

To enumerate staphylococcal load in renal tissue, animals were killed, their kidneys excised and tissue homogenate spread on agar media for colony formation. On day 5 of infection, a mean of 1×10^6 CFU g^{-1} renal tissue for *S. aureus* Newman was observed. To quantify abscess formation, kidneys were visually inspected, and each individual organ was given a score of one or zero. The final sum was divided by the total number of kidneys to calculate percent surface abscesses (Table 4). In addition, randomly chosen kidneys were fixed in formalin, embedded, thin sectioned, and stained with hematoxylin-eosin. For each kidney, four sagittal sections at $200 \mu m$ intervals were viewed by microscopy. The numbers of lesions were counted for each section and averaged to quantify the number of abscesses within the kidneys. *S. aureus* Newman caused 4.364 ± 0.889 abscesses per kidney, and surface abscesses were observed on 14 out of 20 kidneys (70%) (Table 4).

When examined by scanning electron microscopy, *S. aureus* Newman was located in tightly associated lawns at the center of abscesses. Staphylococci were contained by an amorphous pseudocapsule that separated bacteria from the

cuff of abscesses leukocytes. No immune cells were observed in these central nests of staphylococci, however occasional red blood cells were located among the bacteria. Bacterial populations at the abscess center, designated staphylococcal abscess communities (SAC), appeared homogenous and coated by an electron-dense, granular material. The kinetics of the appearance of infectious lesions and the morphological attributes of abscesses formed by *S. aureus* Newman were similar to those observed following mouse infection with *S. aureus* USA300 (LAC), the current epidemic community-acquired methicillin-resistant *S. aureus* (CA-MRSA) clone in the United States (Diep et al., 2006).

TABLE 4

Genotype	Staphylococcal load in kidney tissue		Abscess formation in kidney tissue			
	^a log ₁₀ CFU g ⁻¹ tissue	^b Significance (P-value)	^c Reduction (log ₁₀ CFU g ⁻¹)	^d Surface abscesses (%)	^e Number of abscesses per kidney	^f Significance (P-value)
wild-type	6.141 ± 0.192	—	—	70	4.364 ± 0.889	—
AsrtA	4.095 ± 0.347	6.7 × 10 ⁻⁶	2.046	0	0.000 ± 0.000	0.0216
spa	5.137 ± 0.374	0.0144	1.004	13	0.375 ± 0.374	0.0356

^aMeans of staphylococcal load calculated as log₁₀ CFU g⁻¹ in homogenized renal tissues 5 days following infection in cohorts of fifteen BALB/c mice per challenge strain. Standard error of the means (±SEM) is indicated.

^bStatistical significance was calculated with the Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

^cReduction in bacterial load calculated as log₁₀ CFU g⁻¹.

^dAbscess formation in kidney tissues five days following infection was measured by macroscopic inspection (% positive)

^eHistopathology of hematoxylin-eosin stained, thin sectioned kidneys from eight to ten animals; the average number of abscesses per kidney was recorded and averaged again for the final mean (±SEM).

^fStatistical significance was calculated with the Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

S. aureus Protein a (Spa) Mutants are Avirulent and Cannot Form Abscesses

Sortase A is a transpeptidase that immobilizes nineteen surface proteins in the envelope of *S. aureus* strain Newman (Mazmanian et al., 1999; Mazmanian et al., 2000). Earlier work identified sortase A as a virulence factor in multiple animal model systems, however the contributions of this enzyme and its anchored surface proteins to abscess formation or persistence have not yet been revealed (Jonsson et al., 2002; Weiss et al., 2004). Compared to the wild-type parent (Baba et al., 2007), an isogenic *srtA* variant (AsrtA) failed to form abscess lesions on either macroscopic or histopathology examination on days 2, 5, or 15. In mice infected with the *srtA* mutant, only 1×10⁴ CFU g⁻¹ was recovered from kidney tissue on day 5 of infection, which is a 2.046 log₁₀ CFU g⁻¹ reduction compared to the wild-type parent strain (P=6.73×10⁻⁶). A similar defect was observed for the *srtA* mutant of MRSA strain USA300 (data not shown). Scanning electron microscopy showed that *srtA* mutants were highly dispersed and often associated with leukocytes in otherwise healthy renal tissue. On day fifteen following infection, *srtA* mutants were cleared from renal tissues, a ≥3.5 log₁₀ CFU g⁻¹ reduction compared to the wild-type (Table 3). Thus, sortase A anchored surface proteins enable the formation of abscess lesions and the persistence of bacteria in host tissues, wherein staphylococci replicate as communities embedded in an extracellular matrix and shielded from surrounding leukocytes by an amorphous pseudocapsule.

Sortase A anchors a large spectrum of proteins with LPXTG motif sorting signals to the cell wall envelope, thereby providing for the surface display of many virulence factors (Mazmanian et al., 2002). To identify surface proteins required for staphylococcal abscess formation, bursa

aurealis insertions were introduced in 5' coding sequences of genes that encode polypeptides with LPXTG motif proteins (Bae et al., 2004) and these mutations were transduced into *S. aureus* Newman. Mutations in the structural gene for Protein A (*spa*) reduced the staphylococcal load in infected mouse kidney tissues by 1.004 log₁₀ (P=0.0144). When analyzed for their ability to form abscesses in kidney tissues by histopathology, we observed that the *spa* mutants were unable to form abscesses as compared with the wild-type parent strain *S. aureus* Newman (wild-type *S. aureus* Newman 4.364±0.889 abscesses per kidney vs. the isogenic *spa* mutant with 0.375±0.374 lesions; P=0.0356).

Protein A Blocks Innate and Adaptive Immune Responses.

Studies identified Protein A as a critical virulence factor during the pathogenesis of *S. aureus* infections. Earlier work demonstrated that Protein A impedes phagocytosis of staphylococci by binding the Fc component of immunoglobulin (Jensen 1958; Uhlén et al., 1984), activates platelet aggregation via the von Willebrand factor (Hartleib et al., 2000), functions as a B cell superantigen by capturing the F(ab)₂ region of VH3 bearing IgM (Roben et al., 1995), and, through its activation of TNFR1, can initiate staphylococcal pneumonia (Gomez et al., 2004). Due to the fact that Protein A captures immunoglobulin and displays toxic attributes, the possibility that this surface molecule may function as a vaccine in humans has not been rigorously pursued. The inventors demonstrate for the first time that Protein A variants no longer able to bind to immunoglobulins, vWF and TNFR-1 are removed of their toxic potential and are able to stimulate humoral immune responses that protect against staphylococcal disease.

Molecular Basis of Protein a Surface Display and Function.

Protein A is synthesized as a precursor in the bacterial cytoplasm and secreted via its YSIRK signal peptide at the cross wall, i.e., the cell division septum of staphylococci (FIG. 1). (DeDent et al., 2007; DeDent et al., 2008). Following cleavage of the C-terminal LPXTG sorting signal, Protein A is anchored to bacterial peptidoglycan cross-bridges by sortase A (Schneewind et al., 1995; Mazmanian et al., 1999; Mazmanian et al., 2000). Protein A is the most abundant surface protein of staphylococci; the molecule is expressed by virtually all *S. aureus* strains (Said-Salim et al., 2003; Cespedes et al., 2005; Kennedy et al., 2008). Staphylococci turn over 15-20% of their cell wall per division cycle

(Navarre and Schneewind 1999). Murine hydrolases cleave the glycan strands and wall peptides of peptidoglycan, thereby releasing Protein A with its attached C-terminal cell wall disaccharide tetrapeptide into the extracellular medium (Ton-That et al., 1999). Thus, by physiological design, Protein A is both anchored to the cell wall and displayed on the bacterial surface but also released into surrounding tissues during host infection (Marraffini et al., 2006).

Protein A captures immunoglobulins on the bacterial surface and this biochemical activity enables staphylococcal escape from host innate and acquired immune responses (Jensen 1958; Goodyear and Silverman 2004). Interestingly, region X of Protein A (Guss et al., 1984), a repeat domain that tethers the IgG binding domains to the LPXTG sorting signal/cell wall anchor, is perhaps the most variable portion of the staphylococcal genome (Schneewind et al., 1992; Said-Salim et al., 2003). Each of the five immunoglobulin binding domains of Protein A (SpA), formed from three helix bundles and designated E, D, A, B, and C, exerts similar structural and functional properties (Sjödahl 1977; Jansson et al., 1998). The solution and crystal structure of domain D has been solved both with and without the Fc and V_H3 (Fab) ligands, which bind Protein A in a non-competitive manner at distinct sites (Graille et al., 2000).

In the crystal structure complex, the Fab interacts with helix II and helix III of domain D via a surface composed of four VH region β -strands (Graille et al., 2000). The major axis of helix II of domain D is approximately 50° to the orientation of the strands, and the interhelical portion of domain D is most proximal to the C0 strand. The site of interaction on Fab is remote from the Ig light chain and the heavy chain constant region. The interaction involves the following domain D residues: Asp-36 of helix II as well as Asp-37 and Gln-40 in the loop between helix II and helix III, in addition to several other residues with SpA-D (Graille et al., 2000). Both interacting surfaces are composed predominantly of polar side chains, with three negatively charged residues on domain D and two positively charged residues on the 2A2 Fab buried by the interaction, providing an overall electrostatic attraction between the two molecules. Of the five polar interactions identified between Fab and domain D, three are between side chains. A salt bridge is formed between Arg-H19 and Asp-36 and two hydrogen bonds are made between Tyr-H59 and Asp-37 and between Asn-H82a and Ser-33. Because of the conservation of Asp-36 and Asp-37 in all five IgG binding domains of Protein A, these residues were selected for mutagenesis.

The SpA-D sites responsible for Fab binding are structurally separate from the domain surface that mediates Fc γ binding. The interaction of Fc γ with domain B primarily involves residues in helix I with lesser involvement of helix II (Deisenhofer 1981; Gouda et al., 1992). With the exception of the Gln-32, a minor contact in both complexes, none of the residues that mediate the Fc γ interaction are involved in Fab binding. To examine the spatial relationship between these different Ig-binding sites, the SpA domains in these complexes have been superimposed to construct a model of a complex between Fab, the SpA-domain D, and the Fc γ molecule. In this ternary model, Fab and Fc γ form a sandwich about opposite faces of the helix II without evidence of steric hindrance of either interaction. These findings illustrate how, despite its small size (i.e., 56-61 aa), a SpA domain can simultaneously display both activities, explaining experimental evidence that the interactions of Fab with an individual domain are noncompetitive. Residues for the interaction between SpA-D and Fc γ are Gln-9 and Gln-10.

In contrast, occupancy of the Fc portion of IgG on the domain D blocks its interaction with vWF A1 and probably also TNFR1 (O'Seaghda et al., 2006). Mutations in residues essential for IgG Fc binding (F5, Q9, Q10, S11, F13, Y14, L17, N28, I31 and K35) are also required for vWF A1 and TNFR1 binding (Cedergren et al., 1993; Gomez et al., 2006; O'Seaghda et al. 2006), whereas residues critical for the V_H3 interaction (Q26, G29, F30, S33, D36, D37, Q40, N43, E47) have no impact on the binding activities of IgG Fc, vWF A1 or TNFR1 (Jansson et al., 1998; Graille et al., 2000). The Protein A immunoglobulin Fab binding activity targets a subset of B cells that express VH3 family related IgM on their surface, i.e. these molecules function as VH3 type B cell receptors (Roben et al., 1995). Upon interaction with SpA, these B cells rapidly proliferate and then commit to apoptosis, leading to preferential and prolonged deletion of innate-like B lymphocytes (i.e. marginal zone B cells and follicular B2 cells) (Goodyear and Silverman 2003; Goodyear and Silverman 2004). It is important to note that more than 40% of circulating B cells are targeted by the Protein A interaction and the VH3 family represents the largest family of human B cell receptors to impart protective humoral responses against pathogens (Goodyear and Silverman 2003; Goodyear and Silverman 2004). Thus, Protein A functions analogously to staphylococcal superantigens (Roben et al., 1995), albeit that the latter class of molecules, for example SEB, TSST-1, TSST-2, form complexes with the T cell receptor to inappropriately stimulate host immune responses and thereby precipitating characteristic disease features of staphylococcal infections (Roben et al., 1995; Tiedemann et al., 1995). Together these findings document the contributions of Protein A in establishing staphylococcal infections and in modulating host immune responses.

Non-Toxicogenic Variant of Protein A.

The inventors have developed a non-toxicogenic variant of staphylococcal Protein A and, with this reagent in hand, aimed for the first time to measure the immune response of animals to Protein A immunization. Further, the inventors address whether immunization of animals with a non-toxicogenic variant of Protein A could generate immune responses that raise protective immunity against staphylococcal infection.

To perturb the IgG Fc, vWF A1 and TNFR1 binding activities of Protein A, glutamine (Q) residues 9 and 10 [the numbering here is derived from that established for the SpA domain D] were modified generating lysine or glycine substitutions for both glutamines with the expectation that these substitutions abolish the ion bonds formed between wild-type Protein A and its ligands. The added effect of the dual lysine substitutions may be that these positively charged residues institute a repellent charge for immunoglobulins. To perturb IgM Fab VH3 binding, the inventors selected the aspartate (D) residues 36 and 37 of SpA-D, each of which is required for the association of Protein A with the B cell receptor. D36 and D37 were both substituted with alanine. The Q9,10K and D36,37A mutations were combined in the recombinant molecule SpA-D_{Q9,10K;D36,37A} and examined for the binding attributes of Protein A.

In brief, the Protein A (spa) genomic sequence of *Staphylococcus aureus* N315 was PCR amplified with the primers (GCTGCACATATGGCGCAACACGATGAAGCTCAAC [5'primer](SEQ ID NO:35) and AGTGGATCCTTATGCTTTGTTAGCATCTGC [3' primer] (SEQ ID NO:36)), cloned into the pET15b vector (pYSJ1, codons 48-486) (Stranger-Jones, et al., 2006) and recombinant plasmid transformed into *E. coli* BL21(DE3) (Studier et al., 1990). The Protein A product derived from pYSJ1 harbors SpA

residues 36-265 fused to the N-terminal His tag (MGSSH-HHHHSSGLVPRGS (SEQ ID NO:37)). Following IPTG inducible expression, recombinant N-terminal His₆-tagged SpA was purified by affinity chromatography on Ni-NTA resin (Stranger-Jones et al., 2006). The domain D of SpA (SpA-D) was PCR amplified with a pair of specific primers (AACATATGTTCAACAAAGATCAACAAAGC [5' primer](SEQ ID NO:38) and AAGGATCCAGATTCGTT-TAATTTTTTAGC [3' primer] (SEQ ID NO:39)), subcloned into the pET15b vector (pHAN1, spa codons 212-261) and recombinant plasmid transformed into *E. coli* BL21(DE3) to express and purify recombinant N-terminal His₆-tagged protein. To generate mutations in the SpA-D coding sequence, sets of two pairs of primers were synthesized (for D to A substitutions: CTCATTCAAAGTCT-TAAAGCCGCCCAAGCCAAAGCACTAAC [5' primer] (SEQ ID NO:40) and GTTAGTGCTTTGGCTTGGGGCG-GCTTTAAGACTTTGAATGAAG [3' primer] (SEQ ID NO:41); for Q to K substitutions CATATGTTCAACAAAGATAAAAAAGCGCCTTCTATGAAATC [5' primer] (SEQ ID NO:42) and GATTCATA-GAAGGCGCTTTTTTATCTTTGTTGAACATATG [3' primer] (SEQ ID NO:43); for Q to G substitutions CATATGTTCAACAAAGATGGAGGAAGCGCCTTCTAT-GAAATC [5' primer] (SEQ ID NO:44) and GATTCATA-GAAGGCGCTTCTCCATCTTTGTTGAACATATG [3' primer] (SEQ ID NO:45). Primers were used for quick-change mutagenesis protocols. Following mutagenesis, DNA sequences were confirmed for each of the recombinant proteins: SpA, SpA-D and SpA-D_{Q9,10K;D36,37A} and SpA-D_{Q9,10K;D36,37A}. All proteins were purified from lysates of recombinant *E. coli* using Ni-NTA chromatography and subsequently dialyzed against PBS and stored at 4° C.

To measure binding of immunoglobulin to Protein A and its variants, 200 µg of purified protein was diluted into a 1 ml volume using column buffer (50 mM Tris-HCl, 150 mM NaCl, pH7.5) and then loaded onto a pre-equilibrated Ni-NTA column (1 ml bed volume). Columns were washed with 10 ml of column buffer. 200 µg of purified human IgG was diluted in a total volume of 1 ml column buffer and then applied to each of the columns charged with Protein A and its variants. The columns were subsequently washed with 5 ml wash buffer (10 mM imidazole in column buffer) and 5 ml column buffer. Protein samples were eluted with 2 ml elution buffer (500 mM imidazole in column buffer), fractions collected and aliquots subjected to SDS-PAGE gel electrophoresis, followed by Coomassie-Blue staining. As shown in FIG. 3, wild-type Protein A (SpA) and its SpA-domain D both retained immunoglobulin during chromatography. In contrast, the SpA-D_{Q9,10K;D36,37A} variant did not bind to immunoglobulin.

To quantify the binding of Protein A and its variants to the Fc portion of immunoglobulin and the VH3 domain of Fab, HRP conjugated human immunoglobulin G [hIgG], the Fc portion of human IgG [hFc] and the F(ab)₂ portion of human IgG [hF(ab)₂] as well as ELISA assays were used to quantify the relative amount binding to Protein A and its variants. The data in FIG. 4 demonstrate the binding of SpA and SpA-D to hIgG and hFc, whereas SpA-D_{Q9,10K;D36,37A} and SpA-D_{Q9,10K;D36,37A} displayed only background binding activities. SpA bound similar amounts of hFc and hF(ab)₂, however the binding of SpA-D to hF(ab)₂ was reduced compared to full length SpA. This result suggests that the presence of multiple IgG binding domains may cooperatively increase the ability of Protein A to bind to the B cell receptor. When compared with the reduced binding power of SpA-D for hF(ab)₂, of the two variants only SpA-D_{Q9,10K;D36,37A} dis-

played a significant reduction in the ability to bind the VH3 domain of immunoglobulin. To examine the toxicogenic attributes of SpA-D and its variants, purified proteins were injected into mice, which were sacrificed after 4 hours to remove their spleens. Organ tissue was homogenized, capsular material removed and B cells stained with fluorescent CD19 antibodies. Following FACS analysis to quantify the abundance of B cells in splenic tissues, it was observed that SpA-D caused a 5% drop in the B cell count compared to a mock (PBS) control (FIG. 5). In contrast, SpA-D_{Q9,10K;D36,37A} did not cause a reduction in B-cell counts, indicating that the mutant molecule had lost its toxicogenic attributes of stimulating B cell proliferation and death (FIG. 5). In summary, amino acid substitutions in the SpA-D residues Q9, Q10, D36, and D37 abolished the ability of Protein A domains to bind immunoglobulins or exert toxicogenic functions in human and animal tissues.

Non-Toxicogenic Protein A Variants Elicit Vaccine Protection.

To test whether or not Protein A and its variants can function as vaccine antigens, SpA, SpA-D, SpA-D_{Q9,10K;D36,37A}, and SpA-D_{Q9,10K;D36,37A} were emulsified with complete or incomplete Freund's adjuvant and immunized 4 week old BALB/c mice on day 1 and day 11 with 50 µg of purified protein. Cohort of animals (n=5) were analyzed for humoral immune responses to immunization by bleeding the animals before (day 0) and after the immunization schedule (day 21). Table 5 indicates that immunized mice generated only a modest humoral immune response directed at wild-type Protein A or its SpA-D module, whereas the amount of antibody raised following immunization with SpA-D_{Q9,10K;D36,37A} or SpA-D_{Q9,10K;D36,37A} was increased four to five fold. Following intravenous challenge with 1×10⁷ CFU *S. aureus* Newman, animals were killed on day 4, their kidneys removed and either analyzed for staphylococcal load (by plating tissue homogenate on agar plates and enumerating colony forming units, CFU) or histopathology. As expected, mock (PBS) immunized mice (n=19) harbored 6.46 log₁₀ (±0.25) CFU in kidney tissue and infectious lesions were organized into 3.7 (±1.2) abscesses per organ (n=10)(Table 5). Immunization of animals with SpA led to a 2.51 log₁₀ CFU reduction on day 5 (P=0.0003) with 2.1 (±1.2) abscesses per organ. The latter data indicate that there was no significant reduction in abscess formation (P=0.35). Immunization with SpA-D generated similar results: a 2.03 log₁₀ CFU reduction on day 5 (P=0.0001) with 1.5 (±0.8) abscesses per organ (P=0.15). In contrast, immunization with SpA-D_{Q9,10K;D36,37A} or SpA-D_{Q9,10K;D36,37A} created increased protection, with 3.07 log₁₀ and 3.03 log₁₀ CFU reduction on day 4, respectively (statistical significance P<0.0001 for both observations). Further, immunization with both SpA-D_{Q9,10K;D36,37A} and SpA-D_{Q9,10K;D36,37A} generated significant protection from staphylococcal abscess formation, as only 0.5 (±0.4) and 0.8 (±0.5) infectious lesions per organ (P=0.02 and P=0.04) were identified. Thus, immunization with non-toxicogenic Protein A variants generates increased humoral immune responses for Protein A and provides protective immunity against staphylococcal challenge. These data indicate that Protein A is an ideal candidate for a human vaccine that prevents *S. aureus* disease.

These exciting results have several implications for the design of a human vaccine. First, the generation of substitution mutations that affect the ability of the immunoglobulin binding domains of Protein A, either alone or in combination of two or more domains, can generate non-toxicogenic variants suitable for vaccine development. It seems likely that a combination of mutant IgG binding domains closely

resembling the structure of Protein A can generate even better humoral immune responses as is reported here for the SpA-domain D alone. Further, a likely attribute of Protein A specific antibodies may be that the interaction of antigen binding sites with the microbial surface can neutralize the ability of staphylococci to capture immunoglobulins via their Fc portion or to stimulate the B cell receptor via the VH3 binding activities.

room temperature in 10% formalin for 24 h. Tissues are embedded in paraffin, thin-sectioned, stained with hematoxylin-eosin, and examined by microscopy.

Murine Lethal Infection—

BALB/c mice (24-day-old female, 8-10 mice per group, Charles River Laboratories, Wilmington, Mass.) are immunized by intramuscular injection into the hind leg with purified SpA, SpA-D or SpA-D_{Q9,10K;D36,37A} (50 µg pro-

TABLE 5

Non-toxicogenic Protein A variants as vaccine antigens that prevent <i>S. aureus</i> disease									
Antigen	Bacterial load in kidney (n = number of mice)			IgG titer	Abscess formation in mice (n = number of mice)				
	^a log ₁₀ CFU g ⁻¹	^b Reduction	^c P value		^d Surface abscess	Reduction	^e Histopathology	Reduction	^f P value
Mock	6.46 ± 0.25 (n = 19)	—	—	<100	14/19 (70%)	—	3.7 ± 1.2 (n = 10)	—	—
SpA	3.95 ± 0.56 (n = 20)	2.51	0.0003	1706 ± 370	10/20 (50%)	32%	2.1 ± 1.2 (n = 10)	2.2	0.35
SpA-D	4.43 ± 0.41 (n = 18)	2.03	0.0001	381 ± 27	10/18 (55%)	25%	1.5 ± 0.8 (n = 10)	2.2	0.15
SpA-D1	3.39 ± 0.50 (n = 19)	3.07	<0.0001	5600 ± 801	6/20 (30%)	59%	0.5 ± 0.4 (n = 10)	3.2	0.02
SpA-D2	3.43 ± 0.46 (n = 19)	3.03	<0.0001	3980 ± 676	6/19 (32%)	57%	0.8 ± 0.5 (n = 10)	2.9	0.04

^aMeans of staphylococcal load calculated as log₁₀ CFU g⁻¹ in homogenized renal tissues 4 days following infection in cohorts of 18 to 20 BALB/c mice. Standard error of the means (±SEM) is indicated.

^cStatistical significance was calculated with the Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

^bReduction in bacterial load calculated as log₁₀ CFU g⁻¹.

^dAbscess formation in kidney tissues four days following infection was measured by macroscopic inspection (% positive)

^eHistopathology of hematoxylin-eosin stained, thin sectioned kidneys from ten animals; the number of abscesses per kidney was recorded and averaged for the final mean (±SEM).

^fStatistical significance was calculated with the Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

SpA-D1 and SpA-D2 represent SpA-D_{Q9, 10K; D36, 37A} and SpA-D_{Q9, 10G; D36, 37A}, respectively.

Vaccine Protection in Murine Abscess, Murine Lethal Infection, and Murine Pneumonia Models.

Three animal models have been established for the study of *S. aureus* infectious disease. These models are used here to examine the level of protective immunity provided via the generation of Protein A specific antibodies.

Murine Abscess—

BALB/c mice (24-day-old female, 8-10 mice per group, Charles River Laboratories, Wilmington, Mass.) are immunized by intramuscular injection into the hind leg with purified protein (Chang et al., 2003; Schneewind et al., 1992). Purified SpA, SpA-D or SpA-D_{Q9,10K;D36,37A} (50 µg protein) is administered on days 0 (emulsified 1:1 with complete Freund's adjuvant) and 11 (emulsified 1:1 with incomplete Freund's adjuvant). Blood samples are drawn by retroorbital bleeding on days 0, 11, and 20. Sera are examined by ELISA for IgG titers for specific SpA-D and SpA-D_{Q9,10K;D36,37A} binding activity. Immunized animals are challenged on day 21 by retroorbital injection of 100 µl of *S. aureus* Newman or *S. aureus* USA300 suspension (1×10⁷ cfu). For this, overnight cultures of *S. aureus* Newman are diluted 1:100 into fresh tryptic soy broth and grown for 3 h at 37° C. Staphylococci are centrifuged, washed twice, and diluted in PBS to yield an A₆₀₀ of 0.4 (1×10⁸ cfu per ml). Dilutions are verified experimentally by agar plating and colony formation. Mice are anesthetized by intraperitoneal injection of 80-120 mg of ketamine and 3-6 mg of xylazine per kilogram of body weight and infected by retroorbital injection. On day 5 or 15 following challenge, mice are euthanized by compressed CO₂ inhalation. Kidneys are removed and homogenized in 1% Triton X-100. Aliquots are diluted and plated on agar medium for triplicate determination of cfu. For histology, kidney tissue is incubated at

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Murine Pneumonia Model—

S. aureus strains Newman or USA300 (LAC) are grown at 37° C. in tryptic soy broth/agar to OD₆₆₀ 0.5. 50-ml culture aliquots are centrifuged, washed in PBS, and suspended in 750 µl PBS for mortality studies (3-4×10⁸ CFU per 30-µl volume), or 1,250 µl PBS (2×10⁸ CFU per 30-µl volume) for bacterial load and histopathology experiments (2, 3). For lung infection, 7-wk-old C57BL/6J mice (The Jackson Laboratory) are anesthetized before inoculation of 30 µl of *S. aureus* suspension into the left nare. Animals are placed into the cage in a supine position for recovery and observed for 14 days. For active immunization, 4-wk-old mice receive 20 µg SpA-D or SpA-D_{Q9,10K;D36,37A} in CFA

on day 0 via the i.m. route, followed by a boost with 20 µg SpA-D or SpA-D_{Q9,10K;D36,37A} in incomplete Freund's adjuvant (IFA) on day 10. Animals are challenged with *S. aureus* on day 21. Sera are collected before immunization and on day 20 to assess specific antibody production. For passive immunization studies, 7-wk-old mice receive 100 µl of either NRS (normal rabbit serum) or SpA-D-specific rabbit antisera via i.p. injection 24 h before challenge. To assess the pathological correlates of pneumonia, infected animals are killed via forced CO₂ inhalation before removal of both lungs. The right lung is homogenized for enumeration of lung bacterial load. The left lung is placed in 1% formalin and paraffin embedded, thin sectioned, stained with hematoxylin-eosin, and analyzed by microscopy.

Rabbit Antibodies—

Purified 200 µg SpA-D or SpA-D_{Q9,10K;D36,37A} is used as an immunogen for the production of rabbit antisera. 200 µg protein is emulsified with CFA for injection at day 0, followed by booster injections with 200 µg protein emulsified with IFA on days 21 and 42. Rabbit antibody titers are determined by ELISA. Purified antibodies are obtained by affinity chromatography of rabbit serum on SpA-D or SpA-D_{Q9,10K;D36,37A} sepharose. The concentration of eluted antibodies is measured by absorbance at 400 nm and specific antibody titers are determined by ELISA.

Active Immunization with SpA-Domain D Variants.—

To determine vaccine efficacy, animals are actively immunized with purified SpA-D or SpA-D_{Q9,10K;D36,37A}. As a control, animals are immunized with adjuvant alone. Antibody titers against Protein A preparations are determined using SpA-D or SpA-D_{Q9,10K;D36,37A} as antigens; note that the SpA-D_{Q9,10K;D36,37A} variant cannot bind the Fc or Fab portion of IgG. Using infectious disease models described above, any reduction in bacterial load (murine abscess and pneumonia), histopathology evidence of staphylococcal disease (murine abscess and pneumonia) and protection from lethal disease (murine lethal challenge and pneumonia) is measured.

Passive Immunization with Affinity Purified Rabbit Polyclonal Antibodies Generated Against SpA-Domain D Variants.

To determine protective immunity of Protein A specific rabbit antibodies, mice are passively immunized with 5 mg/kg of purified SpA-D or SpA-D_{Q9,10K;D36,37A} derived rabbit antibodies. Both of these antibody preparations are purified by affinity chromatography using immobilized SpA-D or SpA-D_{Q9,10K;D36,37A}. As a control, animals are passively immunized with rV10 antibodies (a plague protective antigen that has no impact on the outcome of staphylococcal infections). Antibody titers against all Protein A preparations are determined using SpA-D_{Q9,10K;D36,37A} as an antigen, as this variant cannot bind the Fc or Fab portion of IgG. Using the infectious disease models described above, the reduction in bacterial load (murine abscess and pneumonia), histopathology evidence of staphylococcal disease (murine abscess and pneumonia), and the protection from lethal disease (murine lethal challenge and pneumonia) is measured.

Example 2

Non-Toxicogenic Protein A Vaccine for Methicillin-Resistant *Staphylococcus Aureus* Infection

Clinical isolates of *S. aureus* express protein A (Shopsin et al., 1999, whose primary translational product is com-

prised of an N-terminal signal peptide (DeDent et al., 2008), five Ig-BDs (designated E, D, A, B and C)(Sjodahl, 1977), region X with variable repeats of an eight residue peptide (Guss et al., 1984), and C-terminal sorting signal for the cell wall anchoring of SpA (Schneewind et al., 1992; Schneewind et al., 1995) (FIG. 6). Guided by amino acid homology (Uhlen et al., 1984), the triple α -helical bundle structure of IgBDs (Deisenhofer et al., 1978; Deisenhofer et al., 1981) and their atomic interactions with Fab V_H3 (Graille et al., 2000) or Fc γ (Gouda et al., 1998), glutamine 9 and 10 were selected as well as aspartate 36 and 37 as critical for the association of SpA with antibodies or B cell receptor, respectively. Substitutions Gln9Lys, Gln10Lys, Asp36Ala and Asp37Ala were introduced into the D domain to generate SpA-D_{KKAA} (FIG. 6). The ability of isolated SpA-D or SpA-D_{KKAA} to bind human IgG was analyzed by affinity chromatography (FIG. 6). Polyhistidine tagged SpA-D as well as full-length SpA retained human IgG on Ni-NTA, whereas SpA-D_{KKAA} and a negative control (SrtA) did not (FIG. 6). A similar result was observed with von Willebrand factor (Hartleib et al., 2000), which, along with tumor necrosis factor receptor 1 (TNFR1)(Gomez et al., 2004), can also bind protein A via glutamine 9 and 10 (FIG. 6). Human immunoglobulin encompasses 60-70% V_H3-type IgG. The inventors distinguish between Fc domain and B cell receptor activation of Igs and measured association of human Fc γ and F(ab)₂ fragments, both of which bound to full-length SpA or SpA-D, but not to SpA-D_{KKAA} (FIG. 6). Injection of SpA-D into the peritoneal cavity of mice resulted in B cell expansion followed by apoptotic collapse of CD19+ lymphocytes in spleen tissue of BALB/c mice (Goodyear and Silverman, 2003)(FIG. 6). B cell superantigen activity was not observed following injection with SpA-D_{KKAA}, and TUNEL-staining of splenic tissue failed to detect the increase in apoptotic cells that follows injection of SpA or SpA-D (FIG. 6).

Naive six week old BALB/c mice were injected with 50 µg each of purified SpA, SpA-D or SpA-D_{KKAA} emulsified in CFA and boosted with the same antigen emulsified in IFA. In agreement with the hypothesis that SpA-D promotes the apoptotic collapse of activated clonal B cell populations, the inventors observed a ten-fold higher titer of SpA-D_{KKAA} specific antibodies following immunization of mice with the non-toxicogenic variant as compared to the B cell superantigen (SpA-D vs. SpA-D_{KKAA} P<0.0001, Table 6). Antibody titers raised by immunization with full-length SpA were higher than those elicited by SpA-D (P=0.0022), which is likely due to the larger size and reiterative domain structure of this antigen (Table 6). Nevertheless, even SpA elicited lower antibody titers than SpA-D_{KKAA} (P=0.0003), which encompasses only 50 amino acids of protein A (520 residues, SEQ ID NO:33). Immunized mice were challenged by intravenous inoculation with *S. aureus* Newman and the ability of staphylococci to seed abscesses in renal tissues was examined by necropsy four days after challenge. In homogenized renal tissue of mock (PBS/adjuvant) immunized mice, an average staphylococcal load of 6.46 log₁₀ CFU g⁻¹ was enumerated (Table 6). Immunization of mice with SpA or SpA-D led to a reduction in staphylococcal load, however SpA-D_{KKAA} vaccinated animals displayed an even greater, 3.07 log₁₀ CFU g⁻¹ reduction of *S. aureus* Newman in renal tissues (P<0.0001, Table 6). Abscess formation in kidneys was analyzed by histopathology (FIG. 7). Mock immunized animals harbored an average of 3.7 (±1.2) abscesses per kidney (Table 6). Vaccination with SpA-D_{KKAA} reduced the average number of abscesses to 0.5 (±0.4)(P=0.0204), whereas immunization with SpA or SpA-D did not cause a significant reduction in the number of abscess lesions (Table

6). Lesions from SpA-D_{KKAA} vaccinated animals were smaller in size, with fewer infiltrating PMNs and characteristically lacked staphylococcal abscess communities (Cheng et al., 2009)(FIG. 7). Abscesses in animals that had been immunized with SpA or SpA-D displayed the same overall structure of lesions in mock immunized animals (FIG. 7).

The inventors examined whether SpA-D_{KKAA} immunization can protect mice against MRSA strains and selected the USA300 LAC isolate for animal challenge (Diep et al., 2006). This highly virulent CA-MRSA strain spread rapidly throughout the United States, causing significant human morbidity and mortality (Kennedy et al., 2008). Compared to adjuvant control mice, SpA-D_{KKAA} immunized animals harbored a 1.07 log₁₀ CFU g⁻¹ reduction in bacterial load of infected kidney tissues. Histopathology examination of renal tissue following *S. aureus* USA300 challenge revealed that the average number of abscesses was reduced from 4.04 (±0.8) to 1.6 (±0.6)(P=0.02774). In contrast, SpA or SpA-D immunization did not cause a significant reduction in bacterial load or abscess formation (Table 6).

Rabbits were immunized with SpA-D_{KKAA} and specific antibodies were purified on SpA-D_{KKAA} affinity column followed by SDS-PAGE (FIG. 8). SpA-D_{KKAA} specific IgG was cleaved with pepsin to generate Fcγ and F(ab)₂ fragments, the latter of which were purified by chromatography on SpA-D_{KKAA} column (FIG. 8). Binding of human IgG or vWF to SpA or SpA-D was perturbed by SpA-D_{KKAA} specific F(ab)₂, indicating that SpA-D_{KKAA} derived antibodies neutralize the B cell superantigen function of protein A as well as its interactions with Ig (FIG. 8).

To further improve the vaccine properties for non-toxic protein A, the inventors generated SpA_{KKAA}, which includes all five IgBDs with four amino acid substitutions—

substitutions corresponding to Gln9Lys, Gln10Lys, Asp36Ala and Asp37Ala of domain D—in each of its five domains (E, D, A, B and C). Polyhistidine tagged SpA_{KKAA} was purified by affinity chromatography and analyzed by Coomassie Blue-stained SDS-PAGE (FIG. 9). Unlike full-length SpA, SpA_{KKAA} did not bind human IgG, Fc and F(ab)₂ or vWF (FIG. 9). SpA_{KKAA} failed to display B cell superantigen activity, as injection of the variant into BALB/c mice did not cause a depletion of CD19+ B cells in splenic tissue (FIG. 9). SpA_{KKAA} vaccination generated higher specific antibody titers than SpA-D_{KKAA} immunization and provided mice with elevated protection against *S. aureus* USA300 challenge (Table 6). Four days following challenge, SpA_{KKAA} vaccinated animals harbored 3.54 log₁₀ CFU g⁻¹ fewer staphylococci in renal tissues (P=0.0001) and also caused a greater reduction in the number of abscess lesions (P=0.0109) (Table 6).

SpA_{KKAA} was used to immunize rabbits. Rabbit antibodies specific for SpA-D_{KKAA} or SpA_{KKAA} were affinity purified on matrices with immobilized cognate antigen and injected at a concentration of 5 mg kg⁻¹ body weight into the peritoneal cavity of BALB/c mice (Table 7). Twenty-four hours later, specific antibody titers were determined in serum and animals challenged by intravenous inoculation with *S. aureus* Newman. Passive transfer reduced the staphylococcal load in kidney tissues for SpA-D_{KKAA} (P=0.0016) or SpA_{KKAA} (P=0.0005) specific antibodies. On histopathology examination, both antibodies reduced the abundance of abscess lesions in the kidneys of mice challenged with *S. aureus* Newman (Table 7). Together these data reveal that vaccine protection following immunization with SpA-D_{KKAA} or SpA_{KKAA} is conferred by antibodies that neutralize protein A.

TABLE 6

Immunization of mice with protein A vaccines.						
Staphylococcal load and abscess formation in renal tissue						
Antigen	^a log ₁₀ CFU g ⁻¹	^b P-value	^c Reduction (log ₁₀ CFU g ⁻¹)	^d IgG Titer	^e Number of abscesses	^f P-value
<i>S. aureus</i> Newman challenge						
Mock	6.46 ± 0.25	—	—	<100	3.7 ± 1.2	—
SpA	3.95 ± 0.56	0.0003	2.51	1706 ± 370	2.1 ± 1.2	0.3581
SpA-D	4.43 ± 0.41	0.0001	2.03	381 ± 27	1.5 ± 0.8	0.1480
SpA D _{KKAA}	3.39 ± 0.50	<0.0001	3.07	5600 ± 801	0.5 ± 0.4	0.0204
<i>S. aureus</i> USA300 (LAC) challenge						
Mock	7.20 ± 0.24	—	—	<100	4.0 ± 0.8	—
SpA	6.81 ± 0.26	0.2819	0.39	476 ± 60	3.3 ± 1.0	0.5969
SpA-D	6.34 ± 0.52	0.1249	0.86	358 ± 19	2.2 ± 0.6	0.0912
SpA-D _{KKAA}	6.00 ± 0.42	0.0189	1.20	3710 ± 1147	1.6 ± 0.6	0.0277
SpA _{KKAA}	3.66 ± 0.76	0.0001	3.54	10200 ± 2476	1.2 ± 0.5	0.0109

^aMeans of staphylococcal load calculated as log₁₀ CFU g⁻¹ in homogenized renal tissues 4 days following infection in cohorts of fifteen to twenty BALB/c mice per immunization. Representative of two independent and reproducible animal experiments is shown. Standard error of the means (±SEM) is indicated.

^bStatistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

^cReduction in bacterial load calculated as log₁₀ CFU g⁻¹.

^dMeans of five randomly chosen serum IgG titers were measured prior to staphylococcal infection by ELISA

^eHistopathology of hematoxyline-eosin stained, thin sectioned kidneys from ten animals; the average number of abscesses per kidney was recorded and averaged again for the final mean (±SEM).

TABLE 7

Passive immunization of mice with antibodies against protein A.						
Staphylococcal load and abscess formation in renal tissue						
^a Antibody	^b log ₁₀ CFU g ⁻¹	^c P-value	^d Reduction (log ₁₀ CFU g ⁻¹)	^e IgG Titer	^f Number of abscesses	^g P-value
Mock	7.10 ± 0.14	—	—	<100	4.5 ± 0.8	—
α-SpA-D _{KKAA}	5.53 ± 0.43	0.0016	1.57	466 ± 114	1.9 ± 0.7	0.0235
α-SpA _{KKAA}	5.69 ± 0.34	0.0005	1.41	1575 ± 152	1.6 ± 0.5	0.0062

^aAffinity purified antibodies were injected into the peritoneal cavity of BALB/c mice at a concentration of 5 mg · kg⁻¹ twenty-four hours prior to intravenous challenge with 1 × 10⁷ CFU *S. aureus* Newman.

^bMeans of staphylococcal load calculated as log₁₀ CFU g⁻¹ in homogenized renal tissues 4 days following infection in cohorts of fifteen BALB/c mice per immunization. Representative of two independent and reproducible animal experiments is shown. Standard error of the means (±SEM) is indicated.

^cStatistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

^dReduction in bacterial load calculated as log₁₀ CFU g⁻¹.

^eMeans of five randomly chosen serum IgG titers were measured prior to staphylococcal infection by ELISA.

^fHistopathology of hematoxyline-eosin stained, thin sectioned kidneys from ten animals; the average number of abscesses per kidney was recorded and averaged again for the final mean (±SEM).

Following infection with virulent *S. aureus*, mice do not develop protective immunity against subsequent infection with the same strain (Burts et al., 2008)(FIG. 10). The average abundance of SpA-D_{KKAA} specific IgG in these animals was determined by dot blot as 0.20 ml⁻¹ (±0.04) and 0.14 μg ml⁻¹ (±0.01) for strains Newman and USA300 LAC, respectively (FIG. 9). The minimal concentration of protein A-specific IgG required for disease protection in SpA_{KKAA} or SpA-D_{KKAA} vaccinated animals (P 0.05 log₁₀ reduction in staphylococcal CFU g⁻¹ renal tissue) was calculated as 4.05 μg ml⁻¹ (±0.88). Average serum concentration of SpA-specific IgG in adult healthy human volunteers (n=16) was 0.21 μg ml⁻¹ (±0.02). Thus, *S. aureus* infections in mice or humans are not associated with immune responses that raise significant levels of neutralizing antibodies directed against protein A, which is likely due to the B cell superantigen attributes of this molecule. In contrast, the average serum concentration of IgG specific for diphtheria toxin in human volunteers, 0.068 μg ml⁻¹ (±0.20), was within range for protective immunity against diphtheria (Behring, 1890; Lagergard et al., 1992).

Clinical *S. aureus* isolates express protein A, an essential virulence factor whose B cell superantigen activity and evasive attributes towards opsono-phagocytic clearance are absolutely required for staphylococcal abscess formation (Palmqvist et al., 2005; Cheng et al., 2009; Silverman and Goodyear, 2006). Protein A can thus be thought of as a toxin, essential for pathogenesis, whose molecular attributes must be neutralized in order to achieve protective immunity. By generating non-toxic variants unable to bind Igs via Fcγ or VH₃-Fab domains, the inventors measure here for the first time protein A neutralizing immune responses as a correlate for protective immunity against *S. aureus* infection. In contrast to many methicillin-sensitive strains, CA-MRSA isolate USA300 LAC is significantly more virulent (Cheng et al., 2009). For example, immunization of experimental animals with the surface protein IsdB (Kuklin et al., 2006; Stranger-Jones et al., 2006) raises antibodies that confer protection against *S. aureus* Newman (Stranger-Jones et al., 2009) but not against USA300 challenge.

The methods utilized include:

Bacterial Strains and Growth.

Staphylococcus aureus strains Newman and USA300 were grown in tryptic soy broth (TSB) at 37°C. *Escherichia coli* strains DH5α and BL21 (DE3) were grown in Luria-Bertani (LB) broth with 100 μg ml⁻¹ ampicillin at 37°C.

Rabbit Antibodies.

The coding sequence for SpA was PCR-amplified with two primers, gctgcacatattggcgcaacacatgaagctcaac (SEQ ID NO:35) and agtggatccttatgcttgagctttgttagcatctgc (SEQ ID NO:36) using *S. aureus* Newman template DNA. SpA-D was PCR-amplified with two primers, aacatattgtcaacaagaat-caacaaagc (SEQ ID NO:38) and aaggatccagattgcttaatttttagc (SEQ ID NO:39). The sequence for SpA-D_{KKAA} was mutagenized with two sets of primers catatgttcaacaagaataaaaaagcgccttctatgaaatc (SEQ ID NO:42) and gatttcata-gaaggcgtttttttatcttggtaacatag (SEQ ID NO:43) for Q9K, Q10K as well as cttcattcaaatcttaagccgcccccaagc-caaagcactaac (SEQ ID NO:40) and gttagtctttggcttggggcg-gcttaagactttgaatgaag (SEQ ID NO:41) for D36A,D37A. The sequence of SpA_{KKAA} was synthesized by Integrated DNA Technologies, Inc. PCR products were cloned into pET-15b generating N-terminal His₆ tagged recombinant protein. Plasmids were transformed into BL21(DE3). Overnight cultures of transformants were diluted 1:100 into fresh media and grown at 37°C to an OD₆₀₀ 0.5, at which point cultures were induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and grown for an additional three hours. Bacterial cells were sedimented by centrifugation, suspended in column buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl) and disrupted with a French pressure cell at 14,000 psi. Lysates were cleared of membrane and insoluble components by ultracentrifugation at 40,000×g. Proteins in the soluble lysate were subjected to nickel-nitrilotriacetic acid (Ni-NTA, Qiagen) affinity chromatography. Proteins were eluted in column buffer containing successively higher concentrations of imidazole (100-500 mM). Protein concentrations were determined by bicinchonic acid (BCA) assay (Thermo Scientific). For antibody generation, rabbits (6 month old New-Zealand white, female, Charles River Laboratories) were immunized with 500 μg protein emulsified in Complete Freund's Adjuvant (Difco) by subscapular injection. For booster immunizations, proteins emulsified in Incomplete Freund's Adjuvant and injected 24 or 48 days following the initial immunization. On day 60, rabbits were bled and serum recovered.

Purified antigen (5 mg protein) was covalently linked to HiTrap NHS-activated HP columns (GE Healthcare). Antigen-matrix was used for affinity chromatography of 10-20 ml of rabbit serum at 4°C. Charged matrix was washed with 50 column volumes of PBS, antibodies eluted with elution buffer (1 M glycine, pH 2.5, 0.5 M NaCl) and immediately

neutralized with 1M Tris-HCl, pH 8.5. Purified antibodies were dialyzed overnight against PBS at 4° C.

F(Ab)₂ Fragments.

Affinity purified antibodies were mixed with 3 mg of pepsin at 37° C. for 30 minutes. The reaction was quenched with 1 M Tris-HCl, pH 8.5 and F(ab)₂ fragments were affinity purified with specific antigen-conjugated HiTrap NHS-activated HP columns. Purified antibodies were dialyzed overnight against PBS at 4° C., loaded onto SDS-PAGE gel and visualized with Coomassie Blue staining.

Active and Passive Immunization.

BALB/c mice (3 week old, female, Charles River Laboratories) were immunized with 50 µg protein emulsified in Complete Freund's Adjuvant (Difco) by intramuscular injection. For booster immunizations, proteins were emulsified in Incomplete Freund's Adjuvant and injected 11 days following the initial immunization. On day 20 following immunization, 5 mice were bled to obtain sera for specific antibody titers by enzyme-linked immunosorbent assay (ELISA).

Affinity purified antibodies in PBS were injected at a concentration 5 mg kg⁻¹ of experimental animal weight into the peritoneal cavity of BALB/c mice (6 week old, female, Charles River Laboratories) 24 hours prior to challenge with *S. aureus*. Animal blood was collected via periorbital vein puncture. Blood cells were removed with heparinized microhematocrit capillary tubes (Fisher) and Z-gel serum separation micro tubes (Sarstedt) were used to collect and measure antigen specific antibody titers by ELISA.

Mouse Renal Abscess.

Overnight cultures of *S. aureus* Newman or USA300 (LAC) were diluted 1:100 into fresh TSB and grown for 2 hours at 37° C. Staphylococci were sedimented, washed and suspended PBS at OD₆₀₀ of 0.4 (~1×10⁸ CFU ml⁻¹). Inocula were quantified by spreading sample aliquots on TSA and enumerating colonies formed. BALB/c mice (6 week old, female, Charles River Laboratories) were anesthetized via intraperitoneal injection with 100 mg ml⁻¹ ketamine and 20 mg ml⁻¹ xylazine per kilogram of body weight. Mice were infected by retro-obital injection with 1×10⁷ CFU of *S. aureus* Newman or 5×10⁶ CFU of *S. aureus* USA300. On day 4 following challenge, mice were killed by CO₂ inhalation. Both kidneys were removed, and the staphylococcal load in one organ was analyzed by homogenizing renal tissue with PBS, 1% Triton X-100. Serial dilutions of homogenate were spread on TSA and incubated for colony formation. The remaining organ was examined by histopathology. Briefly, kidneys were fixed in 10% formalin for 24 hours at room temperature. Tissues were embedded in paraffin, thin-sectioned, stained with hematoxylin-eosin, and inspected by light microscopy to enumerate abscess lesions. All mouse experiments were performed in accordance with the institutional guidelines following experimental protocol review and approval by the Institutional Biosafety Committee (IBC) and the Institutional Animal Care and Use Committee (IACUC) at the University of Chicago.

Protein A Binding.

For human IgG binding, Ni-NTA affinity columns were pre-charged with 200 µg of purified proteins (SpA, SpA-D, SpA-D_{KKAA}, and SrtA) in column buffer. After washing, 200 µg of human IgG (Sigma) was loaded onto the column. Protein samples were collected from washes and elutions and subjected to SDS-PAGE gel electrophoresis, followed by Coomassie Blue staining. Purified proteins (SpA, SpA_{KKAA}, SpA-D and SpA-D_{KKAA}) were coated onto MaxiSorp ELISA plates (NUNC) in 0.1M carbonate buffer (pH 9.5) at 1 µg ml⁻¹ concentration overnight at 4° C. Plates were

next blocked with 5% whole milk followed by incubation with serial dilutions of peroxidase-conjugated human IgG, Fc or F(ab)₂ fragments for one hour. Plates were washed and developed using OptEIA ELISA reagents (BD). Reactions were quenched with 1 M phosphoric acid and A₄₅₀ readings were used to calculate half maximal titer and percent binding.

Von Willebrand Factor (vWF) Binding Assays.

Purified proteins (SpA, SpA_{KKAA}, SpA D and SpA-D_{KKAA}) were coated and blocked as described above. Plates were incubated with human vWF at 1 µg ml⁻¹ concentration for two hours, then washed and blocked with human IgG for another hour. After washing, plates were incubated with serial dilution of peroxidase-conjugated antibody directed against human vWF for one hour. Plates were washed and developed using OptEIA ELISA reagents (BD). Reactions were quenched with 1 M phosphoric acid and A₄₅₀ readings were used to calculate half maximal titer and percent binding. For inhibition assays, plates were incubated with affinity purified F(ab)₂ fragments specific for SpA-D_{KKAA} at 10 µg ml⁻¹ concentration for one hour prior to ligand binding assays.

Splenocyte Apoptosis.

Affinity purified proteins (150 µg of SpA, SpA-D, SpA_{KKAA}, and SpA-D_{KKAA}) were injected into the peritoneal cavity of BALB/c mice (6 week old, female, Charles River Laboratories). Four hours following injection, animals were killed by CO₂ inhalation. Their spleens were removed and homogenized. Cell debris were removed using cell strainer and suspended cells were transferred to ACK lysis buffer (0.15 M NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA) to lyse red blood cells. White blood cells were sedimented by centrifugation, suspended in PBS and stained with 1:250 diluted R-PE conjugated anti-CD19 monoclonal antibody (Invitrogen) on ice and in the dark for one hour. Cells were washed with 1% FBS and fixed with 4% formalin overnight at 4° C. The following day, cells were diluted in PBS and analyzed by flow cytometry. The remaining organ was examined for histopathology. Briefly, spleens were fixed in 10% formalin for 24 hours at room temperature. Tissues were embedded in paraffin, thin-sectioned, stained with the Apoptosis detection kit (Millipore), and inspected by light microscopy.

Antibody Quantification.

Sera were collected from healthy human volunteers or BALB/c mice that had been either infected with *S. aureus* Newman or USA300 for 30 days or that had been immunized with SpA-D_{KKAA}/SpA_{KKAA} as described above. Human/mouse IgG (Jackson Immunology Laboratory), SpA_{KKAA}, and CRM197 were blotted onto nitrocellulose membrane. Membranes were blocked with 5% whole milk, followed by incubation with either human or mouse sera. IRDye 700DX conjugated affinity purified anti-human/mouse IgG (Rockland) was used to quantify signal intensities using the Odyssey™ infrared imaging system (Li-cor). Experiments with blood from human volunteers involved protocols that were reviewed, approved and performed under regulatory supervision of The University of Chicago's Institutional Review Board (IRB).

Statistical Analysis.

Two tailed Student's t tests were performed to analyze the statistical significance of renal abscess, ELISA, and B cell superantigen data.

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 <211> LENGTH: 52
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (7)..(8)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (34)..(35)
 <223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 7

```

Asn Asn Phe Asn Lys Asp Xaa Xaa Ser Ala Phe Tyr Glu Ile Leu Asn
1           5           10           15
Met Pro Asn Leu Asn Glu Ala Gln Arg Asn Gly Phe Ile Gln Ser Leu
          20           25           30
Lys Xaa Xaa Pro Ser Gln Ser Thr Asn Val Leu Gly Glu Ala Lys Lys
          35           40           45
Leu Asn Glu Ser
          50
  
```

<210> SEQ ID NO 8
 <211> LENGTH: 52
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (7)..(8)
 <223> OTHER INFORMATION: where X is any amino acid other than Q
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (12)..(35)
 <223> OTHER INFORMATION: where Y is any amion acid other than D

<400> SEQUENCE: 8

```

Asn Asn Phe Asn Lys Asp Xaa Xaa Ser Ala Phe Tyr Glu Ile Leu Asn
1           5           10           15
Met Pro Asn Leu Asn Glu Ala Gln Arg Asn Gly Phe Ile Gln Ser Leu
          20           25           30
Lys Tyr Tyr Pro Ser Gln Ser Thr Asn Val Leu Gly Glu Ala Lys Lys
          35           40           45
Leu Asn Glu Ser
          50
  
```

<210> SEQ ID NO 9

-continued

<211> LENGTH: 450

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 9

Met Lys Lys Lys Asn Ile Tyr Ser Ile Arg Lys Leu Gly Val Gly Ile
1 5 10 15
Ala Ser Val Thr Leu Gly Thr Leu Leu Ile Ser Gly Gly Val Thr Pro
20 25 30
Ala Ala Asn Ala Ala Gln His Asp Glu Ala Gln Gln Asn Ala Phe Tyr
35 40 45
Gln Val Leu Asn Met Pro Asn Leu Asn Ala Asp Gln Arg Asn Gly Phe
50 55 60
Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Val Leu Gly
65 70 75 80
Glu Ala Gln Lys Leu Asn Asp Ser Gln Ala Pro Lys Ala Asp Ala Gln
85 90 95
Gln Asn Asn Phe Asn Lys Asp Gln Gln Ser Ala Phe Tyr Glu Ile Leu
100 105 110
Asn Met Pro Asn Leu Asn Glu Ala Gln Arg Asn Gly Phe Ile Gln Ser
115 120 125
Leu Lys Asp Asp Pro Ser Gln Ser Thr Asn Val Leu Gly Glu Ala Lys
130 135 140
Lys Leu Asn Glu Ser Gln Ala Pro Lys Ala Asp Asn Asn Phe Asn Lys
145 150 155 160
Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn Leu Asn
165 170 175
Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser
180 185 190
Gln Ser Ala Asn Leu Leu Ser Glu Ala Lys Lys Leu Asn Glu Ser Gln
195 200 205
Ala Pro Lys Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe
210 215 220
Tyr Glu Ile Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly
225 230 235 240
Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu
245 250 255
Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Glu Glu Asp
260 265 270
Asn Lys Lys Pro Gly Lys Glu Asp Gly Asn Lys Pro Gly Lys Glu Asp
275 280 285
Gly Asn Lys Pro Gly Lys Glu Asp Asn Lys Lys Pro Gly Lys Glu Asp
290 295 300
Gly Asn Lys Pro Gly Lys Glu Asp Asn Asn Lys Pro Gly Lys Glu Asp
305 310 315 320
Gly Asn Lys Pro Gly Lys Glu Asp Asn Asn Lys Pro Gly Lys Glu Asp
325 330 335
Gly Asn Lys Pro Gly Lys Glu Asp Gly Asn Lys Pro Gly Lys Glu Asp
340 345 350
Gly Asn Gly Val His Val Val Lys Pro Gly Asp Thr Val Asn Asp Ile
355 360 365
Ala Lys Ala Asn Gly Thr Thr Ala Asp Lys Ile Ala Ala Asp Asn Lys
370 375 380
Leu Ala Asp Lys Asn Met Ile Lys Pro Gly Gln Glu Leu Val Val Asp

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385                390                395                400
Lys Lys Gln Pro Ala Asn His Ala Asp Ala Asn Lys Ala Gln Ala Leu
                405                410                415
Pro Glu Thr Gly Glu Glu Asn Pro Phe Ile Gly Thr Thr Val Phe Gly
                420                425                430
Gly Leu Ser Leu Ala Leu Gly Ala Ala Leu Leu Ala Gly Arg Arg Arg
                435                440                445
Glu Leu
                450

```

<210> SEQ ID NO 10

<211> LENGTH: 450

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 10

```

Met Lys Lys Lys Asn Ile Tyr Ser Ile Arg Lys Leu Gly Val Gly Ile
 1                5                10                15
Ala Ser Val Thr Leu Gly Thr Leu Leu Ile Ser Gly Gly Val Thr Pro
                20                25                30
Ala Ala Asn Ala Ala Gln His Asp Glu Ala Gln Gln Asn Ala Phe Tyr
                35                40                45
Gln Val Leu Asn Met Pro Asn Leu Asn Ala Asp Gln Arg Asn Gly Phe
 50                55                60
Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Val Leu Gly
 65                70                75                80
Glu Ala Gln Lys Leu Asn Asp Ser Gln Ala Pro Lys Ala Asp Ala Gln
                85                90                95
Gln Asn Asn Phe Asn Lys Asp Gln Gln Ser Ala Phe Tyr Glu Ile Leu
                100                105                110
Asn Met Pro Asn Leu Asn Glu Ala Gln Arg Asn Gly Phe Ile Gln Ser
                115                120                125
Leu Lys Asp Asp Pro Ser Gln Ser Thr Asn Val Leu Gly Glu Ala Lys
                130                135                140
Lys Leu Asn Glu Ser Gln Ala Pro Lys Ala Asp Asn Asn Phe Asn Lys
                145                150                155                160
Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn Leu Asn
                165                170                175
Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser
                180                185                190
Gln Ser Ala Asn Leu Leu Ser Glu Ala Lys Lys Leu Asn Glu Ser Gln
                195                200                205
Ala Pro Lys Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe
                210                215                220
Tyr Glu Ile Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly
                225                230                235                240
Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu
                245                250                255
Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Glu Glu Asp
                260                265                270
Asn Lys Lys Pro Gly Lys Glu Asp Gly Asn Lys Pro Gly Lys Glu Asp
                275                280                285
Gly Asn Lys Pro Gly Lys Glu Asp Asn Lys Lys Pro Gly Lys Glu Asp
                290                295                300

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Gly Asn Lys Pro Gly Lys Glu Asp Asn Asn Lys Pro Gly Lys Glu Asp
305 310 315 320

Gly Asn Lys Pro Gly Lys Glu Asp Asn Asn Lys Pro Gly Lys Glu Asp
325 330 335

Gly Asn Lys Pro Gly Lys Glu Asp Gly Asn Lys Pro Gly Lys Glu Asp
340 345 350

Gly Asn Gly Val His Val Val Lys Pro Gly Asp Thr Val Asn Asp Ile
355 360 365

Ala Lys Ala Asn Gly Thr Thr Ala Asp Lys Ile Ala Ala Asp Asn Lys
370 375 380

Leu Ala Asp Lys Asn Met Ile Lys Pro Gly Gln Glu Leu Val Val Asp
385 390 395 400

Lys Lys Gln Pro Ala Asn His Ala Asp Ala Asn Lys Ala Gln Ala Leu
405 410 415

Pro Glu Thr Gly Glu Glu Asn Pro Phe Ile Gly Thr Thr Val Phe Gly
420 425 430

Gly Leu Ser Leu Ala Leu Gly Ala Ala Leu Leu Ala Gly Arg Arg Arg
435 440 445

Glu Leu
450

<210> SEQ ID NO 11
 <211> LENGTH: 97
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 11

Met Ala Met Ile Lys Met Ser Pro Glu Glu Ile Arg Ala Lys Ser Gln
1 5 10 15

Ser Tyr Gly Gln Gly Ser Asp Gln Ile Arg Gln Ile Leu Ser Asp Leu
20 25 30

Thr Arg Ala Gln Gly Glu Ile Ala Ala Asn Trp Glu Gly Gln Ala Phe
35 40 45

Ser Arg Phe Glu Glu Gln Phe Gln Gln Leu Ser Pro Lys Val Glu Lys
50 55 60

Phe Ala Gln Leu Leu Glu Glu Ile Lys Gln Gln Leu Asn Ser Thr Ala
65 70 75 80

Asp Ala Val Gln Glu Gln Asp Gln Gln Leu Ser Asn Asn Phe Gly Leu
85 90 95

Gln

<210> SEQ ID NO 12
 <211> LENGTH: 102
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 12

Met Gly Gly Tyr Lys Gly Ile Lys Ala Asp Gly Gly Lys Val Asn Gln
1 5 10 15

Ala Lys Gln Leu Ala Ala Lys Ile Ala Lys Asp Ile Glu Ala Cys Gln
20 25 30

Lys Gln Thr Gln Gln Leu Ala Glu Tyr Ile Glu Gly Ser Asp Trp Glu
35 40 45

Gly Gln Phe Ala Asn Lys Val Lys Asp Val Leu Leu Ile Met Ala Lys
50 55 60

Phe Gln Glu Glu Leu Val Gln Pro Met Ala Asp His Gln Lys Ala Ile

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65              70              75              80
Asp Asn Leu Ser  Gln Asn Leu Ala Lys Tyr Asp Thr Leu Ser Ile Lys
            85              90              95

Gln Gly Leu Asp Arg Val
            100

<210> SEQ ID NO 13
<211> LENGTH: 1385
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 13

Met Leu Asn Arg Glu Asn Lys Thr Ala Ile Thr Arg Lys Gly Met Val
1              5              10              15

Ser Asn Arg Leu Asn Lys Phe Ser Ile Arg Lys Tyr Thr Val Gly Thr
            20              25              30

Ala Ser Ile Leu Val Gly Thr Thr Leu Ile Phe Gly Leu Gly Asn Gln
            35              40              45

Glu Ala Lys Ala Ala Glu Ser Thr Asn Lys Glu Leu Asn Glu Ala Thr
            50              55              60

Thr Ser Ala Ser Asp Asn Gln Ser Ser Asp Lys Val Asp Met Gln Gln
65              70              75              80

Leu Asn Gln Glu Asp Asn Thr Lys Asn Asp Asn Gln Lys Glu Met Val
            85              90              95

Ser Ser Gln Gly Asn Glu Thr Thr Ser Asn Gly Asn Lys Ser Ile Glu
            100              105              110

Lys Glu Ser Val Gln Ser Thr Thr Gly Asn Lys Val Glu Val Ser Thr
            115              120              125

Ala Lys Ser Asp Glu Gln Ala Ser Pro Lys Ser Thr Asn Glu Asp Leu
            130              135              140

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

Leu Glu Asn Lys Ser Val Val Asn Val Gln Pro Thr Asn Glu Glu Asn
            165              170              175

Lys Lys Val Asp Ala Lys Thr Glu Ser Thr Thr Leu Asn Val Lys Ser
            180              185              190

Asp Ala Ile Lys Ser Asn Ala Glu Thr Leu Val Asp Asn Asn Ser Asn
            195              200              205

Ser Asn Asn Glu Asn Asn Ala Asp Ile Ile Leu Pro Lys Ser Thr Ala
210              215              220

Pro Lys Ser Leu Asn Thr Arg Met Arg Met Ala Ala Ile Gln Pro Asn
225              230              235              240

Ser Thr Asp Ser Lys Asn Val Asn Asp Leu Ile Thr Ser Asn Thr Thr
            245              250              255

Leu Thr Val Val Asp Ala Asp Asn Ser Lys Thr Ile Val Pro Ala Gln
            260              265              270

Asp Tyr Leu Ser Leu Lys Ser Gln Ile Thr Val Asp Asp Lys Val Lys
            275              280              285

Ser Gly Asp Tyr Phe Thr Ile Lys Tyr Ser Asp Thr Val Gln Val Tyr
290              295              300

Gly Leu Asn Pro Glu Asp Ile Lys Asn Ile Gly Asp Ile Lys Asp Pro
305              310              315              320

Asn Asn Gly Glu Thr Ile Ala Thr Ala Lys His Asp Thr Ala Asn Asn
            325              330              335

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Leu Ile Thr Tyr Thr Phe Thr Asp Tyr Val Asp Arg Phe Asn Ser Val
 340 345 350

Lys Met Gly Ile Asn Tyr Ser Ile Tyr Met Asp Ala Asp Thr Ile Pro
 355 360 365

Val Asp Lys Lys Asp Val Pro Phe Ser Val Thr Ile Gly Asn Gln Ile
 370 375 380

Thr Thr Thr Thr Ala Asp Ile Thr Tyr Pro Ala Tyr Lys Glu Ala Asp
 385 390 395 400

Asn Asn Ser Ile Gly Ser Ala Phe Thr Glu Thr Val Ser His Val Gly
 405 410 415

Asn Val Glu Asp Pro Gly Tyr Tyr Asn Gln Val Val Tyr Val Asn Pro
 420 425 430

Met Asp Lys Asp Leu Lys Gly Ala Lys Leu Lys Val Glu Ala Tyr His
 435 440 445

Pro Lys Tyr Pro Thr Asn Ile Gly Gln Ile Asn Gln Asn Val Thr Asn
 450 455 460

Ile Lys Ile Tyr Arg Val Pro Glu Gly Tyr Thr Leu Asn Lys Gly Tyr
 465 470 475 480

Asp Val Asn Thr Asn Asp Leu Val Asp Val Thr Asp Glu Phe Lys Asn
 485 490 495

Lys Met Thr Tyr Gly Ser Asn Gln Ser Val Asn Leu Asp Phe Gly Asp
 500 505 510

Ile Thr Ser Ala Tyr Val Val Met Val Asn Thr Lys Phe Gln Tyr Thr
 515 520 525

Asn Ser Glu Ser Pro Thr Leu Val Gln Met Ala Thr Leu Ser Ser Thr
 530 535 540

Gly Asn Lys Ser Val Ser Thr Gly Asn Ala Leu Gly Phe Thr Asn Asn
 545 550 555 560

Gln Ser Gly Gly Ala Gly Gln Glu Val Tyr Lys Ile Gly Asn Tyr Val
 565 570 575

Trp Glu Asp Thr Asn Lys Asn Gly Val Gln Glu Leu Gly Glu Lys Gly
 580 585 590

Val Gly Asn Val Thr Val Thr Val Phe Asp Asn Asn Thr Asn Thr Lys
 595 600 605

Val Gly Glu Ala Val Thr Lys Glu Asp Gly Ser Tyr Leu Ile Pro Asn
 610 615 620

Leu Pro Asn Gly Asp Tyr Arg Val Glu Phe Ser Asn Leu Pro Lys Gly
 625 630 635 640

Tyr Glu Val Thr Pro Ser Lys Gln Gly Asn Asn Glu Glu Leu Asp Ser
 645 650 655

Asn Gly Leu Ser Ser Val Ile Thr Val Asn Gly Lys Asp Asn Leu Ser
 660 665 670

Ala Asp Leu Gly Ile Tyr Lys Pro Lys Tyr Asn Leu Gly Asp Tyr Val
 675 680 685

Trp Glu Asp Thr Asn Lys Asn Gly Ile Gln Asp Gln Asp Glu Lys Gly
 690 695 700

Ile Ser Gly Val Thr Val Thr Leu Lys Asp Glu Asn Gly Asn Val Leu
 705 710 715 720

Lys Thr Val Thr Thr Asp Ala Asp Gly Lys Tyr Lys Phe Thr Asp Leu
 725 730 735

Asp Asn Gly Asn Tyr Lys Val Glu Phe Thr Thr Pro Glu Gly Tyr Thr
 740 745 750

Pro Thr Thr Val Thr Ser Gly Ser Asp Ile Glu Lys Asp Ser Asn Gly

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755				760				765							
Leu	Thr	Thr	Thr	Gly	Val	Ile	Asn	Gly	Ala	Asp	Asn	Met	Thr	Leu	Asp
770						775					780				
Ser	Gly	Phe	Tyr	Lys	Thr	Pro	Lys	Tyr	Asn	Leu	Gly	Asn	Tyr	Val	Trp
785					790					795					800
Glu	Asp	Thr	Asn	Lys	Asp	Gly	Lys	Gln	Asp	Ser	Thr	Glu	Lys	Gly	Ile
				805					810					815	
Ser	Gly	Val	Thr	Val	Thr	Leu	Lys	Asn	Glu	Asn	Gly	Glu	Val	Leu	Gln
		820						825					830		
Thr	Thr	Lys	Thr	Asp	Lys	Asp	Gly	Lys	Tyr	Gln	Phe	Thr	Gly	Leu	Glu
		835					840						845		
Asn	Gly	Thr	Tyr	Lys	Val	Glu	Phe	Glu	Thr	Pro	Ser	Gly	Tyr	Thr	Pro
850						855					860				
Thr	Gln	Val	Gly	Ser	Gly	Thr	Asp	Glu	Gly	Ile	Asp	Ser	Asn	Gly	Thr
865					870					875					880
Ser	Thr	Thr	Gly	Val	Ile	Lys	Asp	Lys	Asp	Asn	Asp	Thr	Ile	Asp	Ser
				885					890					895	
Gly	Phe	Tyr	Lys	Pro	Thr	Tyr	Asn	Leu	Gly	Asp	Tyr	Val	Trp	Glu	Asp
			900					905					910		
Thr	Asn	Lys	Asn	Gly	Val	Gln	Asp	Lys	Asp	Glu	Lys	Gly	Ile	Ser	Gly
		915					920						925		
Val	Thr	Val	Thr	Leu	Lys	Asp	Glu	Asn	Asp	Lys	Val	Leu	Lys	Thr	Val
930						935					940				
Thr	Thr	Asp	Glu	Asn	Gly	Lys	Tyr	Gln	Phe	Thr	Asp	Leu	Asn	Asn	Gly
945					950					955					960
Thr	Tyr	Lys	Val	Glu	Phe	Glu	Thr	Pro	Ser	Gly	Tyr	Thr	Pro	Thr	Ser
			965						970					975	
Val	Thr	Ser	Gly	Asn	Asp	Thr	Glu	Lys	Asp	Ser	Asn	Gly	Leu	Thr	Thr
			980					985					990		
Thr	Gly	Val	Ile	Lys	Asp	Ala	Asp	Asn	Met	Thr	Leu	Asp	Ser	Gly	Phe
		995					1000						1005		
Tyr	Lys	Thr	Pro	Lys	Tyr	Ser	Leu	Gly	Asp	Tyr	Val	Trp	Tyr	Asp	
1010						1015						1020			
Ser	Asn	Lys	Asp	Gly	Lys	Gln	Asp	Ser	Thr	Glu	Lys	Gly	Ile	Lys	
1025						1030						1035			
Asp	Val	Lys	Val	Ile	Leu	Leu	Asn	Glu	Lys	Gly	Glu	Val	Ile	Gly	
1040						1045						1050			
Thr	Thr	Lys	Thr	Asp	Glu	Asn	Gly	Lys	Tyr	Arg	Phe	Asp	Asn	Leu	
1055						1060						1065			
Asp	Ser	Gly	Lys	Tyr	Lys	Val	Ile	Phe	Glu	Lys	Pro	Thr	Gly	Leu	
1070						1075						1080			
Thr	Gln	Thr	Gly	Thr	Asn	Thr	Thr	Glu	Asp	Asp	Lys	Asp	Ala	Asp	
1085						1090						1095			
Gly	Gly	Glu	Val	Asp	Val	Thr	Ile	Thr	Asp	His	Asp	Asp	Phe	Thr	
1100						1105						1110			
Leu	Asp	Asn	Gly	Tyr	Tyr	Glu	Glu	Glu	Thr	Ser	Asp	Ser	Asp	Ser	
1115						1120						1125			
Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	
1130						1135						1140			
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	
1145						1150						1155			
Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	
1160						1165						1170			

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Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 1175 1180 1185

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 1190 1195 1200

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 1205 1210 1215

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 1220 1225 1230

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 1235 1240 1245

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 1250 1255 1260

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 1265 1270 1275

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 1280 1285 1290

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 1295 1300 1305

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 1310 1315 1320

Ser Asp Ala Gly Lys His Thr Pro Val Lys Pro Met Ser Thr Thr
 1325 1330 1335

Lys Asp His His Asn Lys Ala Lys Ala Leu Pro Glu Thr Gly Asn
 1340 1345 1350

Glu Asn Ser Gly Ser Asn Asn Ala Thr Leu Phe Gly Gly Leu Phe
 1355 1360 1365

Ala Ala Leu Gly Ser Leu Leu Leu Phe Gly Arg Arg Lys Lys Gln
 1370 1375 1380

Asn Lys
 1385

<210> SEQ ID NO 14
 <211> LENGTH: 1141
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 14

Met Ile Asn Arg Asp Asn Lys Lys Ala Ile Thr Lys Lys Gly Met Ile
 1 5 10 15

Ser Asn Arg Leu Asn Lys Phe Ser Ile Arg Lys Tyr Thr Val Gly Thr
 20 25 30

Ala Ser Ile Leu Val Gly Thr Thr Leu Ile Phe Gly Leu Gly Asn Gln
 35 40 45

Glu Ala Lys Ala Ala Glu Asn Thr Ser Thr Glu Asn Ala Lys Gln Asp
 50 55 60

Asp Ala Thr Thr Ser Asp Asn Lys Glu Val Val Ser Glu Thr Glu Asn
 65 70 75 80

Asn Ser Thr Thr Glu Asn Asp Ser Thr Asn Pro Ile Lys Lys Glu Thr
 85 90 95

Asn Thr Asp Ser Gln Pro Glu Ala Lys Glu Glu Ser Thr Thr Ser Ser
 100 105 110

Thr Gln Gln Gln Gln Asn Asn Val Thr Ala Thr Thr Glu Thr Lys Pro
 115 120 125

Gln Asn Ile Glu Lys Glu Asn Val Lys Pro Ser Thr Asp Lys Thr Ala

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130			135			140									
Thr	Glu	Asp	Thr	Ser	Val	Ile	Leu	Glu	Glu	Lys	Lys	Ala	Pro	Asn	Tyr
145				150				155						160	
Thr	Asn	Asn	Asp	Val	Thr	Thr	Lys	Pro	Ser	Thr	Ser	Glu	Ile	Gln	Thr
			165					170						175	
Lys	Pro	Thr	Thr	Pro	Gln	Glu	Ser	Thr	Asn	Ile	Glu	Asn	Ser	Gln	Pro
			180					185						190	
Gln	Pro	Thr	Pro	Ser	Lys	Val	Asp	Asn	Gln	Val	Thr	Asp	Ala	Thr	Asn
			195				200					205			
Pro	Lys	Glu	Pro	Val	Asn	Val	Ser	Lys	Glu	Glu	Leu	Lys	Asn	Asn	Pro
			210			215					220				
Glu	Lys	Leu	Lys	Glu	Leu	Val	Arg	Asn	Asp	Asn	Thr	Asp	Arg	Ser	
225				230					235					240	
Thr	Lys	Pro	Val	Ala	Thr	Ala	Pro	Thr	Ser	Val	Ala	Pro	Lys	Arg	Leu
			245						250					255	
Asn	Ala	Lys	Met	Arg	Phe	Ala	Val	Ala	Gln	Pro	Ala	Ala	Val	Ala	Ser
			260					265						270	
Asn	Asn	Val	Asn	Asp	Leu	Ile	Thr	Val	Thr	Lys	Gln	Thr	Ile	Lys	Val
			275				280					285			
Gly	Asp	Gly	Lys	Asp	Asn	Val	Ala	Ala	Ala	His	Asp	Gly	Lys	Asp	Ile
290					295						300				
Glu	Tyr	Asp	Thr	Glu	Phe	Thr	Ile	Asp	Asn	Lys	Val	Lys	Lys	Gly	Asp
305				310						315				320	
Thr	Met	Thr	Ile	Asn	Tyr	Asp	Lys	Asn	Val	Ile	Pro	Ser	Asp	Leu	Thr
			325					330						335	
Asp	Lys	Asn	Asp	Pro	Ile	Asp	Ile	Thr	Asp	Pro	Ser	Gly	Glu	Val	Ile
			340					345					350		
Ala	Lys	Gly	Thr	Phe	Asp	Lys	Ala	Thr	Lys	Gln	Ile	Thr	Tyr	Thr	Phe
			355				360						365		
Thr	Asp	Tyr	Val	Asp	Lys	Tyr	Glu	Asp	Ile	Lys	Ala	Arg	Leu	Thr	Leu
370					375						380				
Tyr	Ser	Tyr	Ile	Asp	Lys	Gln	Ala	Val	Pro	Asn	Glu	Thr	Ser	Leu	Asn
385				390						395				400	
Leu	Thr	Phe	Ala	Thr	Ala	Gly	Lys	Glu	Thr	Ser	Gln	Asn	Val	Ser	Val
			405					410						415	
Asp	Tyr	Gln	Asp	Pro	Met	Val	His	Gly	Asp	Ser	Asn	Ile	Gln	Ser	Ile
			420					425					430		
Phe	Thr	Lys	Leu	Asp	Glu	Asn	Lys	Gln	Thr	Ile	Glu	Gln	Gln	Ile	Tyr
			435				440						445		
Val	Asn	Pro	Leu	Lys	Lys	Thr	Ala	Thr	Asn	Thr	Lys	Val	Asp	Ile	Ala
450					455						460				
Gly	Ser	Gln	Val	Asp	Asp	Tyr	Gly	Asn	Ile	Lys	Leu	Gly	Asn	Gly	Ser
465				470						475				480	
Thr	Ile	Ile	Asp	Gln	Asn	Thr	Glu	Ile	Lys	Val	Tyr	Lys	Val	Asn	Pro
			485					490						495	
Asn	Gln	Gln	Leu	Pro	Gln	Ser	Asn	Arg	Ile	Tyr	Asp	Phe	Ser	Gln	Tyr
			500					505					510		
Glu	Asp	Val	Thr	Ser	Gln	Phe	Asp	Asn	Lys	Lys	Ser	Phe	Ser	Asn	Asn
			515				520						525		
Val	Ala	Thr	Leu	Asp	Phe	Gly	Asp	Ile	Asn	Ser	Ala	Tyr	Ile	Ile	Lys
			530				535				540				
Val	Val	Ser	Lys	Tyr	Thr	Pro	Thr	Ser	Asp	Gly	Glu	Leu	Asp	Ile	Ala
545					550					555				560	

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Thr Lys Pro Val Gln Pro Lys Val Glu Lys Val Lys Pro Thr Val Thr
 210 215 220
 Thr Thr Ser Lys Val Glu Asp Asn His Ser Thr Lys Val Val Ser Thr
 225 230 235 240
 Asp Thr Thr Lys Asp Gln Thr Lys Thr Gln Thr Ala His Thr Val Lys
 245 250 255
 Thr Ala Gln Thr Ala Gln Glu Gln Asn Lys Val Gln Thr Pro Val Lys
 260 265 270
 Asp Val Ala Thr Ala Lys Ser Glu Ser Asn Asn Gln Ala Val Ser Asp
 275 280 285
 Asn Lys Ser Gln Gln Thr Asn Lys Val Thr Lys His Asn Glu Thr Pro
 290 295 300
 Lys Gln Ala Ser Lys Ala Lys Glu Leu Pro Lys Thr Gly Leu Thr Ser
 305 310 315 320
 Val Asp Asn Phe Ile Ser Thr Val Ala Phe Ala Thr Leu Ala Leu Leu
 325 330 335
 Gly Ser Leu Ser Leu Leu Leu Phe Lys Arg Lys Glu Ser Lys
 340 345 350

<210> SEQ ID NO 16

<211> LENGTH: 645

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 16

Met Asn Lys Gln Gln Lys Glu Phe Lys Ser Phe Tyr Ser Ile Arg Lys
 1 5 10 15
 Ser Ser Leu Gly Val Ala Ser Val Ala Ile Ser Thr Leu Leu Leu Leu
 20 25 30
 Met Ser Asn Gly Glu Ala Gln Ala Ala Ala Glu Glu Thr Gly Gly Thr
 35 40 45
 Asn Thr Glu Ala Gln Pro Lys Thr Glu Ala Val Ala Ser Pro Thr Thr
 50 55 60
 Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
 65 70 75 80
 Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
 85 90 95
 Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Ala Val Lys
 100 105 110
 Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
 115 120 125
 Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
 130 135 140
 Ala Pro Asn Ser Arg Pro Ile Asp Phe Glu Met Lys Lys Glu Asn Gly
 145 150 155 160
 Glu Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Lys Pro Ala Arg Val
 165 170 175
 Ile Phe Thr Asp Ser Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
 180 185 190
 Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
 195 200 205
 Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
 210 215 220
 Phe Ser Val Ser Asn Gly Thr Lys Ala Val Lys Ile Val Ser Ser Thr

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<210> SEQ ID NO 17
 <211> LENGTH: 80
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 17

Met Asn Gln His Val Lys Val Thr Phe Asp Phe Thr Asn Tyr Asn Tyr
 1 5 10 15
 Gly Thr Tyr Asp Leu Ala Val Pro Ala Tyr Leu Pro Ile Lys Asn Leu
 20 25 30
 Ile Ala Leu Val Leu Asp Ser Leu Asp Ile Ser Ile Phe Asp Val Asn
 35 40 45
 Thr Gln Ile Lys Val Met Thr Lys Gly Gln Leu Leu Val Glu Asn Asp
 50 55 60
 Arg Leu Ile Asp Tyr Gln Ile Ala Asp Gly Asp Ile Leu Lys Leu Leu
 65 70 75 80

<210> SEQ ID NO 18
 <211> LENGTH: 877
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 18

Met Lys Lys Arg Ile Asp Tyr Leu Ser Asn Lys Gln Asn Lys Tyr Ser
 1 5 10 15
 Ile Arg Arg Phe Thr Val Gly Thr Thr Ser Val Ile Val Gly Ala Thr
 20 25 30
 Ile Leu Phe Gly Ile Gly Asn His Gln Ala Gln Ala Ser Glu Gln Ser
 35 40 45
 Asn Asp Thr Thr Gln Ser Ser Lys Asn Asn Ala Ser Ala Asp Ser Glu
 50 55 60
 Lys Asn Asn Met Ile Glu Thr Pro Gln Leu Asn Thr Thr Ala Asn Asp
 65 70 75 80
 Thr Ser Asp Ile Ser Ala Asn Thr Asn Ser Ala Asn Val Asp Ser Thr
 85 90 95
 Thr Lys Pro Met Ser Thr Gln Thr Ser Asn Thr Thr Thr Thr Glu Pro
 100 105 110
 Ala Ser Thr Asn Glu Thr Pro Gln Pro Thr Ala Ile Lys Asn Gln Ala
 115 120 125
 Thr Ala Ala Lys Met Gln Asp Gln Thr Val Pro Gln Glu Ala Asn Ser
 130 135 140
 Gln Val Asp Asn Lys Thr Thr Asn Asp Ala Asn Ser Ile Ala Thr Asn
 145 150 155 160
 Ser Glu Leu Lys Asn Ser Gln Thr Leu Asp Leu Pro Gln Ser Ser Pro
 165 170 175
 Gln Thr Ile Ser Asn Ala Gln Gly Thr Ser Lys Pro Ser Val Arg Thr
 180 185 190
 Arg Ala Val Arg Ser Leu Ala Val Ala Glu Pro Val Val Asn Ala Ala
 195 200 205
 Asp Ala Lys Gly Thr Asn Val Asn Asp Lys Val Thr Ala Ser Asn Phe
 210 215 220
 Lys Leu Glu Lys Thr Thr Phe Asp Pro Asn Gln Ser Gly Asn Thr Phe
 225 230 235 240
 Met Ala Ala Asn Phe Thr Val Thr Asp Lys Val Lys Ser Gly Asp Tyr
 245 250 255

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Asp Ser Glu Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
   675                               680                               685

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
   690                               695                               700

Asp Ser Asp Ser Asp Ser Asp Ser Glu Ser Asp Ser Asp Ser Asp Ser
   705                               710                               715                               720

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
   725                               730                               735

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
   740                               745                               750

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
   755                               760                               765

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
   770                               775                               780

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
   785                               790                               795                               800

Asp Ser Asp Ser Arg Val Thr Pro Pro Asn Asn Glu Gln Lys Ala Pro
   805                               810                               815

Ser Asn Pro Lys Gly Glu Val Asn His Ser Asn Lys Val Ser Lys Gln
   820                               825                               830

His Lys Thr Asp Ala Leu Pro Glu Thr Gly Asp Lys Ser Glu Asn Thr
   835                               840                               845

Asn Ala Thr Leu Phe Gly Ala Met Met Ala Leu Leu Gly Ser Leu Leu
   850                               855                               860

Leu Phe Arg Lys Arg Lys Gln Asp His Lys Glu Lys Ala
   865                               870                               875

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<210> SEQ ID NO 19

<211> LENGTH: 227

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 19

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Met Lys Asn Ile Leu Lys Val Phe Asn Thr Thr Ile Leu Ala Leu Ile
  1      5      10      15

Ile Ile Ile Ala Thr Phe Ser Asn Ser Ala Asn Ala Ala Asp Ser Gly
  20      25      30

Thr Leu Asn Tyr Glu Val Tyr Lys Tyr Asn Thr Asn Asp Thr Ser Ile
  35      40      45

Ala Asn Asp Tyr Phe Asn Lys Pro Ala Lys Tyr Ile Lys Lys Asn Gly
  50      55      60

Lys Leu Tyr Val Gln Ile Thr Val Asn His Ser His Trp Ile Thr Gly
  65      70      75      80

Met Ser Ile Glu Gly His Lys Glu Asn Ile Ile Ser Lys Asn Thr Ala
  85      90      95

Lys Asp Glu Arg Thr Ser Glu Phe Glu Val Ser Lys Leu Asn Gly Lys
  100     105     110

Ile Asp Gly Lys Ile Asp Val Tyr Ile Asp Glu Lys Val Asn Gly Lys
  115     120     125

Pro Phe Lys Tyr Asp His His Tyr Asn Ile Thr Tyr Lys Phe Asn Gly
  130     135     140

Pro Thr Asp Val Ala Gly Ala Asn Ala Pro Gly Lys Asp Asp Lys Asn
  145     150     155     160

Ser Ala Ser Gly Ser Asp Lys Gly Ser Asp Gly Thr Thr Thr Gly Gln
  165     170     175

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Ser Glu Ser Asn Ser Ser Asn Lys Asp Lys Val Glu Asn Pro Gln Thr
 180 185 190
 Asn Ala Gly Thr Pro Ala Tyr Ile Tyr Ala Ile Pro Val Ala Ser Leu
 195 200 205
 Ala Leu Leu Ile Ala Ile Thr Leu Phe Val Arg Lys Lys Ser Lys Gly
 210 215 220
 Asn Val Glu
 225

<210> SEQ ID NO 20
 <211> LENGTH: 635
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 20

Met Ala Lys Tyr Arg Gly Lys Pro Phe Gln Leu Tyr Val Lys Leu Ser
 1 5 10 15
 Cys Ser Thr Met Met Ala Ser Ser Ile Ile Leu Thr Asn Ile Leu Pro
 20 25 30
 Tyr Asp Ala Gln Ala Ala Ser Glu Lys Asp Thr Glu Ile Ser Lys Glu
 35 40 45
 Ile Leu Ser Lys Gln Asp Leu Leu Asp Lys Val Asp Lys Ala Ile Arg
 50 55 60
 Gln Ile Glu Gln Leu Lys Gln Leu Ser Ala Ser Ser Lys Ala His Tyr
 65 70 75 80
 Lys Ala Gln Leu Asn Glu Ala Lys Thr Ala Ser Gln Ile Asp Glu Ile
 85 90 95
 Ile Lys Arg Ala Asn Glu Leu Asp Ser Lys Glu Asn Lys Ser Ser His
 100 105 110
 Thr Glu Met Asn Gly Gln Ser Asp Ile Asp Ser Lys Leu Asp Gln Leu
 115 120 125
 Leu Lys Asp Leu Asn Glu Val Ser Ser Asn Val Asp Arg Gly Gln Gln
 130 135 140
 Ser Gly Glu Asp Asp Leu Asn Ala Met Lys Asn Asp Met Ser Gln Thr
 145 150 155 160
 Ala Thr Thr Lys Tyr Gly Glu Lys Asp Asp Lys Asn Asp Glu Ala Met
 165 170 175
 Val Asn Lys Ala Leu Glu Asp Leu Asp His Leu Asn Gln Gln Ile His
 180 185 190
 Lys Ser Lys Asp Ala Leu Lys Asp Ala Ser Lys Asp Pro Ala Val Ser
 195 200 205
 Thr Thr Asp Ser Asn His Glu Val Ala Lys Thr Pro Asn Asn Asp Gly
 210 215 220
 Ser Gly His Val Val Leu Asn Lys Phe Leu Ser Asn Glu Glu Asn Gln
 225 230 235 240
 Ser His Ser Asn Gln Leu Thr Asp Lys Leu Gln Gly Ser Asp Lys Ile
 245 250 255
 Asn His Ala Met Ile Glu Lys Leu Ala Lys Ser Asn Ala Ser Thr Gln
 260 265 270
 His Tyr Thr Tyr His Lys Leu Asn Thr Leu Gln Ser Leu Asp Gln Arg
 275 280 285
 Ile Ala Asn Thr Gln Leu Pro Lys Asn Gln Lys Ser Asp Leu Met Ser
 290 295 300
 Glu Val Asn Lys Thr Lys Glu Arg Ile Lys Ser Gln Arg Asn Ile Ile

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Lys Ala Ala Glu His Thr Asn Gly Glu Leu Asn Gln Ser Lys Asn Glu
 50 55 60
 Thr Thr Ala Pro Ser Glu Asn Lys Thr Thr Glu Lys Val Asp Ser Arg
 65 70 75 80
 Gln Leu Lys Asp Asn Thr Gln Thr Ala Thr Ala Asp Gln Pro Lys Val
 85 90 95
 Thr Met Ser Asp Ser Ala Thr Val Lys Glu Thr Ser Ser Asn Met Gln
 100 105 110
 Ser Pro Gln Asn Ala Thr Ala Ser Gln Ser Thr Thr Gln Thr Ser Asn
 115 120 125
 Val Thr Thr Asn Asp Lys Ser Ser Thr Thr Tyr Ser Asn Glu Thr Asp
 130 135 140
 Lys Ser Asn Leu Thr Gln Ala Lys Asn Val Ser Thr Thr Pro Lys Thr
 145 150 155 160
 Thr Thr Ile Lys Gln Arg Ala Leu Asn Arg Met Ala Val Asn Thr Val
 165 170 175
 Ala Ala Pro Gln Gln Gly Thr Asn Val Asn Asp Lys Val His Phe Thr
 180 185 190
 Asn Ile Asp Ile Ala Ile Asp Lys Gly His Val Asn Lys Thr Thr Gly
 195 200 205
 Asn Thr Glu Phe Trp Ala Thr Ser Ser Asp Val Leu Lys Leu Lys Ala
 210 215 220
 Asn Tyr Thr Ile Asp Asp Ser Val Lys Glu Gly Asp Thr Phe Thr Phe
 225 230 235 240
 Lys Tyr Gly Gln Tyr Phe Arg Pro Gly Ser Val Arg Leu Pro Ser Gln
 245 250 255
 Thr Gln Asn Leu Tyr Asn Ala Gln Gly Asn Ile Ile Ala Lys Gly Ile
 260 265 270
 Tyr Asp Ser Lys Thr Asn Thr Thr Thr Tyr Thr Phe Thr Asn Tyr Val
 275 280 285
 Asp Gln Tyr Thr Asn Val Ser Gly Ser Phe Glu Gln Val Ala Phe Ala
 290 295 300
 Lys Arg Glu Asn Ala Thr Thr Asp Lys Thr Ala Tyr Lys Met Glu Val
 305 310 315 320
 Thr Leu Gly Asn Asp Thr Tyr Ser Lys Asp Val Ile Val Asp Tyr Gly
 325 330 335
 Asn Gln Lys Gly Gln Gln Leu Ile Ser Ser Thr Asn Tyr Ile Asn Asn
 340 345 350
 Glu Asp Leu Ser Arg Asn Met Thr Val Tyr Val Asn Gln Pro Lys Lys
 355 360 365
 Thr Tyr Thr Lys Glu Thr Phe Val Thr Asn Leu Thr Gly Tyr Lys Phe
 370 375 380
 Asn Pro Asp Ala Lys Asn Phe Lys Ile Tyr Glu Val Thr Asp Gln Asn
 385 390 395 400
 Gln Phe Val Asp Ser Phe Thr Pro Asp Thr Ser Lys Leu Lys Asp Val
 405 410 415
 Thr Gly Gln Phe Asp Val Ile Tyr Ser Asn Asp Asn Lys Thr Ala Thr
 420 425 430
 Val Asp Leu Leu Asn Gly Gln Ser Ser Asp Lys Gln Tyr Ile Ile
 435 440 445
 Gln Gln Val Ala Tyr Pro Asp Asn Ser Ser Thr Asp Asn Gly Lys Ile
 450 455 460
 Asp Tyr Thr Leu Glu Thr Gln Asn Gly Lys Ser Ser Trp Ser Asn Ser

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His Thr Pro Thr Lys Pro Met Ser Thr Val Lys Asp Gln His Lys Thr
 900 905 910

Ala Lys Ala Leu Pro Glu Thr Gly Ser Glu Asn Asn Asn Ser Asn Asn
 915 920 925

Gly Thr Leu Phe Gly Gly Leu Phe Ala Ala Leu Gly Ser Leu Leu Leu
 930 935 940

Phe Gly Arg Arg Lys Lys Gln Asn Lys
 945 950

<210> SEQ ID NO 22
 <211> LENGTH: 989
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 22

Met Asn Met Lys Lys Lys Glu Lys His Ala Ile Arg Lys Lys Ser Ile
 1 5 10 15

Gly Val Ala Ser Val Leu Val Gly Thr Leu Ile Gly Phe Gly Leu Leu
 20 25 30

Ser Ser Lys Glu Ala Asp Ala Ser Glu Asn Ser Val Thr Gln Ser Asp
 35 40 45

Ser Ala Ser Asn Glu Ser Lys Ser Asn Asp Ser Ser Ser Val Ser Ala
 50 55 60

Ala Pro Lys Thr Asp Asp Thr Asn Val Ser Asp Thr Lys Thr Ser Ser
 65 70 75 80

Asn Thr Asn Asn Gly Glu Thr Ser Val Ala Gln Asn Pro Ala Gln Gln
 85 90 95

Glu Thr Thr Gln Ser Ser Ser Thr Asn Ala Thr Thr Glu Glu Thr Pro
 100 105 110

Val Thr Gly Glu Ala Thr Thr Thr Thr Thr Asn Gln Ala Asn Thr Pro
 115 120 125

Ala Thr Thr Gln Ser Ser Asn Thr Asn Ala Glu Glu Leu Val Asn Gln
 130 135 140

Thr Ser Asn Glu Thr Thr Ser Asn Asp Thr Asn Thr Val Ser Ser Val
 145 150 155 160

Asn Ser Pro Gln Asn Ser Thr Asn Ala Glu Asn Val Ser Thr Thr Gln
 165 170 175

Asp Thr Ser Thr Glu Ala Thr Pro Ser Asn Asn Glu Ser Ala Pro Gln
 180 185 190

Asn Thr Asp Ala Ser Asn Lys Asp Val Val Ser Gln Ala Val Asn Pro
 195 200 205

Ser Thr Pro Arg Met Arg Ala Phe Ser Leu Ala Ala Val Ala Ala Asp
 210 215 220

Ala Pro Ala Ala Gly Thr Asp Ile Thr Asn Gln Leu Thr Asp Val Lys
 225 230 235 240

Val Thr Ile Asp Ser Gly Thr Thr Val Tyr Pro His Gln Ala Gly Tyr
 245 250 255

Val Lys Leu Asn Tyr Gly Phe Ser Val Pro Asn Ser Ala Val Lys Gly
 260 265 270

Asp Thr Phe Lys Ile Thr Val Pro Lys Glu Leu Asn Leu Asn Gly Val
 275 280 285

Thr Ser Thr Ala Lys Val Pro Pro Ile Met Ala Gly Asp Gln Val Leu
 290 295 300

Ala Asn Gly Val Ile Asp Ser Asp Gly Asn Val Ile Tyr Thr Phe Thr

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Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 740 745 750
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 755 760 765
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 770 775 780
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 785 790 795 800
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 805 810 815
 Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Glu
 820 825 830
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 835 840 845
 Ser Asp Ser Asp Ser Asp Ser Glu Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 850 855 860
 Ser Asp Ser Glu Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 865 870 875 880
 Ser Ala Ser Asp Ser Asp Ser Gly Ser Asp Ser Asp Ser Ser Ser Asp
 885 890 895
 Ser Asp Ser Asp Ser Thr Ser Asp Thr Gly Ser Asp Asn Asp Ser Asp
 900 905 910
 Ser Asp Ser Asn Ser Asp Ser Glu Ser Gly Ser Asn Asn Asn Val Val
 915 920 925
 Pro Pro Asn Ser Pro Lys Asn Gly Thr Asn Ala Ser Asn Lys Asn Glu
 930 935 940
 Ala Lys Asp Ser Lys Glu Pro Leu Pro Asp Thr Gly Ser Glu Asp Glu
 945 950 955 960
 Ala Asn Thr Ser Leu Ile Trp Gly Leu Leu Ala Ser Leu Gly Ser Leu
 965 970 975
 Leu Leu Phe Arg Arg Lys Lys Glu Asn Lys Asp Lys Lys
 980 985

<210> SEQ ID NO 23
 <211> LENGTH: 584
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 23

Met Lys Phe Lys Ser Leu Ile Thr Thr Thr Leu Ala Leu Gly Val Leu
 1 5 10 15
 Ala Ser Thr Gly Ala Asn Phe Asn Asn Asn Glu Ala Ser Ala Ala Ala
 20 25 30
 Lys Pro Leu Asp Lys Ser Ser Ser Ser Leu His His Gly Tyr Ser Lys
 35 40 45
 Val His Val Pro Tyr Ala Ile Thr Val Asn Gly Thr Ser Gln Asn Ile
 50 55 60
 Leu Ser Ser Leu Thr Phe Asn Lys Asn Gln Asn Ile Ser Tyr Lys Asp
 65 70 75 80
 Leu Glu Asp Arg Val Lys Ser Val Leu Lys Ser Asp Arg Gly Ile Ser
 85 90 95
 Asp Ile Asp Leu Arg Leu Ser Lys Gln Ala Lys Tyr Thr Val Tyr Phe
 100 105 110
 Lys Asn Gly Thr Lys Lys Val Ile Asp Leu Lys Ala Gly Ile Tyr Thr

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Gly Lys Lys Gln Val Val Asn Leu Lys Ser Asp Ile Phe Thr Pro Asn
 545 550 555 560

Leu Phe Ser Ala Lys Asp Ile Lys Lys Ile Asp Ile Asp Val Lys Gln
 565 570 575

Tyr Thr Lys Ser Lys Lys Asn Lys
 580

<210> SEQ ID NO 24
 <211> LENGTH: 10419
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 24

Met Asn Tyr Arg Asp Lys Ile Gln Lys Phe Ser Ile Arg Lys Tyr Thr
 1 5 10 15

Val Gly Thr Phe Ser Thr Val Ile Ala Thr Leu Val Phe Leu Gly Phe
 20 25 30

Asn Thr Ser Gln Ala His Ala Ala Glu Thr Asn Gln Pro Ala Ser Val
 35 40 45

Val Lys Gln Lys Gln Gln Ser Asn Asn Glu Gln Thr Glu Asn Arg Glu
 50 55 60

Ser Gln Val Gln Asn Ser Gln Asn Ser Gln Asn Gly Gln Ser Leu Ser
 65 70 75 80

Ala Thr His Glu Asn Glu Gln Pro Asn Ile Ser Gln Ala Asn Leu Val
 85 90 95

Asp Gln Lys Val Ala Gln Ser Ser Thr Thr Asn Asp Glu Gln Pro Ala
 100 105 110

Ser Gln Asn Val Asn Thr Lys Lys Asp Ser Ala Thr Ala Ala Thr Thr
 115 120 125

Gln Pro Asp Lys Glu Gln Ser Lys His Lys Gln Asn Glu Ser Gln Ser
 130 135 140

Ala Asn Lys Asn Gly Asn Asp Asn Arg Ala Ala His Val Glu Asn His
 145 150 155 160

Glu Ala Asn Val Val Thr Ala Ser Asp Ser Ser Asp Asn Gly Asn Val
 165 170 175

Gln His Asp Arg Asn Glu Leu Gln Ala Phe Phe Asp Ala Asn Tyr His
 180 185 190

Asp Tyr Arg Phe Ile Asp Arg Glu Asn Ala Asp Ser Gly Thr Phe Asn
 195 200 205

Tyr Val Lys Gly Ile Phe Asp Lys Ile Asn Thr Leu Leu Gly Ser Asn
 210 215 220

Asp Pro Ile Asn Asn Lys Asp Leu Gln Leu Ala Tyr Lys Glu Leu Glu
 225 230 235 240

Gln Ala Val Ala Leu Ile Arg Thr Met Pro Gln Arg Gln Gln Thr Ser
 245 250 255

Arg Arg Ser Asn Arg Ile Gln Thr Arg Ser Val Glu Ser Arg Ala Ala
 260 265 270

Glu Pro Arg Ser Val Ser Asp Tyr Gln Asn Ala Asn Ser Ser Tyr Tyr
 275 280 285

Val Glu Asn Ala Asn Asp Gly Ser Gly Tyr Pro Val Gly Thr Tyr Ile
 290 295 300

Asn Ala Ser Ser Lys Gly Ala Pro Tyr Asn Leu Pro Thr Thr Pro Trp
 305 310 315 320

Asn Thr Leu Lys Ala Ser Asp Ser Lys Glu Ile Ala Leu Met Thr Ala

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Val	Ser	Gln	Met	Asn	Ala	Gly	Thr	Gln	Leu	Tyr	His	Ile	Thr	Ala
1160						1165					1170			
Thr	Asn	Ala	Tyr	His	Lys	Asp	Ser	Glu	Asp	Phe	Tyr	Ile	Ser	Leu
1175						1180					1185			
Lys	Ile	Ile	Asp	Val	Lys	Gln	Pro	Glu	Gly	Asp	Gln	Arg	Val	Tyr
1190						1195					1200			
Arg	Thr	Ser	Thr	Tyr	Asp	Leu	Thr	Thr	Asp	Glu	Ile	Ser	Lys	Val
1205						1210					1215			
Lys	Gln	Ala	Phe	Ile	Asn	Ala	Asn	Arg	Asp	Val	Ile	Thr	Leu	Ala
1220						1225					1230			
Glu	Gly	Asp	Ile	Ser	Val	Thr	Asn	Thr	Pro	Asn	Gly	Ala	Asn	Val
1235						1240					1245			
Ser	Thr	Ile	Thr	Val	Asn	Ile	Asn	Lys	Gly	Arg	Leu	Thr	Lys	Ser
1250						1255					1260			
Phe	Ala	Ser	Asn	Leu	Ala	Asn	Met	Asn	Phe	Leu	Arg	Trp	Val	Asn
1265						1270					1275			
Phe	Pro	Gln	Asp	Tyr	Thr	Val	Thr	Trp	Thr	Asn	Ala	Lys	Ile	Ala
1280						1285					1290			
Asn	Arg	Pro	Thr	Asp	Gly	Gly	Leu	Ser	Trp	Ser	Asp	Asp	His	Lys
1295						1300					1305			
Ser	Leu	Ile	Tyr	Arg	Tyr	Asp	Ala	Thr	Leu	Gly	Thr	Gln	Ile	Thr
1310						1315					1320			
Thr	Asn	Asp	Ile	Leu	Thr	Met	Leu	Lys	Ala	Thr	Thr	Thr	Val	Pro
1325						1330					1335			
Gly	Leu	Arg	Asn	Asn	Ile	Thr	Gly	Asn	Glu	Lys	Ser	Gln	Ala	Glu
1340						1345					1350			
Ala	Gly	Gly	Arg	Pro	Asn	Phe	Arg	Thr	Thr	Gly	Tyr	Ser	Gln	Ser
1355						1360					1365			
Asn	Ala	Thr	Thr	Asp	Gly	Gln	Arg	Gln	Phe	Thr	Leu	Asn	Gly	Gln
1370						1375					1380			
Val	Ile	Gln	Val	Leu	Asp	Ile	Ile	Asn	Pro	Ser	Asn	Gly	Tyr	Gly
1385						1390					1395			
Gly	Gln	Pro	Val	Thr	Asn	Ser	Asn	Thr	Arg	Ala	Asn	His	Ser	Asn
1400						1405					1410			
Ser	Thr	Val	Val	Asn	Val	Asn	Glu	Pro	Ala	Ala	Asn	Gly	Ala	Gly
1415						1420					1425			
Ala	Phe	Thr	Ile	Asp	His	Val	Val	Lys	Ser	Asn	Ser	Thr	His	Asn
1430						1435					1440			
Ala	Ser	Asp	Ala	Val	Tyr	Lys	Ala	Gln	Leu	Tyr	Leu	Thr	Pro	Tyr
1445						1450					1455			
Gly	Pro	Lys	Gln	Tyr	Val	Glu	His	Leu	Asn	Gln	Asn	Thr	Gly	Asn
1460						1465					1470			
Thr	Thr	Asp	Ala	Ile	Asn	Ile	Tyr	Phe	Val	Pro	Ser	Asp	Leu	Val
1475						1480					1485			
Asn	Pro	Thr	Ile	Ser	Val	Gly	Asn	Tyr	Thr	Asn	His	Gln	Val	Phe
1490						1495					1500			
Ser	Gly	Glu	Thr	Phe	Thr	Asn	Thr	Ile	Thr	Ala	Asn	Asp	Asn	Phe
1505						1510					1515			
Gly	Val	Gln	Ser	Val	Thr	Val	Pro	Asn	Thr	Ser	Gln	Ile	Thr	Gly
1520						1525					1530			
Thr	Val	Asp	Asn	Asn	His	Gln	His	Val	Ser	Ala	Thr	Ala	Pro	Asn
1535						1540					1545			
Val	Thr	Ser	Ala	Thr	Asn	Lys	Thr	Ile	Asn	Leu	Leu	Ala	Thr	Asp

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1550	1555	1560
Thr Ser Gly Asn Thr Ala Thr	Thr Ser Phe Asn Val	Thr Val Lys
1565	1570	1575
Pro Leu Arg Asp Lys Tyr Arg	Val Gly Thr Ser Ser	Thr Ala Ala
1580	1585	1590
Asn Pro Val Arg Ile Ala Asn	Ile Ser Asn Asn Ala	Thr Val Ser
1595	1600	1605
Gln Ala Asp Gln Thr Thr Ile	Ile Asn Ser Leu Thr	Phe Thr Glu
1610	1615	1620
Thr Val Pro Asn Arg Ser Tyr	Ala Arg Ala Ser Ala	Asn Glu Ile
1625	1630	1635
Thr Ser Lys Thr Val Ser Asn	Val Ser Arg Thr Gly	Asn Asn Ala
1640	1645	1650
Asn Val Thr Val Thr Val Thr	Tyr Gln Asp Gly Thr	Thr Ser Thr
1655	1660	1665
Val Thr Val Pro Val Lys His	Val Ile Pro Glu Ile	Val Ala His
1670	1675	1680
Ser His Tyr Thr Val Gln Gly	Gln Asp Phe Pro Ala	Gly Asn Gly
1685	1690	1695
Ser Ser Ala Ser Asp Tyr Phe	Lys Leu Ser Asn Gly	Ser Asp Ile
1700	1705	1710
Ala Asp Ala Thr Ile Thr Trp	Val Ser Gly Gln Ala	Pro Asn Lys
1715	1720	1725
Asp Asn Thr Arg Ile Gly Glu	Asp Ile Thr Val Thr	Ala His Ile
1730	1735	1740
Leu Ile Asp Gly Glu Thr Thr	Pro Ile Thr Lys Thr	Ala Thr Tyr
1745	1750	1755
Lys Val Val Arg Thr Val Pro	Lys His Val Phe Glu	Thr Ala Arg
1760	1765	1770
Gly Val Leu Tyr Pro Gly Val	Ser Asp Met Tyr Asp	Ala Lys Gln
1775	1780	1785
Tyr Val Lys Pro Val Asn Asn	Ser Trp Ser Thr Asn	Ala Gln His
1790	1795	1800
Met Asn Phe Gln Phe Val Gly	Thr Tyr Gly Pro Asn	Lys Asp Val
1805	1810	1815
Val Gly Ile Ser Thr Arg Leu	Ile Arg Val Thr Tyr	Asp Asn Arg
1820	1825	1830
Gln Thr Glu Asp Leu Thr Ile	Leu Ser Lys Val Lys	Pro Asp Pro
1835	1840	1845
Pro Arg Ile Asp Ala Asn Ser	Val Thr Tyr Lys Ala	Gly Leu Thr
1850	1855	1860
Asn Gln Glu Ile Lys Val Asn	Asn Val Leu Asn Asn	Ser Ser Val
1865	1870	1875
Lys Leu Phe Lys Ala Asp Asn	Thr Pro Leu Asn Val	Thr Asn Ile
1880	1885	1890
Thr His Gly Ser Gly Phe Ser	Ser Val Val Thr Val	Ser Asp Ala
1895	1900	1905
Leu Pro Asn Gly Gly Ile Lys	Ala Lys Ser Ser Ile	Ser Met Asn
1910	1915	1920
Asn Val Thr Tyr Thr Thr Gln	Asp Glu His Gly Gln	Val Val Thr
1925	1930	1935
Val Thr Arg Asn Glu Ser Val	Asp Ser Asn Asp Ser	Ala Thr Val
1940	1945	1950

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Thr	Val	Thr	Pro	Gln	Leu	Gln	Ala	Thr	Thr	Glu	Gly	Ala	Val	Phe
1955						1960					1965			
Ile	Lys	Gly	Gly	Asp	Gly	Phe	Asp	Phe	Gly	His	Val	Glu	Arg	Phe
1970						1975					1980			
Ile	Gln	Asn	Pro	Pro	His	Gly	Ala	Thr	Val	Ala	Trp	His	Asp	Ser
1985						1990					1995			
Pro	Asp	Thr	Trp	Lys	Asn	Thr	Val	Gly	Asn	Thr	His	Lys	Thr	Ala
2000						2005					2010			
Val	Val	Thr	Leu	Pro	Asn	Gly	Gln	Gly	Thr	Arg	Asn	Val	Glu	Val
2015						2020					2025			
Pro	Val	Lys	Val	Tyr	Pro	Val	Ala	Asn	Ala	Lys	Ala	Pro	Ser	Arg
2030						2035					2040			
Asp	Val	Lys	Gly	Gln	Asn	Leu	Thr	Asn	Gly	Thr	Asp	Ala	Met	Asn
2045						2050					2055			
Tyr	Ile	Thr	Phe	Asp	Pro	Asn	Thr	Asn	Thr	Asn	Gly	Ile	Thr	Ala
2060						2065					2070			
Ala	Trp	Ala	Asn	Arg	Gln	Gln	Pro	Asn	Asn	Gln	Gln	Ala	Gly	Val
2075						2080					2085			
Gln	His	Leu	Asn	Val	Asp	Val	Thr	Tyr	Pro	Gly	Ile	Ser	Ala	Ala
2090						2095					2100			
Lys	Arg	Val	Pro	Val	Thr	Val	Asn	Val	Tyr	Gln	Phe	Glu	Phe	Pro
2105						2110					2115			
Gln	Thr	Thr	Tyr	Thr	Thr	Thr	Val	Gly	Gly	Thr	Leu	Ala	Ser	Gly
2120						2125					2130			
Thr	Gln	Ala	Ser	Gly	Tyr	Ala	His	Met	Gln	Asn	Ala	Thr	Gly	Leu
2135						2140					2145			
Pro	Thr	Asp	Gly	Phe	Thr	Tyr	Lys	Trp	Asn	Arg	Asp	Thr	Thr	Gly
2150						2155					2160			
Thr	Asn	Asp	Ala	Asn	Trp	Ser	Ala	Met	Asn	Lys	Pro	Asn	Val	Ala
2165						2170					2175			
Lys	Val	Val	Asn	Ala	Lys	Tyr	Asp	Val	Ile	Tyr	Asn	Gly	His	Thr
2180						2185					2190			
Phe	Ala	Thr	Ser	Leu	Pro	Ala	Lys	Phe	Val	Val	Lys	Asp	Val	Gln
2195						2200					2205			
Pro	Ala	Lys	Pro	Thr	Val	Thr	Glu	Thr	Ala	Ala	Gly	Ala	Ile	Thr
2210						2215					2220			
Ile	Ala	Pro	Gly	Ala	Asn	Gln	Thr	Val	Asn	Thr	His	Ala	Gly	Asn
2225						2230					2235			
Val	Thr	Thr	Tyr	Ala	Asp	Lys	Leu	Val	Ile	Lys	Arg	Asn	Gly	Asn
2240						2245					2250			
Val	Val	Thr	Thr	Phe	Thr	Arg	Arg	Asn	Asn	Thr	Ser	Pro	Trp	Val
2255						2260					2265			
Lys	Glu	Ala	Ser	Ala	Ala	Thr	Val	Ala	Gly	Ile	Ala	Gly	Thr	Asn
2270						2275					2280			
Asn	Gly	Ile	Thr	Val	Ala	Ala	Gly	Thr	Phe	Asn	Pro	Ala	Asp	Thr
2285						2290					2295			
Ile	Gln	Val	Val	Ala	Thr	Gln	Gly	Ser	Gly	Glu	Thr	Val	Ser	Asp
2300						2305					2310			
Glu	Gln	Arg	Ser	Asp	Asp	Phe	Thr	Val	Val	Ala	Pro	Gln	Pro	Asn
2315						2320					2325			
Gln	Ala	Thr	Thr	Lys	Ile	Trp	Gln	Asn	Gly	His	Ile	Asp	Ile	Thr
2330						2335					2340			

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Pro	Asn	Asn	Pro	Ser	Gly	His	Leu	Ile	Asn	Pro	Thr	Gln	Ala	Met
2345						2350					2355			
Asp	Ile	Ala	Tyr	Thr	Glu	Lys	Val	Gly	Asn	Gly	Ala	Glu	His	Ser
2360						2365					2370			
Lys	Thr	Ile	Asn	Val	Val	Arg	Gly	Gln	Asn	Asn	Gln	Trp	Thr	Ile
2375						2380					2385			
Ala	Asn	Lys	Pro	Asp	Tyr	Val	Thr	Leu	Asp	Ala	Gln	Thr	Gly	Lys
2390						2395					2400			
Val	Thr	Phe	Asn	Ala	Asn	Thr	Ile	Lys	Pro	Asn	Ser	Ser	Ile	Thr
2405						2410					2415			
Ile	Thr	Pro	Lys	Ala	Gly	Thr	Gly	His	Ser	Val	Ser	Ser	Asn	Pro
2420						2425					2430			
Ser	Thr	Leu	Thr	Ala	Pro	Ala	Ala	His	Thr	Val	Asn	Thr	Thr	Glu
2435						2440					2445			
Ile	Val	Lys	Asp	Tyr	Gly	Ser	Asn	Val	Thr	Ala	Ala	Glu	Ile	Asn
2450						2455					2460			
Asn	Ala	Val	Gln	Val	Ala	Asn	Lys	Arg	Thr	Ala	Thr	Ile	Lys	Asn
2465						2470					2475			
Gly	Thr	Ala	Met	Pro	Thr	Asn	Leu	Ala	Gly	Gly	Ser	Thr	Thr	Thr
2480						2485					2490			
Ile	Pro	Val	Thr	Val	Thr	Tyr	Asn	Asp	Gly	Ser	Thr	Glu	Glu	Val
2495						2500					2505			
Gln	Glu	Ser	Ile	Phe	Thr	Lys	Ala	Asp	Lys	Arg	Glu	Leu	Ile	Thr
2510						2515					2520			
Ala	Lys	Asn	His	Leu	Asp	Asp	Pro	Val	Ser	Thr	Glu	Gly	Lys	Lys
2525						2530					2535			
Pro	Gly	Thr	Ile	Thr	Gln	Tyr	Asn	Asn	Ala	Met	His	Asn	Ala	Gln
2540						2545					2550			
Gln	Gln	Ile	Asn	Thr	Ala	Lys	Thr	Glu	Ala	Gln	Gln	Val	Ile	Asn
2555						2560					2565			
Asn	Glu	Arg	Ala	Thr	Pro	Gln	Gln	Val	Ser	Asp	Ala	Leu	Thr	Lys
2570						2575					2580			
Val	Arg	Ala	Ala	Gln	Thr	Lys	Ile	Asp	Gln	Ala	Lys	Ala	Leu	Leu
2585						2590					2595			
Gln	Asn	Lys	Glu	Asp	Asn	Ser	Gln	Leu	Val	Thr	Ser	Lys	Asn	Asn
2600						2605					2610			
Leu	Gln	Ser	Ser	Val	Asn	Gln	Val	Pro	Ser	Thr	Ala	Gly	Met	Thr
2615						2620					2625			
Gln	Gln	Ser	Ile	Asp	Asn	Tyr	Asn	Ala	Lys	Lys	Arg	Glu	Ala	Glu
2630						2635					2640			
Thr	Glu	Ile	Thr	Ala	Ala	Gln	Arg	Val	Ile	Asp	Asn	Gly	Asp	Ala
2645						2650					2655			
Thr	Ala	Gln	Gln	Ile	Ser	Asp	Glu	Lys	His	Arg	Val	Asp	Asn	Ala
2660						2665					2670			
Leu	Thr	Ala	Leu	Asn	Gln	Ala	Lys	His	Asp	Leu	Thr	Ala	Asp	Thr
2675						2680					2685			
His	Ala	Leu	Glu	Gln	Ala	Val	Gln	Gln	Leu	Asn	Arg	Thr	Gly	Thr
2690						2695					2700			
Thr	Thr	Gly	Lys	Lys	Pro	Ala	Ser	Ile	Thr	Ala	Tyr	Asn	Asn	Ser
2705						2710					2715			
Ile	Arg	Ala	Leu	Gln	Ser	Asp	Leu	Thr	Ser	Ala	Lys	Asn	Ser	Ala
2720						2725					2730			
Asn	Ala	Ile	Ile	Gln	Lys	Pro	Ile	Arg	Thr	Val	Gln	Glu	Val	Gln

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2735	2740	2745
Ser Ala Leu Thr Asn Val	Asn Arg Val Asn Glu	Arg Leu Thr Gln
2750	2755	2760
Ala Ile Asn Gln Leu Val	Pro Leu Ala Asp Asn	Ser Ala Leu Lys
2765	2770	2775
Thr Ala Lys Thr Lys Leu	Asp Glu Glu Ile Asn	Lys Ser Val Thr
2780	2785	2790
Thr Asp Gly Met Thr Gln	Ser Ser Ile Gln Ala	Tyr Glu Asn Ala
2795	2800	2805
Lys Arg Ala Gly Gln Thr	Glu Ser Thr Asn Ala	Gln Asn Val Ile
2810	2815	2820
Asn Asn Gly Asp Ala Thr	Asp Gln Gln Ile Ala	Ala Glu Lys Thr
2825	2830	2835
Lys Val Glu Glu Lys Tyr	Asn Ser Leu Lys Gln	Ala Ile Ala Gly
2840	2845	2850
Leu Thr Pro Asp Leu Ala	Pro Leu Gln Thr Ala	Lys Thr Gln Leu
2855	2860	2865
Gln Asn Asp Ile Asp Gln	Pro Thr Ser Thr Thr	Gly Met Thr Ser
2870	2875	2880
Ala Ser Ile Ala Ala Phe	Asn Glu Lys Leu Ser	Ala Ala Arg Thr
2885	2890	2895
Lys Ile Gln Glu Ile Asp	Arg Val Leu Ala Ser	His Pro Asp Val
2900	2905	2910
Ala Thr Ile Arg Gln Asn	Val Thr Ala Ala Asn	Ala Ala Lys Ser
2915	2920	2925
Ala Leu Asp Gln Ala Arg	Asn Gly Leu Thr Val	Asp Lys Ala Pro
2930	2935	2940
Leu Glu Asn Ala Lys Asn	Gln Leu Gln His Ser	Ile Asp Thr Gln
2945	2950	2955
Thr Ser Thr Thr Gly Met	Thr Gln Asp Ser Ile	Asn Ala Tyr Asn
2960	2965	2970
Ala Lys Leu Thr Ala Ala	Arg Asn Lys Ile Gln	Gln Ile Asn Gln
2975	2980	2985
Val Leu Ala Gly Ser Pro	Thr Val Glu Gln Ile	Asn Thr Asn Thr
2990	2995	3000
Ser Thr Ala Asn Gln Ala	Lys Ser Asp Leu Asp	His Ala Arg Gln
3005	3010	3015
Ala Leu Thr Pro Asp Lys	Ala Pro Leu Gln Thr	Ala Lys Thr Gln
3020	3025	3030
Leu Glu Gln Ser Ile Asn	Gln Pro Thr Asp Thr	Thr Thr Gly Met Thr
3035	3040	3045
Thr Ala Ser Leu Asn Ala	Tyr Asn Gln Lys Leu	Gln Ala Ala Arg
3050	3055	3060
Gln Lys Leu Thr Glu Ile	Asn Gln Val Leu Asn	Gly Asn Pro Thr
3065	3070	3075
Val Gln Asn Ile Asn Asp	Lys Val Thr Glu Ala	Asn Gln Ala Lys
3080	3085	3090
Asp Gln Leu Asn Thr Ala	Arg Gln Gly Leu Thr	Leu Asp Arg Gln
3095	3100	3105
Pro Ala Leu Thr Thr Leu	His Gly Ala Ser Asn	Leu Asn Gln Ala
3110	3115	3120
Gln Gln Asn Asn Phe Thr	Gln Gln Ile Asn Ala	Ala Gln Asn His
3125	3130	3135

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Ala Ala	Leu Glu Thr Ile Lys	Ser Asn Ile Thr	Ala Leu Asn Thr
3140	3145		3150
Ala Met	Thr Lys Leu Lys Asp	Ser Val Ala Asp	Asn Asn Thr Ile
3155	3160		3165
Lys Ser	Asp Gln Asn Tyr Thr	Asp Ala Thr Pro	Ala Asn Lys Gln
3170	3175		3180
Ala Tyr	Asp Asn Ala Val Asn	Ala Ala Lys Gly Val	Ile Gly Glu
3185	3190		3195
Thr Thr	Asn Pro Thr Met Asp	Val Asn Thr Val	Asn Gln Lys Ala
3200	3205		3210
Ala Ser	Val Lys Ser Thr Lys	Asp Ala Leu Asp	Gly Gln Gln Asn
3215	3220		3225
Leu Gln	Arg Ala Lys Thr Glu	Ala Thr Asn Ala	Ile Thr His Ala
3230	3235		3240
Ser Asp	Leu Asn Gln Ala Gln	Lys Asn Ala Leu	Thr Gln Gln Val
3245	3250		3255
Asn Ser	Ala Gln Asn Val Gln	Ala Val Asn Asp	Ile Lys Gln Thr
3260	3265		3270
Thr Gln	Ser Leu Asn Thr Ala	Met Thr Gly Leu	Lys Arg Gly Val
3275	3280		3285
Ala Asn	His Asn Gln Val Val	Gln Ser Asp Asn	Tyr Val Asn Ala
3290	3295		3300
Asp Thr	Asn Lys Lys Asn Asp	Tyr Asn Asn Ala	Tyr Asn His Ala
3305	3310		3315
Asn Asp	Ile Ile Asn Gly Asn	Ala Gln His Pro	Val Ile Thr Pro
3320	3325		3330
Ser Asp	Val Asn Asn Ala Leu	Ser Asn Val Thr	Ser Lys Glu His
3335	3340		3345
Ala Leu	Asn Gly Glu Ala Lys	Leu Asn Ala Ala	Lys Gln Glu Ala
3350	3355		3360
Asn Thr	Ala Leu Gly His Leu	Asn Asn Leu Asn	Asn Ala Gln Arg
3365	3370		3375
Gln Asn	Leu Gln Ser Gln Ile	Asn Gly Ala His	Gln Ile Asp Ala
3380	3385		3390
Val Asn	Thr Ile Lys Gln Asn	Ala Thr Asn Leu	Asn Ser Ala Met
3395	3400		3405
Gly Asn	Leu Arg Gln Ala Val	Ala Asp Lys Asp	Gln Val Lys Arg
3410	3415		3420
Thr Glu	Asp Tyr Ala Asp Ala	Asp Thr Ala Lys	Gln Asn Ala Tyr
3425	3430		3435
Asn Ser	Ala Val Ser Ser Ala	Glu Thr Ile Ile	Asn Gln Thr Thr
3440	3445		3450
Asn Pro	Thr Met Ser Val Asp	Asp Val Asn Arg	Ala Thr Ser Ala
3455	3460		3465
Val Thr	Ser Asn Lys Asn Ala	Leu Asn Gly Tyr	Glu Lys Leu Ala
3470	3475		3480
Gln Ser	Lys Thr Asp Ala Ala	Arg Ala Ile Asp	Ala Leu Pro His
3485	3490		3495
Leu Asn	Asn Ala Gln Lys Ala	Asp Val Lys Ser	Lys Ile Asn Ala
3500	3505		3510
Ala Ser	Asn Ile Ala Gly Val	Asn Thr Val Lys	Gln Gln Gly Thr
3515	3520		3525

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Asp	Leu	Asn	Thr	Ala	Met	Gly	Asn	Leu	Gln	Gly	Ala	Ile	Asn	Asp
3530						3535					3540			
Glu	Gln	Thr	Thr	Leu	Asn	Ser	Gln	Asn	Tyr	Gln	Asp	Ala	Thr	Pro
3545						3550					3555			
Ser	Lys	Lys	Thr	Ala	Tyr	Thr	Asn	Ala	Val	Gln	Ala	Ala	Lys	Asp
3560						3565					3570			
Ile	Leu	Asn	Lys	Ser	Asn	Gly	Gln	Asn	Lys	Thr	Lys	Asp	Gln	Val
3575						3580					3585			
Thr	Glu	Ala	Met	Asn	Gln	Val	Asn	Ser	Ala	Lys	Asn	Asn	Leu	Asp
3590						3595					3600			
Gly	Thr	Arg	Leu	Leu	Asp	Gln	Ala	Lys	Gln	Thr	Ala	Lys	Gln	Gln
3605						3610					3615			
Leu	Asn	Asn	Met	Thr	His	Leu	Thr	Thr	Ala	Gln	Lys	Thr	Asn	Leu
3620						3625					3630			
Thr	Asn	Gln	Ile	Asn	Ser	Gly	Thr	Thr	Val	Ala	Gly	Val	Gln	Thr
3635						3640					3645			
Val	Gln	Ser	Asn	Ala	Asn	Thr	Leu	Asp	Gln	Ala	Met	Asn	Thr	Leu
3650						3655					3660			
Arg	Gln	Ser	Ile	Ala	Asn	Lys	Asp	Ala	Thr	Lys	Ala	Ser	Glu	Asp
3665						3670					3675			
Tyr	Val	Asp	Ala	Asn	Asn	Asp	Lys	Gln	Thr	Ala	Tyr	Asn	Asn	Ala
3680						3685					3690			
Val	Ala	Ala	Ala	Glu	Thr	Ile	Ile	Asn	Ala	Asn	Ser	Asn	Pro	Glu
3695						3700					3705			
Met	Asn	Pro	Ser	Thr	Ile	Thr	Gln	Lys	Ala	Glu	Gln	Val	Asn	Ser
3710						3715					3720			
Ser	Lys	Thr	Ala	Leu	Asn	Gly	Asp	Glu	Asn	Leu	Ala	Ala	Ala	Lys
3725						3730					3735			
Gln	Asn	Ala	Lys	Thr	Tyr	Leu	Asn	Thr	Leu	Thr	Ser	Ile	Thr	Asp
3740						3745					3750			
Ala	Gln	Lys	Asn	Asn	Leu	Ile	Ser	Gln	Ile	Thr	Ser	Ala	Thr	Arg
3755						3760					3765			
Val	Ser	Gly	Val	Asp	Thr	Val	Lys	Gln	Asn	Ala	Gln	His	Leu	Asp
3770						3775					3780			
Gln	Ala	Met	Ala	Ser	Leu	Gln	Asn	Gly	Ile	Asn	Asn	Glu	Ser	Gln
3785						3790					3795			
Val	Lys	Ser	Ser	Glu	Lys	Tyr	Arg	Asp	Ala	Asp	Thr	Asn	Lys	Gln
3800						3805					3810			
Gln	Glu	Tyr	Asp	Asn	Ala	Ile	Thr	Ala	Ala	Lys	Ala	Ile	Leu	Asn
3815						3820					3825			
Lys	Ser	Thr	Gly	Pro	Asn	Thr	Ala	Gln	Asn	Ala	Val	Glu	Ala	Ala
3830						3835					3840			
Leu	Gln	Arg	Val	Asn	Asn	Ala	Lys	Asp	Ala	Leu	Asn	Gly	Asp	Ala
3845						3850					3855			
Lys	Leu	Ile	Ala	Ala	Gln	Asn	Ala	Ala	Lys	Gln	His	Leu	Gly	Thr
3860						3865					3870			
Leu	Thr	His	Ile	Thr	Thr	Ala	Gln	Arg	Asn	Asp	Leu	Thr	Asn	Gln
3875						3880					3885			
Ile	Ser	Gln	Ala	Thr	Asn	Leu	Ala	Gly	Val	Glu	Ser	Val	Lys	Gln
3890						3895					3900			
Asn	Ala	Asn	Ser	Leu	Asp	Gly	Ala	Met	Gly	Asn	Leu	Gln	Thr	Ala
3905						3910					3915			
Ile	Asn	Asp	Lys	Ser	Gly	Thr	Leu	Ala	Ser	Gln	Asn	Phe	Leu	Asp

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3920	3925	3930
Ala Asp Glu Gln Lys Arg Asn 3935	Ala Tyr Asn Gln Ala Val Ser Ala 3940	Ala Val Ser Ala 3945
Ala Glu Thr Ile Leu Asn Lys 3950	Gln Thr Gly Pro Asn Thr Ala Lys 3955	3960
Thr Ala Val Glu Gln Ala Leu 3965	Asn Asn Val Asn Asn Ala Lys His 3970	3975
Ala Leu Asn Gly Thr Gln Asn 3980	Leu Asn Asn Ala Lys Gln Ala Ala 3985	3990
Ile Thr Ala Ile Asn Gly Ala 3995	Ser Asp Leu Asn Gln Lys Gln Lys 4000	4005
Asp Ala Leu Lys Ala Gln Ala 4010	Asn Gly Ala Gln Arg Val Ser Asn 4015	4020
Ala Gln Asp Val Gln His Asn 4025	Ala Thr Glu Leu Asn Thr Ala Met 4030	4035
Gly Thr Leu Lys His Ala Ile 4040	Ala Asp Lys Thr Asn Thr Leu Ala 4045	4050
Ser Ser Lys Tyr Val Asn Ala 4055	Asp Ser Thr Lys Gln Asn Ala Tyr 4060	4065
Thr Thr Lys Val Thr Asn Ala 4070	Glu His Ile Ile Ser Gly Thr Pro 4075	4080
Thr Val Val Thr Thr Pro Ser 4085	Glu Val Thr Ala Ala Ala Asn Gln 4090	4095
Val Asn Ser Ala Lys Gln Glu 4100	Leu Asn Gly Asp Glu Arg Leu Arg 4105	4110
Glu Ala Lys Gln Asn Ala Asn 4115	Thr Ala Ile Asp Ala Leu Thr Gln 4120	4125
Leu Asn Thr Pro Gln Lys Ala 4130	Lys Leu Lys Glu Gln Val Gly Gln 4135	4140
Ala Asn Arg Leu Glu Asp Val 4145	Gln Thr Val Gln Thr Asn Gly Gln 4150	4155
Ala Leu Asn Asn Ala Met Lys 4160	Gly Leu Arg Asp Ser Ile Ala Asn 4165	4170
Glu Thr Thr Val Lys Thr Ser 4175	Gln Asn Tyr Thr Asp Ala Ser Pro 4180	4185
Asn Asn Gln Ser Thr Tyr Asn 4190	Ser Ala Val Ser Asn Ala Lys Gly 4195	4200
Ile Ile Asn Gln Thr Asn Asn 4205	Pro Thr Met Asp Thr Ser Ala Ile 4210	4215
Thr Gln Ala Thr Thr Gln Val 4220	Asn Asn Ala Lys Asn Gly Leu Asn 4225	4230
Gly Ala Glu Asn Leu Arg Asn 4235	Ala Gln Asn Thr Ala Lys Gln Asn 4240	4245
Leu Asn Thr Leu Ser His Leu 4250	Thr Asn Asn Gln Lys Ser Ala Ile 4255	4260
Ser Ser Gln Ile Asp Arg Ala 4265	Gly His Val Ser Glu Val Thr Ala 4270	4275
Thr Lys Asn Ala Ala Thr Glu 4280	Leu Asn Thr Gln Met Gly Asn Leu 4285	4290
Glu Gln Ala Ile His Asp Gln 4295	Asn Thr Val Lys Gln Ser Val Lys 4300	4305
Phe Thr Asp Ala Asp Lys Ala 4310	Lys Arg Asp Ala Tyr Thr Asn Ala 4315	4320

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Val Ser Arg Ala Glu Ala Ile Leu Asn Lys Thr Gln Gly Ala Asn 4325 4330 4335
Thr Ser Lys Gln Asp Val Glu Ala Ala Ile Gln Asn Val Ser Ser 4340 4345 4350
Ala Lys Asn Ala Leu Asn Gly Asp Gln Asn Val Thr Asn Ala Lys 4355 4360 4365
Asn Ala Ala Lys Asn Ala Leu Asn Asn Leu Thr Ser Ile Asn Asn 4370 4375 4380
Ala Gln Lys Arg Asp Leu Thr Thr Lys Ile Asp Gln Ala Thr Thr 4385 4390 4395
Val Ala Gly Val Glu Ala Val Ser Asn Thr Ser Thr Gln Leu Asn 4400 4405 4410
Thr Ala Met Ala Asn Leu Gln Asn Gly Ile Asn Asp Lys Thr Asn 4415 4420 4425
Thr Leu Ala Ser Glu Asn Tyr His Asp Ala Asp Ser Asp Lys Lys 4430 4435 4440
Thr Ala Tyr Thr Gln Ala Val Thr Asn Ala Glu Asn Ile Leu Asn 4445 4450 4455
Lys Asn Ser Gly Ser Asn Leu Asp Lys Thr Ala Val Glu Asn Ala 4460 4465 4470
Leu Ser Gln Val Ala Asn Ala Lys Gly Ala Leu Asn Gly Asn His 4475 4480 4485
Asn Leu Glu Gln Ala Lys Ser Asn Ala Asn Thr Thr Ile Asn Gly 4490 4495 4500
Leu Gln His Leu Thr Thr Ala Gln Lys Asp Lys Leu Lys Gln Gln 4505 4510 4515
Val Gln Gln Ala Gln Asn Val Ala Gly Val Asp Thr Val Lys Ser 4520 4525 4530
Ser Ala Asn Thr Leu Asn Gly Ala Met Gly Thr Leu Arg Asn Ser 4535 4540 4545
Ile Gln Asp Asn Thr Ala Thr Lys Asn Gly Gln Asn Tyr Leu Asp 4550 4555 4560
Ala Thr Glu Arg Asn Lys Thr Asn Tyr Asn Asn Ala Val Asp Ser 4565 4570 4575
Ala Asn Gly Val Ile Asn Ala Thr Ser Asn Pro Asn Met Asp Ala 4580 4585 4590
Asn Ala Ile Asn Gln Ile Ala Thr Gln Val Thr Ser Thr Lys Asn 4595 4600 4605
Ala Leu Asp Gly Thr His Asn Leu Thr Gln Ala Lys Gln Thr Ala 4610 4615 4620
Thr Asn Ala Ile Asp Gly Ala Thr Asn Leu Asn Lys Ala Gln Lys 4625 4630 4635
Asp Ala Leu Lys Ala Gln Val Thr Ser Ala Gln Arg Val Ala Asn 4640 4645 4650
Val Thr Ser Ile Gln Gln Thr Ala Asn Glu Leu Asn Thr Ala Met 4655 4660 4665
Gly Gln Leu Gln His Gly Ile Asp Asp Glu Asn Ala Thr Lys Gln 4670 4675 4680
Thr Gln Lys Tyr Arg Asp Ala Glu Gln Ser Lys Lys Thr Ala Tyr 4685 4690 4695
Asp Gln Ala Val Ala Ala Ala Lys Ala Ile Leu Asn Lys Gln Thr 4700 4705 4710

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Gly	Ser	Asn	Ser	Asp	Lys	Ala	Ala	Val	Asp	Arg	Ala	Leu	Gln	Gln
4715						4720					4725			
Val	Thr	Ser	Thr	Lys	Asp	Ala	Leu	Asn	Gly	Asp	Ala	Lys	Leu	Ala
4730						4735					4740			
Glu	Ala	Lys	Ala	Ala	Ala	Lys	Gln	Asn	Leu	Gly	Thr	Leu	Asn	His
4745						4750					4755			
Ile	Thr	Asn	Ala	Gln	Arg	Thr	Asp	Leu	Glu	Gly	Gln	Ile	Asn	Gln
4760						4765					4770			
Ala	Thr	Thr	Val	Asp	Gly	Val	Asn	Thr	Val	Lys	Thr	Asn	Ala	Asn
4775						4780					4785			
Thr	Leu	Asp	Gly	Ala	Met	Asn	Ser	Leu	Gln	Gly	Ser	Ile	Asn	Asp
4790						4795					4800			
Lys	Asp	Ala	Thr	Leu	Arg	Asn	Gln	Asn	Tyr	Leu	Asp	Ala	Asp	Glu
4805						4810					4815			
Ser	Lys	Arg	Asn	Ala	Tyr	Thr	Gln	Ala	Val	Thr	Ala	Ala	Glu	Gly
4820						4825					4830			
Ile	Leu	Asn	Lys	Gln	Thr	Gly	Gly	Asn	Thr	Ser	Lys	Ala	Asp	Val
4835						4840					4845			
Asp	Asn	Ala	Leu	Asn	Ala	Val	Thr	Arg	Ala	Lys	Ala	Ala	Leu	Asn
4850						4855					4860			
Gly	Ala	Asp	Asn	Leu	Arg	Asn	Ala	Lys	Thr	Ser	Ala	Thr	Asn	Thr
4865						4870					4875			
Ile	Asp	Gly	Leu	Pro	Asn	Leu	Thr	Gln	Leu	Gln	Lys	Asp	Asn	Leu
4880						4885					4890			
Lys	His	Gln	Val	Glu	Gln	Ala	Gln	Asn	Val	Ala	Gly	Val	Asn	Gly
4895						4900					4905			
Val	Lys	Asp	Lys	Gly	Asn	Thr	Leu	Asn	Thr	Ala	Met	Gly	Ala	Leu
4910						4915					4920			
Arg	Thr	Ser	Ile	Gln	Asn	Asp	Asn	Thr	Thr	Lys	Thr	Ser	Gln	Asn
4925						4930					4935			
Tyr	Leu	Asp	Ala	Ser	Asp	Ser	Asn	Lys	Asn	Asn	Tyr	Asn	Thr	Ala
4940						4945					4950			
Val	Asn	Asn	Ala	Asn	Gly	Val	Ile	Asn	Ala	Thr	Asn	Asn	Pro	Asn
4955						4960					4965			
Met	Asp	Ala	Asn	Ala	Ile	Asn	Gly	Met	Ala	Asn	Gln	Val	Asn	Thr
4970						4975					4980			
Thr	Lys	Ala	Ala	Leu	Asn	Gly	Ala	Gln	Asn	Leu	Ala	Gln	Ala	Lys
4985						4990					4995			
Thr	Asn	Ala	Thr	Asn	Thr	Ile	Asn	Asn	Ala	His	Asp	Leu	Asn	Gln
5000						5005					5010			
Lys	Gln	Lys	Asp	Ala	Leu	Lys	Thr	Gln	Val	Asn	Asn	Ala	Gln	Arg
5015						5020					5025			
Val	Ser	Asp	Ala	Asn	Asn	Val	Gln	His	Thr	Ala	Thr	Glu	Leu	Asn
5030						5035					5040			
Ser	Ala	Met	Thr	Ala	Leu	Lys	Ala	Ala	Ile	Ala	Asp	Lys	Glu	Arg
5045						5050					5055			
Thr	Lys	Ala	Ser	Gly	Asn	Tyr	Val	Asn	Ala	Asp	Gln	Glu	Lys	Arg
5060						5065					5070			
Gln	Ala	Tyr	Asp	Ser	Lys	Val	Thr	Asn	Ala	Glu	Asn	Ile	Ile	Ser
5075						5080					5085			
Gly	Thr	Pro	Asn	Ala	Thr	Leu	Thr	Val	Asn	Asp	Val	Asn	Ser	Ala
5090						5095					5100			
Ala	Ser	Gln	Val	Asn	Ala	Ala	Lys	Thr	Ala	Leu	Asn	Gly	Asp	Asn

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5105	5110	5115
Asn Leu Arg Val Ala Lys Glu His Ala Asn Asn Thr Ile Asp Gly 5120 5125 5130		
Leu Ala Gln Leu Asn Asn Ala Gln Lys Ala Lys Leu Lys Glu Gln 5135 5140 5145		
Val Gln Ser Ala Thr Thr Leu Asp Gly Val Gln Thr Val Lys Asn 5150 5155 5160		
Ser Ser Gln Thr Leu Asn Thr Ala Met Lys Gly Leu Arg Asp Ser 5165 5170 5175		
Ile Ala Asn Glu Ala Thr Ile Lys Ala Gly Gln Asn Tyr Thr Asp 5180 5185 5190		
Ala Ser Pro Asn Asn Arg Asn Glu Tyr Asp Ser Ala Val Thr Ala 5195 5200 5205		
Ala Lys Ala Ile Ile Asn Gln Thr Ser Asn Pro Thr Met Glu Pro 5210 5215 5220		
Asn Thr Ile Thr Gln Val Thr Ser Gln Val Thr Thr Lys Glu Gln 5225 5230 5235		
Ala Leu Asn Gly Ala Arg Asn Leu Ala Gln Ala Lys Thr Thr Ala 5240 5245 5250		
Lys Asn Asn Leu Asn Asn Leu Thr Ser Ile Asn Asn Ala Gln Lys 5255 5260 5265		
Asp Ala Leu Thr Arg Ser Ile Asp Gly Ala Thr Thr Val Ala Gly 5270 5275 5280		
Val Asn Gln Glu Thr Ala Lys Ala Thr Glu Leu Asn Asn Ala Met 5285 5290 5295		
His Ser Leu Gln Asn Gly Ile Asn Asp Glu Thr Gln Thr Lys Gln 5300 5305 5310		
Thr Gln Lys Tyr Leu Asp Ala Glu Pro Ser Lys Lys Ser Ala Tyr 5315 5320 5325		
Asp Gln Ala Val Asn Ala Ala Lys Ala Ile Leu Thr Lys Ala Ser 5330 5335 5340		
Gly Gln Asn Val Asp Lys Ala Ala Val Glu Gln Ala Leu Gln Asn 5345 5350 5355		
Val Asn Ser Thr Lys Thr Ala Leu Asn Gly Asp Ala Lys Leu Asn 5360 5365 5370		
Glu Ala Lys Ala Ala Ala Lys Gln Thr Leu Gly Thr Leu Thr His 5375 5380 5385		
Ile Asn Asn Ala Gln Arg Thr Ala Leu Asp Asn Glu Ile Thr Gln 5390 5395 5400		
Ala Thr Asn Val Glu Gly Val Asn Thr Val Lys Ala Lys Ala Gln 5405 5410 5415		
Gln Leu Asp Gly Ala Met Gly Gln Leu Glu Thr Ser Ile Arg Asp 5420 5425 5430		
Lys Asp Thr Thr Leu Gln Ser Gln Asn Tyr Gln Asp Ala Asp Asp 5435 5440 5445		
Ala Lys Arg Thr Ala Tyr Ser Gln Ala Val Asn Ala Ala Ala Thr 5450 5455 5460		
Ile Leu Asn Lys Thr Ala Gly Gly Asn Thr Pro Lys Ala Asp Val 5465 5470 5475		
Glu Arg Ala Met Gln Ala Val Thr Gln Ala Asn Thr Ala Leu Asn 5480 5485 5490		
Gly Ile Gln Asn Leu Asp Arg Ala Lys Gln Ala Ala Asn Thr Ala 5495 5500 5505		

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Ile Thr Asn Ala Ser Asp Leu Asn Thr Lys Gln Lys Glu Ala Leu 5510 5515 5520
Lys Ala Gln Val Thr Ser Ala Gly Arg Val Ser Ala Ala Asn Gly 5525 5530 5535
Val Glu His Thr Ala Thr Glu Leu Asn Thr Ala Met Thr Ala Leu 5540 5545 5550
Lys Arg Ala Ile Ala Asp Lys Ala Glu Thr Lys Ala Ser Gly Asn 5555 5560 5565
Tyr Val Asn Ala Asp Ala Asn Lys Arg Gln Ala Tyr Asp Glu Lys 5570 5575 5580
Val Thr Ala Ala Glu Asn Ile Val Ser Gly Thr Pro Thr Pro Thr 5585 5590 5595
Leu Thr Pro Ala Asp Val Thr Asn Ala Ala Thr Gln Val Thr Asn 5600 5605 5610
Ala Lys Thr Gln Leu Asn Gly Asn His Asn Leu Glu Val Ala Lys 5615 5620 5625
Gln Asn Ala Asn Thr Ala Ile Asp Gly Leu Thr Ser Leu Asn Gly 5630 5635 5640
Pro Gln Lys Ala Lys Leu Lys Glu Gln Val Gly Gln Ala Thr Thr 5645 5650 5655
Leu Pro Asn Val Gln Thr Val Arg Asp Asn Ala Gln Thr Leu Asn 5660 5665 5670
Thr Ala Met Lys Gly Leu Arg Asp Ser Ile Ala Asn Glu Ala Thr 5675 5680 5685
Ile Lys Ala Gly Gln Asn Tyr Thr Asp Ala Ser Gln Asn Lys Gln 5690 5695 5700
Thr Asp Tyr Asn Ser Ala Val Thr Ala Ala Lys Ala Ile Ile Gly 5705 5710 5715
Gln Thr Thr Ser Pro Ser Met Asn Ala Gln Glu Ile Asn Gln Ala 5720 5725 5730
Lys Asp Gln Val Thr Ala Lys Gln Gln Ala Leu Asn Gly Gln Glu 5735 5740 5745
Asn Leu Arg Thr Ala Gln Thr Asn Ala Lys Gln His Leu Asn Gly 5750 5755 5760
Leu Ser Asp Leu Thr Asp Ala Gln Lys Asp Ala Val Lys Arg Gln 5765 5770 5775
Ile Glu Gly Ala Thr His Val Asn Glu Val Thr Gln Ala Gln Asn 5780 5785 5790
Asn Ala Asp Ala Leu Asn Thr Ala Met Thr Asn Leu Lys Asn Gly 5795 5800 5805
Ile Gln Asp Gln Asn Thr Ile Lys Gln Gly Val Asn Phe Thr Asp 5810 5815 5820
Ala Asp Glu Ala Lys Arg Asn Ala Tyr Thr Asn Ala Val Thr Gln 5825 5830 5835
Ala Glu Gln Ile Leu Asn Lys Ala Gln Gly Pro Asn Thr Ser Lys 5840 5845 5850
Asp Gly Val Glu Thr Ala Leu Glu Asn Val Gln Arg Ala Lys Asn 5855 5860 5865
Glu Leu Asn Gly Asn Gln Asn Val Ala Asn Ala Lys Thr Thr Ala 5870 5875 5880
Lys Asn Ala Leu Asn Asn Leu Thr Ser Ile Asn Asn Ala Gln Lys 5885 5890 5895

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Glu	Ala	Leu	Lys	Ser	Gln	Ile	Glu	Gly	Ala	Thr	Thr	Val	Ala	Gly
5900						5905					5910			
Val	Asn	Gln	Val	Ser	Thr	Thr	Ala	Ser	Glu	Leu	Asn	Thr	Ala	Met
5915						5920					5925			
Ser	Asn	Leu	Gln	Asn	Gly	Ile	Asn	Asp	Glu	Ala	Ala	Thr	Lys	Ala
5930						5935					5940			
Ala	Gln	Lys	Tyr	Thr	Asp	Ala	Asp	Arg	Glu	Lys	Gln	Thr	Ala	Tyr
5945						5950					5955			
Asn	Asp	Ala	Val	Thr	Ala	Ala	Lys	Thr	Leu	Leu	Asp	Lys	Thr	Ala
5960						5965					5970			
Gly	Ser	Asn	Asp	Asn	Lys	Ala	Ala	Val	Glu	Gln	Ala	Leu	Gln	Arg
5975						5980					5985			
Val	Asn	Thr	Ala	Lys	Thr	Ala	Leu	Asn	Gly	Asp	Glu	Arg	Leu	Asn
5990						5995					6000			
Glu	Ala	Lys	Asn	Thr	Ala	Lys	Gln	Gln	Val	Ala	Thr	Met	Ser	His
6005						6010					6015			
Leu	Thr	Asp	Ala	Gln	Lys	Ala	Asn	Leu	Thr	Ser	Gln	Ile	Glu	Ser
6020						6025					6030			
Gly	Thr	Thr	Val	Ala	Gly	Val	Gln	Gly	Ile	Gln	Ala	Asn	Ala	Gly
6035						6040					6045			
Thr	Leu	Asp	Gln	Ala	Met	Asn	Gln	Leu	Arg	Gln	Ser	Ile	Ala	Ser
6050						6055					6060			
Lys	Asp	Ala	Thr	Lys	Ser	Ser	Glu	Asp	Tyr	Gln	Asp	Ala	Asn	Ala
6065						6070					6075			
Asp	Leu	Gln	Asn	Ala	Tyr	Asn	Asp	Ala	Val	Thr	Asn	Ala	Glu	Gly
6080						6085					6090			
Ile	Ile	Ser	Ala	Thr	Asn	Asn	Pro	Glu	Met	Asn	Pro	Asp	Thr	Ile
6095						6100					6105			
Asn	Gln	Lys	Ala	Ser	Gln	Val	Asn	Ser	Ala	Lys	Ser	Ala	Leu	Asn
6110						6115					6120			
Gly	Asp	Glu	Lys	Leu	Ala	Ala	Ala	Lys	Gln	Thr	Ala	Lys	Ser	Asp
6125						6130					6135			
Ile	Gly	Arg	Leu	Thr	Asp	Leu	Asn	Asn	Ala	Gln	Arg	Thr	Ala	Ala
6140						6145					6150			
Asn	Ala	Glu	Val	Asp	Gln	Ala	Pro	Asn	Leu	Ala	Ala	Val	Thr	Ala
6155						6160					6165			
Ala	Lys	Asn	Lys	Ala	Thr	Ser	Leu	Asn	Thr	Ala	Met	Gly	Asn	Leu
6170						6175					6180			
Lys	His	Ala	Leu	Ala	Glu	Lys	Asp	Asn	Thr	Lys	Arg	Ser	Val	Asn
6185						6190					6195			
Tyr	Thr	Asp	Ala	Asp	Gln	Pro	Lys	Gln	Gln	Ala	Tyr	Asp	Thr	Ala
6200						6205					6210			
Val	Thr	Gln	Ala	Glu	Ala	Ile	Thr	Asn	Ala	Asn	Gly	Ser	Asn	Ala
6215						6220					6225			
Asn	Glu	Thr	Gln	Val	Gln	Ala	Ala	Leu	Asn	Gln	Leu	Asn	Gln	Ala
6230						6235					6240			
Lys	Asn	Asp	Leu	Asn	Gly	Asp	Asn	Lys	Val	Ala	Gln	Ala	Lys	Glu
6245						6250					6255			
Ser	Ala	Lys	Arg	Ala	Leu	Ala	Ser	Tyr	Ser	Asn	Leu	Asn	Asn	Ala
6260						6265					6270			
Gln	Ser	Thr	Ala	Ala	Ile	Ser	Gln	Ile	Asp	Asn	Ala	Thr	Thr	Val
6275						6280					6285			
Ala	Gly	Val	Thr	Ala	Ala	Gln	Asn	Thr	Ala	Asn	Glu	Leu	Asn	Thr

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6290	6295	6300
Ala Met Gly Gln Leu Gln Asn Gly Ile Asn Asp Gln Asn Thr Val 6305	6310	6315
Lys Gln Gln Val Asn Phe Thr Asp Ala Asp Gln Gly Lys Lys Asp 6320	6325	6330
Ala Tyr Thr Asn Ala Val Thr Asn Ala Gln Gly Ile Leu Asp Lys 6335	6340	6345
Ala His Gly Gln Asn Met Thr Lys Ala Gln Val Glu Ala Ala Leu 6350	6355	6360
Asn Gln Val Thr Thr Ala Lys Asn Ala Leu Asn Gly Asp Ala Asn 6365	6370	6375
Val Arg Gln Ala Lys Ser Asp Ala Lys Ala Asn Leu Gly Thr Leu 6380	6385	6390
Thr His Leu Asn Asn Ala Gln Lys Gln Asp Leu Thr Ser Gln Ile 6395	6400	6405
Glu Gly Ala Thr Thr Val Asn Gly Val Asn Gly Val Lys Thr Lys 6410	6415	6420
Ala Gln Asp Leu Asp Gly Ala Met Gln Arg Leu Gln Ser Ala Ile 6425	6430	6435
Ala Asn Lys Asp Gln Thr Lys Ala Ser Glu Asn Tyr Ile Asp Ala 6440	6445	6450
Asp Pro Thr Lys Lys Thr Ala Phe Asp Asn Ala Ile Thr Gln Ala 6455	6460	6465
Glu Ser Tyr Leu Asn Lys Asp His Gly Ala Asn Lys Asp Lys Gln 6470	6475	6480
Ala Val Glu Gln Ala Ile Gln Ser Val Thr Ser Thr Glu Asn Ala 6485	6490	6495
Leu Asn Gly Asp Ala Asn Leu Gln Arg Ala Lys Thr Glu Ala Ile 6500	6505	6510
Gln Ala Ile Asp Asn Leu Thr His Leu Asn Thr Pro Gln Lys Thr 6515	6520	6525
Ala Leu Lys Gln Gln Val Asn Ala Ala Gln Arg Val Ser Gly Val 6530	6535	6540
Thr Asp Leu Lys Asn Ser Ala Thr Ser Leu Asn Asn Ala Met Asp 6545	6550	6555
Gln Leu Lys Gln Ala Ile Ala Asp His Asp Thr Ile Val Ala Ser 6560	6565	6570
Gly Asn Tyr Thr Asn Ala Ser Pro Asp Lys Gln Gly Ala Tyr Thr 6575	6580	6585
Asp Ala Tyr Asn Ala Ala Lys Asn Ile Val Asn Gly Ser Pro Asn 6590	6595	6600
Val Ile Thr Asn Ala Ala Asp Val Thr Ala Ala Thr Gln Arg Val 6605	6610	6615
Asn Asn Ala Glu Thr Gly Leu Asn Gly Asp Thr Asn Leu Ala Thr 6620	6625	6630
Ala Lys Gln Gln Ala Lys Asp Ala Leu Arg Gln Met Thr His Leu 6635	6640	6645
Ser Asp Ala Gln Lys Gln Ser Ile Thr Gly Gln Ile Asp Ser Ala 6650	6655	6660
Thr Gln Val Thr Gly Val Gln Ser Val Lys Asp Asn Ala Thr Asn 6665	6670	6675
Leu Asp Asn Ala Met Asn Gln Leu Arg Asn Ser Ile Ala Asn Lys 6680	6685	6690

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Asp	Asp	Val	Lys	Ala	Ser	Gln	Pro	Tyr	Val	Asp	Ala	Asp	Arg	Asp
6695						6700					6705			
Lys	Gln	Asn	Ala	Tyr	Asn	Thr	Ala	Val	Thr	Asn	Ala	Glu	Asn	Ile
6710						6715					6720			
Ile	Asn	Ala	Thr	Ser	Gln	Pro	Thr	Leu	Asp	Pro	Ser	Ala	Val	Thr
6725						6730					6735			
Gln	Ala	Ala	Asn	Gln	Val	Ser	Thr	Asn	Lys	Thr	Ala	Leu	Asn	Gly
6740						6745					6750			
Ala	Gln	Asn	Leu	Ala	Asn	Lys	Lys	Gln	Glu	Thr	Thr	Ala	Asn	Ile
6755						6760					6765			
Asn	Gln	Leu	Ser	His	Leu	Asn	Asn	Ala	Gln	Lys	Gln	Asp	Leu	Asn
6770						6775					6780			
Thr	Gln	Val	Thr	Asn	Ala	Pro	Asn	Ile	Ser	Thr	Val	Asn	Gln	Val
6785						6790					6795			
Lys	Thr	Lys	Ala	Glu	Gln	Leu	Asp	Gln	Ala	Met	Glu	Arg	Leu	Ile
6800						6805					6810			
Asn	Gly	Ile	Gln	Asp	Lys	Asp	Gln	Val	Lys	Gln	Ser	Val	Asn	Phe
6815						6820					6825			
Thr	Asp	Ala	Asp	Pro	Glu	Lys	Gln	Thr	Ala	Tyr	Asn	Asn	Ala	Val
6830						6835					6840			
Thr	Ala	Ala	Glu	Asn	Ile	Ile	Asn	Gln	Ala	Asn	Gly	Thr	Asn	Ala
6845						6850					6855			
Asn	Gln	Ser	Gln	Val	Glu	Ala	Ala	Leu	Ser	Thr	Val	Thr	Thr	Thr
6860						6865					6870			
Lys	Gln	Ala	Leu	Asn	Gly	Asp	Arg	Lys	Val	Thr	Asp	Ala	Lys	Asn
6875						6880					6885			
Asn	Ala	Asn	Gln	Thr	Leu	Ser	Thr	Leu	Asp	Asn	Leu	Asn	Asn	Ala
6890						6895					6900			
Gln	Lys	Gly	Ala	Val	Thr	Gly	Asn	Ile	Asn	Gln	Ala	His	Thr	Val
6905						6910					6915			
Ala	Glu	Val	Thr	Gln	Ala	Ile	Gln	Thr	Ala	Gln	Glu	Leu	Asn	Thr
6920						6925					6930			
Ala	Met	Gly	Asn	Leu	Lys	Asn	Ser	Leu	Asn	Asp	Lys	Asp	Thr	Thr
6935						6940					6945			
Leu	Gly	Ser	Gln	Asn	Phe	Ala	Asp	Ala	Asp	Pro	Glu	Lys	Lys	Asn
6950						6955					6960			
Ala	Tyr	Asn	Glu	Ala	Val	His	Asn	Ala	Glu	Asn	Ile	Leu	Asn	Lys
6965						6970					6975			
Ser	Thr	Gly	Thr	Asn	Val	Pro	Lys	Asp	Gln	Val	Glu	Ala	Ala	Met
6980						6985					6990			
Asn	Gln	Val	Asn	Ala	Thr	Lys	Ala	Ala	Leu	Asn	Gly	Thr	Gln	Asn
6995						7000					7005			
Leu	Glu	Lys	Ala	Lys	Gln	His	Ala	Asn	Thr	Ala	Ile	Asp	Gly	Leu
7010						7015					7020			
Ser	His	Leu	Thr	Asn	Ala	Gln	Lys	Glu	Ala	Leu	Lys	Gln	Leu	Val
7025						7030					7035			
Gln	Gln	Ser	Thr	Thr	Val	Ala	Glu	Ala	Gln	Gly	Asn	Glu	Gln	Lys
7040						7045					7050			
Ala	Asn	Asn	Val	Asp	Ala	Ala	Met	Asp	Lys	Leu	Arg	Gln	Ser	Ile
7055						7060					7065			
Ala	Asp	Asn	Ala	Thr	Thr	Lys	Gln	Asn	Gln	Asn	Tyr	Thr	Asp	Ala
7070						7075					7080			

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Ser	Gln	Asn	Lys	Lys	Asp	Ala	Tyr	Asn	Asn	Ala	Val	Thr	Thr	Ala
7085						7090					7095			
Gln	Gly	Ile	Ile	Asp	Gln	Thr	Thr	Ser	Pro	Thr	Leu	Asp	Pro	Thr
7100					7105						7110			
Val	Ile	Asn	Gln	Ala	Ala	Gly	Gln	Val	Ser	Thr	Thr	Lys	Asn	Ala
7115						7120					7125			
Leu	Asn	Gly	Asn	Glu	Asn	Leu	Glu	Ala	Ala	Lys	Gln	Gln	Ala	Ser
7130						7135					7140			
Gln	Ser	Leu	Gly	Ser	Leu	Asp	Asn	Leu	Asn	Asn	Ala	Gln	Lys	Gln
7145						7150					7155			
Thr	Val	Thr	Asp	Gln	Ile	Asn	Gly	Ala	His	Thr	Val	Asp	Glu	Ala
7160						7165					7170			
Asn	Gln	Ile	Lys	Gln	Asn	Ala	Gln	Asn	Leu	Asn	Thr	Ala	Met	Gly
7175						7180					7185			
Asn	Leu	Lys	Gln	Ala	Ile	Ala	Asp	Lys	Asp	Ala	Thr	Lys	Ala	Thr
7190						7195					7200			
Val	Asn	Phe	Thr	Asp	Ala	Asp	Gln	Ala	Lys	Gln	Gln	Ala	Tyr	Asn
7205						7210					7215			
Thr	Ala	Val	Thr	Asn	Ala	Glu	Asn	Ile	Ser	Lys	Ala	Asn	Gly	Asn
7220						7225					7230			
Ala	Thr	Gln	Ala	Glu	Val	Glu	Gln	Ala	Ile	Lys	Gln	Val	Asn	Ala
7235						7240					7245			
Ala	Lys	Gln	Ala	Leu	Asn	Gly	Asn	Ala	Asn	Val	Gln	His	Ala	Lys
7250						7255					7260			
Asp	Glu	Ala	Thr	Ala	Leu	Ile	Asn	Ser	Ser	Asn	Asp	Leu	Asn	Gln
7265						7270					7275			
Ala	Gln	Lys	Asp	Ala	Leu	Lys	Gln	Gln	Val	Gln	Asn	Ala	Thr	Thr
7280						7285					7290			
Val	Ala	Gly	Val	Asn	Asn	Val	Lys	Gln	Thr	Ala	Gln	Glu	Leu	Asn
7295						7300					7305			
Asn	Ala	Met	Thr	Gln	Leu	Lys	Gln	Gly	Ile	Ala	Asp	Lys	Glu	Gln
7310						7315					7320			
Thr	Lys	Ala	Asp	Gly	Asn	Phe	Val	Asn	Ala	Asp	Pro	Asp	Lys	Gln
7325						7330					7335			
Asn	Ala	Tyr	Asn	Gln	Ala	Val	Ala	Lys	Ala	Glu	Ala	Leu	Ile	Ser
7340						7345					7350			
Ala	Thr	Pro	Asp	Val	Val	Val	Thr	Pro	Ser	Glu	Ile	Thr	Ala	Ala
7355						7360					7365			
Leu	Asn	Lys	Val	Thr	Gln	Ala	Lys	Asn	Asp	Leu	Asn	Gly	Asn	Thr
7370						7375					7380			
Asn	Leu	Ala	Thr	Ala	Lys	Gln	Asn	Val	Gln	His	Ala	Ile	Asp	Gln
7385						7390					7395			
Leu	Pro	Asn	Leu	Asn	Gln	Ala	Gln	Arg	Asp	Glu	Tyr	Ser	Lys	Gln
7400						7405					7410			
Ile	Thr	Gln	Ala	Thr	Leu	Val	Pro	Asn	Val	Asn	Ala	Ile	Gln	Gln
7415						7420					7425			
Ala	Ala	Thr	Thr	Leu	Asn	Asp	Ala	Met	Thr	Gln	Leu	Lys	Gln	Gly
7430						7435					7440			
Ile	Ala	Asn	Lys	Ala	Gln	Ile	Lys	Gly	Ser	Glu	Asn	Tyr	His	Asp
7445						7450					7455			
Ala	Asp	Thr	Asp	Lys	Gln	Thr	Ala	Tyr	Asp	Asn	Ala	Val	Thr	Lys
7460						7465					7470			
Ala	Glu	Glu	Leu	Leu	Lys	Gln	Thr	Thr	Asn	Pro	Thr	Met	Asp	Pro

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7475	7480	7485
Asn Thr Ile Gln Gln Ala Leu Thr Lys Val Asn Asp Thr Asn Gln 7490 7495 7500		
Ala Leu Asn Gly Asn Gln Lys Leu Ala Asp Ala Lys Gln Asp Ala 7505 7510 7515		
Lys Thr Thr Leu Gly Thr Leu Asp His Leu Asn Asp Ala Gln Lys 7520 7525 7530		
Gln Ala Leu Thr Thr Gln Val Glu Gln Ala Pro Asp Ile Ala Thr 7535 7540 7545		
Val Asn Asn Val Lys Gln Asn Ala Gln Asn Leu Asn Asn Ala Met 7550 7555 7560		
Thr Asn Leu Asn Asn Ala Leu Gln Asp Lys Thr Glu Thr Leu Asn 7565 7570 7575		
Ser Ile Asn Phe Thr Asp Ala Asp Gln Ala Lys Lys Asp Ala Tyr 7580 7585 7590		
Thr Asn Ala Val Ser His Ala Glu Gly Ile Leu Ser Lys Ala Asn 7595 7600 7605		
Gly Ser Asn Ala Ser Gln Thr Glu Val Glu Gln Ala Met Gln Arg 7610 7615 7620		
Val Asn Glu Ala Lys Gln Ala Leu Asn Gly Asn Asp Asn Val Gln 7625 7630 7635		
Arg Ala Lys Asp Ala Ala Lys Gln Val Ile Thr Asn Ala Asn Asp 7640 7645 7650		
Leu Asn Gln Ala Gln Lys Asp Ala Leu Lys Gln Gln Val Asp Ala 7655 7660 7665		
Ala Gln Thr Val Ala Asn Val Asn Thr Ile Lys Gln Thr Ala Gln 7670 7675 7680		
Asp Leu Asn Gln Ala Met Thr Gln Leu Lys Gln Gly Ile Ala Asp 7685 7690 7695		
Lys Asp Gln Thr Lys Ala Asn Gly Asn Phe Val Asn Ala Asp Thr 7700 7705 7710		
Asp Lys Gln Asn Ala Tyr Asn Asn Ala Val Ala His Ala Glu Gln 7715 7720 7725		
Ile Ile Ser Gly Thr Pro Asn Ala Asn Val Asp Pro Gln Gln Val 7730 7735 7740		
Ala Gln Ala Leu Gln Gln Val Asn Gln Ala Lys Gly Asp Leu Asn 7745 7750 7755		
Gly Asn His Asn Leu Gln Val Ala Lys Asp Asn Ala Asn Thr Ala 7760 7765 7770		
Ile Asp Gln Leu Pro Asn Leu Asn Gln Pro Gln Lys Thr Ala Leu 7775 7780 7785		
Lys Asp Gln Val Ser His Ala Glu Leu Val Thr Gly Val Asn Ala 7790 7795 7800		
Ile Lys Gln Asn Ala Asp Ala Leu Asn Asn Ala Met Gly Thr Leu 7805 7810 7815		
Lys Gln Gln Ile Gln Ala Asn Ser Gln Val Pro Gln Ser Val Asp 7820 7825 7830		
Phe Thr Gln Ala Asp Gln Asp Lys Gln Gln Ala Tyr Asn Asn Ala 7835 7840 7845		
Ala Asn Gln Ala Gln Gln Ile Ala Asn Gly Ile Pro Thr Pro Val 7850 7855 7860		
Leu Thr Pro Asp Thr Val Thr Gln Ala Val Thr Thr Met Asn Gln 7865 7870 7875		

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Ala Lys Asp Ala Leu Asn Gly Asp Glu Lys Leu Ala Gln Ala Lys 7880 7885 7890
Gln Glu Ala Leu Ala Asn Leu Asp Thr Leu Arg Asp Leu Asn Gln 7895 7900 7905
Pro Gln Arg Asp Ala Leu Arg Asn Gln Ile Asn Gln Ala Gln Ala 7910 7915 7920
Leu Ala Thr Val Glu Gln Thr Lys Gln Asn Ala Gln Asn Val Asn 7925 7930 7935
Thr Ala Met Ser Asn Leu Lys Gln Gly Ile Ala Asn Lys Asp Thr 7940 7945 7950
Val Lys Ala Ser Glu Asn Tyr His Asp Ala Asp Ala Asp Lys Gln 7955 7960 7965
Thr Ala Tyr Thr Asn Ala Val Ser Gln Ala Glu Gly Ile Ile Asn 7970 7975 7980
Gln Thr Thr Asn Pro Thr Leu Asn Pro Asp Glu Ile Thr Arg Ala 7985 7990 7995
Leu Thr Gln Val Thr Asp Ala Lys Asn Gly Leu Asn Gly Glu Ala 8000 8005 8010
Lys Leu Ala Thr Glu Lys Gln Asn Ala Lys Asp Ala Val Ser Gly 8015 8020 8025
Met Thr His Leu Asn Asp Ala Gln Lys Gln Ala Leu Lys Gly Gln 8030 8035 8040
Ile Asp Gln Ser Pro Glu Ile Ala Thr Val Asn Gln Val Lys Gln 8045 8050 8055
Thr Ala Thr Ser Leu Asp Gln Ala Met Asp Gln Leu Ser Gln Ala 8060 8065 8070
Ile Asn Asp Lys Ala Gln Thr Leu Ala Asp Gly Asn Tyr Leu Asn 8075 8080 8085
Ala Asp Pro Asp Lys Gln Asn Ala Tyr Lys Gln Ala Val Ala Lys 8090 8095 8100
Ala Glu Ala Leu Leu Asn Lys Gln Ser Gly Thr Asn Glu Val Gln 8105 8110 8115
Ala Gln Val Glu Ser Ile Thr Asn Glu Val Asn Ala Ala Lys Gln 8120 8125 8130
Ala Leu Asn Gly Asn Asp Asn Leu Ala Asn Ala Lys Gln Gln Ala 8135 8140 8145
Lys Gln Gln Leu Ala Asn Leu Thr His Leu Asn Asp Ala Gln Lys 8150 8155 8160
Gln Ser Phe Glu Ser Gln Ile Thr Gln Ala Pro Leu Val Thr Asp 8165 8170 8175
Val Thr Thr Ile Asn Gln Lys Ala Gln Thr Leu Asp His Ala Met 8180 8185 8190
Glu Leu Leu Arg Asn Ser Val Ala Asp Asn Gln Thr Thr Leu Ala 8195 8200 8205
Ser Glu Asp Tyr His Asp Ala Thr Ala Gln Arg Gln Asn Asp Tyr 8210 8215 8220
Asn Gln Ala Val Thr Ala Ala Asn Asn Ile Ile Asn Gln Thr Thr 8225 8230 8235
Ser Pro Thr Met Asn Pro Asp Asp Val Asn Gly Ala Thr Thr Gln 8240 8245 8250
Val Asn Asn Thr Lys Val Ala Leu Asp Gly Asp Glu Asn Leu Ala 8255 8260 8265

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Ala	Ala	Lys	Gln	Gln	Ala	Asn	Asn	Arg	Leu	Asp	Gln	Leu	Asp	His
8270						8275					8280			
Leu	Asn	Asn	Ala	Gln	Lys	Gln	Gln	Leu	Gln	Ser	Gln	Ile	Thr	Gln
8285						8290					8295			
Ser	Ser	Asp	Ile	Ala	Ala	Val	Asn	Gly	His	Lys	Gln	Thr	Ala	Glu
8300						8305					8310			
Ser	Leu	Asn	Thr	Ala	Met	Gly	Asn	Leu	Ile	Asn	Ala	Ile	Ala	Asp
8315						8320					8325			
His	Gln	Ala	Val	Glu	Gln	Arg	Gly	Asn	Phe	Ile	Asn	Ala	Asp	Thr
8330						8335					8340			
Asp	Lys	Gln	Thr	Ala	Tyr	Asn	Thr	Ala	Val	Asn	Glu	Ala	Ala	Ala
8345						8350					8355			
Met	Ile	Asn	Lys	Gln	Thr	Gly	Gln	Asn	Ala	Asn	Gln	Thr	Glu	Val
8360						8365					8370			
Glu	Gln	Ala	Ile	Thr	Lys	Val	Gln	Thr	Thr	Leu	Gln	Ala	Leu	Asn
8375						8380					8385			
Gly	Asp	His	Asn	Leu	Gln	Val	Ala	Lys	Thr	Asn	Ala	Thr	Gln	Ala
8390						8395					8400			
Ile	Asp	Ala	Leu	Thr	Ser	Leu	Asn	Asp	Pro	Gln	Lys	Thr	Ala	Leu
8405						8410					8415			
Lys	Asp	Gln	Val	Thr	Ala	Ala	Thr	Leu	Val	Thr	Ala	Val	His	Gln
8420						8425					8430			
Ile	Glu	Gln	Asn	Ala	Asn	Thr	Leu	Asn	Gln	Ala	Met	His	Gly	Leu
8435						8440					8445			
Arg	Gln	Ser	Ile	Gln	Asp	Asn	Ala	Ala	Thr	Lys	Ala	Asn	Ser	Lys
8450						8455					8460			
Tyr	Ile	Asn	Glu	Asp	Gln	Pro	Glu	Gln	Gln	Asn	Tyr	Asp	Gln	Ala
8465						8470					8475			
Val	Gln	Ala	Ala	Asn	Asn	Ile	Ile	Asn	Glu	Gln	Thr	Ala	Thr	Leu
8480						8485					8490			
Asp	Asn	Asn	Ala	Ile	Asn	Gln	Ala	Ala	Thr	Thr	Val	Asn	Thr	Thr
8495						8500					8505			
Lys	Ala	Ala	Leu	His	Gly	Asp	Val	Lys	Leu	Gln	Asn	Asp	Lys	Asp
8510						8515					8520			
His	Ala	Lys	Gln	Thr	Val	Ser	Gln	Leu	Ala	His	Leu	Asn	Asn	Ala
8525						8530					8535			
Gln	Lys	His	Met	Glu	Asp	Thr	Leu	Ile	Asp	Ser	Glu	Thr	Thr	Arg
8540						8545					8550			
Thr	Ala	Val	Lys	Gln	Asp	Leu	Thr	Glu	Ala	Gln	Ala	Leu	Asp	Gln
8555						8560					8565			
Leu	Met	Asp	Ala	Leu	Gln	Gln	Ser	Ile	Ala	Asp	Lys	Asp	Ala	Thr
8570						8575					8580			
Arg	Ala	Ser	Ser	Ala	Tyr	Val	Asn	Ala	Glu	Pro	Asn	Lys	Lys	Gln
8585						8590					8595			
Ser	Tyr	Asp	Glu	Ala	Val	Gln	Asn	Ala	Glu	Ser	Ile	Ile	Ala	Gly
8600						8605					8610			
Leu	Asn	Asn	Pro	Thr	Ile	Asn	Lys	Gly	Asn	Val	Ser	Ser	Ala	Thr
8615						8620					8625			
Gln	Ala	Val	Ile	Ser	Ser	Lys	Asn	Ala	Leu	Asp	Gly	Val	Glu	Arg
8630						8635					8640			
Leu	Ala	Gln	Asp	Lys	Gln	Thr	Ala	Gly	Asn	Ser	Leu	Asn	His	Leu
8645						8650					8655			
Asp	Gln	Leu	Thr	Pro	Ala	Gln	Gln	Gln	Ala	Leu	Glu	Asn	Gln	Ile

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8660	8665	8670
Asn Asn Ala Thr Thr Arg Gly Glu Val Ala Gln Lys Leu Thr Glu 8675 8680 8685		
Ala Gln Ala Leu Asn Gln Ala Met Glu Ala Leu Arg Asn Ser Ile 8690 8695 8700		
Gln Asp Gln Gln Gln Thr Glu Ala Gly Ser Lys Phe Ile Asn Glu 8705 8710 8715		
Asp Lys Pro Gln Lys Asp Ala Tyr Gln Ala Ala Val Gln Asn Ala 8720 8725 8730		
Lys Asp Leu Ile Asn Gln Thr Asn Asn Pro Thr Leu Asp Lys Ala 8735 8740 8745		
Gln Val Glu Gln Leu Thr Gln Ala Val Asn Gln Ala Lys Asp Asn 8750 8755 8760		
Leu His Gly Asp Gln Lys Leu Ala Asp Asp Lys Gln His Ala Val 8765 8770 8775		
Thr Asp Leu Asn Gln Leu Asn Gly Leu Asn Asn Pro Gln Arg Gln 8780 8785 8790		
Ala Leu Glu Ser Gln Ile Asn Asn Ala Ala Thr Arg Gly Glu Val 8795 8800 8805		
Ala Gln Lys Leu Ala Glu Ala Lys Ala Leu Asp Gln Ala Met Gln 8810 8815 8820		
Ala Leu Arg Asn Ser Ile Gln Asp Gln Gln Gln Thr Glu Ser Gly 8825 8830 8835		
Ser Lys Phe Ile Asn Glu Asp Lys Pro Gln Lys Asp Ala Tyr Gln 8840 8845 8850		
Ala Ala Val Gln Asn Ala Lys Asp Leu Ile Asn Gln Thr Gly Asn 8855 8860 8865		
Pro Thr Leu Asp Lys Ser Gln Val Glu Gln Leu Thr Gln Ala Val 8870 8875 8880		
Thr Thr Ala Lys Asp Asn Leu His Gly Asp Gln Lys Leu Ala Arg 8885 8890 8895		
Asp Gln Gln Gln Ala Val Thr Thr Val Asn Ala Leu Pro Asn Leu 8900 8905 8910		
Asn His Ala Gln Gln Gln Ala Leu Thr Asp Ala Ile Asn Ala Ala 8915 8920 8925		
Pro Thr Arg Thr Glu Val Ala Gln His Val Gln Thr Ala Thr Glu 8930 8935 8940		
Leu Asp His Ala Met Glu Thr Leu Lys Asn Lys Val Asp Gln Val 8945 8950 8955		
Asn Thr Asp Lys Ala Gln Pro Asn Tyr Thr Glu Ala Ser Thr Asp 8960 8965 8970		
Lys Lys Glu Ala Val Asp Gln Ala Leu Gln Ala Ala Glu Ser Ile 8975 8980 8985		
Thr Asp Pro Thr Asn Gly Ser Asn Ala Asn Lys Asp Ala Val Asp 8990 8995 9000		
Gln Val Leu Thr Lys Leu Gln Glu Lys Glu Asn Glu Leu Asn Gly 9005 9010 9015		
Asn Glu Arg Val Ala Glu Ala Lys Thr Gln Ala Lys Gln Thr Ile 9020 9025 9030		
Asp Gln Leu Thr His Leu Asn Ala Asp Gln Ile Ala Thr Ala Lys 9035 9040 9045		
Gln Asn Ile Asp Gln Ala Thr Lys Leu Gln Pro Ile Ala Glu Leu 9050 9055 9060		

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Val Asp	Gln Ala Thr	Gln Leu	Asn Gln Ser Met	Asp	Gln Leu Gln
9065		9070		9075	
Gln Ala	Val Asn Glu His	Ala	Asn Val Glu Gln Thr	Val Asp Tyr	
9080		9085		9090	
Thr Gln	Ala Asp Ser Asp	Lys	Gln Asn Ala Tyr	Lys	Gln Ala Ile
9095		9100		9105	
Ala Asp	Ala Glu Asn Val	Leu	Lys Gln Asn Ala	Asn	Lys Gln Gln
9110		9115		9120	
Val Asp	Gln Ala Leu Gln	Asn	Ile Leu Asn Ala	Lys	Gln Ala Leu
9125		9130		9135	
Asn Gly	Asp Glu Arg Val	Ala	Leu Ala Lys Thr	Asn	Gly Lys His
9140		9145		9150	
Asp Ile	Asp Gln Leu Asn	Ala	Leu Asn Asn Ala	Gln	Gln Asp Gly
9155		9160		9165	
Phe Lys	Gly Arg Ile Asp	Gln	Ser Asn Asp Leu	Asn	Gln Ile Gln
9170		9175		9180	
Gln Ile	Val Asp Glu Ala	Lys	Ala Leu Asn Arg	Ala	Met Asp Gln
9185		9190		9195	
Leu Ser	Gln Glu Ile Thr	Asp	Asn Glu Gly Arg	Thr	Lys Gly Ser
9200		9205		9210	
Thr Asn	Tyr Val Asn Ala	Asp	Thr Gln Val Lys	Gln	Val Tyr Asp
9215		9220		9225	
Glu Thr	Val Asp Lys Ala	Lys	Gln Ala Leu Asp	Lys	Ser Thr Gly
9230		9235		9240	
Gln Asn	Leu Thr Ala Lys	Gln	Val Ile Lys Leu	Asn	Asp Ala Val
9245		9250		9255	
Thr Ala	Ala Lys Lys Ala	Leu	Asn Gly Glu Glu	Arg	Leu Asn Asn
9260		9265		9270	
Arg Lys	Ala Glu Ala Leu	Gln	Arg Leu Asp Gln	Leu	Thr His Leu
9275		9280		9285	
Asn Asn	Ala Gln Arg Gln	Leu	Ala Ile Gln Gln	Ile	Asn Asn Ala
9290		9295		9300	
Glu Thr	Leu Asn Lys Ala	Ser	Arg Ala Ile Asn	Arg	Ala Thr Lys
9305		9310		9315	
Leu Asp	Asn Ala Met Gly	Ala	Val Gln Gln Tyr	Ile	Asp Glu Gln
9320		9325		9330	
His Leu	Gly Val Ile Ser	Ser	Thr Asn Tyr Ile	Asn	Ala Asp Asp
9335		9340		9345	
Asn Leu	Lys Ala Asn Tyr	Asp	Asn Ala Ile Ala	Asn	Ala Ala His
9350		9355		9360	
Glu Leu	Asp Lys Val Gln	Gly	Asn Ala Ile Ala	Lys	Ala Glu Ala
9365		9370		9375	
Glu Gln	Leu Lys Gln Asn	Ile	Ile Asp Ala Gln	Asn	Ala Leu Asn
9380		9385		9390	
Gly Asp	Gln Asn Leu Ala	Asn	Ala Lys Asp Lys	Ala	Asn Ala Phe
9395		9400		9405	
Val Asn	Ser Leu Asn Gly	Leu	Asn Gln Gln Gln	Gln	Asp Leu Ala
9410		9415		9420	
His Lys	Ala Ile Asn Asn	Ala	Asp Thr Val Ser	Asp	Val Thr Asp
9425		9430		9435	
Ile Val	Asn Asn Gln Ile	Asp	Leu Asn Asp Ala	Met	Glu Thr Leu
9440		9445		9450	

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Lys 9455	His	Leu	Val	Asp	Asn	Glu 9460	Ile	Pro	Asn	Ala	Glu 9465	Gln	Thr	Val
Asn 9470	Tyr	Gln	Asn	Ala	Asp	Asp 9475	Asn	Ala	Lys	Thr	Asn 9480	Phe	Asp	Asp
Ala 9485	Lys	Arg	Leu	Ala	Asn	Thr 9490	Leu	Leu	Asn	Ser	Asp 9495	Asn	Thr	Asn
Val 9500	Asn	Asp	Ile	Asn	Gly	Ala 9505	Ile	Gln	Ala	Val	Asn 9510	Asp	Ala	Ile
His 9515	Asn	Leu	Asn	Gly	Asp	Gln 9520	Arg	Leu	Gln	Asp	Ala 9525	Lys	Asp	Lys
Ala 9530	Ile	Gln	Ser	Ile	Asn	Gln 9535	Ala	Leu	Ala	Asn	Lys 9540	Leu	Lys	Glu
Ile 9545	Glu	Ala	Ser	Asn	Ala	Thr 9550	Asp	Gln	Asp	Lys	Leu 9555	Ile	Ala	Lys
Asn 9560	Lys	Ala	Glu	Glu	Leu	Ala 9565	Asn	Ser	Ile	Ile	Asn 9570	Asn	Ile	Asn
Lys 9575	Ala	Thr	Ser	Asn	Gln	Ala 9580	Val	Ser	Gln	Val	Gln 9585	Thr	Ala	Gly
Asn 9590	His	Ala	Ile	Glu	Gln	Val 9595	His	Ala	Asn	Glu	Ile 9600	Pro	Lys	Ala
Lys 9605	Ile	Asp	Ala	Asn	Lys	Asp 9610	Val	Asp	Lys	Gln	Val 9615	Gln	Ala	Leu
Ile 9620	Asp	Glu	Ile	Asp	Arg	Asn 9625	Pro	Asn	Leu	Thr	Asp 9630	Lys	Glu	Lys
Gln 9635	Ala	Leu	Lys	Asp	Arg	Ile 9640	Asn	Gln	Ile	Leu	Gln 9645	Gln	Gly	His
Asn 9650	Gly	Ile	Asn	Asn	Ala	Met 9655	Thr	Lys	Glu	Glu	Ile 9660	Glu	Gln	Ala
Lys 9665	Ala	Gln	Leu	Ala	Gln	Ala 9670	Leu	Gln	Asp	Ile	Lys 9675	Asp	Leu	Val
Lys 9680	Ala	Lys	Glu	Asp	Ala	Lys 9685	Gln	Asp	Val	Asp	Lys 9690	Gln	Val	Gln
Ala 9695	Leu	Ile	Asp	Glu	Ile	Asp 9700	Gln	Asn	Pro	Asn	Leu 9705	Thr	Asp	Lys
Glu 9710	Lys	Gln	Ala	Leu	Lys	Tyr 9715	Arg	Ile	Asn	Gln	Ile 9720	Leu	Gln	Gln
Gly 9725	His	Asn	Asp	Ile	Asn	Asn 9730	Ala	Leu	Thr	Lys	Glu 9735	Glu	Ile	Glu
Gln 9740	Ala	Lys	Ala	Gln	Leu	Ala 9745	Gln	Ala	Leu	Gln	Asp 9750	Ile	Lys	Asp
Leu 9755	Val	Lys	Ala	Lys	Glu	Asp 9760	Ala	Lys	Asn	Ala	Ile 9765	Lys	Ala	Leu
Ala 9770	Asn	Ala	Lys	Arg	Asp	Gln 9775	Ile	Asn	Ser	Asn	Pro 9780	Asp	Leu	Thr
Pro 9785	Glu	Gln	Lys	Ala	Lys	Ala 9790	Leu	Lys	Glu	Ile	Asp 9795	Glu	Ala	Glu
Lys 9800	Arg	Ala	Leu	Gln	Asn	Val 9805	Glu	Asn	Ala	Gln	Thr 9810	Ile	Asp	Gln
Leu 9815	Asn	Arg	Gly	Leu	Asn	Leu 9820	Gly	Leu	Asp	Asp	Ile 9825	Arg	Asn	Thr
His 9830	Val	Trp	Glu	Val	Asp	Glu 9835	Gln	Pro	Ala	Val	Asn 9840	Glu	Ile	Phe
Glu 9845	Ala	Thr	Pro	Glu	Gln	Ile	Leu	Val	Asn	Gly	Glu	Leu	Ile	Val

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9845	9850	9855
His Arg Asp Asp Ile Ile Thr 9860	Glu Gln Asp Ile 9865	Leu Ala His Ile 9870
Asn Leu Ile Asp Gln Leu Ser 9875	Ala Glu Val Ile 9880	Asp Thr Pro Ser 9885
Thr Ala Thr Ile Ser Asp Ser 9890	Leu Thr Ala Lys 9895	Val Glu Val Thr 9900
Leu Leu Asp Gly Ser Lys Val 9905	Ile Val Asn Val 9910	Pro Val Lys Val 9915
Val Glu Lys Glu Leu Ser Val 9920	Val Lys Gln Gln 9925	Ala Ile Glu Ser 9930
Ile Glu Asn Ala Ala Gln Gln 9935	Lys Ile Asn Glu 9940	Ile Asn Asn Ser 9945
Val Thr Leu Thr Leu Glu Gln 9950	Lys Glu Ala Ala 9955	Ile Ala Glu Val 9960
Asn Lys Leu Lys Gln Gln Ala 9965	Ile Asp His Val 9970	Asn Asn Ala Pro 9975
Asp Val His Ser Val Glu Glu 9980	Ile Gln Gln Gln 9985	Glu Gln Ala His 9990
Ile Glu Gln Phe Asn Pro Glu 9995	Gln Phe Thr Ile 10000	Glu Gln Ala Lys 10005
Ser Asn Ala Ile Lys Ser Ile 10010	Glu Asp Ala Ile 10015	Gln His Met Ile 10020
Asp Glu Ile Lys Ala Arg Thr 10025	Asp Leu Thr 10030	Asp Lys Glu Lys Gln 10035
Glu Ala Ile Ala Lys Leu Asn 10040	Gln Leu Lys Glu 10045	Gln Ala Ile Gln 10050
Ala Ile Gln Arg Ala Gln Ser 10055	Ile Asp Glu Ile 10060	Ser Glu Gln Leu 10065
Glu Gln Phe Lys Ala Gln Met 10070	Lys Ala Ala Asn 10075	Pro Thr Ala Lys 10080
Glu Leu Ala Lys Arg Lys Gln 10085	Glu Ala Ile Ser 10090	Arg Ile Lys Asp 10095
Phe Ser Asn Glu Lys Ile Asn 10100	Ser Ile Arg Asn 10105	Ser Glu Ile Gly 10110
Thr Ala Asp Glu Lys Gln Ala 10115	Ala Met Asn Gln 10120	Ile Asn Glu Ile 10125
Val Leu Glu Thr Ile Arg Asp 10130	Ile Asn Asn Ala 10135	His Thr Leu Gln 10140
Gln Val Glu Ala Ala Leu Asn 10145	Asn Gly Ile Ala 10150	Arg Ile Ser Ala 10155
Val Gln Ile Val Thr Ser Asp 10160	Arg Ala Lys Gln 10165	Ser Ser Ser Thr 10170
Gly Asn Glu Ser Asn Ser His 10175	Leu Thr Ile Gly 10180	Tyr Gly Thr Ala 10185
Asn His Pro Phe Asn Ser Ser 10190	Thr Ile Gly His 10195	Lys Lys Lys Leu 10200
Asp Glu Asp Asp Asp Ile Asp 10205	Pro Leu His Met 10210	Arg His Phe Ser 10215
Asn Asn Phe Gly Asn Val Ile 10220	Lys Asn Ala Ile 10225	Gly Val Val Gly 10230
Ile Ser Gly Leu Leu Ala Ser 10235	Phe Trp Phe Phe 10240	Ile Ala Lys Arg 10245

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Arg Arg Lys Glu Asp Glu Glu Glu Glu Leu Glu Ile Arg Asp Asn
 10250 10255 10260

Asn Lys Asp Ser Ile Lys Glu Thr Leu Asp Asp Thr Lys His Leu
 10265 10270 10275

Pro Leu Leu Phe Ala Lys Arg Arg Arg Lys Glu Asp Glu Glu Asp
 10280 10285 10290

Val Thr Val Glu Glu Lys Asp Ser Leu Asn Asn Gly Glu Ser Leu
 10295 10300 10305

Asp Lys Val Lys His Thr Pro Phe Phe Leu Pro Lys Arg Arg Arg
 10310 10315 10320

Lys Glu Asp Glu Glu Asp Val Glu Val Thr Asn Glu Asn Thr Asp
 10325 10330 10335

Glu Lys Val Leu Lys Asp Asn Glu His Ser Pro Leu Leu Phe Ala
 10340 10345 10350

Lys Arg Arg Lys Asp Lys Glu Glu Asp Val Glu Thr Thr Thr Ser
 10355 10360 10365

Ile Glu Ser Lys Asp Glu Asp Val Pro Leu Leu Leu Ala Lys Lys
 10370 10375 10380

Lys Asn Gln Lys Asp Asn Gln Ser Lys Asp Lys Lys Ser Ala Ser
 10385 10390 10395

Lys Asn Thr Ser Lys Lys Val Ala Ala Lys Lys Lys Lys Lys Lys
 10400 10405 10410

Ala Lys Lys Asn Lys Lys
 10415

<210> SEQ ID NO 25
 <211> LENGTH: 340
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 25

Met Lys Lys Lys Leu Leu Val Leu Thr Met Ser Thr Leu Phe Ala Thr
 1 5 10 15

Gln Ile Met Asn Ser Asn His Ala Lys Ala Ser Val Thr Glu Ser Val
 20 25 30

Asp Lys Lys Phe Val Val Pro Glu Ser Gly Ile Asn Lys Ile Ile Pro
 35 40 45

Ala Tyr Asp Glu Phe Lys Asn Ser Pro Lys Val Asn Val Ser Asn Leu
 50 55 60

Thr Asp Asn Lys Asn Phe Val Ala Ser Glu Asp Lys Leu Asn Lys Ile
 65 70 75 80

Ala Asp Ser Ser Ala Ala Ser Lys Ile Val Asp Lys Asn Phe Val Val
 85 90 95

Pro Glu Ser Lys Leu Gly Asn Ile Val Pro Glu Tyr Lys Glu Ile Asn
 100 105 110

Asn Arg Val Asn Val Ala Thr Asn Asn Pro Ala Ser Gln Gln Val Asp
 115 120 125

Lys His Phe Val Ala Lys Gly Pro Glu Val Asn Arg Phe Ile Thr Gln
 130 135 140

Asn Lys Val Asn His His Phe Ile Thr Thr Gln Thr His Tyr Lys Lys
 145 150 155 160

Val Ile Thr Ser Tyr Lys Ser Thr His Val His Lys His Val Asn His
 165 170 175

Ala Lys Asp Ser Ile Asn Lys His Phe Ile Val Lys Pro Ser Glu Ser

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180				185				190							
Pro	Arg	Tyr	Thr	His	Pro	Ser	Gln	Ser	Leu	Ile	Ile	Lys	His	His	Phe
	195						200						205		
Ala	Val	Pro	Gly	Tyr	His	Ala	His	Lys	Phe	Val	Thr	Pro	Gly	His	Ala
	210					215					220				
Ser	Ile	Lys	Ile	Asn	His	Phe	Cys	Val	Val	Pro	Gln	Ile	Asn	Ser	Phe
	225				230						235				240
Lys	Val	Ile	Pro	Pro	Tyr	Gly	His	Asn	Ser	His	Arg	Met	His	Val	Pro
			245						250					255	
Ser	Phe	Gln	Asn	Asn	Thr	Thr	Ala	Thr	His	Gln	Asn	Ala	Lys	Val	Asn
		260							265					270	
Lys	Ala	Tyr	Asp	Tyr	Lys	Tyr	Phe	Tyr	Ser	Tyr	Lys	Val	Val	Lys	Gly
		275					280						285		
Val	Lys	Lys	Tyr	Phe	Ser	Phe	Ser	Gln	Ser	Asn	Gly	Tyr	Lys	Ile	Gly
	290					295					300				
Lys	Pro	Ser	Leu	Asn	Ile	Lys	Asn	Val	Asn	Tyr	Gln	Tyr	Ala	Val	Pro
	305				310						315				320
Ser	Tyr	Ser	Pro	Thr	His	Tyr	Val	Pro	Glu	Phe	Lys	Gly	Ser	Leu	Pro
			325						330					335	
Ala	Pro	Arg	Val												
			340												

<210> SEQ ID NO 26

<211> LENGTH: 130

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 26

Met	Asn	Phe	Asn	Asp	Ile	Glu	Thr	Met	Val	Lys	Ser	Lys	Phe	Lys	Asp
1				5					10					15	
Ile	Lys	Lys	His	Ala	Glu	Glu	Ile	Ala	His	Glu	Ile	Glu	Val	Arg	Ser
			20					25					30		
Gly	Tyr	Leu	Arg	Lys	Ala	Glu	Gln	Tyr	Lys	Arg	Leu	Glu	Phe	Asn	Leu
		35					40					45			
Ser	Phe	Ala	Leu	Asp	Asp	Ile	Glu	Ser	Thr	Ala	Lys	Asp	Val	Gln	Thr
	50				55						60				
Ala	Lys	Ser	Ser	Ala	Asn	Lys	Asp	Ser	Val	Thr	Val	Lys	Gly	Lys	Ala
	65				70					75					80
Pro	Asn	Thr	Leu	Tyr	Ile	Glu	Lys	Arg	Asn	Leu	Met	Lys	Gln	Lys	Leu
			85						90					95	
Glu	Met	Leu	Gly	Glu	Asp	Ile	Asp	Lys	Asn	Lys	Glu	Ser	Leu	Gln	Lys
		100						105					110		
Ala	Lys	Glu	Ile	Ala	Gly	Glu	Lys	Ala	Ser	Glu	Tyr	Phe	Asn	Lys	Ala
		115						120					125		
Met	Asn														
	130														

<210> SEQ ID NO 27

<211> LENGTH: 636

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 27

Met	Lys	Lys	Gln	Ile	Ile	Ser	Leu	Gly	Ala	Leu	Ala	Val	Ala	Ser	Ser
1				5						10				15	
Leu	Phe	Thr	Trp	Asp	Asn	Lys	Ala	Asp	Ala	Ile	Val	Thr	Lys	Asp	Tyr

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20			25			30									
Ser	Gly	Lys	Ser	Gln	Val	Asn	Ala	Gly	Ser	Lys	Asn	Gly	Thr	Leu	Ile
	35						40					45			
Asp	Ser	Arg	Tyr	Leu	Asn	Ser	Ala	Leu	Tyr	Tyr	Leu	Glu	Asp	Tyr	Ile
	50						55					60			
Ile	Tyr	Ala	Ile	Gly	Leu	Thr	Asn	Lys	Tyr	Glu	Tyr	Gly	Asp	Asn	Ile
	65				70					75					80
Tyr	Lys	Glu	Ala	Lys	Asp	Arg	Leu	Leu	Glu	Lys	Val	Leu	Arg	Glu	Asp
				85						90					95
Gln	Tyr	Leu	Leu	Glu	Arg	Lys	Lys	Ser	Gln	Tyr	Glu	Asp	Tyr	Lys	Gln
		100								105					110
Trp	Tyr	Ala	Asn	Tyr	Lys	Lys	Glu	Asn	Pro	Arg	Thr	Asp	Leu	Lys	Met
		115						120							125
Ala	Asn	Phe	His	Lys	Tyr	Asn	Leu	Glu	Glu	Leu	Ser	Met	Lys	Glu	Tyr
		130						135							140
Asn	Glu	Leu	Gln	Asp	Ala	Leu	Lys	Arg	Ala	Leu	Asp	Asp	Phe	His	Arg
		145			150						155				160
Glu	Val	Lys	Asp	Ile	Lys	Asp	Lys	Asn	Ser	Asp	Leu	Lys	Thr	Phe	Asn
				165						170					175
Ala	Ala	Glu	Glu	Asp	Lys	Ala	Thr	Lys	Glu	Val	Tyr	Asp	Leu	Val	Ser
				180											190
Glu	Ile	Asp	Thr	Leu	Val	Val	Ser	Tyr	Tyr	Gly	Asp	Lys	Asp	Tyr	Gly
		195						200							205
Glu	His	Ala	Lys	Glu	Leu	Arg	Ala	Lys	Leu	Asp	Leu	Ile	Leu	Gly	Asp
		210						215							220
Thr	Asp	Asn	Pro	His	Lys	Ile	Thr	Asn	Glu	Arg	Ile	Lys	Lys	Glu	Met
		225			230						235				240
Ile	Asp	Asp	Leu	Asn	Ser	Ile	Ile	Asp	Asp	Phe	Phe	Met	Glu	Thr	Lys
				245						250					255
Gln	Asn	Arg	Pro	Lys	Ser	Ile	Thr	Lys	Tyr	Asn	Pro	Thr	Thr	His	Asn
				260						265					270
Tyr	Lys	Thr	Asn	Ser	Asp	Asn	Lys	Pro	Asn	Phe	Asp	Lys	Leu	Val	Glu
		275						280							285
Glu	Thr	Lys	Lys	Ala	Val	Lys	Glu	Ala	Asp	Asp	Ser	Trp	Lys	Lys	Lys
		290						295							300
Thr	Val	Lys	Lys	Tyr	Gly	Glu	Thr	Glu	Thr	Lys	Ser	Pro	Val	Val	Lys
		305			310						315				320
Glu	Glu	Lys	Lys	Val	Glu	Glu	Pro	Gln	Ala	Pro	Lys	Val	Asp	Asn	Gln
				325						330					335
Gln	Glu	Val	Lys	Thr	Thr	Ala	Gly	Lys	Ala	Glu	Glu	Thr	Thr	Gln	Pro
				340						345					350
Val	Ala	Gln	Pro	Leu	Val	Lys	Ile	Pro	Gln	Gly	Thr	Ile	Thr	Gly	Glu
		355						360							365
Ile	Val	Lys	Gly	Pro	Glu	Tyr	Pro	Thr	Met	Glu	Asn	Lys	Thr	Val	Gln
		370						375							380
Gly	Glu	Ile	Val	Gln	Gly	Pro	Asp	Phe	Leu	Thr	Met	Glu	Gln	Ser	Gly
		385			390						395				400
Pro	Ser	Leu	Ser	Asn	Asn	Tyr	Thr	Asn	Pro	Pro	Leu	Thr	Asn	Pro	Ile
				405							410				415
Leu	Glu	Gly	Leu	Glu	Gly	Ser	Ser	Ser	Lys	Leu	Glu	Ile	Lys	Pro	Gln
				420							425				430
Gly	Thr	Glu	Ser	Thr	Leu	Lys	Gly	Thr	Gln	Gly	Glu	Ser	Ser	Asp	Ile
		435						440							445

-continued

Glu Val Lys Pro Gln Ala Thr Glu Thr Thr Glu Ala Ser Gln Tyr Gly
 450 455 460
 Pro Arg Pro Gln Phe Asn Lys Thr Pro Lys Tyr Val Lys Tyr Arg Asp
 465 470 475 480
 Ala Gly Thr Gly Ile Arg Glu Tyr Asn Asp Gly Thr Phe Gly Tyr Glu
 485 490 495
 Ala Arg Pro Arg Phe Asn Lys Pro Ser Glu Thr Asn Ala Tyr Asn Val
 500 505 510
 Thr Thr His Ala Asn Gly Gln Val Ser Tyr Gly Ala Arg Pro Thr Tyr
 515 520 525
 Lys Lys Pro Ser Glu Thr Asn Ala Tyr Asn Val Thr Thr His Ala Asn
 530 535 540
 Gly Gln Val Ser Tyr Gly Ala Arg Pro Thr Gln Asn Lys Pro Ser Lys
 545 550 555 560
 Thr Asn Ala Tyr Asn Val Thr Thr His Gly Asn Gly Gln Val Ser Tyr
 565 570 575
 Gly Ala Arg Pro Thr Gln Asn Lys Pro Ser Lys Thr Asn Ala Tyr Asn
 580 585 590
 Val Thr Thr His Ala Asn Gly Gln Val Ser Tyr Gly Ala Arg Pro Thr
 595 600 605
 Tyr Lys Lys Pro Ser Lys Thr Asn Ala Tyr Asn Val Thr Thr His Ala
 610 615 620
 Asp Gly Thr Ala Thr Tyr Gly Pro Arg Val Thr Lys
 625 630 635

<210> SEQ ID NO 28

<211> LENGTH: 745

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 28

Ala Glu Gln His Thr Pro Met Lys Ala His Ala Val Thr Thr Ile Asp
 1 5 10 15
 Lys Ala Thr Thr Asp Lys Gln Gln Val Pro Pro Thr Lys Glu Ala Ala
 20 25 30
 His His Ser Gly Lys Glu Ala Ala Thr Asn Val Ser Ala Ser Ala Gln
 35 40 45
 Gly Thr Ala Asp Asp Thr Asn Ser Lys Val Thr Ser Asn Ala Pro Ser
 50 55 60
 Asn Lys Pro Ser Thr Val Val Ser Thr Lys Val Asn Glu Thr Arg Asp
 65 70 75 80
 Val Asp Thr Gln Gln Ala Ser Thr Gln Lys Pro Thr His Thr Ala Thr
 85 90 95
 Phe Lys Leu Ser Asn Ala Lys Thr Ala Ser Leu Ser Pro Arg Met Phe
 100 105 110
 Ala Ala Asn Ala Pro Gln Thr Thr Thr His Lys Ile Leu His Thr Asn
 115 120 125
 Asp Ile His Gly Arg Leu Ala Glu Glu Lys Gly Arg Val Ile Gly Met
 130 135 140
 Ala Lys Leu Lys Thr Val Lys Glu Gln Glu Lys Pro Asp Leu Met Leu
 145 150 155 160
 Asp Ala Gly Asp Ala Phe Gln Gly Leu Pro Leu Ser Asn Gln Ser Lys
 165 170 175
 Gly Glu Glu Met Ala Lys Ala Met Asn Ala Val Gly Tyr Asp Ala Met

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180				185				190							
Ala	Val	Gly	Asn	His	Glu	Phe	Asp	Phe	Gly	Tyr	Asp	Gln	Leu	Lys	Lys
		195					200					205			
Leu	Glu	Gly	Met	Leu	Asp	Phe	Pro	Met	Leu	Ser	Thr	Asn	Val	Tyr	Lys
	210					215					220				
Asp	Gly	Lys	Arg	Ala	Phe	Lys	Pro	Ser	Thr	Ile	Val	Thr	Lys	Asn	Gly
	225				230					235				240	
Ile	Arg	Tyr	Gly	Ile	Ile	Gly	Val	Thr	Thr	Pro	Glu	Thr	Lys	Thr	Lys
			245						250					255	
Thr	Arg	Pro	Glu	Gly	Ile	Lys	Gly	Val	Glu	Phe	Arg	Asp	Pro	Leu	Gln
			260						265					270	
Ser	Val	Thr	Ala	Glu	Met	Met	Arg	Ile	Tyr	Lys	Asp	Val	Asp	Thr	Phe
		275					280					285			
Val	Val	Ile	Ser	His	Leu	Gly	Ile	Asp	Pro	Ser	Thr	Gln	Glu	Thr	Trp
	290					295					300				
Arg	Gly	Asp	Tyr	Leu	Val	Lys	Gln	Leu	Ser	Gln	Asn	Pro	Gln	Leu	Lys
	305				310					315				320	
Lys	Arg	Ile	Thr	Val	Ile	Asp	Gly	His	Ser	His	Thr	Val	Leu	Gln	Asn
			325						330					335	
Gly	Gln	Ile	Tyr	Asn	Asn	Asp	Ala	Leu	Ala	Gln	Thr	Gly	Thr	Ala	Leu
			340						345					350	
Ala	Asn	Ile	Gly	Lys	Ile	Thr	Phe	Asn	Tyr	Arg	Asn	Gly	Glu	Val	Ser
		355					360					365			
Asn	Ile	Lys	Pro	Ser	Leu	Ile	Asn	Val	Lys	Asp	Val	Glu	Asn	Val	Thr
	370					375					380				
Pro	Asn	Lys	Ala	Leu	Ala	Glu	Gln	Ile	Asn	Gln	Ala	Asp	Gln	Thr	Phe
	385				390					395				400	
Arg	Ala	Gln	Thr	Ala	Glu	Val	Ile	Ile	Pro	Asn	Asn	Thr	Ile	Asp	Phe
			405						410					415	
Lys	Gly	Glu	Arg	Asp	Asp	Val	Arg	Thr	Arg	Glu	Thr	Asn	Leu	Gly	Asn
			420						425				430		
Ala	Ile	Ala	Asp	Ala	Met	Glu	Ala	Tyr	Gly	Val	Lys	Asn	Phe	Ser	Lys
		435					440					445			
Lys	Thr	Asp	Phe	Ala	Val	Thr	Asn	Gly	Gly	Gly	Ile	Arg	Ala	Ser	Ile
	450					455					460				
Ala	Lys	Gly	Lys	Val	Thr	Arg	Tyr	Asp	Leu	Ile	Ser	Val	Leu	Pro	Phe
	465				470					475				480	
Gly	Asn	Thr	Ile	Ala	Gln	Ile	Asp	Val	Lys	Gly	Ser	Asp	Val	Trp	Thr
			485						490					495	
Ala	Phe	Glu	His	Ser	Leu	Gly	Ala	Pro	Thr	Thr	Gln	Lys	Asp	Gly	Lys
			500						505					510	
Thr	Val	Leu	Thr	Ala	Asn	Gly	Gly	Leu	Leu	His	Ile	Ser	Asp	Ser	Ile
		515					520						525		
Arg	Val	Tyr	Tyr	Asp	Ile	Asn	Lys	Pro	Ser	Gly	Lys	Arg	Ile	Asn	Ala
	530					535					540				
Ile	Gln	Ile	Leu	Asn	Lys	Glu	Thr	Gly	Lys	Phe	Glu	Asn	Ile	Asp	Leu
	545				550					555				560	
Lys	Arg	Val	Tyr	His	Val	Thr	Met	Asn	Asp	Phe	Thr	Ala	Ser	Gly	Gly
			565						570					575	
Asp	Gly	Tyr	Ser	Met	Phe	Gly	Gly	Pro	Arg	Glu	Glu	Gly	Ile	Ser	Leu
			580						585					590	
Asp	Gln	Val	Leu	Ala	Ser	Tyr	Leu	Lys	Thr	Ala	Asn	Leu	Ala	Lys	Tyr
		595					600							605	

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Asp Thr Thr Glu Pro Gln Arg Met Leu Leu Gly Lys Pro Ala Val Ser
 610 615 620
 Glu Gln Pro Ala Lys Gly Gln Gln Gly Ser Lys Gly Ser Lys Ser Gly
 625 630 635 640
 Lys Asp Thr Gln Pro Ile Gly Asp Asp Lys Val Met Asp Pro Ala Lys
 645 650 655
 Lys Pro Ala Pro Gly Lys Val Val Leu Leu Leu Ala His Arg Gly Thr
 660 665 670
 Val Ser Ser Gly Thr Glu Gly Ser Gly Arg Thr Ile Glu Gly Ala Thr
 675 680 685
 Val Ser Ser Lys Ser Gly Lys Gln Leu Ala Arg Met Ser Val Pro Lys
 690 695 700
 Gly Ser Ala His Glu Lys Gln Leu Pro Lys Thr Gly Thr Asn Gln Ser
 705 710 715 720
 Ser Ser Pro Glu Ala Met Phe Val Leu Leu Ala Gly Ile Gly Leu Ile
 725 730 735
 Ala Thr Val Arg Arg Arg Lys Ala Ser
 740 745

<210> SEQ ID NO 29
 <211> LENGTH: 628
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 29

Met Ser Asp Arg Phe Ile Lys Phe Asn Asp Glu Gln Leu Asp Ala Lys
 1 5 10 15
 Gln Val Met Met Leu Gln Asp Leu Ala Arg Leu Leu Leu Lys Asn Glu
 20 25 30
 Gln Thr Gln Val Lys Ile Gln Lys Phe Pro Tyr Tyr Asn Pro Val Gln
 35 40 45
 Asn Val Leu Ile Thr Ser Trp Phe Trp Ser His Arg Pro Ser His Ile
 50 55 60
 Glu Met Ala Gly Leu Lys Thr Asp Val Met Leu Ala Ala Tyr Gly Tyr
 65 70 75 80
 His Met Met Asp Val Gln Ile Val Asn Glu Val Val Gln Asp Lys Thr
 85 90 95
 Phe Lys His Pro Lys Phe Tyr Gln Gln Leu Phe Lys Leu Leu Glu Asp
 100 105 110
 Met Arg Val Leu Asn Ser Ile Lys Val Glu Arg Pro Ser Thr Ala Lys
 115 120 125
 Leu Ile Asp Leu Arg Leu Asp Thr Arg Ile Ser Tyr Thr Glu Ser Gln
 130 135 140
 Ile Lys Val Tyr Arg Thr Lys Thr Gln Tyr Thr Asp Leu Leu Phe Leu
 145 150 155 160
 Tyr Leu Glu His Ala Phe Leu Ser Gln Asp Phe Phe Asp Ile Pro Ser
 165 170 175
 Ile His Ser Asp Leu Asp Asp Ile Leu Val Asn Met Phe Leu Tyr Leu
 180 185 190
 Pro Asn Phe Phe Gln Asn Gln Asn Ser Glu Asp Asn Met Tyr Leu Ala
 195 200 205
 Gln Arg Ile Met Tyr Gln Val Asp Asp Ile Leu Lys Glu Asp Met Leu
 210 215 220
 Asn Glu Tyr Tyr Tyr Leu Pro Lys Thr Leu Tyr Asn Thr Leu Ala Ser

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225	230	235	240
Pro Glu Phe Asp Asp Leu Lys Arg Thr Asp Ala Ser Gln Val Asp Gly	245	250	255
Gln Asp Asp Thr Ser Glu Asp Asp Asp Asn Glu Ser Glu Lys Ala Asp	260	265	270
Ser Lys Ser Ala Asp Ser Glu Ser Lys Gly Gly Ala Tyr Leu Glu Met	275	280	285
Glu Leu His Glu Gly Gln Asn Ser Glu Thr Leu Gly Asn Asp Glu Ala	290	295	300
Arg Glu Gly Asp Ala Thr Asp Asp Met Thr Asp Met Met Thr Lys Lys	305	310	315
Gly Lys Gly Ser Asn Asp Thr Leu Asn Arg Glu Glu Gly Asp Ala Val	325	330	335
Gly Gln Ser Gln Ala Phe Gln Leu Asp Gly Val Asn Lys Asn Val Glu	340	345	350
Ile Lys Trp Gln Ile Pro Glu Ile Glu Pro Gln Tyr Val Leu Glu Tyr	355	360	365
Gln Glu Ser Lys Gln Asp Val Gln Tyr Glu Ile Lys Asp Leu Ile Gln	370	375	380
Ile Ile Lys Lys Thr Ile Glu Arg Glu Gln Arg Asp Ala Arg Phe Asn	385	390	395
Leu Thr Lys Gly Arg Leu Gln Lys Asp Leu Ile Asn Trp Phe Ile Asp	405	410	415
Asp Gln Tyr Lys Leu Phe Tyr Lys Lys Gln Asp Leu Ser Lys Ser Phe	420	425	430
Asp Ala Thr Phe Thr Leu Leu Ile Asp Ala Ser Ala Ser Met His Asp	435	440	445
Lys Met Ala Glu Thr Lys Lys Gly Val Val Leu Phe His Glu Thr Leu	450	455	460
Lys Ala Leu Asn Ile Lys His Glu Ile Leu Ser Phe Ser Glu Asp Ala	465	470	475
Phe Asp Ser Asp Glu His Ala Gln Pro Asn Ile Ile Asn Glu Ile Ile	485	490	495
Asn Tyr Asp Tyr Ser Thr Phe Glu Lys Asp Gly Pro Arg Ile Met Ala	500	505	510
Leu Glu Pro Gln Asp Asp Asn Arg Asp Gly Val Ala Ile Arg Val Ala	515	520	525
Ser Glu Arg Leu Met Arg Arg Asn Gln His Gln Arg Phe Leu Ile Val	530	535	540
Phe Ser Asp Gly Glu Pro Ser Ala Phe Asn Tyr Ser Gln Asp Gly Ile	545	550	555
Ile Asp Thr Tyr Glu Ala Val Glu Met Ser Arg Lys Phe Gly Ile Glu	565	570	575
Val Phe Asn Val Phe Leu Ser Gln Asp Pro Ile Thr Glu Asp Val Glu	580	585	590
Gln Thr Ile His Asn Ile Tyr Gly Gln Tyr Ala Ile Phe Val Glu Gly	595	600	605
Val Ala His Leu Pro Gly His Leu Ser Pro Leu Leu Lys Lys Leu Leu	610	615	620
Leu Lys Ser Leu			
625			

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<211> LENGTH: 154

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 30

Ala Glu Ile Asn Lys Gln Thr Thr Ser Gln Gly Val Thr Thr Glu Lys
 1 5 10 15
 Asn Asn Gly Ile Ala Val Leu Glu Gln Asp Val Ile Thr Pro Thr Val
 20 25 30
 Lys Pro Gln Ala Lys Gln Asp Ile Ile Gln Ala Val Thr Thr Arg Lys
 35 40 45
 Gln Gln Ile Lys Lys Ser Asn Ala Ser Leu Gln Asp Glu Lys Asp Val
 50 55 60
 Ala Asn Asp Lys Ile Gly Lys Ile Glu Thr Lys Ala Ile Lys Asp Ile
 65 70 75 80
 Asp Ala Ala Thr Thr Asn Ala Gln Val Glu Ala Ile Lys Thr Lys Ala
 85 90 95
 Ile Asn Asp Ile Asn Gln Thr Thr Pro Ala Thr Thr Ala Lys Ala Ala
 100 105 110
 Ala Leu Glu Glu Phe Asp Glu Val Val Gln Ala Gln Ile Asp Gln Ala
 115 120 125
 Pro Leu Asn Pro Asp Thr Thr Asn Glu Glu Val Ala Glu Ala Ile Glu
 130 135 140
 Arg Ile Asn Ala Ala Lys Val Ser Gly Val
 145 150

<210> SEQ ID NO 31

<211> LENGTH: 584

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 31

Met Lys Phe Lys Ser Leu Ile Thr Thr Thr Leu Ala Leu Gly Val Leu
 1 5 10 15
 Ala Ser Thr Gly Ala Asn Phe Asn Asn Asn Glu Ala Ser Ala Ala Ala
 20 25 30
 Lys Pro Leu Asp Lys Ser Ser Ser Ser Leu His His Gly Tyr Ser Lys
 35 40 45
 Val His Val Pro Tyr Ala Ile Thr Val Asn Gly Thr Ser Gln Asn Ile
 50 55 60
 Leu Ser Ser Leu Thr Phe Asn Lys Asn Gln Asn Ile Ser Tyr Lys Asp
 65 70 75 80
 Leu Glu Asp Arg Val Lys Ser Val Leu Lys Ser Asp Arg Gly Ile Ser
 85 90 95
 Asp Ile Asp Leu Arg Leu Ser Lys Gln Ala Lys Tyr Thr Val Tyr Phe
 100 105 110
 Lys Asn Gly Thr Lys Lys Val Ile Asp Leu Lys Ala Gly Ile Tyr Thr
 115 120 125
 Ala Asp Leu Ile Asn Thr Ser Glu Ile Lys Ala Ile Asn Ile Asn Val
 130 135 140
 Asp Thr Lys Lys Gln Val Glu Asp Lys Lys Lys Asp Lys Ala Asn Tyr
 145 150 155 160
 Gln Val Pro Tyr Thr Ile Thr Val Asn Gly Thr Ser Gln Asn Ile Leu
 165 170 175
 Ser Asn Leu Thr Phe Asn Lys Asn Gln Asn Ile Ser Tyr Lys Asp Leu
 180 185 190

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Glu Asp Lys Val Lys Ser Val Leu Glu Ser Asn Arg Gly Ile Thr Asp
 195 200 205
 Val Asp Leu Arg Leu Ser Lys Gln Ala Lys Tyr Thr Val Asn Phe Lys
 210 215 220
 Asn Gly Thr Lys Lys Val Ile Asp Leu Lys Ser Gly Ile Tyr Thr Ala
 225 230 235 240
 Asn Leu Ile Asn Ser Ser Asp Ile Lys Ser Ile Asn Ile Asn Val Asp
 245 250 255
 Thr Lys Lys His Ile Glu Asn Lys Ala Lys Arg Asn Tyr Gln Val Pro
 260 265 270
 Tyr Ser Ile Asn Leu Asn Gly Thr Ser Thr Asn Ile Leu Ser Asn Leu
 275 280 285
 Ser Phe Ser Asn Lys Pro Trp Thr Asn Tyr Lys Asn Leu Thr Ser Gln
 290 295 300
 Ile Lys Ser Val Leu Lys His Asp Arg Gly Ile Ser Glu Gln Asp Leu
 305 310 315 320
 Lys Tyr Ala Lys Lys Ala Tyr Tyr Thr Val Tyr Phe Lys Asn Gly Gly
 325 330 335
 Lys Arg Ile Leu Gln Leu Asn Ser Lys Asn Tyr Thr Ala Asn Leu Val
 340 345 350
 His Ala Lys Asp Val Lys Arg Ile Glu Ile Thr Val Lys Thr Gly Thr
 355 360 365
 Lys Ala Lys Ala Asp Arg Tyr Val Pro Tyr Thr Ile Ala Val Asn Gly
 370 375 380
 Thr Ser Thr Pro Ile Leu Ser Asp Leu Lys Phe Thr Gly Asp Pro Arg
 385 390 395 400
 Val Gly Tyr Lys Asp Ile Ser Lys Lys Val Lys Ser Val Leu Lys His
 405 410 415
 Asp Arg Gly Ile Gly Glu Arg Glu Leu Lys Tyr Ala Lys Lys Ala Thr
 420 425 430
 Tyr Thr Val His Phe Lys Asn Gly Thr Lys Lys Val Ile Asn Ile Asn
 435 440 445
 Ser Asn Ile Ser Gln Leu Asn Leu Leu Tyr Val Gln Asp Ile Lys Lys
 450 455 460
 Ile Asp Ile Asp Val Lys Thr Gly Thr Lys Ala Lys Ala Asp Ser Tyr
 465 470 475 480
 Val Pro Tyr Thr Ile Ala Val Asn Gly Thr Ser Thr Pro Ile Leu Ser
 485 490 495
 Lys Leu Lys Ile Ser Asn Lys Gln Leu Ile Ser Tyr Lys Tyr Leu Asn
 500 505 510
 Asp Lys Val Lys Ser Val Leu Lys Ser Glu Arg Gly Ile Ser Asp Leu
 515 520 525
 Asp Leu Lys Phe Ala Lys Gln Ala Lys Tyr Thr Val Tyr Phe Lys Asn
 530 535 540
 Gly Lys Lys Gln Val Val Asn Leu Lys Ser Asp Ile Phe Thr Pro Asn
 545 550 555 560
 Leu Phe Ser Ala Lys Asp Ile Lys Lys Ile Asp Ile Asp Val Lys Gln
 565 570 575
 Tyr Thr Lys Ser Lys Lys Asn Lys
 580

<210> SEQ ID NO 32

<211> LENGTH: 508

-continued

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 32

Met Lys Asn Lys Leu Leu Val Leu Ser Leu Gly Ala Leu Cys Val Ser
1 5 10 15
Gln Ile Trp Glu Ser Asn Arg Ala Ser Ala Val Val Ser Gly Glu Lys
20 25 30
Asn Pro Tyr Val Ser Glu Ser Leu Lys Leu Thr Asn Asn Lys Asn Lys
35 40 45
Ser Arg Thr Val Glu Glu Tyr Lys Lys Ser Leu Asp Asp Leu Ile Trp
50 55 60
Ser Phe Pro Asn Leu Asp Asn Glu Arg Phe Asp Asn Pro Glu Tyr Lys
65 70 75 80
Glu Ala Met Lys Lys Tyr Gln Gln Arg Phe Met Ala Glu Asp Glu Ala
85 90 95
Leu Lys Lys Phe Phe Ser Glu Glu Lys Lys Ile Lys Asn Gly Asn Thr
100 105 110
Asp Asn Leu Asp Tyr Leu Gly Leu Ser His Glu Arg Tyr Glu Ser Val
115 120 125
Phe Asn Thr Leu Lys Lys Gln Ser Glu Glu Phe Leu Lys Glu Ile Glu
130 135 140
Asp Ile Lys Lys Asp Asn Pro Glu Leu Lys Asp Phe Asn Glu Glu Glu
145 150 155 160
Gln Leu Lys Cys Asp Leu Glu Leu Asn Lys Leu Glu Asn Gln Ile Leu
165 170 175
Met Leu Gly Lys Thr Phe Tyr Gln Asn Tyr Arg Asp Asp Val Glu Ser
180 185 190
Leu Tyr Ser Lys Leu Asp Leu Ile Met Gly Tyr Lys Asp Glu Glu Arg
195 200 205
Ala Asn Lys Lys Ala Val Asn Lys Arg Met Leu Glu Asn Lys Lys Glu
210 215 220
Asp Leu Glu Thr Ile Ile Asp Glu Phe Phe Ser Asp Ile Asp Lys Thr
225 230 235 240
Arg Pro Asn Asn Ile Pro Val Leu Glu Asp Glu Lys Gln Glu Glu Lys
245 250 255
Asn His Lys Asn Met Ala Gln Leu Lys Ser Asp Thr Glu Ala Ala Lys
260 265 270
Ser Asp Glu Ser Lys Arg Ser Lys Arg Ser Lys Arg Ser Leu Asn Thr
275 280 285
Gln Asn His Lys Pro Ala Ser Gln Glu Val Ser Glu Gln Gln Lys Ala
290 295 300
Glu Tyr Asp Lys Arg Ala Glu Glu Arg Lys Ala Arg Phe Leu Asp Asn
305 310 315 320
Gln Lys Ile Lys Lys Thr Pro Val Val Ser Leu Glu Tyr Asp Phe Glu
325 330 335
His Lys Gln Arg Ile Asp Asn Glu Asn Asp Lys Lys Leu Val Val Ser
340 345 350
Ala Pro Thr Lys Lys Pro Thr Ser Pro Thr Thr Tyr Thr Glu Thr Thr
355 360 365
Thr Gln Val Pro Met Pro Thr Val Glu Arg Gln Thr Gln Gln Gln Ile
370 375 380
Ile Tyr Asn Ala Pro Lys Gln Leu Ala Gly Leu Asn Gly Glu Ser His
385 390 395 400

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260					265					270					
Lys	Leu	Asn	Asp	Ala	Gln	Ala	Pro	Lys	Ala	Asp	Asn	Lys	Phe	Asn	Lys
	275						280					285			
Glu	Gln	Gln	Asn	Ala	Phe	Tyr	Glu	Ile	Leu	His	Leu	Pro	Asn	Leu	Thr
	290					295						300			
Glu	Glu	Gln	Arg	Asn	Gly	Phe	Ile	Gln	Ser	Leu	Lys	Asp	Asp	Pro	Ser
	305					310						315			320
Val	Ser	Lys	Glu	Ile	Leu	Ala	Glu	Ala	Lys	Lys	Leu	Asn	Asp	Ala	Gln
				325					330					335	
Ala	Pro	Lys	Glu	Glu	Asp	Asn	Asn	Lys	Pro	Gly	Lys	Glu	Asp	Gly	Asn
			340					345					350		
Lys	Pro	Gly	Lys	Glu	Asp	Asn	Asn	Lys	Pro	Gly	Lys	Glu	Asp	Asn	Lys
		355					360						365		
Lys	Pro	Gly	Lys	Glu	Asp	Asn	Asn	Lys	Pro	Gly	Lys	Glu	Asp	Asn	Asn
	370					375						380			
Lys	Pro	Gly	Lys	Glu	Asp	Gly	Asn	Lys	Pro	Gly	Lys	Glu	Asp	Asn	Lys
	385					390					395				400
Lys	Pro	Gly	Lys	Glu	Asp	Asn	Asn	Lys	Pro	Gly	Lys	Glu	Asp	Gly	Asn
			405						410					415	
Lys	Pro	Gly	Lys	Glu	Asp	Gly	Asn	Gly	Val	His	Val	Val	Lys	Pro	Gly
			420					425						430	
Asp	Thr	Val	Asn	Asp	Ile	Ala	Lys	Ala	Asn	Gly	Thr	Thr	Ala	Asp	Lys
		435					440						445		
Ile	Ala	Ala	Asp	Asn	Lys	Leu	Ala	Asp	Lys	Asn	Met	Ile	Lys	Pro	Gly
	450					455					460				
Gln	Glu	Leu	Val	Val	Asp	Lys	Lys	Gln	Pro	Ala	Asn	His	Ala	Asp	Ala
	465					470					475				480
Asn	Lys	Ala	Gln	Ala	Leu	Pro	Glu	Thr	Gly	Glu	Glu	Asn	Pro	Phe	Ile
			485						490					495	
Gly	Thr	Thr	Val	Phe	Gly	Gly	Leu	Ser	Leu	Ala	Leu	Gly	Ala	Ala	Leu
			500					505					510		
Leu	Ala	Gly	Arg	Arg	Arg	Glu	Leu								
		515					520								

<210> SEQ ID NO 34

<211> LENGTH: 291

<212> TYPE: PRT

<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 34

Ala	Gln	His	Asp	Glu	Ala	Lys	Lys	Asn	Ala	Phe	Tyr	Gln	Val	Leu	Asn
1				5					10					15	
Met	Pro	Asn	Leu	Asn	Ala	Asp	Gln	Arg	Asn	Gly	Phe	Ile	Gln	Ser	Leu
			20					25					30		
Lys	Ala	Ala	Pro	Ser	Gln	Ser	Ala	Asn	Val	Leu	Gly	Glu	Ala	Gln	Lys
			35				40						45		
Leu	Asn	Asp	Ser	Gln	Ala	Pro	Lys	Ala	Asp	Ala	Gln	Gln	Asn	Asn	Phe
	50					55					60				
Asn	Lys	Asp	Lys	Lys	Ser	Ala	Phe	Tyr	Glu	Ile	Leu	Asn	Met	Pro	Asn
	65				70						75				80
Leu	Asn	Glu	Ala	Gln	Arg	Asn	Gly	Phe	Ile	Gln	Ser	Leu	Lys	Ala	Ala
				85					90					95	
Pro	Ser	Gln	Ser	Thr	Asn	Val	Leu	Gly	Glu	Ala	Lys	Lys	Leu	Asn	Glu
				100				105						110	

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Ser Gln Ala Pro Lys Ala Asp Asn Asn Phe Asn Lys Glu Lys Lys Asn
 115 120 125

Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn Leu Asn Glu Glu Gln Arg
 130 135 140

Asn Gly Phe Ile Gln Ser Leu Lys Ala Ala Pro Ser Gln Ser Ala Asn
 145 150 155 160

Leu Leu Ser Glu Ala Lys Lys Leu Asn Glu Ser Gln Ala Pro Lys Ala
 165 170 175

Asp Asn Lys Phe Asn Lys Glu Lys Lys Asn Ala Phe Tyr Glu Ile Leu
 180 185 190

His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser
 195 200 205

Leu Lys Ala Ala Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala Lys
 210 215 220

Lys Leu Asn Asp Ala Gln Ala Pro Lys Ala Asp Asn Lys Phe Asn Lys
 225 230 235 240

Glu Lys Lys Asn Ala Phe Tyr Glu Ile Leu His Leu Pro Asn Leu Thr
 245 250 255

Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Ala Ala Pro Ser
 260 265 270

Val Ser Lys Glu Ile Leu Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln
 275 280 285

Ala Pro Lys
 290

<210> SEQ ID NO 35
 <211> LENGTH: 34
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 35

gctgcacata tggcgcaaca cgatgaagct caac

34

<210> SEQ ID NO 36
 <211> LENGTH: 30
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 36

agtggatcct tatgctttgt tagcatctgc

30

<210> SEQ ID NO 37
 <211> LENGTH: 19
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 37

Met Gly Ser Ser His His His His His Ser Ser Gly Leu Val Pro
 1 5 10 15

Arg Gly Ser

<210> SEQ ID NO 38
 <211> LENGTH: 29
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 38

aacatattgtt caacaaagat caacaaagc

29

-continued

<210> SEQ ID NO 39
 <211> LENGTH: 29
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

 <400> SEQUENCE: 39

 aaggatccag attcgtttaa ttttttagc 29

<210> SEQ ID NO 40
 <211> LENGTH: 43
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

 <400> SEQUENCE: 40

 cttcattcaa agtcttaaag cgcgcccaag ccaaagcact aac 43

<210> SEQ ID NO 41
 <211> LENGTH: 43
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

 <400> SEQUENCE: 41

 gttagtgett tggcttgggg cggctttaag actttgaatg aag 43

<210> SEQ ID NO 42
 <211> LENGTH: 42
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

 <400> SEQUENCE: 42

 catatgttca acaaagataa aaaaagcgcc ttctatgaaa tc 42

<210> SEQ ID NO 43
 <211> LENGTH: 42
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

 <400> SEQUENCE: 43

 gatttcatag aaggcgcttt ttttatcttt gttgaacata tg 42

<210> SEQ ID NO 44
 <211> LENGTH: 42
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

 <400> SEQUENCE: 44

 catatgttca acaaagatgg aggaagcgcc ttctatgaaa tc 42

<210> SEQ ID NO 45
 <211> LENGTH: 42
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

 <400> SEQUENCE: 45

 gatttcatag aaggcgcttc ctccatcttt gttgaacata tg 42

<210> SEQ ID NO 46
 <211> LENGTH: 52
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

 <400> SEQUENCE: 46

 ggggacaagt ttgtacaaaa aagcaggctg atgactaagt tgaaaaaaga ag 52

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<210> SEQ ID NO 47
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.
<400> SEQUENCE: 47
aaggatcccc tccaaaatgt aattgccc 28

<210> SEQ ID NO 48
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.
<400> SEQUENCE: 48
aaggatccgt ttgtaactct atccaaagac 30

<210> SEQ ID NO 49
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.
<400> SEQUENCE: 49
ggggaccact ttgtacaaga aagctgggtg acacctattg caggattcg 49

<210> SEQ ID NO 50
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.
<400> SEQUENCE: 50
ggggacaagt ttgtacaaaa aagcaggctc agatagcgat tcagattcag 50

<210> SEQ ID NO 51
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.
<400> SEQUENCE: 51
aaggatccct gtattttctc cttaattttc c 31

<210> SEQ ID NO 52
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.
<400> SEQUENCE: 52
aaggatccca tggctgcaaa gcaaataatg 30

<210> SEQ ID NO 53
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.
<400> SEQUENCE: 53
ggggaccact ttgtacaaga aagctgggtg ccoctgggtgta acaaatttat g 51

<210> SEQ ID NO 54
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.
<400> SEQUENCE: 54

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gaaggatccg tttattctag ttaatatata gttaatg	37
<210> SEQ ID NO 55 <211> LENGTH: 33 <212> TYPE: DNA <213> ORGANISM: Staphylococcus sp.	
<400> SEQUENCE: 55 gaactgcagc tgtatgtctt tggatagagt tac	
	33
<210> SEQ ID NO 56 <211> LENGTH: 33 <212> TYPE: DNA <213> ORGANISM: Staphylococcus sp.	
<400> SEQUENCE: 56 gaaggatccg gtggcttttt tacttggatt ttc	
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<210> SEQ ID NO 57 <211> LENGTH: 33 <212> TYPE: DNA <213> ORGANISM: Staphylococcus sp.	
<400> SEQUENCE: 57 gaactgcagc gacaaactca ttatttgcctt tgc	
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<210> SEQ ID NO 58 <211> LENGTH: 27 <212> TYPE: DNA <213> ORGANISM: Staphylococcus sp.	
<400> SEQUENCE: 58 gaactcgagt ctagcttatt tacatgg	
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<210> SEQ ID NO 59 <211> LENGTH: 45 <212> TYPE: DNA <213> ORGANISM: Staphylococcus sp.	
<400> SEQUENCE: 59 gaactcgaga tagaaggcag aatagtaaca aaggattata gtggg	
	45
<210> SEQ ID NO 60 <211> LENGTH: 27 <212> TYPE: DNA <213> ORGANISM: Staphylococcus sp.	
<400> SEQUENCE: 60 gtaggatcct gggatagagt tacaaaac	
	27
<210> SEQ ID NO 61 <211> LENGTH: 34 <212> TYPE: DNA <213> ORGANISM: Staphylococcus sp.	
<400> SEQUENCE: 61 gaactcgagg cattatgtgt atcacaatt tggg	
	34
<210> SEQ ID NO 62 <211> LENGTH: 43 <212> TYPE: DNA <213> ORGANISM: Staphylococcus sp.	
<400> SEQUENCE: 62	

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gaactcgaga tagaaggcag agtggtttct ggggagaaga atc 43

<210> SEQ ID NO 63
 <211> LENGTH: 33
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 63

gaactcgagg cagccatgca ttaattattt gcc 33

<210> SEQ ID NO 64
 <211> LENGTH: 677
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 64

Met Lys Ser Asn Leu Arg Tyr Gly Ile Arg Lys His Lys Leu Gly Ala
 1 5 10 15

Ala Ser Val Phe Leu Gly Thr Met Ile Val Val Gly Met Gly Gln Glu
 20 25 30

Lys Glu Ala Ala Ala Ser Glu Gln Asn Asn Thr Thr Val Glu Glu Ser
 35 40 45

Gly Ser Ser Ala Thr Glu Ser Lys Ala Ser Glu Thr Gln Thr Thr Thr
 50 55 60

Asn Asn Val Asn Thr Ile Asp Glu Thr Gln Ser Tyr Ser Ala Thr Ser
 65 70 75 80

Thr Glu Gln Pro Ser Gln Ser Thr Gln Val Thr Thr Glu Glu Ala Pro
 85 90 95

Lys Thr Val Gln Ala Pro Lys Val Glu Thr Ser Arg Val Asp Leu Pro
 100 105 110

Ser Glu Lys Val Ala Asp Lys Glu Thr Thr Gly Thr Gln Val Asp Ile
 115 120 125

Ala Gln Pro Ser Asn Val Ser Glu Ile Lys Pro Arg Met Lys Arg Ser
 130 135 140

Thr Asp Val Thr Ala Val Ala Glu Lys Glu Val Val Glu Glu Thr Lys
 145 150 155 160

Ala Thr Gly Thr Asp Val Thr Asn Lys Val Glu Val Glu Glu Gly Ser
 165 170 175

Glu Ile Val Gly His Lys Gln Asp Thr Asn Val Val Asn Pro His Asn
 180 185 190

Ala Glu Arg Val Thr Leu Lys Tyr Lys Trp Lys Phe Gly Glu Gly Ile
 195 200 205

Lys Ala Gly Asp Tyr Phe Asp Phe Thr Leu Ser Asp Asn Val Glu Thr
 210 215 220

His Gly Ile Ser Thr Leu Arg Lys Val Pro Glu Ile Lys Ser Thr Asp
 225 230 235 240

Gly Gln Val Met Ala Thr Gly Glu Ile Ile Gly Glu Arg Lys Val Arg
 245 250 255

Tyr Thr Phe Lys Glu Tyr Val Gln Glu Lys Lys Asp Leu Thr Ala Glu
 260 265 270

Leu Ser Leu Asn Leu Phe Ile Asp Pro Thr Thr Val Thr Gln Lys Gly
 275 280 285

Asn Gln Asn Val Glu Val Lys Leu Gly Glu Thr Thr Val Ser Lys Ile
 290 295 300

Phe Asn Ile Gln Tyr Leu Gly Gly Val Arg Asp Asn Trp Gly Val Thr

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305		310		315		320
Ala Asn Gly Arg Ile Asp Thr Leu Asn Lys Val Asp Gly Lys Phe Ser						
		325		330		335
His Phe Ala Tyr Met Lys Pro Asn Asn Gln Ser Leu Ser Ser Val Thr						
		340		345		350
Val Thr Gly Gln Val Thr Lys Gly Asn Lys Pro Gly Val Asn Asn Pro						
		355		360		365
Thr Val Lys Val Tyr Lys His Ile Gly Ser Asp Asp Leu Ala Glu Ser						
		370		375		380
Val Tyr Ala Lys Leu Asp Asp Val Ser Lys Phe Glu Asp Val Thr Asp						
		385		390		395
Asn Met Ser Leu Asp Phe Asp Thr Asn Gly Gly Tyr Ser Leu Asn Phe						
		405		410		415
Asn Asn Leu Asp Gln Ser Lys Asn Tyr Val Ile Lys Tyr Glu Gly Tyr						
		420		425		430
Tyr Asp Ser Asn Ala Ser Asn Leu Glu Phe Gln Thr His Leu Phe Gly						
		435		440		445
Tyr Tyr Asn Tyr Tyr Tyr Thr Ser Asn Leu Thr Trp Lys Asn Gly Val						
		450		455		460
Ala Phe Tyr Ser Asn Asn Ala Gln Gly Asp Gly Lys Asp Lys Leu Lys						
		465		470		475
Glu Pro Ile Ile Glu His Ser Thr Pro Ile Glu Leu Glu Phe Lys Ser						
		485		490		495
Glu Pro Pro Val Glu Lys His Glu Leu Thr Gly Thr Ile Glu Glu Ser						
		500		505		510
Asn Asp Ser Lys Pro Ile Asp Phe Glu Tyr His Thr Ala Val Glu Gly						
		515		520		525
Ala Glu Gly His Ala Glu Gly Thr Ile Glu Thr Glu Glu Asp Ser Ile						
		530		535		540
His Val Asp Phe Glu Glu Ser Thr His Glu Asn Ser Lys His His Ala						
		545		550		555
Asp Val Val Glu Tyr Glu Glu Asp Thr Asn Pro Gly Gly Gly Gln Val						
		565		570		575
Thr Thr Glu Ser Asn Leu Val Glu Phe Asp Glu Asp Ser Thr Lys Gly						
		580		585		590
Ile Val Thr Gly Ala Val Ser Asp His Thr Thr Ile Glu Asp Thr Lys						
		595		600		605
Glu Tyr Thr Thr Glu Ser Asn Leu Ile Glu Leu Val Asp Glu Leu Pro						
		610		615		620
Glu Glu His Gly Gln Ala Gln Gly Pro Ile Glu Glu Ile Thr Glu Asn						
		625		630		635
Asn His His Ile Ser His Ser Gly Leu Gly Thr Glu Asn Gly His Gly						
		645		650		655
Asn Tyr Gly Val Ile Glu Glu Ile Glu Glu Asn Ser His Val Asp Ile						
		660		665		670
Lys Ser Glu Leu Gly						
		675				

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The invention claimed is:

1. A variant Protein A (SpA) comprising a domain E having alanine residue substitutions at amino acid positions 33 and 34 of SEQ ID NO: 3.

2. The variant SpA of claim 1, wherein the domain E further comprises lysine residue substitutions at amino acid positions 6 and 7 of SEQ ID NO: 3; and/or further wherein the SpA variant further comprises a domain D having a lysine residue substitution at amino acid positions 9 and 10 of SEQ ID NO: 2; a domain A having a lysine residue substitution at amino acid positions 7 and 8 of SEQ ID NO: 4; a domain B having a lysine residue substitution at amino acid positions 7 and 8 of SEQ ID NO: 6, and/or a domain C having a lysine residue substitution at amino acid positions 7 and 8 of SEQ ID NO: 5.

3. The variant SpA of claim 1, comprising a domain D having an alanine residue substitution at amino acid positions 36 and 37 of SEQ ID NO: 2; a domain A having an alanine residue substitution at amino acid positions 34 and 35 of SEQ ID NO: 4; a domain B having an alanine residue substitution at amino acid positions 34 and 35 of SEQ ID NO: 6, and/or a domain C having an alanine residue substitution at amino acid positions 34 and 35 of SEQ ID NO: 5.

4. The variant SpA of claim 1,

(i) wherein the domain E comprises a lysine residue substitution at amino acid positions 6 and 7 of SEQ ID NO: 3; and/or wherein the variant SpA further comprises a domain D having a lysine residue substitution at amino acid positions 9 and 10 of SEQ ID NO: 2; a domain A having a lysine residue substitution at amino acid positions 7 and 8 of SEQ ID NO: 4; a domain B having a lysine residue substitution at

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amino acid positions 7 and 8 of SEQ ID NO: 6, and/or a domain C having a lysine residue substitution at amino acid positions 7 and 8 of SEQ ID NO: 5; and

(ii) wherein the domain E comprises an alanine residue substitution at amino acid positions 33 and 34 of SEQ ID NO: 3; and/or wherein the variant SpA further comprises a domain D having an alanine residue substitution at amino acid positions 36 and 37 of SEQ ID NO: 2; a domain A having an alanine residue substitution at amino acid positions 34 and 35 of SEQ ID NO: 4; a domain B having an alanine residue substitution at amino acid positions 34 and 35 of SEQ ID NO: 6, and/or a domain C having an alanine residue substitution at amino acid positions 34 and 35 of SEQ ID NO: 5.

5. An immunogenic composition comprising the variant SpA of claim 1.

6. An immunogenic composition according to claim 5, further comprising at least a second staphylococcal antigen.

7. An immunogenic composition according to claim 6, wherein the second staphylococcal antigen is selected from the group consisting of EsaB, Emp, EsxA, EsxB, EsaC, Eap, Ebh, Coa, vWh, Hla, SdrC, SdrE, IsdA, IsdB, IsdC, ClfA, ClfB, SasF peptide, a type V and/or a type VIII capsular polysaccharide or oligosaccharide from *S. aureus*.

8. A vaccine comprising a pharmaceutically acceptable composition having an isolated SpA variant polypeptide of claim 1, wherein the composition is capable of stimulating an immune response against a *staphylococcus* bacterium.

9. The variant SpA of claim 1, wherein the variant SpA comprises a segment of SpA comprising 5 or more IgG binding domains.

* * * * *