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

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(57) **ABSTRACT**

Disclosed are methods and compositions for treating or preventing a *Staphylococcus* bacterial infection using a non-toxigenic Protein A (SpA) variant.

14 Claims, 15 Drawing Sheets

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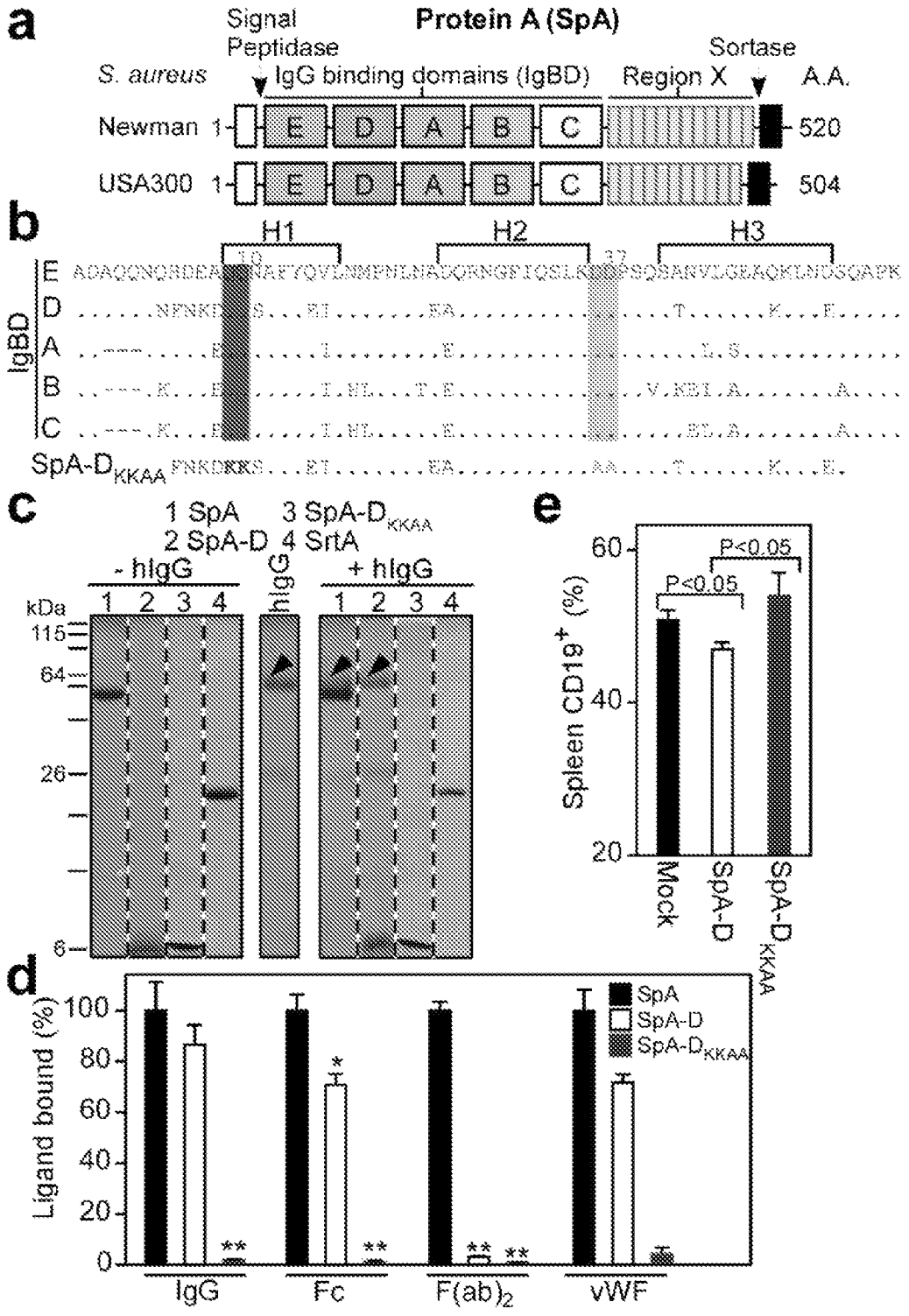

a
Signal Peptidase
Protein A (SpA)
Sortase
S. aureus
IgG binding domains (IgBD)
Region X
A.A.
Newman 1
E D A B C
520
USA300 1
E D A B C
504
b
H1
H2
H3
IgBD
E
D
A
B
C
SpA-D KKAA
c
1 SpA
3 SpA-D KKAA
2 SpA-D
4 SrtA
- hIgG
+ hIgG
kDa
115
64
26
6
hIgG
e
P<0.05
P<0.05
Spleen CD19+ (%)
60
40
20
Mock
SpA-D
SpA-D KKAA
d
Ligand bound (%)
100
80
60
40
20
0
SpA
SpA-D
SpA-D KKAA
IgG
Fc
F(ab)2
vWF

FIG. 1

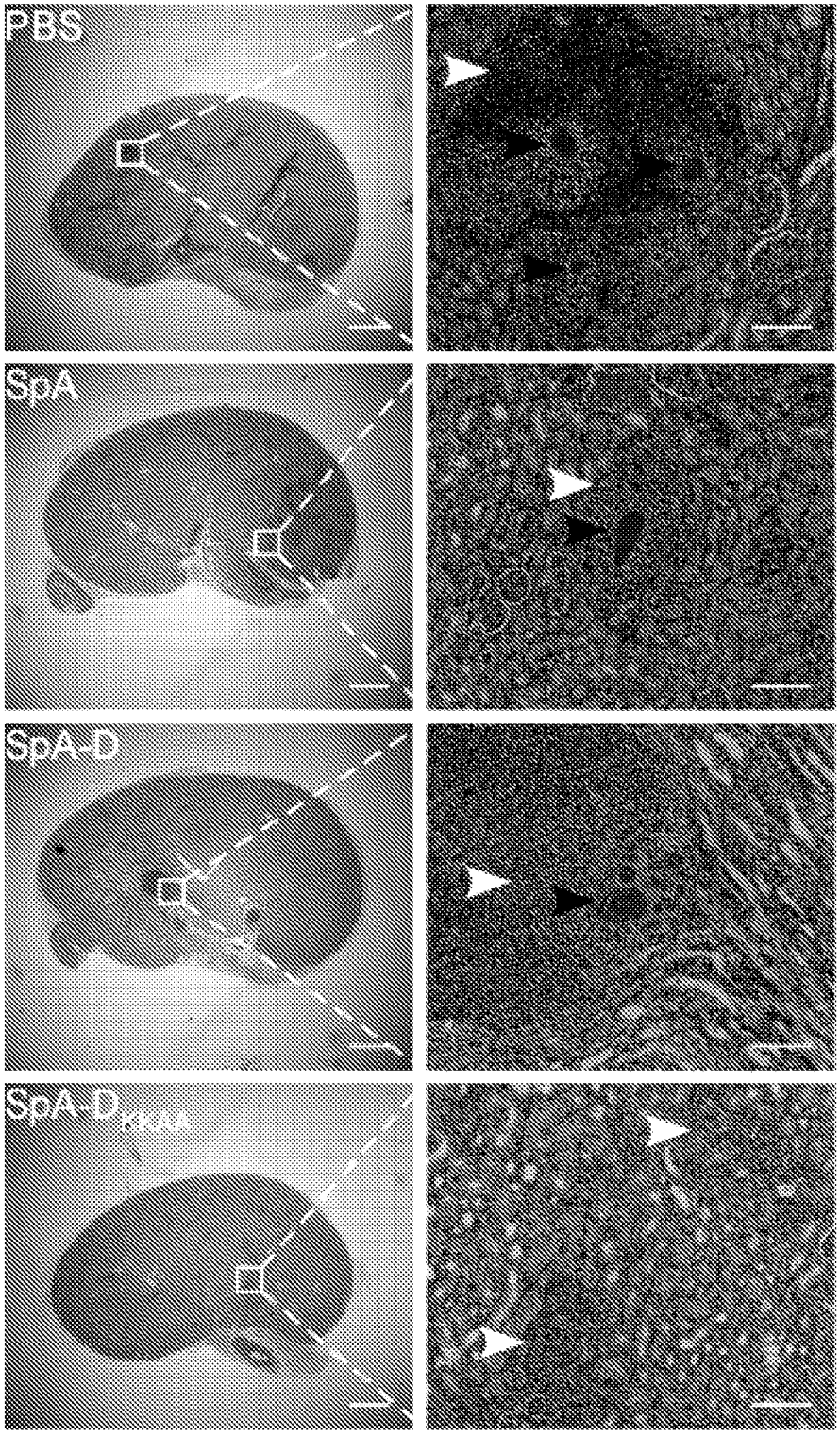


FIG. 2

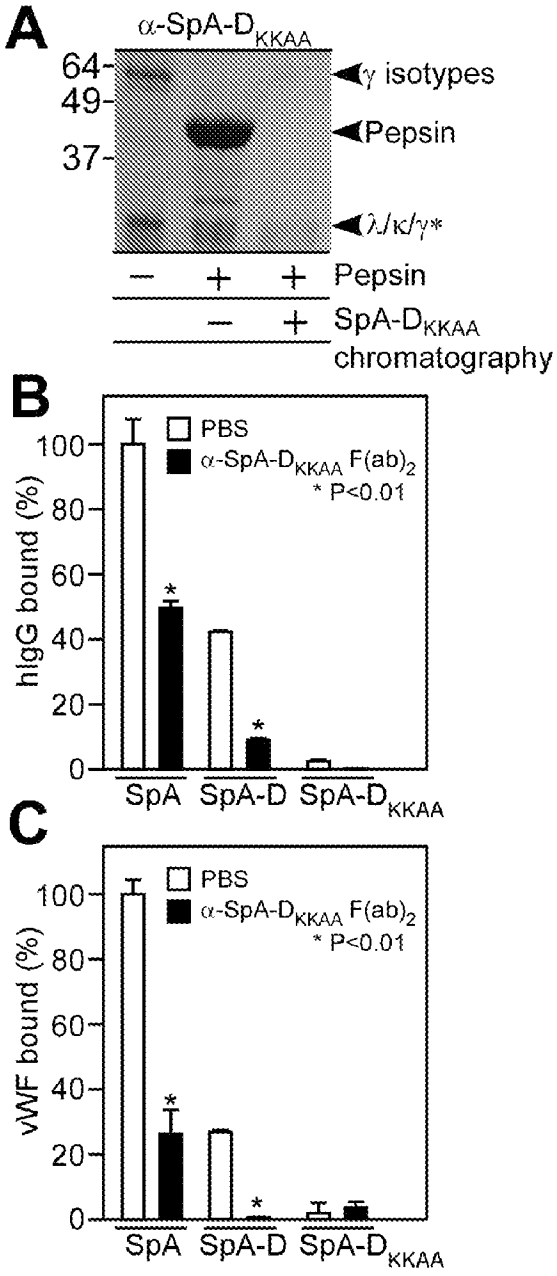

A
α-SpA-D$_{KKAA}$
64-
49-
37-
γ isotypes
Pepsin
λ/κ/γ*
− + + Pepsin
− + SpA-D$_{KKAA}$
chromatography
B
PBS
α-SpA-D$_{KKAA}$ F(ab)$_2$
* P<0.01
hIgG bound (%)
100
80
60
40
20
0
SpA
SpA-D
SpA-D$_{KKAA}$
C
PBS
α-SpA-D$_{KKAA}$ F(ab)$_2$
* P<0.01
vWF bound (%)
100
80
60
40
20
0
SpA
SpA-D
SpA-D$_{KKAA}$

FIG. 3

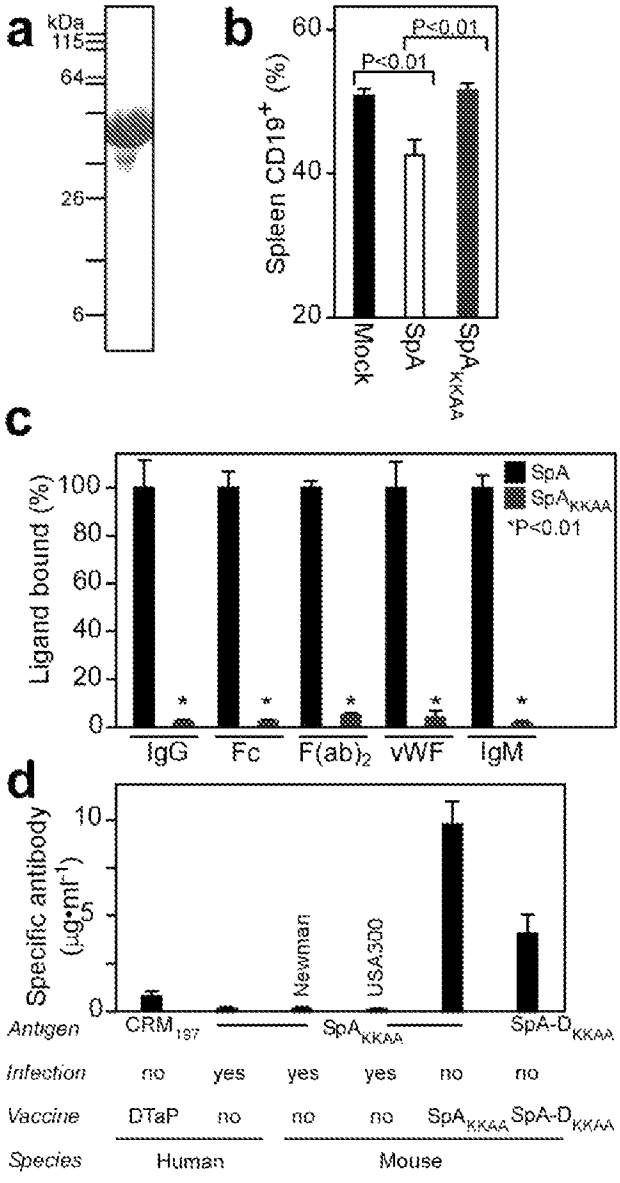

a
kDa
115
64
26
6
b
Spleen CD19+ (%)
60
40
20
P<0.01
P<0.01
Mock
SpA
SpAKKAA
c
Ligand bound (%)
100
80
60
40
20
0
SpA
SpAKKAA
*P<0.01
IgG
Fc
F(ab)2
vWF
IgM
d
Specific antibody (μg•ml-1)
10
5
0
Newman
USA300
Antigen
CRM197
SpAKKAA
SpA-DKKAA
Infection
no
yes
yes
yes
no
no
Vaccine
DTaP
no
no
no
SpAKKAA
SpA-DKKAA
Species
Human
Mouse

FIG. 4

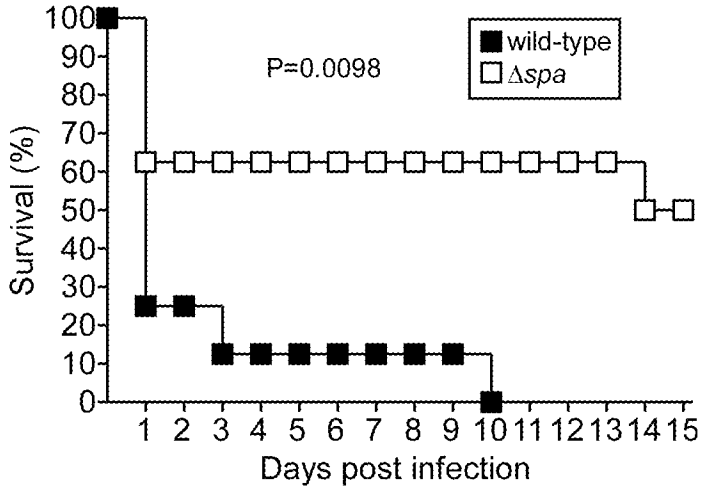

P=0.0098
wild-type
Δspa
Survival (%)
Days post infection

FIG. 5

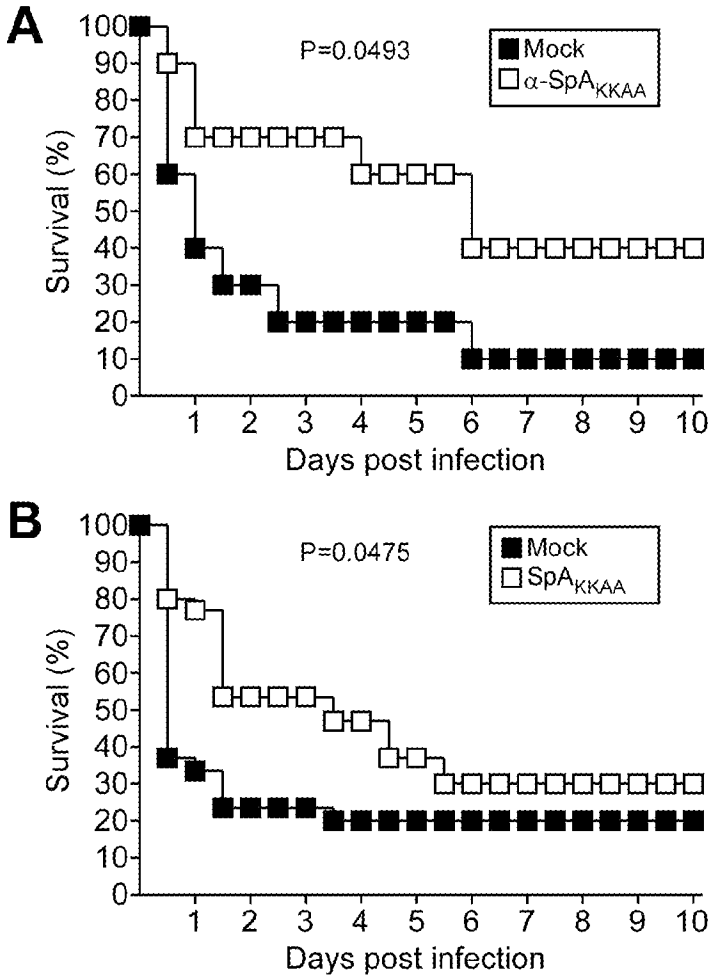

A
P=0.0493
Mock
α-SpA$_{KKAA}$
Survival (%)
Days post infection
B
P=0.0475
Mock
SpA$_{KKAA}$
Survival (%)
Days post infection

FIG. 6

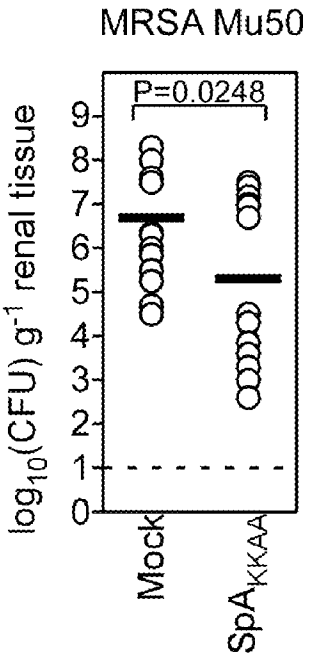

MRSA Mu50
P=0.0248
log10(CFU) g-1 renal tissue
0
1
2
3
4
5
6
7
8
9
Mock
SpAKKAA

FIG. 7

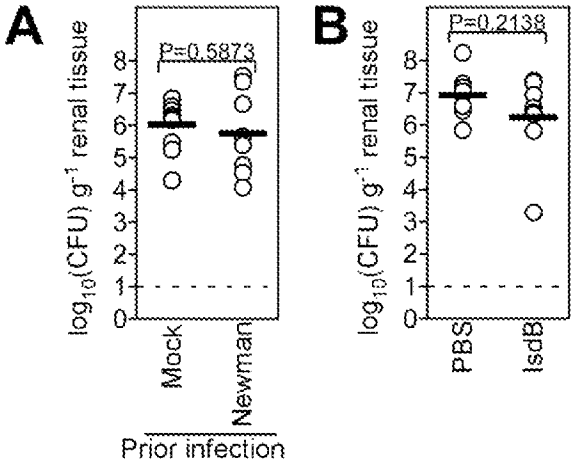

A
P=0.5873
log10(CFU) g-1 renal tissue
Mock
Newman
Prior infection
B
P=0.2138
log10(CFU) g-1 renal tissue
PBS
IsdB

FIG. 8

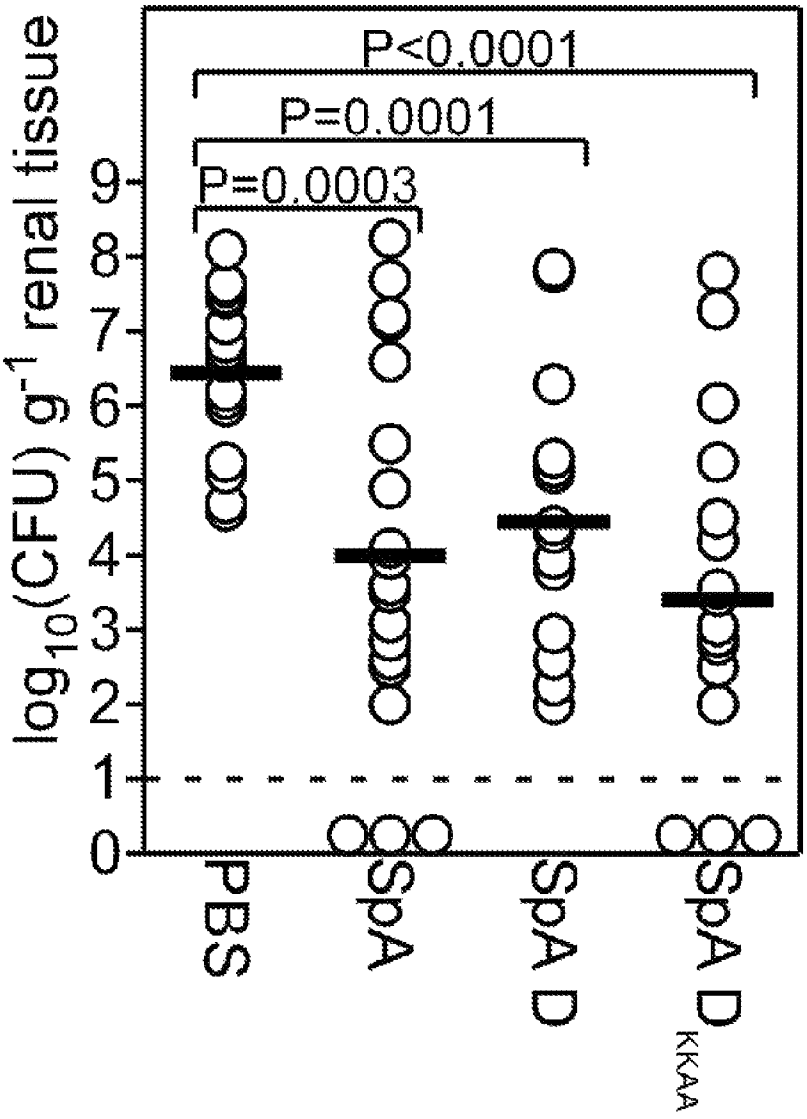

P<0.0001
P=0.0001
P=0.0003
log10(CFU) g-1 renal tissue
0
1
2
3
4
5
6
7
8
9
PBS
SpA
SpA D
SpA DKKAA

FIG. 9

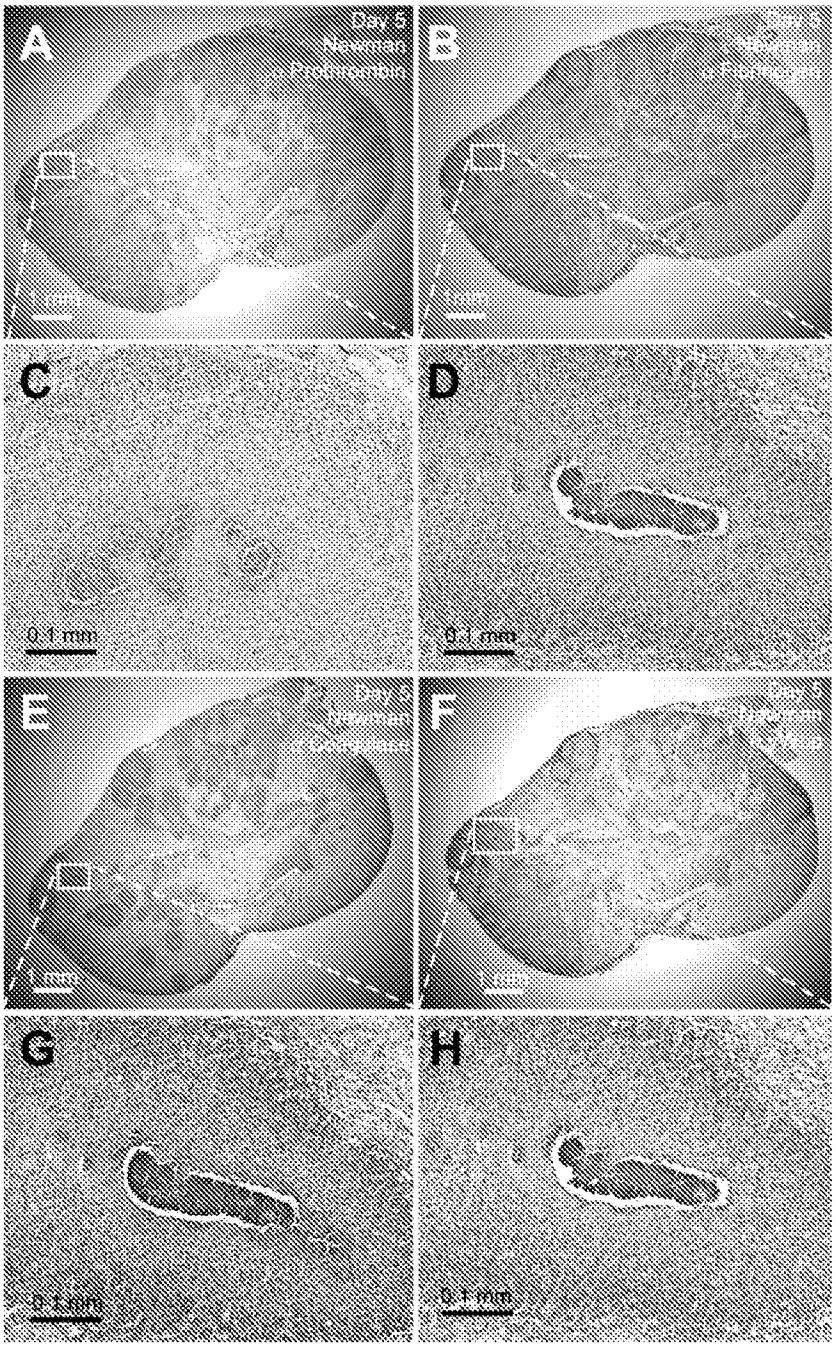
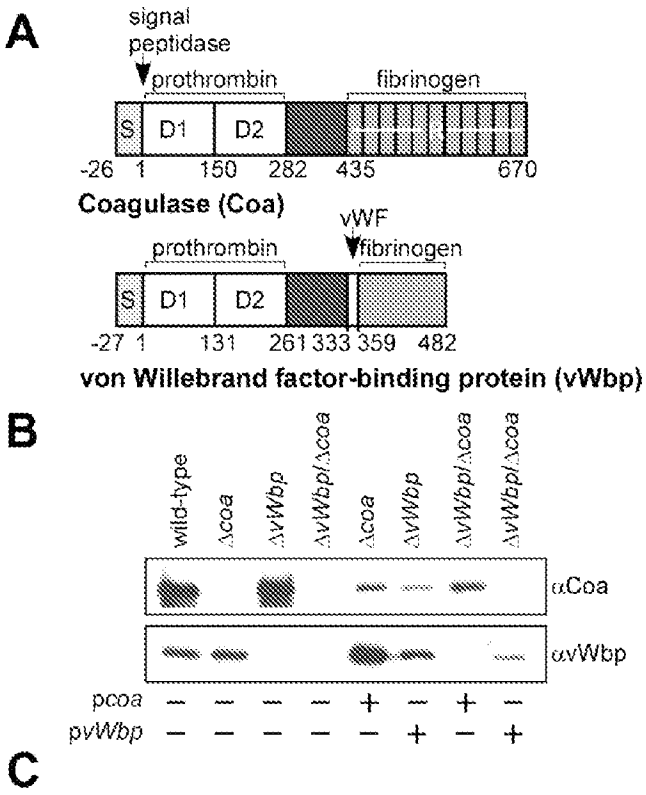


FIG. 10



S. aureus strain	*24 hours*	*Coagulation (hours)*
Mock		>48
Newman		<12
Δ*vWbp*		24
Δ*coa*		36
Δ*vWbp*/Δ*coa*		>48
Δ*vWbp*/Δ*coa*+ p*vWbp*		<12
Δ*vWbp*/Δ*coa* + p*coa*		24
Δ*vWbp* + p*vWbp*		36
Δ*coa* + p*coa*		24

FIG. 11

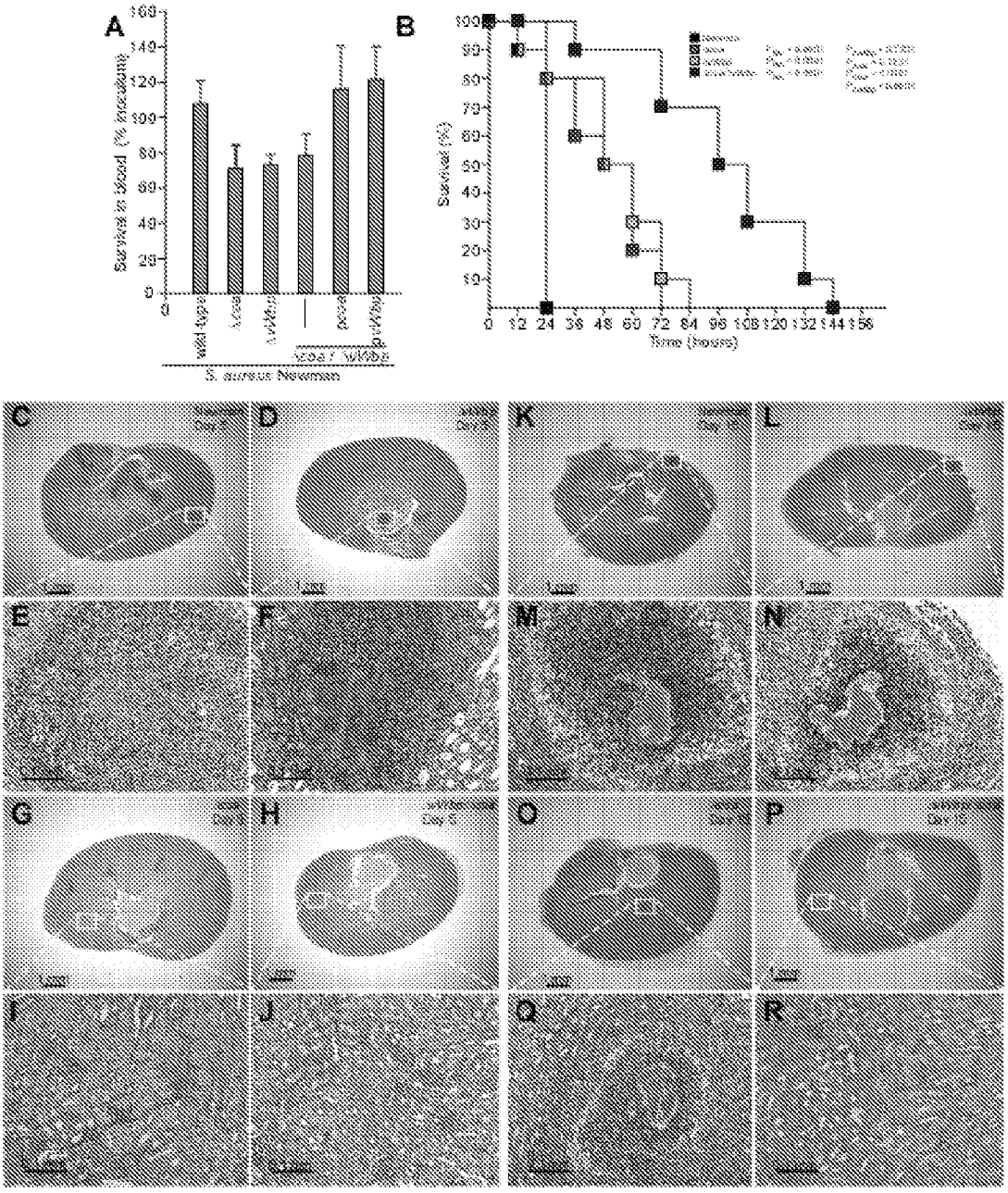
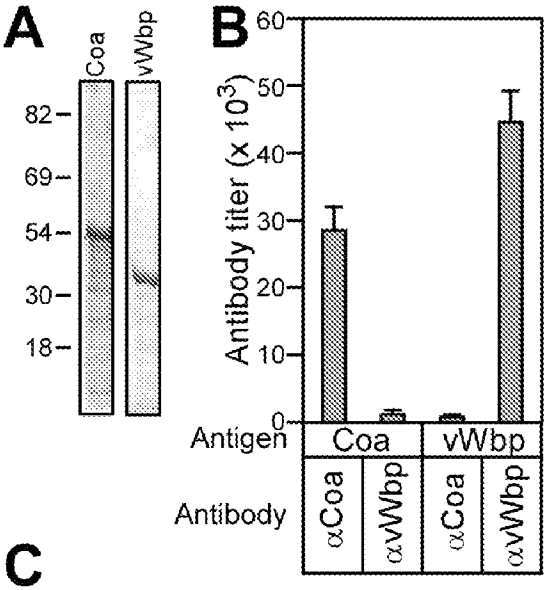


FIG. 12



C

***S. aureus* infection of mouse blood**

Antibody	Coagulation 24 hours	Coagulation Completed
Mock	—	24
αV10		24
αCoa		>48
αvWbp		>48
αCoa/αvWbp		>48

D

Mouse blood with	Coagulation time
PBS	indefinite
Coagulase	< 30 min
Coagulase + αV10	< 30 min
Coagulase + αCoa	2 hrs
Coagulase + αvWbp	1 hr
vWbp	< 30 min
vWbp + αV10	< 30 min
vWbp + αCoa	2 hrs
vWbp + αvWbp	2 hrs

FIG. 13

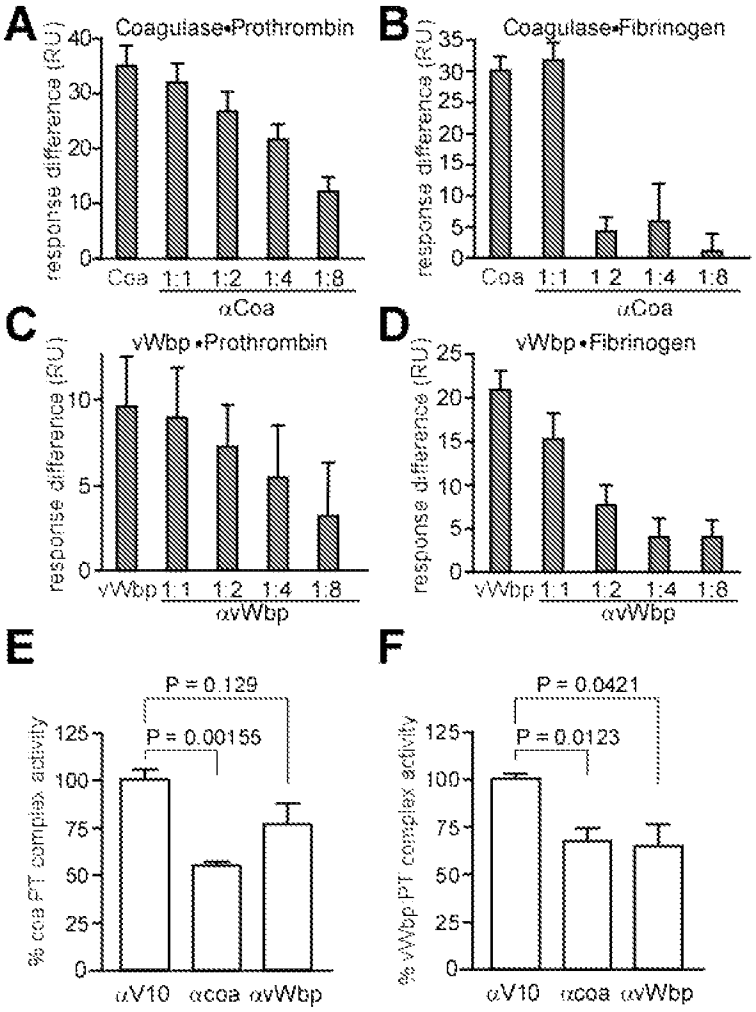

A
Coagulase•Prothrombin
response difference (RU)
Coa 1:1 1:2 1:4 1:8
αCoa
B
Coagulase•Fibrinogen
response difference (RU)
Coa 1:1 1:2 1:4 1:8
αCoa
C
vWbp•Prothrombin
response difference (RU)
vWbp 1:1 1:2 1:4 1:8
αvWbp
D
vWbp•Fibrinogen
response difference (RU)
vWbp 1:1 1:2 1:4 1:8
αvWbp
E
P = 0.129
P = 0.00156
% coa PT complex activity
αV10 αcoa αvWbp
F
P = 0.0421
P = 0.0123
% vWbp PT complex activity
αV10 αcoa αvWbp

FIG. 14

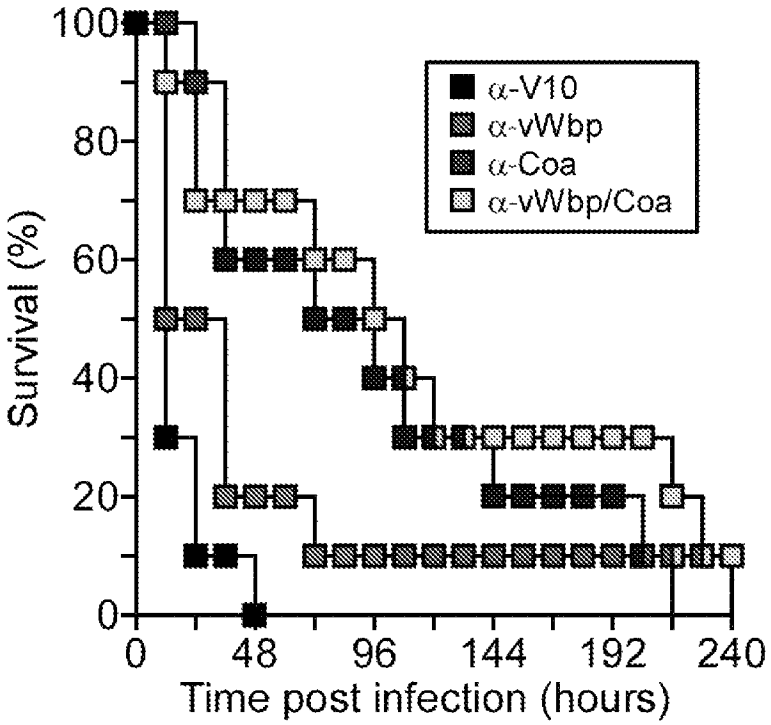

100
80
60
40
20
0
Survival (%)
0
48
96
144
192
240
Time post infection (hours)
α-V10
α-vWbp
α-Coa
α-vWbp/Coa

FIG. 15

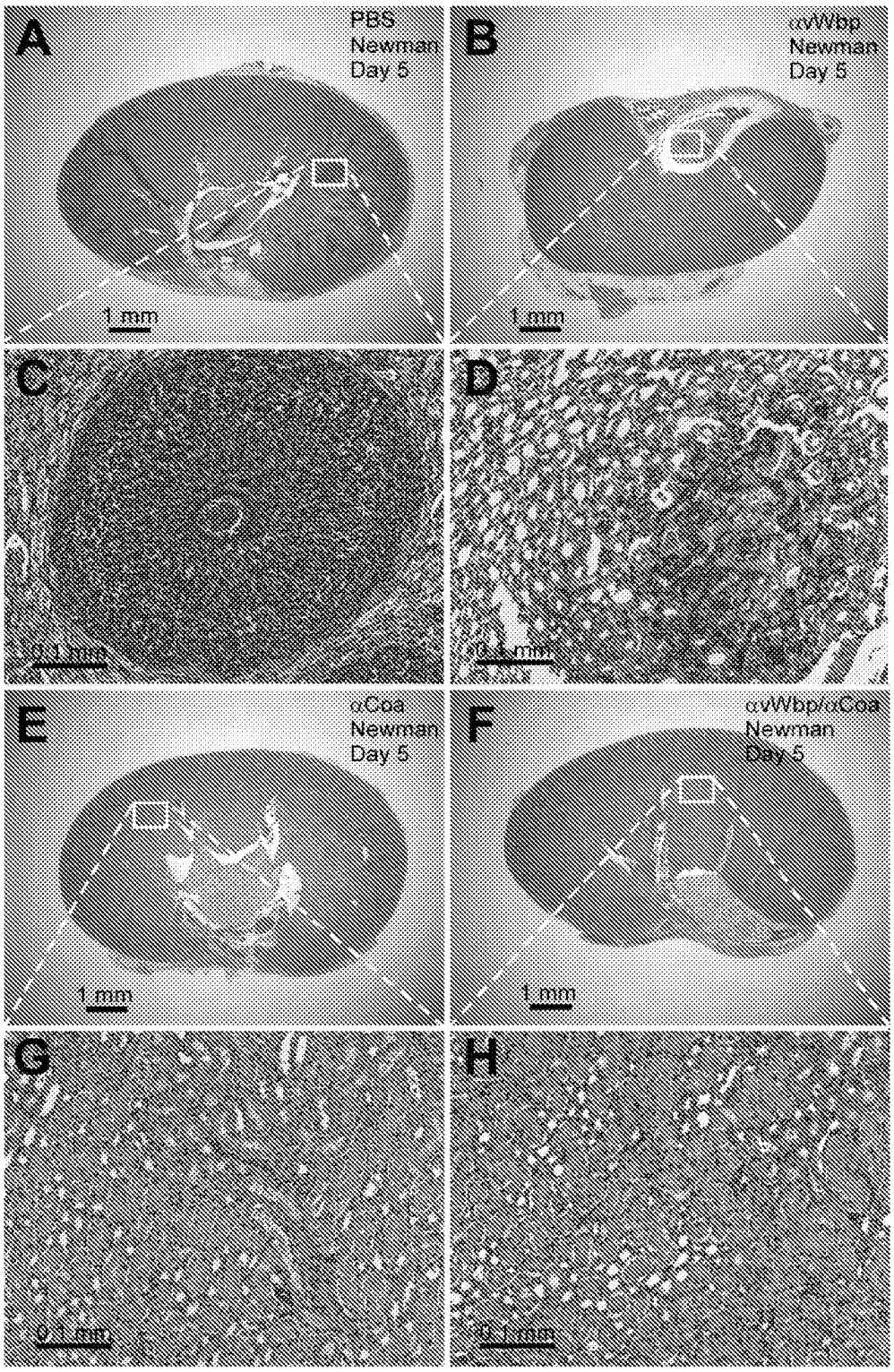

A
PBS
Newman
Day 5
1 mm
B
αvWbp
Newman
Day 5
1 mm
C
0.1 mm
D
0.1 mm
E
αCoa
Newman
Day 5
1 mm
F
αvWbp/αCoa
Newman
Day 5
1 mm
G
0.1 mm
H
0.1 mm

FIG. 16

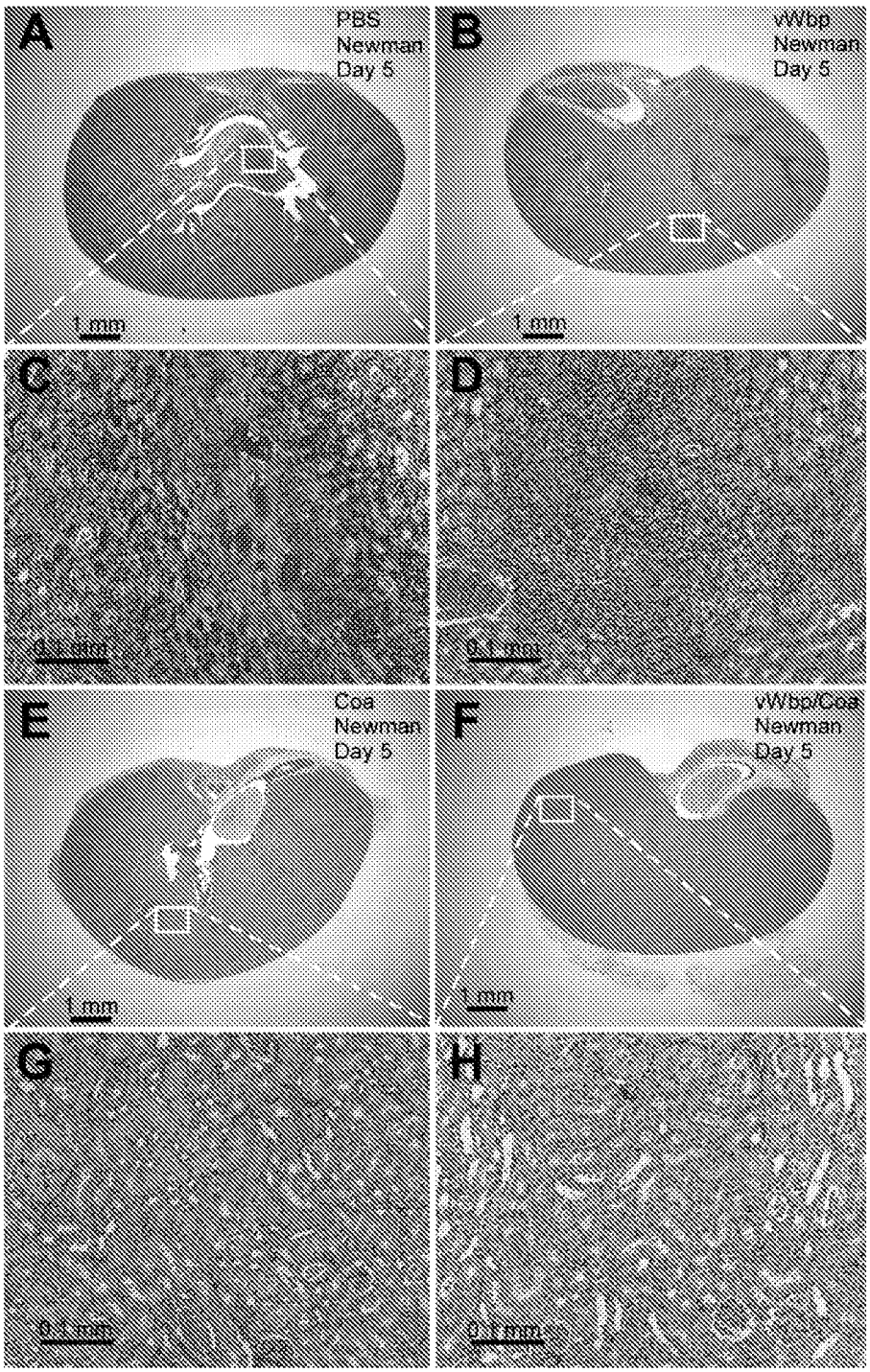

A
PBS
Newman
Day 5
1 mm
B
vWbp
Newman
Day 5
1 mm
C
0.1 mm
D
0.1 mm
E
Coa
Newman
Day 5
1 mm
F
vWbp/Coa
Newman
Day 5
1 mm
G
0.1 mm
H
0.1 mm

FIG. 17

COMPOSITIONS AND METHODS RELATED TO PROTEIN A (SPA) VARIANTS

The present application is a national phase application under 35 U.S.C. §371 of International Patent Application No. PCT/US2010/029959 filed Apr. 5, 2010, which claims benefit of priority to U.S. Provisional Application Ser. No. 61/166,432, filed Apr. 3, 2009, U.S. Provisional Application Ser. No. 61/237,956, filed Aug. 28, 2009, and U.S. Provisional Application Ser. No. 61/287,996, filed Dec. 18, 2009, the entire contents of each of which are hereby incorporated by reference in their entirety without disclaimer.

This invention was made with government support under AI057153, AI75258, 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 mortality. 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 bacterial 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 apotosis, 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 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 anther 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, 37, and/or 38 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 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, QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNES) 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. **1**. 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-toxigenic 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-toxigenic 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-toxigenic 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-toxigenic 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/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 (see PCT publications WO2007/113222, WO2007/113223, WO2006/032472, WO2006/032475, WO2006/032500, each of which is incorporated herein by reference in their entirety). The staphylococcal antigen or immunogenic fragment can be administered concurrently with the Protein A variant. The staphylococcal antigen or immunogenic fragment and the Protein A variant can be administered in the same composition. The Protein A variant can also be a recombinant nucleic acid molecule encoding a Protein A variant. A recombinant nucleic acid molecule can encode the Protein A variant and at least one staphylococcal antigen or immunogenic fragment thereof. As used herein, the term "modulate" or "modulation" encompasses the meanings of the words "enhance," or "inhibit." "Modulation" of activity may be either an increase or a decrease in activity. As used herein, the term "modulator" refers to compounds that effect the function of a moiety, including up-regulation, induction, stimulation, potentiation, inhibition, down-regulation, or suppression of a protein, nucleic acid, gene, organism or the like.

In certain embodiments the methods and compositions use or include or encode all or part of the Protein A variant or antigen. In other aspects, the Protein A variant may be used in combination with secreted factors or surface antigens including, but not limited to one or more of an isolated Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh polypeptide or immunogenic segment thereof. Additional staphylococcal antigens that can be used in combination with a Protein A variant include, but are not limited to 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, MEW 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 certain embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, CHB, 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 formulation of the invention.

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, CHB, 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-toxigenic 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. 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 bacterial 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 97597664.1 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 ClfB; (3) SpA variant and SdrD; (4) SpA variant and Hla or Hla variant; (5) SpA variant and ClfB, SdrD, and Hla or Hla variant; (6) SpA variant, IsdA, SdrD, and Hla or Hla variant; (7) SpA variant, IsdA, ClfB, and Hla or Hla variant; (8) SpA variant, IsdA, ClfB, and SdrD; (9) SpA variant, IsdA, ClfB, SdrD and Hla or Hla variant; (10) SpA variant, IsdA, ClfB, and SdrD; (11) SpA variant, IsdA, SdrD, and Hla or Hla variant; (12) SpA variant, IsdA, and Hla or Hla variant; (13) SpA variant, IsdA, ClfB, and Hla or Hla variant; (14) SpA variant, ClfB, and SdrD; (15) SpA variant, ClfB, and Hla or Hla variant; or (16) SpA variant, SdrD, and Hla or Hla variant.

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.

Certain embodiments are directed to methods for eliciting an immune response against a *staphylococcus* bacterium in a subject comprising providing to the subject an effective amount of a peptide comprising a coagulase polypeptide or an immunogenic segment thereof having an amino acid sequence that is at least 80, 85, 90, 95, 98, to 100% identical to SEQ ID NO:27 or a segment thereof or at least 80, 85, 90, 95, 98, to 100% identical to amino acids 27-508 of SEQ ID NO:32 or a segment thereof.

In certain aspects, the subject is provided with an effective amount of an coagulase polypeptide by administering to the subject a composition comprising: (i) an isolated coagulase polypeptide or segment thereof having an amino acid sequence that is at least 90% identical to SEQ ID NO:27 or a segment thereof or is at least 90% identical to amino acids 27-508 of SEQ ID NO:32 or a segment thereof; or (ii) at least one isolated recombinant nucleic acid molecule encoding a coagulase polypeptide or a segment thereof having an amino acid sequence that is at least 90% identical to SEQ ID NO:27 or a segment thereof or is at least 90% identical to amino acids 27-508 of SEQ ID NO:32 or a segment thereof. In a further aspect, the composition comprises an isolated coagulase polypeptide having the amino acid sequence of SEQ ID NO:27 or the amino acid sequence of amino acids 27-508 of SEQ ID NO:32.

Certain embodiments are directed to methods for treating a staphylococcal infection in a subject comprising providing to a subject having or suspected of having or at risk of developing a staphylococcal infection an effective amount of an isolated peptide comprising a coagulase polypeptide having an amino acid sequence that is at least 80, 85, 90, 95, 98, to 100% identical to SEQ ID NO:27 or is at least 80, 85, 90, 95, 98, to 100% identical to amino acids 27-508 of SEQ ID NO:32. In a particular aspect, the coagulase polypeptide has an amino acid sequence of SEQ ID NO:27 or has an amino acid identical to amino acids 27-508 of SEQ ID NO:32. In certain aspects, the subject is diagnosed with a persistent staphylococcal infection. In a further aspect, the coagulase polypeptide elicits production of an antibody that binds Coa or vWbpvWh in the subject.

Embodiments include methods of preventing or treating staphylococcal infection comprising the step of administering an immunogenic composition comprising a Staphylococcal coagulase or an immunogenic segment thereof.

Certain embodiments are directed to methods of preparing an immunoglobulin for use in prevention or treatment of staphylococcal infection comprising the steps of immunizing a recipient with a coagulase polypeptide and isolating immunoglobulin from the recipient.

A further embodiment is directed to an immunoglobulin prepared by the method described herein.

A further embodiment is directed to methods for treatment or prevention of staphylococcal infection comprising a step of administering to a patient an effective amount of pharmaceutical preparation of immunoglobulin that binds a coagulase.

Other embodiments are directed to a use of the pharmaceutical preparation of coagulase immunoglobulins in the manufacture of a medicament for the treatment or prevention of staphylococcal infection.

Yet still further embodiments include vaccines comprising a pharmaceutically acceptable composition having an isolated coagulase 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 coagulase polypeptide, or any other combination or permutation of protein(s) or peptide(s) described. In certain aspects of the invention the isolated coagulase 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-coagulase 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 coagulase 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 composition may comprise a recombinant nucleic acid encoding all

or part of a coagulase 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 coagulase 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 coagulase compositions that contain one or more of 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, SpA and variants thereof, 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 Coa and/or vWbp, and IsdA; (2) a Coa and/or vWbp, and ClfB; (3) a Coa and/or vWbp, and SdrD; (4) a Coa and/or vWbp, and Hla or Hla variant; (5) a Coa and/or vWbp, and ClfB, SdrD, and Hla or Hla variant; (6) a Coa and/or vWbp, and IsdA, SdrD, and Hla or Hla variant; (7) a Coa and/or vWbp, and IsdA, ClfB, and Hla or Hla variant; (8) a Coa and/or vWbp, and IsdA, ClfB, and SdrD; (9) a Coa and/or vWbp, and IsdA, ClfB, SdrD and Hla or Hla variant; (10) a Coa and/or vWbp, and IsdA, ClfB, and SdrD; (11) a Coa and/or vWbp, and IsdA, SdrD, and Hla or Hla variant; (12) a Coa and/or vWbp, and IsdA, and Hla or Hla variant; (13) a Coa and/or vWbp, and IsdA, ClfB, and Hla or Hla variant; (14) a Coa and/or vWbp, and ClfB, and SdrD; (15) a Coa and/or vWbp, and ClfB, and Hla or Hla variant; or (16) a Coa and/or vWbp, and SdrD, and Hla or Hla variant.

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, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, SpA, vWbp, or vWh protein or peptide or variant thereof. Additional staphylococcal antigens that can be used in combination with the polypeptides described herein include, but are not limited to 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 *staphylo-*

coccus 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, Ebh, 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, MEW 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, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, CHB, 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, MEW 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 pharmaceutically 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 embodiments of the invention briefly summarized above are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate certain embodiments of the invention and therefore are not to be considered limiting in their scope.

FIGS. **1**A-**1**E Generation of a non-toxigenic protein A vaccine. FIG. **1**A Translational protein A (SpA) product of *S. aureus* Newman and USA300 LAC with an N-terminal signal peptide (white box), five immunoglobin binding domains (IgBDs designated E, D, A, B, and C), variable region X and C-terminal sorting signal (black box). FIG. **1**B (SEQ ID NOs. 64-69), Amino acid sequence of the five IgBDs as well as nontoxigenic SpA-D_{KKAA} (SEQ ID NO. 69), 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. FIG. **1**C, COOMASSIE-BLUE-stained SDS-PAGE of SpA, SpA-D, SpA-D_{KKAA} (SEQ ID NO. 69) or SrtA purified on Ni-NTA sepharose in the presence or absence of human immunoglobulin (hIgG). FIG. **1**D, ELISA examining the association of immobilized SpA, SpA-D or SpA-D_{KKAA} (SEQ ID NO. 69) with human IgG as well as its Fc or $F(ab)_2$ fragments and von Willebrand factor (vWF). FIG. **1**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} (SEQ ID NO. 69) were quantified by FACS.

FIG. **2** Non-toxigenic 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} (SEQ ID NO. 69) 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.

FIGS. **3**A-C Antibodies raised by the non-toxigenic protein A vaccine block the B cell superantigen function of SpA. FIG. **3**A, Rabbit antibodies raised against SpA-D_{KKAA} (SEQ ID NO. 69) 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} (SEQ ID NO. 69) matrix. FIG. **3**B, SpA-D_{KKAA} (SEQ ID NO. 69) specific F(ab)2 interfere with the binding of SpA or SpA-D to human immunoglobulin (hIgG) or, FIG. **3**C, to von Willebrand Factor (vWF).

FIGS. **4**A-D Full-length non-toxigenic protein A generates improved immune responses. FIG. **4**A, Full-length SpA_{KKAA} (SEQ ID NO. 34) was purified on Ni-NTA sepharose and analyzed by COOMASSIE-BLUE stained SDS-PAGE. FIG. **4**B, CD19+B lymphocytes in splenic tissue of BALB/c mice that had been mock immunized or treated with SpA or SpA_{KKAA} (SEQ ID NO. 34) were quantified by FACS. FIG. **4**C, ELISA examining the association of immobilized SpA or SpA_{KKAA} (SEQ ID NO. 34) with human IgG as well as its Fc or F(ab)2 fragments or von Willebrand factor (vWF). FIG. **4**D, Human or mouse serum antibody titers to diphtheria toxoid (CRM197) and non-toxigenic SpA_{KKAA} (SEQ ID NO. 34) or SpA-D_{KKAA} (SEQ ID NO. 69). 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} (SEQ ID NO. 34) or SpA-D_{KKAA} (SEQ ID NO. 69) were examined by quantitative dot blot.

FIG. **5** Protein A is required for the pathogenesis of lethal *S. aureus* infections in mice. Cohorts of BALB/c mice (n=8) were injected with suspensions of 2×10^8 CFU *S. aureus* Newman or its isogenic protein A deletion variant (Δspa) in PBS. Infected animals were monitored for survival over a period of 15 days.

FIGS. **6**A-B Antibodies against protein A protect mice against lethal *S. aureus* infections. FIG. **6**A Cohorts of BALB/c mice (n=10) were injected with 5 mg kg^{-1} affinity purified rabbit IgG specific for SpA_{KKAA} (α-Sp_{AKKAA})(SEQ ID NO. 34) or the plague vaccine antigen rV10 (DeBord et al., 2006) (mock). Four hours later, each animal was infected by intraperitoneal injection with a suspension of 3×10^8 CFU *S. aureus* Newman and monitored for survival over a period of 10 days. Data are representative of three independent experiments FIG. **6**B Cohorts of BALB/c mice (n=10) were prime-booster immunized with SpA_{KKAA} (SEQ ID NO. 34) or PBS/adjuvant control (mock). Each animal was subsequently infected by intraperitoneal injection with a suspension of 6×10^8 CFU *S. aureus* Newman and monitored for survival over a period of 10 days. Statistical significance (P) was analyzed with the unpaired two-tailed log-rank test. Data are representative of all three independent experiments.

FIG. **7** SpA_{KKAA} (SEQ ID NO. 34) immunization protects mice against challenge with the vancomycin-resistant MRSA isolated Mu50. Cohorts of BALB/c mice (n=15) were prime-booster immunized with SpA_{KKAA} (SEQ ID NO. 34) or PBS/adjuvant control (mock). Each animal was subsequently infected by intravenous injection with a suspension of 3×10^7 CFU *S. aureus* Mu50. Staphylococcal load, calculated as log 10 CFU g^{-1}, was determined in homogenized renal tissues 4 days following infection. Sta-

tistical significance was calculated with the unpaired two-tailed Students t-test and P-value recorded.

FIGS. **8**A-B Lack of protective immune responses to staphylococcal infections. FIG. **8**A Staphylococcal infection does not generate protective immunity. BALB/c mice (n=10) 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. Data are representative of three independent analyses. FIG. **8**B IsdB immunization does not protect mice against *S. aureus* USA300 (LAC) challenge. BALB/c mice (n=10) were immunized with IsdB (100 μg IsdB emulsified in CFA followed by IFA/IsdB booster on day 11) and challenged by retro-orbital injection with 5×10^6 CFU *S. aureus* USA300 (LAC) on day 21. Four days following challenge, kidneys were removed during necropsy and staphylococcal load per gram of homogenized tissue enumerated by colony formation on agar plates. Compared to mock immunized (PBS/adjuvant) animals with 6.93 (±0.24) log 10 CFU g^{-1}, IsdB vaccination was associated with 6.25 (±0.46) log 10 CFU g^{-1} and did not generate statistically significant protection (P=0.2138, two-tailed Student's t-test) from USA300 (LAC) challenge. Data are representative of three independent analyses.

FIG. **9** Comparison of abscess formation in mice treated with PBS, SpA, SpA-D and SpA-D_{KKAA} (SEQ ID NO. 69).

FIGS. **10**A-**10**H Localization of prothrombin, fibrinogen, coagulase (Coa), and von Willebrand factor binding protein (vWbp) in staphylococcal abscesses. BALB/c mice infected by intravenous inoculation with 1×10^7 CFU *S. aureus* Newman were killed 5 days post infection. Kidneys were removed, embedded in paraffin, thin-sectioned and stained by immunochemistry using rabbit antibodies (a) specific for mouse prothrombin (FIG. **10**A, **10**C), mouse fibrinogen/fibrin (FIG. **10**B, **10**D), *S. aureus* Coa (FIG. **10**E, **10**G) or *S. aureus* vWbp (FIG. **10**F, **10**H). Displayed images are representative of three sampled kidneys. Panels FIGS. **10**C, **10**D, **10**G, and **10**H illustrate antibody staining within a single abscess analyzed as four sequential sections, enlarged from an area in panels FIGS. **10**A, **10**B, **10**E, and **10**F that is defined by box with white margins.

FIGS. **11**A-**11**C *Staphylococcus aureus* coa and vWbp mutants display defects in blood clotting. (FIG. **11**A) Diagram illustrating the primary translational product of coa and vWbp including signal peptide (S), the D1 and D2 domain from prothrombin binding, a domain of unknown function, von Willebrand factor (vWF) binding site on vWbp, and the fibrinogen binding repeats (R) of Coa. Numbers indicate amino acid residues. (FIG. **11**B) Culture supernatants from *S. aureus* Newman (wild-type) or isogenic variants lacking coa (Δcoa), vWbp (ΔvWbp) or both genes (Δcoa, ΔvWbp) were examined by immunoblotting with antibodies specific for Coa (αCoa) or vWbp (αvWbp). For complementation studies, plasmids expressing the wild-type alleles of coa (pcoa) or vWbp (pvWbp) were electroporated into staphylococcal strains and subsequently analyzed by immunoblotting. (FIG. **11**C) Lepirudin-treated mouse blood was mock treated or infected with *S. aureus* Newman or its isogenic coagulase variants and incubated for up to 48 hours at 25° C. Tubes were tilted to assess for coagulation. Data are representative of four independent determinations.

FIGS. **12**A-**12**R Contributions of coa and vWbp to bacterial survival in blood and *S. aureus* induced lethal bacteremia of mice. (FIG. **12**A) Staphylococcal strains Newman, Δcoa, ΔvWbp or Δcoa, ΔvWbp and the complemented variants were incubated with lepirudin anticoagulated mouse blood for 30 minutes and bacterial survival assessed by colony formation on agar plates. Data were generated from three separate trials. (FIG. **12**B) Cohorts of 10 mice were injected into the retro-orbital plexus with 1×10^8 CFU of *S. aureus* Newman (wild-type) as well as Δcoa, ΔvWbp or Δcoa, ΔvWbp. Animal survival over time was recorded over 10 days. Similar to B, mice were given 1×10^7 CFU of staphylococcal strains Newman (FIGS. **12**C, E and K, M), ΔvWbp (FIGS. **14**D, F and M, L), Δcoa (FIGS. **14**G, I and O, Q) or Δcoa, ΔvWbp (FIGS. **12**H, J and P, R), harvested on days 5 (FIG. **12**C-J) or 15 (FIG. **12**K-R) and assessed for bacterial load in the renal tissue (Table 7) and histopathological abscess formation. All animal data are representative of two independent experiments.

FIGS. **13**A-**13**D Antibodies against Coa and vWbp block the clotting of blood by staphylococcal coagulases. (FIG. **13**A) His_6-Coa and His_6-vWbp were purified by affinity chromatography from *E. coli* and analyzed on COOMASSIE-stained SDS-PAGE. (FIG. **13**B) Rabbit antibodies raised against His_6-Coa or His_6-vWbp were affinity purified and analyzed by ELISA for immune reactivity with purified coagulases. Data are averaged from three independent experimental determinations. (FIG. **13**C) Lepirudin-treated mouse blood was treated with PBS (mock), irrelevant antibodies (αV10) or antibodies directed against Coa (αCoa), vWbp (αvWbp) or both coagulases (αCoa/αvWbp) prior to infection with *S. aureus* Newman and incubation for 48 hours at 25° C. (FIG. **13**D) Lepirudin-treated mouse blood was treated with antibodies as above. Blood samples were then incubated with functionally active Coa or vWbp and coagulation time recorded.

FIGS. **14**A-**14**F Biological effects of antibodies directed against staphylococcal coagulases. Surface plasmon resonance measurement of antibody perturbing the association between Coa or vWbp and prothrombin or fibrinogen. Response differences upon addition of coagulase (Coa) to either prothrombin (FIG. **14**A) or fibrinogen (FIG. **14**B) were compared to response differences in the presence of increasing amounts of antibodies (αCoa—1:1, 1:2, 1:4, 1:8). Response differences upon addition of vWbp to either prothrombin (FIG. **16**A) or fibrinogen (FIG. **14**B) were compared to response differences in the presence of increasing amounts of antibodies (αvWbp—1:1, 1:2, 1:4, 1:8). (FIG. **14**E, F) Purified active Coa or vWbp was incubated in a 1:1 molar ratio with human prothrombin. The enzymatic ability of the complex was assessed by monitoring the rate of S-2238 cleavage (fibrinogen substitute chromogenic substrate, given in excess). The assay was repeated in presence of specific or cross antibodies added in 3M excess and the data was normalized to the % average activity without inhibition. Data are an average of three independent trials.

FIG. **15** Contribution of coagulase specific antibodies to the survival of mice with staphylococcal bacteremia. Twenty-four hours prior to infection, BALB/c mice (n=15) were injected into the peritoneum with purified rabbit antibodies (5 mg antibody/kg body weight). Animals were then challenged with 1×10^8 CFU *S. aureus* Newman injected into the retro-orbital plexus and monitored for survival. Data are representative of two independent experiments.

FIGS. **16**A-**16**H Passive transfer of coagulase antibodies confers protection against *S. aureus* abscess formation. An experimental mock (PBS, FIGS. **18**A and **18**C) or purified rabbit antibodies directed against vWbp (αvWbp, FIGS. **18**B and **18**D), Coa (αCoa, FIGS. **18**E and **18**G) or both coagulases (αCoa/αvWbp, FIGS. **18**F and **18**H) were injected into the peritoneal cavity of BALB/c mice (n=10)

and antibody titers analyzed by ELISA (Table 8). Passively immunized animals were infected by injecting 1×10^7 CFU *S. aureus* Newman into the retro-orbital plexus. Bacterial load and abscess formation were determined following necropsy in the kidneys of animals that had been killed five days following infection. Renal tissues were fixed with paraformaldehyde, embedded in paraffin, thin sectioned, stained with hematoxylin-eosin and histopathology images acquired by light microscopy. Data are representative of two separate experiments.

FIG. **17**s A-H Immunization with coagulases protects mice against *S. aureus* abscess formation. BALB/c mice (n=15) were immunized with 50 μg His_6-Coa, His_6-vWbp, His_6-Coa and His_6-vWbp or mock (PBS) emulsified with adjuvant on day 0 and 11 and antibody titers analyzed by ELISA on day 21 (Table 8). On day 21, animals were challenged by injecting 1×10^7 CFU *S. aureus* Newman into the retro-orbital plexus. Bacterial load and abscess formation were determined following necropsy in the kidneys of animals that had been killed five days following infection. Renal tissues were fixed with paraformaldehyde, embedded in paraffin, thin sectioned, stained with hematoxylin-eosin and histopathology images acquired by light microscopy. Data are representative of two separate experiments.

DETAILED DESCRIPTION

Staphylococcus aureus is a commensal of the human skin and nares, and the leading cause of bloodstream, skin and soft tissue infections (Klevens 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).

III. STAPHYLOCOCCAL ANTIGENS

A. Staphylcoccal 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 (SEQ ID NO.: 70) signal peptide and a C-terminal LPXTG (SEQ ID NO.: 71) 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) (Graille et al., 2000), the von Willebrand factor at its A1 domain [vWF AI is a ligand for platelets] (O'Seaghdha 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 AI domains (Hartleib et al., 2000). Plasma proteins such as fibrinogen and fibronectin act as bridges between staphylococci (ClfA and ClfB) and the platelet integrin GPIIb/IIIa (O'Brien et al., 2002), an activity that is supplemented through Protein A association with vWF AI, which allows staphylococci to capture platelets via the GPIb-α platelet receptor (Foster, 2005; O'Seaghdha 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 frgament 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 Fc_1. 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'Seaghdha 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, I31 and K35) are also required for vWF AI and TNFR1 binding (Cedergren et al., 1993; Gomez et al., 2006; O'Seaghdha 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 CO 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 Fcγ binding. The interaction of Fcγ 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 Fcγ 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 Fcγ molecule. In this ternary model, Fab and Fcγ 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 Fcγ 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'Seaghdha 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 (O'Seaghdha 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 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 Fcγ, 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-Val^2 N-terminus into the Ile^{16} 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 Aα-, Bβ-, 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 Bβ- 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•prothrombin 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 Δcoa 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 ΔvWbp 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, Δcoa 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 microorganisms 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 essA 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 sur-

face 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 Esx polypeptides include the amino acid sequence of Esx proteins from bacteria in the *Staphylococcus* genus. The Esx 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 EsxA sequence is SAV0282 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number Q99WU4 (gi|68565539), which is hereby incorporated by reference. In other embodiments, the EsxB sequence is SAV0290 from strain Mu50 (which is the same amino acid sequence for Newman) and can be accessed using Genbank Accession Number Q99WT7 (gi|68565532), 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 (gi|15926240), 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 (gi|15926241), 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 (gi|15924120), 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 (gi|15924119), 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, particularly 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 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 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 1

Exemplary surface proteins of *S. aureus* 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

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 2

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
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-, hgprt- or aprt-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 proten 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.

IV. 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 2 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 conjunction 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.

E. 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), MEW Class II HLA-DRα (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), α1-Antitrypain (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; Sleigh 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-2κb—Interferon (Blanar 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 α 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'□ 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.

F. 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).

G. 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.

V. 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.

H. 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 adhesin and is composed of a polymer of β-(1→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 a 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→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→6)-linked glucosamine substituted with N-acetyl and O-succinyl constituents). Any size of PIA polysaccharide or oligosaccharide may be use 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 agroups are acetylated. In certain aspects, PNAG is deaceylated 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 NH_4OH. 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.

I. 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:

Type 5

→4)-β-D-ManNAcA(3OAc)-(1→4)-α-L-FucNAc (1→3)-β-D-FucNAc-(1→

Type 8

→3)-β-D-ManNAcA(4OAc)-(1→3)-α-L-FucNAc (1→3)-β-D-FucNAc-(1→

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

Type 5

→4)-β-D-ManNAcA-(1→4)-α-L-FucNAc(3OAc)- (1→3)-β-D-FucNAc-(1→

Type 8

→3)-β-D-ManNAcA(4OAc)-(1→3)-α-L-FucNAc (1→3)-α-D-FucNAc(1→

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.

J. *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.

K. 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. influenza* will preferably contain the N-terminal ⅓ 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 (CDAP) 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 in 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 primatized 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 ^{3}H-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; Mernaugh 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.

VI. VACCINE AND OTHER PHARMACEUTICAL COMPOSITIONS AND ADMINISTRATION

E. 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, carbodiimyde, 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, GMCSP, 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, PA); or low-dose Cyclophosphamide (CYP; 300 mg/m^2) (Johnson/Mead, NJ) and cytokines such as γ-interferon, IL-2, or IL-12 or genes encoding proteins involved in immune helper functions, such as B-7.

F. 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.

G. 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 "B":

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

BBB/A BB/AB A/A/B/B AB/AB 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.

H. 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, subcutaneous, or even intraperitoneal routes. The preparation of an aqueous composition that contains a compound or compounds that increase the expression of an MHC 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.

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 cogaulase 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.

J. 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 of 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.

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

Example 1

Non-Toxigenic Protein A Variants as Subunit Vaccines to Prevent *Staphylococcus aureus* Infections

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 μM (±65 μM); 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 1,524 μM 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 retroperito-

neal 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 3). 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 μM 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 3).

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 3

Genetic requirements for *S. aureus* Newman abscess formation in mice

	Staphylococcal load in kidney tissue			Abscess formation in kidney tissue		
Genotype	[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	—
ΔsrtA	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

[a]Means of staphylococcal load calculated as $\log_{10}$ 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.
[b]Statistical significance was calculated with the Students t-test and P-values recorded; P-values < 0.05 were deemed significant.
[c]Reduction in bacterial load calculated as $\log_{10}$ CFU g^{-1}.
[d]Abscess formation in kidney tissues five days following infection was measured by macroscopic inspection (% positive)
[e]Histopathology 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).
[f]Statistical 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 (ΔsrtA) failed to form abscess lesions on either macroscopic or histopathology examination on days 2, 5, or 15. In mice infected with the strA mutant, only 1×10^4 CFU g^{-1} was recovered from kidney tissue on day 5 of infection, which is a 2.046 $\log_{10}$ CFU g^{-1} reduction compared to the wild-type parent strain ($P=6.73\times10^{-6}$). 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 $\log_{10}$ 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 $\log_{10}$ (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 toxigenic 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 (SEQ ID NO.: 72) signal peptide at the cross wall, i.e., the cell division septum of staphylococci (FIG. 1A). (DeDent et al., 2007; DeDent et al., 2008). Following cleavage of the C-terminal LPXTG (SEQ ID NO.: 71) sorting signal, Protein A is anchored to bacterial peptidoglycan crossbridges 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 (Saïd-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 CO 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 Fcγ binding. The interaction of Fcγ 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 Fcγ 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 Fcγ molecule. In this ternary model, Fab and Fcγ 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 Fcγ 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'Seaghdha 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; Gómez et al., 2006; O'Seaghdha 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-Toxigenic Variant of Protein A.

The inventors have developed a non-toxigenic 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-toxi-

genic 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 (MGSSHHHHHHSSGLVPRGS (SEQ ID NO:37)). Following IPTG inducible expression, recombinant N-terminal His_6-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 AAGGATCCAGATTCGTTTAATTTTTTAGC [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 His_6-tagged protein. To generate mutations in the SpA-D coding sequence, sets of two pairs of primers were synthesized (for D to A substitutions: CTTCATTCAAAGTCTTAAAGCCGCCCCAAGCCAAAGCACTAAC [5' primer] (SEQ ID NO:40) and GTTAGTGCTTTGGCTTGGGGCGGCTTTAAGACTTTGAATGAAG [3' primer] (SEQ ID NO:41); for Q to K substitutions CATATGTTCAACAAAGATAAAAAAAGCGCCTTCTATGAAATC [5' primer] (SEQ ID NO:42) and GATTTCATAGAAGGCGCTTTTTTTATCTTTGTTGAACATATG [3' primer] (SEQ ID NO:43); for Q to G substitutions CATATGTTCAACAAAGATGGAGGAAGCGCCTTCTATGAAATC [5' primer] (SEQ ID NO:44) and GATTTCATAGAAGGCGCTTCCTCCATCTTTGTTGAACATATG' [3' primer] (SEQ ID NO:45). Primers were used for quikchange mutagenesis protocols. Following mutagenesis, DNA sequences were confirmed for each of the recombinant proteins: SpA, SpA-D and SPA-$D_{Q9,10G;D36,37A}$ 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. **1**C, wild-type Protein A (SpA) and its SpA-domain D both retained immunoglobulin during chromatography. In contrast, the SpA-DQ9,10K;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. **1**D demonstrate the binding of SpA and SpA-D to hIgG and hFc, whereas SpA-$D_{Q9,10G;D36,37A}$ 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-$D_{Q9,10K;D36,37A}$ displayed a significant reduction in the ability to bind the VH3 domain of immunoglobulin. To examine the toxigenic 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. **1**E). 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 toxigenic attributes of stimulating B cell proliferation and death (FIG. **1**E). 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 toxigenic functions in human and animal tissues.

Non-toxigenic 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 4 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 1×10^7 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 $\log_{10}$ (±0.25) CFU in kidney tissue and infectious lesions were organized into 3.7 (±1.2) abscesses per organ (n=10)(Table

4). Immunization of animals with SpA led to a 2.51 $\log_{10}$ 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 $\log_{10}$ 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-$D_{Q9,10G;D36,37A}$ created increased protection, with 3.07 $\log_{10}$ and 3.03 $\log_{10}$ 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-$D_{Q9,10G;D36,37A}$ 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-toxigenic 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-toxigenic 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.

TABLE 4

Non-toxigenic Protein A variants as vaccine antigens that prevent *S. aureus* disease

	Bacterial load in kidney (n = number of mice)				Abscess formation in mice (n = number of mice)				
Antigen	[a]$\log_{10}$ CFU g^{-1}	[b]Reduction	[c]P value	IgG titer	[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

[a]Means of staphylococcal load calculated as $\log_{10}$ 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.
[c]Statistical significance was calculated with the Students t-test and P-values recorded; P-values < 0.05 were deemed significant.
[b]Reduction in bacterial load calculated as $\log_{10}$ CFU g^{-1}.
[d]Abscess formation in kidney tissues four days following infection was measured by macroscopic inspection (% positive)
[e]Histopathology 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).
[f]Statistical 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.

Materials and Methods

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-DQ9,10K;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-DQ9,10K;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 (1×10^7 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 room temperature in 10% formalin for h. Tissues are embedded in paraffin, thin-sectioned, stained with hematoxylinleosin, 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 protein). Vaccine 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 with 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 (15×10^7 cfu) (34). 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, diluted in PBS to yield an A_{600} of 0.4 (1×10^8 cfu per ml) and concentrated. 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. Immunized animals are challenged on day 21 by intraperitoneal inject with 2×10^{10} cfu of *S. aureus* Newman or $3\text{-}10\times10^9$ cfu of clinical *S. aureus* isolates. Animals are monitored for 14 days, and lethal disease is recorded.

Murine Pneumonia Model—

S. aureus strains Newman or USA300 (LAC) are grown at 37° C. in tryptic soy broth/agar to OD_{660} 0.5. 50-ml culture aliquots are centrifuged, washed in PBS, and suspended in 750 μl PBS for mortality studies ($3\text{-}4\times10^8$ CFU per 30 μl volume), or 1,250 μl PBS (2×10^8 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 CO_2 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 A_{280} 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 $SpAD_{Q9,10K;D36,37A}$. 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-Toxigenic 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 comprised 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. **1**A-**1**B). 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 Fcγ (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. **1**B) (SEQ ID NO.: 69). The ability of isolated SpA-D or SpA-D_{KKAA} (SEQ ID NO.: 69) to bind human IgG was analyzed by affinity chromatography (FIG. **1**D). Polyhistidine tagged SpA-D as well as full-length SpA retained human IgG on Ni-NTA, whereas SpA-D_{KKAA} (SEQ ID NO.: 69) and a negative control (SrtA) did not (FIG. **1**C). 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. **1**D). 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 Fcγ and $F(ab)_2$ fragments, both of which bound to full-length SpA or SpA-D, but not to SpA-D_{KKAA} (FIG. **1**D) (SEQ ID NO.: 69). 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. **1**E). B cell superantigen activity was not observed following injection with SpA-D_{KKAA} (SEQ ID NO.: 69), and TUNEL-staining of splenic tissue failed to detect the increase in apoptotic cells that follows injection of SpA or SpA-D (FIG. **1**E).

Antibodies Against SpA-D_{KKAA} (SEQ ID NO.: 69) Protect Against MSSA and MRSA Infections.

Naive six week old BALB/c mice were injected with 50 μg is each of purified SpA, SpA-D or SpA-D_{KKAA} (SEQ ID NO.: 69) 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} (SEQ ID NO.: 69) specific antibodies following immunization of mice with the non-toxigenic variant as compared to the B cell superantigen (Spa-D vs. SpA-D_{KKAA} (SEQ ID NO.: 69) P<0.0001, Table 5). 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 5). Nevertheless, even SpA elicited lower antibody titers than SpA-D_{KKAA} (SEQ ID NO.: 69) (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 $\log_{10}$ CFU was enumerated (Table 5). Immunization of mice with SpA or SpA-D led to a reduction in staphylococcal load, however SpA-D_{KKAA} (SEQ ID NO.: 69) vaccinated animals displayed an even greater, 3.07 $\log_{10}$ CFU g^{-1} reduction of *S. aureus* Newman in renal tissues (P<0.0001, Table 5). Abscess formation in kidneys was analyzed by histopathology (FIG. **2**). Mock immunized animals harbored an average of 3.7 (±1.2) abscesses per kidney (Table 5). Vaccination with SpA-D_{KKAA} (SEQ ID NO.: 69) 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 5). Lesions from SpA-D_{KKAA} (SEQ ID NO.: 69) vaccinated animals were smaller in size, with fewer infiltrating PMNs and characteristically lacked staphylococcal abscess communities (Cheng et al., 2009) (FIG. **2**). Abscesses in animals that had been immunized with SpA or SpA-D displayed the same overall structure of lesions in mock immunized animals (FIG. **2**).

The inventors examined whether SpA-D_{KKAA} (SEQ ID NO.: 69) 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} (SEQ ID NO.: 69) immunized animals harbored a 1.07 $\log_{10}$ 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 5).

SpA-D_{KKAA} (SEQ ID NO.: 69) Antibodies Prevent Immunoglobulin-Protein A Interaction.

Rabbits were immunized with SpA-D_{KKAA} (SEQ ID NO.: 69) and specific antibodies were purified on SpA-D_{KKAA} (SEQ ID NO.: 69) affinity column followed by SDS-PAGE (FIG. **3**). SpA-D_{KKAA} (SEQ ID NO.: 69) 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} (SEQ ID NO.: 69) column (FIG. **3**). Binding of human IgG or vWF to SpA or SpA-D was perturbed by SpA-D_{KKAA} (SEQ ID NO.: 69) specific $F(ab)_2$, indicating that SpA-D_{KKAA} (SEQ ID NO.: 69) derived antibodies neutralize the B cell superantigen function of protein A as well as its interactions with Ig (FIG. **3**).

SpA_{KKAA} (SEQ ID NO.: 34) generates improved protective immune responses. To further improve the vaccine properties for non-toxigenic protein A, the inventors generated SpA_{KKAA} (SEQ ID NO.: 34) 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} (SEQ ID NO.: 34) was purified by affinity chromatography and analyzed by COOMASSIE-BLUE-stained SDS-PAGE (FIG. **4**). Unlike full-length SpA, SpA_{KKAA} (SEQ ID NO.: 34) did not bind human IgG, Fc and $F(ab)_2$ or vWF (FIG. **4**). SpA_{KKAA} (SEQ ID NO.: 34) 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. **4**). SpA_{KKAA} (SEQ ID NO.: 34) vaccination generated higher specific antibody titers than SpA-D_{KKAA} (SEQ ID NO.: 69) immunization and provided mice with elevated protection against *S. aureus* USA300 challenge (Table 5). Four days following challenge, SpA_{KKAA} (SEQ ID NO.: 34) vaccinated animals harbored 3.54 $\log_{10}$ 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 5). As a test whether protein A vaccines impact other MRSA strains, mice were challenged with the Japanese vancomycin-resistant MRSA isolate Mu50 (Hiramatsu et al., 1997). Similar to the data observed with the MRSA isolate USA300, SpA_{KKAA} (SEQ ID NO.: 34) vaccinated animals harbored fewer Mu50 staphylococci in renal tissues than mock immunized animals (P=0.0248, FIG. **7**).

Passive Transfer of SpA-Specific Antibodies Prevents Staphylococcal Disease.

SpA_{KKAA} (SEQ ID NO.: 34) was used to immunize rabbits. Rabbit antibodies specific for SpA-D_{KKAA} (SEQ ID NO.: 69) or SpA_{KKAA} (SEQ ID NO.: 34) 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 6). 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} (SEQ ID NO.: 69) (P=0.0016) or SpA_{KKAA} (SEQ ID NO.: 34) (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 6). Together these data reveal that vaccine protection following immunization with SpA-D_{KKAA} (SEQ ID NO.: 69) or SpA_{KKAA} (SEQ ID NO.: 34) is conferred by antibodies that neutralize protein A.

The inventors also sought to ascertain whether protein A-specific antibodies can protect animals against lethal challenge. BALB/c mice were actively or passively immunized to raise antibodies against SpA_{KKAA} (SEQ ID NO.: 34) and then challenged by intraperitoneal injection with lethal doses of *S. aureus* Newman (FIG. **6**). Antibodies against SpA_{KKAA} (SEQ ID NO.: 34), whether raised by active (P=0.0475, SpA_{KKAA} (SEQ ID NO.: 34) vs. mock) or passive immunization (P=0.0493, SpA_{KKAA} (SEQ ID NO.: 34) vs. mock), conferred protection against lethal challenge with *S. aureus* Newman (FIG. **6**).

TABLE 5

Active immunization of mice with protein A vaccines.

Antigen	Staphylococcal load and abscess formation in renal tissue: [a]log_{10} CFU g^{-1}	[b]P-value	[c]Reduction (log_{10} CFU g^{-1})	[d]IgG Titer	[e]Number of abscesses	[b]P-value
S. aureus 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} (SEQ ID NO.: 69)	3.39 ± 0.50	<0.000	3.07	5600 ± 801	0.5 ± 0.4	0.0204
S. aureus 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} (SEQ ID NO.: 69)	6.00 ± 0.42	0.0189	1.20	3710 ± 1147	1.6 ± 0.6	0.0277
SpA_{KKAA} (SEQ ID NO.: 34)	3.66 ± 0.76	0.0001	3.54	10200 ± 2476	1.2 ± 0.5	0.0109

[a]Means of staphylococcal load calculated as log_{10} CFU g^{-1} in homogenized renal tissues 4 days following infection in cohorts of fifteen to twenty BALB/c mice per immunization. A representative of three independent and reproducible animal experiments is shown. Standard error of the means (±SEM) is indicated.
[b]Statistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.
[c]Reduction in bacterial load calculated as log_{10} CFU g^{-1}.
[d]Means of five randomly chosen serum IgG titers were measured prior to staphylococcal infection by ELISA.
[e]Histopathology of hematoxylin-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 6

Passive immunization of mice with antibodies against protein A.

[a]Antibody	Staphylococcal load and abscess formation in renal tissue: [b]log_{10} CFU g^{-1}	[c]P-value	[d]Reduction (log_{10} CFU g^{-1})	[e]IgG Titer	[f]Number of abscesses	[c]P-value
Mock	7.10 ± 0.14	—	—	<100	4.5 ± 0.8	—
α-SpA-D_{KKAA} (SEQ ID NO.: 69)	5.53 ± 0.43	0.0016	1.57	466 ± 114	1.9 ± 0.7	0.0235
α-SpA_{KKAA} (SEQ ID NO.: 34)	5.69 ± 0.34	0.0005	1.41	1575 ± 152	1.6 ± 0.5	0.0062

[a]Affinity 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 1×10^7 CFU *S. aureus* Newman.
[b]Means of staphylococcal load calculated as log_{10} CFU g^{-1} in homogenized renal tissues 4 days following infection in cohorts of fifteen BALB/c mice per immunization. A representative of two independent and reproducible animal experiments is shown. Standard error of the means (±SEM) is indicated.
[c]Statistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.
[d]Reduction in bacterial load calculated as log_{10} CFU g^{-1}.
[e]Means of five randomly chosen serum IgG titers were measured prior to staphylococcal infection by ELISA.
[f]Histopathology of hematoxylin-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).

Immune Response to Protein A Following Staphyloccal Infection or SpA_{KKAA} (SEQ ID NO.: 34) Immunization.

Following infection with virulent *S. aureus*, mice do not develop protective immunity against subsequent infection with the same strain (Burts et al., 2008) (FIG. **8**). The average abundance of SpA-D_{KKAA} (SEQ ID NO.: 69) specific IgG in these animals was determined by dot blot as 0.20 μg ml^{-1} (±0.04) and 0.14 μg ml^{-1} (±0.01) for strains Newman and USA300 LAC, respectively (FIG. **4**). The minimal concentration of protein A-specific IgG required for disease protection in SpA_{KKAA} (SEQ ID NO.: 34) or SpA-D_{KKAA} (SEQ ID NO.: 69) vaccinated animals (P 0.0.05 log_{10} 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-toxigenic variants unable to bind Igs via Fcγ or VH_3—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.

Material and Methods

Bacterial Strains and Growth.

Staphylococcus aureus strains Newman and USA300 were grown in tryptic soy broth (TSB) at 37° ° C. *Escherichia coli* strains DH5a 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, gctgcacatatggcgcaacacgatgaagctcaac (SEQ ID NO:35) and agtggatccttatgcttgagctttgttagcatctgc (SEQ ID NO:36) using *S. aureus* Newman template DNA. SpA-D was PCR-amplified with two primers, aacatatgttcaacaaagatcaacaaagc (SEQ ID NO:38) and aaggatccagattcgtttaatttttagc (SEQ ID NO:39). The sequence for SpA-D_{KKAA} (SEQ ID NO.: 69) was mutagenized with two sets of primers catatgttcaacaaagataaaaaaagcgccttctatgaaatc (SEQ ID NO:42) and gatttcatagaaggcgctttttttatctttgttgaacatatg (SEQ ID NO:43) for Q9K, Q10K as well as cttcattcaaagtcttaaagccgccccaagccaaagcactaac (SEQ ID NO:40) and gttagtgctttggcttggggcggctttaagactttgaatgaag (SEQ ID NO:41) for D36A,D37A. The sequence of SpA_{KKAA} (SEQ ID NO.: 34) was synthesized by Integrated DNA Technologies, Inc. PCR products were cloned into pET-15b generating N-terminal His_6 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 OD_{600} 0.5, at which point cultures were induced with 1 mM isopropyl β-D-1-thiogalatopyranoside (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.

Antibody Isolation.

Purified antigen (5 mg protein) was covalently linked to HiTrap NETS-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 NETS-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).

BALB/c mice were immunized by intramuscular injection and boosted with the same antigen in Alum after 11 and 25 days. On day 34, mice were bled to obtain serum for specific antibody titers. Affinity purified antibodies were injected into the peritoneal cavity of BALB/c mice either 24 hours or 4 hours prior to sub-lethal or lethal challenge, respectively. Animal blood was collected via periorbital vein puncture and antigen specific serum antibody titers measured 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 (~1×10^8 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-ketamine and 20 mg ml^{-1} xylazine per kilogram of body weight. Mice were infected by retroobital injection with 1×10^7 CFU of *S. aureus* Newman or 5×10^6 CFU of *S. aureus* USA300. On day 4 following challenge, mice were killed by CO_2 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.

Mouse Infection.

Staphylococci were used to infect anesthetized mice by retro-orbital injection (1×10^7 CFU of *S. aureus* Newman,

5×10^6 CFU of *S. aureus* USA300 or 3×10^7 CFU of *S. aureus* Mu50). On day 4, 15 or 30, mice were killed, kidneys removed, and homogenized tissue spread on agar for colony formation. Organ tissue was also thin-sectioned, stained with hematoxylin-eosin, and viewed by microscopy. Animal 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.

For lethal challenge experiments, BALB/c mice (cohorts of 8-10 animals per experiment) were injected with a suspension of $2\text{-}6\times10^8$ CFU of *S. aureus* Newman or its isogenic Δspa variant into the peritoneal cavity. Animal survival was monitored over a period of 15 days and statistical significance of survival data analyzed with the log-rank test.

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} (SEQ ID NO.: 69), 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} (SEQ ID NO.: 34), SpA-D and SpA-D_{KKAA} (SEQ ID NO.: 69)) 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 A_{450} readings were used to calculate half maximal titer and percent binding.

von Willebrand Factor (vWF) Binding Assays.

Purified proteins (SpA, SpA_{KKAA} (SEQ ID NO.: 34), SpA D and SpA-D_{KKAA} (SEQ ID NO.: 69)) 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 A_{450} 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} (SEQ ID NO.: 69) 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} (SEQ ID NO.: 34), and SpA-D_{KKAA} (SEQ ID NO.: 69)) 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 CO_2 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 NH_4Cl, 10 mM $KHCO_3$, 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} (SEQ ID NO.: 69)/SpA_{KKAA} (SEQ ID NO.: 34) as described above. Human/mouse IgG (Jackson Immunology Laboratory), SpA_{KKAA} (SEQ ID NO.: 34), and CRM_{197} 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. Animal survival data were analyzed with the log-rank test (Prism).

Example 3

Coagulases of *Staphylococcus aureus* Contribute to Abscesses Formation and Function as Protective Antigens

All clinical *S. aureus* isolates display coagulase activity—the clotting of blood or plasma through non-proteolytic activation of prothrombin to cleave fibrinogen. The inventors identified prothrombin, fibrinogen, coagulase (Coa) and von Willebrand-factor binding protein (vWbp) in staphylococcal abscess lesions of infected mice. Secreted Coa and vWbp both contributed to *S. aureus* Newman coagulase activity, thereby enabling abscess formation as well as lethal disease in mice. Antibodies raised against purified Coa or vWbp specifically block association of the corresponding polypeptide with prothrombin and fibrinogen. Coa- and vWbp-specific antibodies, whether raised by active or passive immunization, prevented abscess formation and mortality of mice infected with staphylococci.

VIII. RESULTS

Localization of Coagulase and Coagulation Factors in Staphylococcal Abscesses.

Previous work established the mouse renal abscess model, whereby 1×10^7 CFU of the human clinical isolate *S. aureus* Newman (Baba et al., 2007) are injected into the blood stream of BALB/c mice (Albus et al., 1991). Forty-eight hours following infection, mice develop disseminated abscesses in multiple organs, detectable by light microscopy of hematoxylin-eosin stained, thin-sectioned kidney tissue initially as an accumulation of polymorphonuclear leukocytes (PMNs) with few bacteria (Cheng et al., 2009). By day 5 of infection, abscesses increase in size and enclose a central population of staphylococci (staphylococcal abscess community—SAC), surrounded by a layer of eosinophilic,

amorphous material (the pseudocapsule) and a large cuff of PMNs (Cheng et al., 2009). Histopathology reveals massive necrosis of PMNs in proximity to the staphylococcal nidus at the center of abscess lesions as well as a mantle of healthy phagocytes. At later time intervals, SACs increase and abscesses rupture, releasing necrotic material and staphylococci into the bloodstream. A new round of abscess formation is initiated, eventually precipitating a lethal outcome of infections (Cheng et al., 2009).

To localize coagulases in abscess lesions, kidneys of mice that had been infected for 5 days with *S. aureus* Newman were thin-sectioned and stained by immuno-histochemistry with affinity purified Coa- or vWbp-specific rabbit antibodies (FIG. **10**). The inventors observed intense Coa staining in the pseudocapsule surrounding SACs and in the periphery of abscess lesions, i.e., the fibrin capsule bordering uninfected tissue. vWbp staining occurred throughout abscess lesions, but also with accumulation at the periphery. Prothrombin specific antibodies revealed staining of the zymogen in the pseudocapsule and in the periphery, whereas fibrinogen/fibrin specific staining occurred throughout abscess lesions. Together these data indicate that the eosinophilic pseudocapsule of staphylococcal abscesses harbors prothrombin and fibrinogen, which co-localize with Coa. At the periphery of abscess lesions, Coa, vWbp, prothrombin and fibrinogen/fibrin are co-localized. These observations prompted further investigation in to whether Coa and vWbp are crucial contributors to the establishment of abscesses by triggering prothombin-mediated conversion of fibrinogen to fibrin.

Staphylococcus aureus Coa and vWbp Contribute to the Clotting of Mouse Blood.

The coa and/or vWbp genes on the chromosome of *S. aureus* Newman were deleted by allelic replacement using pKOR1 technology (Bae and Schneewind, 2005). Two complementing plasmids, pcoa and pvWbp, were generated by cloning coa or vWbp structural genes as well as their upstream promoter sequences into p051 (Schneewind et al., 1993). Plasmids were electroporated into staphylococci and their continued replication selected on media supplemented with chloramphenicol (Schneewind et al., 1992). When probed for coagulases with specific antibodies, the inventors observed Coa secretion by the wild-type as well as the ΔvWbp strain, but not by Δcoa or Δcoa/ΔvWbp variants (FIG. **11**). The phenotypic defect of Δcoa and Δcoa/ΔvWbp mutants was restored by electroporation with pcoa but not by pvWbp (FIG. **11**). Similarly, secretion of vWbp was observed in *S. aureus* Newman (wild-type) as well as Δcoa mutant cultures, but not in ΔvWbp or Δcoa/ΔvWbp variants (FIG. **11**). This defect was restored by electroporation with pvWbp, but not by pcoa.

Clotting of blood is effectively inhibited by hirudin (lepirudin) (Harvey et al., 1986), a 65 residue peptide from leech that forms a 1:1 complex with thrombin, thereby blocking proteolytic conversion of fibrinogen to fibrin (Markwardt, 1955). Inoculation of fresh lepirudin-treated mouse blood with *S. aureus* Newman triggered clotting in less than 12 hours, whereas mock infected blood remained without clots for more than 48 hours (FIG. **11**C). Using this assay, it was observed that staphylococcal variants lacking coagulase activity displayed delays in clotting time, Δcoa 36 hours and ΔvWbp 24 hours (FIG. **11**C). The double mutant, Δcoa/ΔvWbp, was unable to clot mouse blood. These defects were complemented by electroporation with plasmids pvWbp as well as pcoa. Taken together, these data indicate that the two coagulases, Coa and vWbp, contribute to the ability of *S. aureus* Newman to clot mouse blood (FIG. **11**C).

Coa and vWbp are Required for Staphylococcal Survival in Blood, Abscess Formation and Lethal Bacteremia in Mice.

To analyze the virulence contributions of coagulases, the inventors first examined staphylococcal survival in lepirudin-treated blood. Wild-type strain *S. aureus* Newman was not killed in mouse blood, however isogenic variants lacking Coa, i.e. Δcoa and Δcoa/ΔvWbp, each displayed a significant reduction in CFU after 30 min incubation. This defect in survival was restored by pcoa, but not by pvWbp, suggesting that only Coa is required for staphylococcal survival in mouse blood.

Staphylococcal bacteremia is a frequent cause of human mortality in hospital settings (Klevens et al., 2007). The inventors sought to ascertain whether coagulases are required for lethal challenge of BALB/c mice, following intravenous injection of 1×10^8 CFU *S. aureus* Newman. All animals infected with the wild-type parent strain Newman succumbed to infection within 24 hours (FIG. **12**B). Animals infected with single mutants, Δcoa or ΔvWbp, each displayed a short but statistically significant delay in time-to-death (FIG. **12**B). The double mutant strain was significantly more impaired than mutants with single deletions and animals infected with the Δcoa/ΔvWbp strain displayed the largest reduction in virulence as compared to the wild-type (FIG. **12**B).

The inventors next analyzed abscess formation in renal tissues of infected mice and observed that Δcoa variants were impaired in their ability to form abscesses by day 5 and 15 of infection (Table 7, FIG. **12**G, **12**I)). The ΔvWbp mutant continued to form abscesses, although the bacterial load, the overall size of staphylococcal abscess communities and the amount of immune cell infiltrates were reduced in these variants (Table 7, FIG. **12**D, **12**F)). Mutants in coagulase are slightly more attenuated in virulence than those in vWbp, as Δcoa has lower abscess formation and bacterial load by day 15. However, the Δcoa/ΔvWbp double mutants markedly incapacitated in their ability to form abscesses and persist in infected tissues (Table 7, FIG. **12**H, **12**K)). Thus, both coagulase and von Willebrand factor binding protein are important for staphylococcal survival in the host, whether in the bloodstream or end organ tissues.

TABLE 7

Virulence of *S. aureus* Newman coa, vWbp, and coa/vWbp mutants

Strain	Staphylococcal load in kidney tissue* [a]log_{10} CFU g^{-1} of kidney tissue	[b]Significance (P-value)	[c]Reduction in [a]log_{10} CFU g^{-1}	Abscess formation in kidney tissue* [e]Surface abscesses (%)	[f]Number of abscesses per kidney	[g]Significance (P-value)
Day 5 analysis of staphylococcal load and abscess formation						
PBS	6.034 ± 0.899	—	—	75	2.333 ± 0.623	—
Coa	5.538 ± 0.560	0.3750	0.492	38	1.111 ± 0.389	0.1635
vWbp	5.247 ± 0.311	0.0859	0.783	56	1.750 ± 0.650	0.6085
coa/vWbp	4.908 ± 0.251	0.0044	1.395	25	0.750 ± 0.342	0.0786
Day 15 analysis of staphylococcal load and abscess formation						
PBS	5.380 ± 0.294	—	—	81	3.000 ± 1.234	—
Coa	4.023 ± 0.324	0.0077	1.357	44	1.400 ± 0.452	0.1862
vWbp	5.140 ± 0.689	0.0688	0.240	50	1.625 ± 0.298	0.2974
coa/vWbp	3.300 ± 0.552	0.0056	2.080	20	0.556 ± 0.154	0.0341

*BALB/c mice (n = 18-20) were injected into the peritoneum with 100 μl each of affinity purified rabbit antibodies against vWbp (α-vWbp), Coa (α-Coa) or vWbp and Coa (α-vWbp/Coa) on day 0. Twenty four hours later, animals were examined for IgG antibody titers in serum and were challenged by intravenous inoculation with 1×10^7 colony forming units (CFU) *S. aureus* Newman or mutants thereof. Five or fifteen days later, animals were killed and both kidneys removed. One kidney was fixed in formaldehyde, embedded in paraffin, thin sectioned, hemaotoxylin-eosin stained and four saggital sections per kidney were analyzed for abscess formation. The other kidney was homogenized in PBS buffer, homogenate spread on agar medium for colony formation, and staphylococcal load enumerated as CFU.

[a]Means of staphylococcal load calculated as log_{10} CFU g^{-1} in homogenized renal tissues 4 days following infection in cohorts of eighteen to twenty BALB/c mice per immunization. Standard error of the means (±SEM) is indicated.

[b]Statistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

[c]Reduction in bacterial load calculated as log_{10} CFU g^{-1}.

[e]Abscess formation in kidney tissues four days following infection was measured by macroscopic inspection (% positive)

[f]Histopathology of hematoxylene-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).

[g]Statistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

Antibodies Against Coagulases and their Effect on Blood Clotting Induced During Staphylococcal Infection.

Recombinant His_6-Coa and His_6-vWbp were purified by affinity chromatography on Ni-NTA (FIG. **13**A), emulsified in adjuvant and injected into rabbits to raise specific antibodies that were purified on affinity matrices harboring recombinant protein. Antibodies directed against Coa preferentially bound to Coa, not to vWbp (FIG. **13**B). The reciprocal was true for antibodies directed against vWbp (FIG. **13**B). When added to lepirudin-treated mouse blood infected with *S. aureus* Newman, the inventors observed that antibodies directed against Coa, vWbp or Coa and vWbp each blocked the coagulation of blood (FIG. **13**C). As controls, mock treated samples or the irrelevant V10 antibody (which provides protection against *Yersinia pestis* type III injection (DeBord et al., 2006)) had no effect (FIG. **13**C).

To examine the role of antibodies on isolated Coa or vWbp, the inventors purified recombinant, functionally active proteins (Friedrich et al., 2003) that were then added to lepirudin treated mouse blood. Coa or vWbp treated mouse blood coagulated in less than 30 minutes (FIG. **13**D). As a control, mock (PBS) or treatment with irrelevant V10 antibody did not affect clotting. Antibodies directed against Coa or vWbp delayed clotting of mouse blood treated with recombinant proteins and this occurred even for the cross-reacting homologous factor (FIG. **13**D). Minimal cross reactivity of the antibodies was observed by ELISA and western blot, yet there is cross inhibition of function.

Antibodies that block association between coagulases and prothrombin or fibrinogen. Surface plasmon resonance (SPR) was used to investigate how αCoa and αvWbp antibodies interfere with the physiological functions of coagulases. Prothrombin was immobilized on a CM5 chip. Flowing purified Coa over the sample, a dissociation constant K_D 28 nM was calculated, a measurement that is commensurate with other reports in the literature (Friedrich et al., 2003). The addition of αCoa led to a concentration-dependent decrease in response signal for the formation of prothrombin•Coa, indicating that these antibodies block association of Coa with prothrombin (FIG. **14**A). SPR further confirmed association between coagulase and fibrinogen (K_D 93.1 nM, FIG. **14**B). Upon pre-incubation with αCoa, the inventors observed a dramatic decrease in the binding of Coa to fibrinogen (FIG. **14**B). Taken together, these results indicate that antibodies directed against Coa block the association of this molecule with blood coagulation factors.

Purified vWbp displayed strong affinity for prothrombin (K_D 38.4 nM, FIG. **13**C) and fibrinogen (484 nM, FIG. **13**D), the latter of which had hitherto not been appreciated (Kroh et al., 2009). Further, pre-incubation with antibodies raised against vWbp blocked the association between vWbp and prothrombin or fibrinogen in a dose-dependent manner (FIG. **13**C, **13**D). These findings support results from the blood coagulation assays, demonstrating that specific polyclonal antibodies can block the interaction between Coa or vWbp and specific components of the coagulation cascade (FIG. **12**).

To test whether antibodies specific for coagulases block the conversion of fibrinogen to fibrin, the ability of prothrombin•coagulase complexes to cleave S-2238 was measured, a surrogate for the cleavage of fibrinogen to fibrin (FIG. **14**E, **14**F). Addition of specific antibodies to prothrombin•Coa or prothrombin•vWbp reduced the ability of these complexes to convert substrate to product. Further, cross-inhibition of coagulase-specific antibodies was observed, where the addition of cross-reacting antibodies caused a reduction in activity of the prothrombin•vWbp complex. These data suggest that specific antibodies

directed against Coa or vWbp neutralize the pathophysiological effect of the secreted product to which they bind.

Antibodies Against Coagulases Provide Protection Against Staphylococcal Disease.

IgG type antibodies specific for Coa or vWbp were isolated from rabbit serum by chromatography over an affinity column, generated by covalent crosslinking of the antigen to CNBr SEPHAROSE. The inventors attempted to perturb staphylococcal pathogenesis by administration of neutralizing antibodies, directed against Coa and/or vWbp. Mice were administered rabbit antibodies and challenged with a lethal dose of *S. aureus* strain Newman. Injection of Coa or vWbp specific antibodies significantly prolonged murine survival (FIG. **15**).

lesions (P=0.039); abscess lesions with staphylococcal communities at the nidus of large PMN infiltrates were not detected (FIG. **16** and Table 8). Animals that received both antibodies, αWbp and αCoa, displayed an even greater reduction in staphylococcal load (P=0.013) and a reduction in the abundance of inflammatory lesions (P=0.0078) (Table 8). Together, these data indicate that antibodies against coagulases, administered by passive immunization, protect mice against abscess formation and enable clearance of the invading pathogen from host tissues. Antibodies against vWbp contribute relatively little to vaccine protection, in agreement with the finding that vWbp does not play the same critical role as Coa during the pathogenesis of *S. aureus* infections in mice (Table 8).

TABLE 8

Passive immunization of mice with rabbit antibodies against Coa and/or vWbp

Purified Rabbit Antibody	Staphylococcal load in kidney tissue* [a]log_{10} CFU g^{-1} of kidney tissue	[b]Significance (P-value)	[c]Reduction in log_{10} CFU g^{-1}	[d]IgG Titer	Abscess formation in kidney tissue* [e]Surface abscesses (%)	[f]Number of abscesses per kidney	[g]Significance (P-value)
Mock	5.86 ± 0.29	—	—	<100	75	4.6 ± 1.4	—
α-vWbp	5.25 ± 0.36	0.3554	0.60	1,100 ± 200	39	1.4 ± 0.5	0.0592
α-Coa	4.68 ± 0.47	0.0420	1.18	1,300 ± 250	20	1.2 ± 0.7	0.0396
α-vWbp/Coa	4.29 ± 0.52	0.0130	1.53	1,000 ± 300	25	0.3 ± 0.2	0.0078

*BALB/c mice (n = 18-20) were injected into the peritoneum with 100 μl each of affinity purified rabbit antibodies against vWbp (α-vWbp), Coa (α-Coa) or vWbp and Coa (α-vWbp/Coa) on day 0. Twenty four hours later, animals were examined for IgG antibody titers in serum and were challenged by intravenous inoculation with 1×10^7 colony forming units (CFU) *S. aureus* Newman. Five days later, animals were killed and both kidneys removed. One kidney was fixed in formaldehyde, embedded in paraffin, thin sectioned, hemaotoxylin-eosin stained and four saggital sections per kidney were analyzed for abscess formation. The other kidney was homogenized in PBS buffer, homogenate spread on agar medium for colony formation, and staphylococcal load enumerated as CFU.

[a]Means of staphylococcal load calculated as log_{10} CFU g^{-1} in homogenized renal tissues 4 days following infection in cohorts of eighteen to twenty BALB/c mice per immunization. Standard error of the means (±SEM) is indicated.

[b]Statistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

[c]Reduction in bacterial load calculated as log_{10} CFU g^{-1}.

[e]Abscess formation in kidney tissues four days following infection was measured by macroscopic inspection (% positive)

[f]Histopathology of hematoxylene-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).

[g]Statistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

To test antibody reagents for possible vaccine protection against lethal bacteremia, affinity purified IgG (5 mg kg^1 body weight) were injected into the peritoneal cavity of mice. Twenty-four hours later, animals were injected with a suspension of 1×10^8 CFU *S. aureus* Newman in PBS into the retro-orbital plexus. Monitoring animals over time, the inventors observed that antibodies directed against vWbp (αvWbp) led to increased time-to-death and to 10% survival, as compared to animals that had received irrelevant αV10 antibodies and died within 12-48 hours (FIG. **15**). Antibodies against Coa (αCoa) further increased the time-to-death of passively immunized mice (FIG. **15**). A mixture of both antibodies (αCoa/αvWbp) did not generate a statistically significant improvement in survival or time-to-death over αCoa antibodies.

To examine the passive immunization for protection against staphylococcal abscess formation, purified antibodies (5 mg kg-1 body weight) were injected into the peritoneal cavity of mice and abscess formation was monitored for five days after intravenous challenge with 1×10^7 CFU *S. aureus* Newman. Antibodies against vWbp did not lead to a significant reduction in staphylococcal load or in the number of inflammatory lesions (Table 8), although the observed lesions harbored smaller abscess communities and reduced PMN infiltrates as compared to mock immunized mice (FIG. **16**). Antibodies against coagulase reduced the staphylococcal load (P=0.042) as well as the number of inflammatory

Coagulases Function as Protective Antigens for Staphylococcal Infections.

Poly-histidine tagged CoA and vWbp were purified from *E. coli* and used as subunit vaccine antigens. Proteins (100 μg emulsified in CFA or IFA) were injected into naïve BALB/c mice on day 0 (CFA) or 11 (IFA). Animals were challenged on day 21 by intravenous inoculation of *S. aureus* Newman. Five control animals were bled at the time of challenge and serum antibody titers against vaccine antigens were determined by ELISA (Table 9). Animals were killed five or fifteen days following challenge staphylococcal load and histopathology of abscess lesions were analyzed. Immunization with Coa reduced the bacterial load by day 5 (P=0.03, PBS mock vs. Coa) and day 15 ($P=4.286\times10^{-5}$, PBS mock vs. Coa, see Table 9). Coa vaccination also diminished the number of infectious lesions that formed in kidney tissues, mock vs. Coa, P=0.03 (day 5) and P=0.0522 (day 15) (Table 9). Of note, none of the Coa-immunized mice developed typical abscess lesions (FIG. **17**). On occasion small accumulations of PMNs that were not associated with staphylococcal abscess communities were observed (FIG. **17**). Immunization with vWbp did not significantly reduce staphylococcal load on day 5 (P=0.39, PBS mock vs. vWbp) or on day 15 (P=0.09, PBS mock vs. vWbp). The total number of inflammatory lesions was not reduced. Nevertheless, the architecture of abscesses had changed following immunization with vWbp. Staphylococcal communities were not detected at the center of abscesses and

instead PMN infiltrations were observed (FIG. **17**). The combination vaccine, vWbp-Coa, further reduced the number of inflammatory cells in kidney tissues and infected animals did not display abscess lesions on day 5 or 15 (Table 9).

TABLE 9

Active immunization of mice with Coa and/or vWbp

		Staphylococcal load in kidney tissue*				Abscess formation in kidney tissue*		
	Purified Vaccine Antigen	[a]$\log_{10}$ CFU g^{-1} of kidney tissue	[b]Significance (P-value)	[c]Reduction in [a]$\log_{10}$ CFU g^{-1}	[d]IgG Titer	[e]Surface abscesses (%)	[f]Number of abscesses per kidney	[g]Significance (P-value)
Day 5[h]	Mock	5.75 ± 0.42	—	—	<100	56	1.3 ± 0.3	—
	vWbp	4.94 ± 0.46	0.1413	0.81	14,000 ± 5,000	45	1.8 ± 0.5	0.39
	Coa	4.86 ± 0.50	0.1417	0.88	19,000 ± 4,000	25	0.3 ± 0.3	0.03
	vWbp/Coa	4.84 ± 0.38	0.1195	0.90	7,000 ± 1,500	25	0.3 ± 0.3	0.03
Day 15[i]	Mock	6.68 ± 0.22	—	—	<100	75	6.0 ± 1.9	—
	vWbp	3.41 ± 0.47	0.4503	3.27	14,000 ± 5,000	20	1.8 ± 1.1	0.09
	Coa	3.43 ± 0.54	0.1681	3.25	19,000 ± 4,000	20	1.2 ± 0.8	0.05
	vWbp/Coa	3.79 ± 0.37	0.0263	2.89	7,000 ± 1,500	30	0.7 ± 0.5	0.01

*BALB/c mice (n = 18-20) were injected with 100 μg each of purified vWbp, Coa or vWbp and Coa emulsified in CFA on day 0 and boosted with the same antigen emulsified in IFA on day 11. On day 20, animals were examined for IgG antibody titers and on day 21 animals were challenged by intravenous inoculation with either 1×10^7 colony forming units (CFU) *S. aureus* Newman. On day 25, animals were killed and both kidneys removed. One kidney was fixed in formaldehyde, embedded in paraffin, thin sectioned, hemaotoxylin-eosin stained and four saggital sections per kidney were analyzed for abscess formation. The other kidney was homogenized in PBS buffer, homogenate spread on agar medium for colony formation, and staphylococcal load enumerated as CFU.

[a]Means of staphylococcal load calculated as $\log_{10}$ CFU g^{-1} in homogenized renal tissues 4 days following infection in cohorts of eighteen to twenty BALB/c mice per immunization. Standard error of the means (±SEM) is indicated.

[b]Statistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

[c]Reduction in bacterial load calculated as $\log_{10}$ CFU g^{-1}.

[d]Means of five randomly chosen serum IgG titers were measured prior to staphylococcal infection by ELISA with SpA-D_{KKAA} (SEQ ID NO.: 69) antigen

[e]Abscess formation in kidney tissues four days following infection was measured by macroscopic inspection (% positive)

[f]Histopathology of hematoxylene-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).

[g]Statistical significance was calculated with the unpaired two-tailed Students t-test and P-values recorded; P-values < 0.05 were deemed significant.

[h]Analysis of mice 5 days following infection with *S. aureus* Newman.

[i]Analysis of mice 15 days following infection with *S. aureus* Newman.

IX. MATERIALS AND METHODS

Bacterial Strains and Growth of Cultures.

Staphylococci were cultured on tryptic soy agar or broth at 37° C. *E. coli* strains DH5a and BL21(DE3) (Studier et al., 1990) were cultured on Luria agar or broth at 37° C. Ampicillin (100 μg/ml) and chloramphenicol (10 μg/ml) were used for pET15b (Studier et al., 1990) and p051 (Schneewind et al., 1993) plasmid selection, respectively.

Generation of Mutants.

DNA sequences 1 kb upstream and downstream of coa and vWbp were PCR amplified using the primers attB1_Coa, Coa1_BamHI, Coa2_BamHI, attbB2_Coa and attB1_vWF, vWF1_BamHI, vWF2_BamHI, attbB2_vWF (Table 10). The fragments were exchanged onto pKOR1 using the BP clonase II kit (Invitrogen) (Bae and Schneewind, 2005). These vectors were electroporated into *S. aureus* Newman and subjected to temperature shift induced allelic exchange to generate the corresponding deletion (Bae and Schneewind, 2005). Mutants were verified by PCR amplification of the gene locus, DNA sequencing, and immunoblot analysis.

To generate complementing plasmids, the primers Coa_promoter_BamHI_F, Coa_out_PstI_R, vWbp_promoter_BamHI_F, vWbp_out_PstI_R (Table 10) were designed to include the upstream promoter region of vWbp or coa and the amplified regions were cloned into pOS1. These plasmids were verified by sequencing and then electroporated into staphylococcal strains. For immunoblot analysis, overnight cultures of staphylococci grown in tryptic soy broth (Difco) were refreshed 1:100 and grown with shaking at 37° C. until they reached OD_{600} of 0.4. One ml samples of each culture centrifuged at 13,000×g for 10 min in a table top centrifuge and the supernatant was recovered. Trichloroacetic acid, 75 μl of 100% w/v solution, was added and samples were incubated on ice for 10 min, followed by centrifugation and wash with 1 ml ice-cold 100% acetone. Samples were air dried overnight and solubilized in 50 μl sample buffer (4% SDS, 50 mM Tris-HCl, pH8, 10% glycerol, and bromophenol blue).

Blood Survival Assay and Blood Coagulation.

Overnight cultures of staphylococcal strains were diluted 1:100 into fresh TSB and grown at 37° C. until they reached an OD_{600} 0.4. One ml of culture was centrifuged, and staphylococci washed and suspended in 10 ml of sterile PBS to generate a suspension of 1×10^7 CFU/ml. Whole blood from naïve 6 week old Balb/c mice was collected and REFLUDAN™ (lepirudin, Bayer) was added to a final concentration 50 μg/ml. 450 μL blood was aliquoted into a 1 ml eppendorf tube and mixed with 50 μl bacterial sample (1×10^5 CFU/ml). Samples were incubated at 37° C. with slow rotation. 100 μl aliquots were removed at times 0 min and 30 min, mixed 1:1 with 2% saponin/PBS and incubated on ice for 30 minutes. Five 1:10 serial dilutions were prepared and 10 μl aliquots spread on TSA agar for colony formation and enumeration.

To assess bacterial blood coagulating activity, 10 μl of the above stock bacterial culture was added to 100 μl of anti-coagulated mouse blood in a sterile plastic test tube (BD falcon) to achieve an end concentration of 1×10^5 CFU/ml. For antibody perturbation, an additional 10 ul of PBS containing 3×10^{-5} Mol of antibody was added to the mixture. To assess recombinant proteins, 10 μl of protein in PBS buffer added to an end concentration of 50 μM. Test tubes were incubated at room temperature and blood liquidity or coagulation was verified by tipping the tubes to 45° angles in timed intervals.

Protein purification. For vaccination studies, full-length coding sequence of mature Coa or vWbp was cloned into

pET15b vector using the primers Coa_foward_XhoI, Coa_reverse_BamHI, vWbp_forward_XhoI, vWbp_reverse_BamHI (Table 10) to obtain His6-Coa and His6-vWbp. *E. coli* BL21(DE3) harboring expression vectors were grown at 37° C. and induced with 1 mM IPTG after two hours. Four hours after induction, cells were centrifuged at 6,000×g, suspended in 1× column buffer (0.1 M Tris-HCl pH 7.5, 0.5 M NaCl) and lysed in a French press at 14,0000 lb/in^2. Lysates were subjected to ultracentrifugation at 40,000×g for 30 min and the supernatant was subjected to Ni-NTA chromatography, washed with column buffer containing 25 μM imidazole, followed by elution with 500 μM imidazole. Eluate was dialyzed with 1×PBS. To remove endotoxin, 1:1,000 TRITON-X114 was added and the solution was chilled for 5 min, incubated at 37° C. for 10 min, and centrifuged at 13,000×g. Supernatant was loaded onto a HiTrap desalting column to remove any remnant of TRITON-X114.

TABLE 10

Primers used in this study

Primer name	Sequence
attB1_Coa	GGGGACAAGTTTGTACAAAAAAGCAGGCTgatgactaagttgaaaaaagaag (SEQ ID NO: 46)
Coa1_BamHI	aaGGATCCcctccaaaatgtaattgccc (SEQ ID NO: 47)
Coa2_BamHI	aaGGATCCgtttgtaactctatccaaagac (SEQ ID NO: 48)
attbB2_Coa	GGGGACCACTTTGTACAAGAAAGCTGGGTgacacctattgcacgattcg (SEQ ID NO: 49)
attB1_vWF	GGGGACAAGTTTGTACAAAAAAGCAGGCTcagatagcgattcagattcag (SEQ ID NO: 50)
vWF1_BamHI	aaGGATCCctgtatttttctccttaatttttcc (SEQ ID NO: 51)
vWF2_BamHI	aaGGATCCcatggctgcaaagcaaataatg (SEQ ID NO: 52)
attbB2_vWF	GGGGACCACTTTGTACAAGAAAGCTGGGTgccctggtgtaacaaatttatg (SEQ ID NO: 53)
Coa_promoter_BamHI_F	gaaGGATCCgtttattctagttaatatatagttaatg (SEQ ID NO: 54)
Coa_out_PstI_R	gaaCTGCAGctgtatgtctttggatagagttac (SEQ ID NO: 55)
vWbp_promoter_BamHI_F	gaaGGATCCggtggctttttacttggattttc (SEQ ID NO: 56)
vWbp_out_PstI_R	gaaCTGCAGcgacaaactcattatttgctttgc (SEQ ID NO: 57)
Coa_foward_XhoI	GAACTCGAGTCTAGCTTATTTACATGG (SEQ ID NO: 58)
Coa_Xho_factorXa_F	GAACTCGAGatagaaggcagaatagtaacaaaggattatagtggg (SEQ ID NO: 59)
Coa_reverse_BamHI	GTAGGATCCTGGGATAGAGTTACAAAC (SEQ ID NO: 60)
vWbp_forward_XhoI	GAACTCGAGgcattatgtgtatcacaaatttggg (SEQ ID NO: 61)
vWbp_Xho_factorXa_F	GAACTCGAGatagaaggcagagtggtttctggggagaagaatc (SEQ ID NO: 62)
vWbp_reverse_BamHI	GAACTCGAGgcagccatgcattaattatttgcc (SEQ ID NO: 63)

For enzymatic studies, ELISA, and SPR, full-length coding sequence of mature Coa or vWbp was cloned into pET15b with primers Coa_Xho_factorXa_F, Coa_reverse_BamHI, vWbp_Xho_factorXa_F, vWbp_reverse_BamHI (Table 10) which contain a Factor Xa site preceding the initial Ile-Val-Thr-Lys (SEQ ID NO.: 73) of coagulase and Val-Val-Ser-Gly (SEQ ID NO.: 74) of vWbp. These proteins were expressed and purified using the above protocol, then cleaved with 10 units Factor Xa/1 ml for 1 hour at 25° C. to remove the His_6 tag from the N-terminus. Proteins were then loaded onto a Superdex 75 (GE Healthcare) column for final purification. All eluted proteins were stored in 1×PBS.

Rabbit Antibodies.

Protein concentration was determined using a BCA kit (Thermo Scientific). Purity was verified by SDS page gel analysis and COOMASSIE BRILLIANT BLUE staining. Six month old New-Zealand white female rabbits (Charles River Laboratories) were immunized with 500 μg protein emulsified in CFA (Difco) for initial immunization or IFA for booster immunizations on day 24 and 48. On day 60, rabbits were bled and serum recovered for immunoblotting or passive transfer experiments. For antibody purification, recombinant His6-Coa or His6-vWbp (5 mg) was covalently linked to HiTrap NETS-activated HP columns (GE Healthcare). This antigen-matrix was then 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.

Surface Plasmon Resonance.

Affinity and rates of association and dissociation were measured on a BIAcore 3000. Buffers were sterile filtered and degassed. A CM5 chip was prepared for amine linkage by injection of human prothrombin (500 nM, pH 4.0) (Innovative Research) and human fibrinogen (200 nM, pH 4.5) (Innovative Research) in presence of 0.2 M EDC and 0.05 M NHS. To measure the interaction of coagulase with

prothrombin and fibrinogen, Coa was diluted into HBS-P buffer (20 mM HEPES [pH 7.4], 150 mM NaCl, 0.005% [vol/vol] surfactant P20) at concentrations 0-75 nM with successive injections of coagulase for 300 seconds followed by 300 seconds for dissociation followed by regeneration with NaOH (50 μL, 30 seconds). K_D and χ^2 were determined using the BiaEvaluation software and best fit was determined with a 1:1 binding model with drifting baseline and local R_{max}. The interaction of von Willebrand factor with prothrombin and fibrinogen was measured in the same way. All experiments were repeated in triplicate. Inhibition experiments with polyclonal antibodies were conducted by successive injections of coagulase (25 nM) incubated with αCoa at 0 nM-200 nM under the same injection conditions described above. vWF (50 nM) was similarly incubated with αvWF at 0 nM-400 nM. Response difference was measured as the change in response units from before the injection to the end of the injection.

Measurements of Coagulase Activity.

1×10^{-16} M prothrombin (Innovative Research) was pre-incubated for 20 min with an equimolar amount of functional coagulase or vWbp at room temperature, followed by addition of S-2238 (a chromogenic substrate) to an end concentration of 1 mM in a total reaction buffer of 100 μl 1×PBS. The change in absorbance was measured at 450 nm for 10 minutes in a spectrophotometer, plotted as a function of time and fitted to a linear curve. The slope of the curve (dA/dt) was interpreted to be the rate of S-2238 hydrolysis, and thus reflective of enzymatic function (% coagulase-prothrombin or vWbp-prothrombin complex activity). The assay was repeated in presence of specific or cross antibodies added in 3M excess (3×10^{-16}M) and the data was normalized to the % average activity without inhibition.

Renal Abscess Model and Lethal Challenge.

Overnight cultures of staphylococcal strains were diluted 1:100 into fresh TSB and grown until they reached an OD_{600} of 0.4. 10 ml of bacteria were centrifuged at 7,500×g, washed, and suspended in 10 ml of 1×PBS. Six week old female BALB/c mice (Charles River) were injected retro-orbitally with 1×10^7 CFU staphylococcal suspension in 100 μl of PBS. Cohorts of 10 mice were used. On the fifth day post infection, these mice were killed by CO_2 asphyxiation and their kidneys were excised. All organs were examined for surface lesions and 8-10 right kidneys were sent for histopathology sectioning and hematoxylin-eosin staining. These slides were examined by light microscopy for internal abscesses. For the lethal challenge model, all experimental conditions remain the same except that 1×10^8 CFU staphylococci were administered and that the mice were monitored for 10 days post infection for survival.

Immunohistochemistry Staining of Renal Sections.

Sectioned kidneys were deparafinized and rehydrated through xylene and serial dilutions of EtOH to distilled water. They were incubated in antigen retrieval buffer (DAKO, pH 6.0) and heated in steamer at over 96° C. for 20 minutes. After rinsing, the slides were incubated in 3% hydrogen peroxide for 5 minutes and then 10% normal serum in 0.025% TRITON X-100-PBS for 30 minutes. 10% human IgG was used as blocking reagent for 30 minutes incubation (Sigma-Aldrich). Primary antibody was applied on the slides for over night incubation at 4° C. degree in a humidity chamber. The primary antibodies used were 1:500 rat anti-mouse Prothrombin (Innovative Research), 1:500 rabbit anti-mouse fibrinogen (Innovative Research), 1:250 rabbit anti-staphylocoagulase, or 1:250 rabbit anti-*staphylococcus* vwbp. Following TBS wash, the slides were incubated with biotinylated secondary antibody (1:50 dilution of biotinylated anti-rat IgG, BA-4001 from Vector Laboratories; or 1:200 dilution of biotinylated anti-rabbit IgG, BA-1000 from Vector), and then ABC reagents (Vector Laboratories). The antigen-antibody binding was detected by DAB substrate chromogen system. The slide were briefly immersed in hematoxylin for counterstaining and evaluated under light microscope.

Active Immunization.

Three week old BALB/c mice were injected with 50 μg protein each, emulsified in 100 μl CFA. Cohorts of 15 mice were used, with 5 mice reserved for bleeding and antibody titers. Eleven days post vaccination, these mice were boosted with 50 μg protein each, emulsified in 100 μl IFA. On day 21, mice were injected with 1×107 CFU of staphylococci for the renal abscess model or 1×108 CFU for lethal challenge. At the time of infection 5 mice were bled to obtain antibody titers.

Passive Transfer of Antibodies.

Twenty four hours prior to infection, six week old BALB/c mice were injected with purified antibodies against Coa and/or vWbp at a dose of 5 mg/kg body weight. Cohorts of 10 mice were used. These mice were challenged by retro-orbital injection with 1×10^7 CFU (renal abscess model) or 1×10^8 CFU staphylococci (lethal bacteremia).

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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
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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
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
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        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

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
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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
        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
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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

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
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    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
    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 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
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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

Gln Gly Thr Ser Met Arg Thr Thr Asp Lys Tyr Gly Tyr Tyr Asn Tyr
                565                 570                 575

Ala Gly Tyr Ser Asn Phe Ile Val Thr Ser Asn Asp Thr Gly Gly Gly
            580                 585                 590

Asp Gly Thr Val Lys Pro Glu Glu Lys Leu Tyr Lys Ile Gly Asp Tyr
        595                 600                 605

Val Trp Glu Asp Val Asp Lys Asp Gly Val Gln Gly Thr Asp Ser Lys
    610                 615                 620

Glu Lys Pro Met Ala Asn Val Leu Val Thr Leu Thr Tyr Pro Asp Gly
625                 630                 635                 640

Thr Thr Lys Ser Val Arg Thr Asp Ala Asn Gly His Tyr Glu Phe Gly
                645                 650                 655

Gly Leu Lys Asp Gly Glu Thr Tyr Thr Val Lys Phe Glu Thr Pro Ala
            660                 665                 670

Gly Tyr Leu Pro Thr Lys Val Asn Gly Thr Thr Asp Gly Glu Lys Asp
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        675                 680                 685

Ser Asn Gly Ser Ser Ile Thr Val Lys Ile Asn Gly Lys Asp Asp Met
    690                 695                 700

Ser Leu Asp Thr Gly Phe Tyr Lys Glu Pro Lys Tyr Asn Leu Gly Asp
705                 710                 715                 720

Tyr Val Trp Glu Asp Thr Asn Lys Asp Gly Ile Gln Asp Ala Asn Glu
                725                 730                 735

Pro Gly Ile Lys Asp Val Lys Val Thr Leu Lys Asp Ser Thr Gly Lys
            740                 745                 750

Val Ile Gly Thr Thr Thr Thr Asp Ala Ser Gly Lys Tyr Lys Phe Thr
        755                 760                 765

Asp Leu Asp Asn Gly Asn Tyr Thr Val Glu Phe Glu Thr Pro Ala Gly
    770                 775                 780

Tyr Thr Pro Thr Val Lys Asn Thr Thr Ala Glu Asp Lys Asp Ser Asn
785                 790                 795                 800

Gly Leu Thr Thr Thr Gly Val Ile Lys Asp Ala Asp Asn Met Thr Leu
                805                 810                 815

Asp Ser Gly Phe Tyr Lys Thr Pro Lys Tyr Ser Leu Gly Asp Tyr Val
            820                 825                 830

Trp Tyr Asp Ser Asn Lys Asp Gly Lys Gln Asp Ser Thr Glu Lys Gly
        835                 840                 845

Ile Lys Asp Val Lys Val Thr Leu Leu Asn Glu Lys Gly Glu Val Ile
    850                 855                 860

Gly Thr Thr Lys Thr Asp Glu Asn Gly Lys Tyr Arg Phe Asp Asn Leu
865                 870                 875                 880

Asp Ser Gly Lys Tyr Lys Val Ile Phe Glu Lys Pro Ala Gly Leu Thr
                885                 890                 895

Gln Thr Val Thr Asn Thr Thr Glu Asp Asp Lys Asp Ala Asp Gly Gly
            900                 905                 910

Glu Val Asp Val Thr Ile Thr Asp His Asp Asp Phe Thr Leu Asp Asn
        915                 920                 925

Gly Tyr Phe Glu Glu Asp Thr Ser Asp Ser Asp Ser Asp Ser Asp Ser
    930                 935                 940

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
945                 950                 955                 960

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
                965                 970                 975

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
            980                 985                 990

Asp Ser Asp Ser Asp Ser Asp Ser  Asp Ser Asp Ser Asp  Ser Asp Ser
        995                 1000                1005

Asp Ser  Asp Ser Asp Ser Asp  Ser Asp Ser Asp Ser  Asp Ser Asp
    1010                1015                1020

Ser Asp  Ser Asp Ser Asp Ser  Asp Ser Asp Ser Asp  Ser Asp Ser
    1025                1030                1035

Asp Ser  Asp Ser Asp Ser Asp  Ser Asp Ser Asp Ser  Asp Ser Asp
    1040                1045                1050

Ser Asp  Ser Asp Ser Asp Ser  Asp Ser Asp Ser Asp  Ser Asp Ser
    1055                1060                1065

Asp Ser  Asp Ser Asp Ser Asp  Ser Asp Ser Asp Ser  Asp Ala Gly
    1070                1075                1080

Lys His  Thr Pro Val Lys Pro  Met Ser Thr Thr Lys  Asp His His
    1085                1090                1095
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Asn Lys  Ala Lys Ala Leu Pro  Glu Thr Gly Ser Glu  Asn Asn Gly
    1100                1105                1110

Ser Asn  Asn Ala Thr Leu Phe  Gly Gly Leu Phe Ala  Ala Leu Gly
    1115                1120                1125

Ser Leu  Leu Leu Phe Gly Arg  Arg Lys Lys Gln Asn  Lys
    1130                1135                1140


<210> SEQ ID NO 15
<211> LENGTH: 350
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 15

Met Thr Lys His Tyr Leu Asn Ser Lys Tyr Gln Ser Glu Gln Arg Ser
1               5                   10                  15

Ser Ala Met Lys Lys Ile Thr Met Gly Thr Ala Ser Ile Ile Leu Gly
            20                  25                  30

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

Glu Ala Thr Asn Ala Thr Asn Asn Gln Ser Thr Gln Val Ser Gln Ala
    50                  55                  60

Thr Ser Gln Pro Ile Asn Phe Gln Val Gln Lys Asp Gly Ser Ser Glu
65                  70                  75                  80

Lys Ser His Met Asp Asp Tyr Met Gln His Pro Gly Lys Val Ile Lys
                85                  90                  95

Gln Asn Asn Lys Tyr Tyr Phe Gln Thr Val Leu Asn Asn Ala Ser Phe
            100                 105                 110

Trp Lys Glu Tyr Lys Phe Tyr Asn Ala Asn Asn Gln Glu Leu Ala Thr
        115                 120                 125

Thr Val Val Asn Asp Asn Lys Lys Ala Asp Thr Arg Thr Ile Asn Val
    130                 135                 140

Ala Val Glu Pro Gly Tyr Lys Ser Leu Thr Thr Lys Val His Ile Val
145                 150                 155                 160

Val Pro Gln Ile Asn Tyr Asn His Arg Tyr Thr Thr His Leu Glu Phe
                165                 170                 175

Glu Lys Ala Ile Pro Thr Leu Ala Asp Ala Ala Lys Pro Asn Asn Val
            180                 185                 190

Lys Pro Val Gln Pro Lys Pro Ala Gln Pro Lys Thr Pro Thr Glu Gln
        195                 200                 205

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
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                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
225                 230                 235                 240

His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
                245                 250                 255

Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
            260                 265                 270

Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
        275                 280                 285

Glu Arg Gln Val Tyr Glu Leu Asn Lys Ile Gln Asp Lys Leu Pro Glu
    290                 295                 300

Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
305                 310                 315                 320

Leu Asp Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
                325                 330                 335

Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
            340                 345                 350
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Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
        355                 360                 365

His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
    370                 375                 380

Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
385                 390                 395                 400

Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
                405                 410                 415

Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
            420                 425                 430

Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
        435                 440                 445

Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
    450                 455                 460

Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
465                 470                 475                 480

Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
                485                 490                 495

Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
            500                 505                 510

Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
        515                 520                 525

Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
    530                 535                 540

Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
545                 550                 555                 560

Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
                565                 570                 575

Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
            580                 585                 590

His Thr Gln Ser Gln Asn Asn Lys Asn Thr Gln Glu Asn Lys Ala Lys
        595                 600                 605

Ser Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro
    610                 615                 620

Leu Met Ala Leu Leu Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro
625                 630                 635                 640

Arg Lys Arg Lys Asn
                645


<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
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<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

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

Tyr Ser Asn Ser Asn Asn Thr Met Pro Ile Ala Asp Ile Lys Ser Thr
275 280 285

Asn Gly Asp Val Val Ala Lys Ala Thr Tyr Asp Ile Leu Thr Lys Thr
290 295 300

Tyr Thr Phe Val Phe Thr Asp Tyr Val Asn Asn Lys Glu Asn Ile Asn
305 310 315 320

Gly Gln Phe Ser Leu Pro Leu Phe Thr Asp Arg Ala Lys Ala Pro Lys
325 330 335

Ser Gly Thr Tyr Asp Ala Asn Ile Asn Ile Ala Asp Glu Met Phe Asn
340 345 350

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

Pro Asn Gly Ala Asn Ile Ser Ser Gln Ile Ile Gly Val Asp Thr Ala

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    370                 375                 380

Ser Gly Gln Asn Thr Tyr Lys Gln Thr Val Phe Val Asn Pro Lys Gln
385                 390                 395                 400

Arg Val Leu Gly Asn Thr Trp Val Tyr Ile Lys Gly Tyr Gln Asp Lys
                405                 410                 415

Ile Glu Glu Ser Ser Gly Lys Val Ser Ala Thr Asp Thr Lys Leu Arg
            420                 425                 430

Ile Phe Glu Val Asn Asp Thr Ser Lys Leu Ser Asp Ser Tyr Tyr Ala
        435                 440                 445

Asp Pro Asn Asp Ser Asn Leu Lys Glu Val Thr Asp Gln Phe Lys Asn
    450                 455                 460

Arg Ile Tyr Tyr Glu His Pro Asn Val Ala Ser Ile Lys Phe Gly Asp
465                 470                 475                 480

Ile Thr Lys Thr Tyr Val Val Leu Val Glu Gly His Tyr Asp Asn Thr
                485                 490                 495

Gly Lys Asn Leu Lys Thr Gln Val Ile Gln Glu Asn Val Asp Pro Val
            500                 505                 510

Thr Asn Arg Asp Tyr Ser Ile Phe Gly Trp Asn Asn Glu Asn Val Val
        515                 520                 525

Arg Tyr Gly Gly Gly Ser Ala Asp Gly Asp Ser Ala Val Asn Pro Lys
    530                 535                 540

Asp Pro Thr Pro Gly Pro Pro Val Asp Pro Glu Pro Ser Pro Asp Pro
545                 550                 555                 560

Glu Pro Glu Pro Thr Pro Asp Pro Glu Pro Ser Pro Asp Pro Glu Pro
                565                 570                 575

Glu Pro Ser Pro Asp Pro Asp Pro Asp Ser Asp Ser Asp Ser Asp Ser
            580                 585                 590

Gly Ser Asp Ser Asp Ser Gly Ser Asp Ser Asp Ser Glu Ser Asp Ser
        595                 600                 605

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Glu Ser
    610                 615                 620

Asp Ser Asp Ser Glu Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
625                 630                 635                 640

Asp Ser Asp Ser Asp Ser Glu Ser Asp Ser Asp Ser Asp Ser Asp Ser
                645                 650                 655

Asp Ser Asp Ser Asp Ser Asp Ser Glu Ser Asp Ser Asp Ser Glu Ser
            660                 665                 670

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
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```
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


<210> SEQ ID NO 19
<211> LENGTH: 227
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 19

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

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
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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
305                 310                 315                 320

Leu Glu Glu Leu Ala Arg Thr Asp Asp Lys Lys Tyr Ala Thr Gln Ser
                325                 330                 335

Ile Leu Glu Ser Ile Phe Asn Lys Asp Glu Ala Asp Lys Ile Leu Lys
            340                 345                 350

Asp Ile Arg Val Asp Gly Lys Thr Asp Gln Gln Ile Ala Asp Gln Ile
        355                 360                 365

Thr Arg His Ile Asp Gln Leu Ser Leu Thr Thr Ser Asp Asp Leu Leu
    370                 375                 380

Thr Ser Leu Ile Asp Gln Ser Gln Asp Lys Ser Leu Leu Ile Ser Gln
385                 390                 395                 400

Ile Leu Gln Thr Lys Leu Gly Lys Ala Glu Ala Asp Lys Leu Ala Lys
                405                 410                 415

Asp Trp Thr Asn Lys Gly Leu Ser Asn Arg Gln Ile Val Asp Gln Leu
            420                 425                 430
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Lys Lys His Phe Ala Ser Thr Gly Asp Thr Ser Ser Asp Asp Ile Leu
        435                 440                 445

Lys Ala Ile Leu Asn Asn Ala Lys Asp Lys Lys Gln Ala Ile Glu Thr
    450                 455                 460

Ile Leu Ala Thr Arg Ile Glu Arg Gln Lys Ala Lys Leu Leu Ala Asp
465                 470                 475                 480

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

Lys Ser Ala Leu Asn Gly Lys Ala Asp Asp Leu Leu Asn Leu Gln Lys
            500                 505                 510

Arg Leu Asn Gln Thr Lys Lys Asp Ile Asp Tyr Ile Leu Ser Pro Ile
        515                 520                 525

Val Asn Arg Pro Ser Leu Leu Asp Arg Leu Asn Lys Asn Gly Lys Thr
    530                 535                 540

Thr Asp Leu Asn Lys Leu Ala Asn Leu Met Asn Gln Gly Ser Asn Leu
545                 550                 555                 560

Leu Asp Ser Ile Pro Asp Ile Pro Thr Pro Lys Pro Glu Lys Thr Leu
                565                 570                 575

Thr Leu Gly Lys Gly Asn Gly Leu Leu Ser Gly Leu Leu Asn Ala Asp
            580                 585                 590

Gly Asn Val Ser Leu Pro Lys Ala Gly Glu Thr Ile Lys Glu His Trp
        595                 600                 605

Leu Pro Ile Ser Val Ile Val Gly Ala Met Gly Val Leu Met Ile Trp
    610                 615                 620

Leu Ser Arg Arg Asn Lys Leu Lys Asn Lys Ala
625                 630                 635


<210> SEQ ID NO 21
<211> LENGTH: 953
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 21

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

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

Ile Leu Val Gly Thr Thr Leu Ile Phe Gly Leu Ser Gly His Glu Ala
        35                  40                  45

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
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```
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 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
465                 470                 475                 480

Tyr Ser Asn Val Asn Gly Ser Ser Thr Ala Asn Gly Asp Gln Lys Lys
                485                 490                 495

Tyr Asn Leu Gly Asp Tyr Val Trp Glu Asp Thr Asn Lys Asp Gly Lys
            500                 505                 510

Gln Asp Ala Asn Glu Lys Gly Ile Lys Gly Val Tyr Val Ile Leu Lys
        515                 520                 525

Asp Ser Asn Gly Lys Glu Leu Asp Arg Thr Thr Thr Asp Glu Asn Gly
    530                 535                 540

Lys Tyr Gln Phe Thr Gly Leu Ser Asn Gly Thr Tyr Ser Val Glu Phe
545                 550                 555                 560

Ser Thr Pro Ala Gly Tyr Thr Pro Thr Thr Ala Asn Ala Gly Thr Asp
                565                 570                 575

Asp Ala Val Asp Ser Asp Gly Leu Thr Thr Thr Gly Val Ile Lys Asp
            580                 585                 590
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```
Ala Asp Asn Met Thr Leu Asp Ser Gly Phe Tyr Lys Thr Pro Lys Tyr
        595                 600                 605

Ser Leu Gly Asp Tyr Val Trp Tyr Asp Ser Asn Lys Asp Gly Lys Gln
    610                 615                 620

Asp Ser Thr Glu Lys Gly Ile Lys Gly Val Lys Val Thr Leu Gln Asn
625                 630                 635                 640

Glu Lys Gly Glu Val Ile Gly Thr Thr Glu Thr Asp Glu Asn Gly Lys
                645                 650                 655

Tyr Arg Phe Asp Asn Leu Asp Ser Gly Lys Tyr Lys Val Ile Phe Glu
            660                 665                 670

Lys Pro Ala Gly Leu Thr Gln Thr Gly Thr Asn Thr Thr Glu Asp Asp
        675                 680                 685

Lys Asp Ala Asp Gly Gly Glu Val Asp Val Thr Ile Thr Asp His Asp
    690                 695                 700

Asp Phe Thr Leu Asp Asn Gly Tyr Tyr Glu Glu Glu Thr 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 Glu 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 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
                805                 810                 815

Asp Ser Asp Ser Asp Ser Asp Asn Asp Ser Asp Ser Asp Ser Asp Ser
            820                 825                 830

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
        835                 840                 845

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
    850                 855                 860

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
865                 870                 875                 880

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ala Gly Lys
                885                 890                 895

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
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```
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
305                 310                 315                 320

Asp Tyr Val Asp Asn Lys Glu Asn Val Thr Ala Asn Ile Thr Met Pro
                325                 330                 335

Ala Tyr Ile Asp Pro Glu Asn Val Thr Lys Thr Gly Asn Val Thr Leu
            340                 345                 350

Thr Thr Gly Ile Gly Thr Asn Thr Ala Ser Lys Thr Val Leu Ile Asp
        355                 360                 365

Tyr Glu Lys Tyr Gly Gln Phe His Asn Leu Ser Ile Lys Gly Thr Ile
    370                 375                 380

Asp Gln Ile Asp Lys Thr Asn Asn Thr Tyr Arg Gln Thr Ile Tyr Val
385                 390                 395                 400

Asn Pro Ser Gly Asp Asn Val Val Leu Pro Ala Leu Thr Gly Asn Leu
                405                 410                 415

Ile Pro Asn Thr Lys Ser Asn Ala Leu Ile Asp Ala Lys Asn Thr Asp
            420                 425                 430
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```
Ile Lys Val Tyr Arg Val Asp Asn Ala Asn Asp Leu Ser Glu Ser Tyr
        435                 440                 445

Tyr Val Asn Pro Ser Asp Phe Glu Asp Val Thr Asn Gln Val Arg Ile
    450                 455                 460

Ser Phe Pro Asn Ala Asn Gln Tyr Lys Val Glu Phe Pro Thr Asp Asp
465                 470                 475                 480

Asp Gln Ile Thr Thr Pro Tyr Ile Val Val Val Asn Gly His Ile Asp
                485                 490                 495

Pro Ala Ser Thr Gly Asp Leu Ala Leu Arg Ser Thr Phe Tyr Gly Tyr
            500                 505                 510

Asp Ser Asn Phe Ile Trp Arg Ser Met Ser Trp Asp Asn Glu Val Ala
        515                 520                 525

Phe Asn Asn Gly Ser Gly Ser Gly Asp Gly Ile Asp Lys Pro Val Val
    530                 535                 540

Pro Glu Gln Pro Asp Glu Pro Gly Glu Ile Glu Pro Ile Pro Glu Asp
545                 550                 555                 560

Ser Asp Ser Asp Pro Gly Ser Asp Ser Gly Ser Asp Ser Asn Ser Asp
                565                 570                 575

Ser Gly Ser Asp Ser Gly Ser Asp Ser Thr Ser Asp Ser Gly Ser Asp
            580                 585                 590

Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp
        595                 600                 605

Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala
    610                 615                 620

Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp
625                 630                 635                 640

Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp
                645                 650                 655

Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Asp
            660                 665                 670

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

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

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

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

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
        755                 760                 765

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
785                 790                 795                 800

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 Glu
            820                 825                 830

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
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    850                 855                 860

Ser Asp Ser Glu 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
        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

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
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```
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 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
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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
                325                 330                 335

Lys Gln Thr Gly Asp Gly Tyr Gln Trp Val Ile Lys Phe Asn Lys Gly
            340                 345                 350

His Ala Pro His Gln Asn Met Ile Phe Trp Phe Ala Leu Pro Ala Asp
        355                 360                 365

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

Thr Asn Val Gln Trp Ser His Gly Ala Gly Ala Gly Ala Asn Lys Pro
385                 390                 395                 400

Leu Gln Gln Met Trp Glu Tyr Gly Val Asn Asp Pro His Arg Ser His
                405                 410                 415

Asp Phe Lys Ile Arg Asn Arg Ser Gly Gln Val Ile Tyr Asp Trp Pro
            420                 425                 430

Thr Val His Ile Tyr Ser Leu Glu Asp Leu Ser Arg Ala Ser Asp Tyr
        435                 440                 445
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Phe Ser Glu Ala Gly Ala Thr Pro Ala Thr Lys Ala Phe Gly Arg Gln
    450                 455                 460

Asn Phe Glu Tyr Ile Asn Gly Gln Lys Pro Ala Glu Ser Pro Gly Val
465                 470                 475                 480

Pro Lys Val Tyr Thr Phe Ile Gly Gln Gly Asp Ala Ser Tyr Thr Ile
                485                 490                 495

Ser Phe Lys Thr Gln Gly Pro Thr Val Asn Lys Leu Tyr Tyr Ala Ala
            500                 505                 510

Gly Gly Arg Ala Leu Glu Tyr Asn Gln Leu Phe Met Tyr Ser Gln Leu
        515                 520                 525

Tyr Val Glu Ser Thr Gln Asp His Gln Gln Arg Leu Asn Gly Leu Arg
    530                 535                 540

Gln Val Val Asn Arg Thr Tyr Arg Ile Gly Thr Thr Lys Arg Val Glu
545                 550                 555                 560

Val Ser Gln Gly Asn Val Gln Thr Lys Lys Val Leu Glu Ser Thr Asn
                565                 570                 575

Leu Asn Ile Asp Asp Phe Val Asp Asp Pro Leu Ser Tyr Val Lys Thr
            580                 585                 590

Pro Ser Asn Lys Val Leu Gly Phe Tyr Ser Asn Asn Ala Asn Thr Asn
        595                 600                 605

Ala Phe Arg Pro Gly Gly Ala Gln Gln Leu Asn Glu Tyr Gln Leu Ser
    610                 615                 620

Gln Leu Phe Thr Asp Gln Lys Leu Gln Glu Ala Ala Arg Thr Arg Asn
625                 630                 635                 640

Pro Ile Arg Leu Met Ile Gly Phe Asp Tyr Pro Asp Ala Tyr Gly Asn
                645                 650                 655

Ser Glu Thr Leu Val Pro Val Asn Leu Thr Val Leu Pro Glu Ile Gln
            660                 665                 670

His Asn Ile Lys Phe Phe Lys Asn Asp Asp Thr Gln Asn Ile Ala Glu
        675                 680                 685

Lys Pro Phe Ser Lys Gln Ala Gly His Pro Val Phe Tyr Val Tyr Ala
    690                 695                 700

Gly Asn Gln Gly Asn Ala Ser Val Asn Leu Gly Gly Ser Val Thr Ser
705                 710                 715                 720

Ile Gln Pro Leu Arg Ile Asn Leu Thr Ser Asn Glu Asn Phe Thr Asp
                725                 730                 735

Lys Asp Trp Gln Ile Thr Gly Ile Pro Arg Thr Leu His Ile Glu Asn
            740                 745                 750

Ser Thr Asn Arg Pro Asn Asn Ala Arg Glu Arg Asn Ile Glu Leu Val
        755                 760                 765

Gly Asn Leu Leu Pro Gly Asp Tyr Phe Gly Thr Ile Arg Phe Gly Arg
    770                 775                 780

Lys Glu Gln Leu Phe Glu Ile Arg Val Lys Pro His Thr Pro Thr Ile
785                 790                 795                 800

Thr Thr Thr Ala Glu Gln Leu Arg Gly Thr Ala Leu Gln Lys Val Pro
                805                 810                 815

Val Asn Ile Ser Gly Ile Pro Leu Asp Pro Ser Ala Leu Val Tyr Leu
            820                 825                 830

Val Ala Pro Thr Asn Gln Thr Thr Asn Gly Gly Ser Glu Ala Asp Gln
        835                 840                 845

Ile Pro Ser Gly Tyr Thr Ile Leu Ala Thr Gly Thr Pro Asp Gly Val
    850                 855                 860

His Asn Thr Ile Thr Ile Arg Pro Gln Asp Tyr Val Val Phe Ile Pro
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865                 870                 875                 880

Pro Val Gly Lys Gln Ile Arg Ala Val Val Tyr Tyr Asn Lys Val Val
                885                 890                 895

Ala Ser Asn Met Ser Asn Ala Val Thr Ile Leu Pro Asp Asp Ile Pro
            900                 905                 910

Pro Thr Ile Asn Asn Pro Val Gly Ile Asn Ala Lys Tyr Tyr Arg Gly
        915                 920                 925

Asp Glu Val Asn Phe Thr Met Gly Val Ser Asp Arg His Ser Gly Ile
    930                 935                 940

Lys Asn Thr Thr Ile Thr Thr Leu Pro Asn Gly Trp Thr Ser Asn Leu
945                 950                 955                 960

Thr Lys Ala Asp Lys Asn Asn Gly Ser Leu Ser Ile Thr Gly Arg Val
                965                 970                 975

Ser Met Asn Gln Ala Phe Asn Ser Asp Ile Thr Phe Lys Val Ser Ala
            980                 985                 990

Thr Asp Asn Val Asn Asn Thr Thr  Asn Asp Ser Gln Ser  Lys His Val
        995                 1000                1005

Ser Ile  His Val Gly Lys Ile  Ser Glu Asp Ala His  Pro Ile Val
    1010                1015                1020

Leu Gly  Asn Thr Glu Lys Val  Val Val Val Asn Pro  Thr Ala Val
    1025                1030                1035

Ser Asn  Asp Glu Lys Gln Ser  Ile Ile Thr Ala Phe  Met Asn Lys
    1040                1045                1050

Asn Gln  Asn Ile Arg Gly Tyr  Leu Ala Ser Thr Asp  Pro Val Thr
    1055                1060                1065

Val Asp  Asn Asn Gly Asn Val  Thr Leu His Tyr Arg  Asp Gly Ser
    1070                1075                1080

Ser Thr  Thr Leu Asp Ala Thr  Asn Val Met Thr Tyr  Glu Pro Val
    1085                1090                1095

Val Lys  Pro Glu Tyr Gln Thr  Val Asn Ala Ala Lys  Thr Ala Thr
    1100                1105                1110

Val Thr  Ile Ala Lys Gly Gln  Ser Phe Ser Ile Gly  Asp Ile Lys
    1115                1120                1125

Gln Tyr  Phe Thr Leu Ser Asn  Gly Gln Pro Ile Pro  Ser Gly Thr
    1130                1135                1140

Phe Thr  Asn Ile Thr Ser Asp  Arg Thr Ile Pro Thr  Ala Gln Glu
    1145                1150                1155

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
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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
    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
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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

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
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    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

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
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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
    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
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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  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

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
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    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

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
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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
    3920                 3925                 3930

Ala Asp  Glu Gln Lys Arg Asn  Ala Tyr Asn Gln Ala  Val Ser Ala
    3935                 3940                 3945

Ala Glu  Thr Ile Leu Asn Lys  Gln Thr Gly Pro Asn  Thr Ala Lys
    3950                 3955                 3960

Thr Ala  Val Glu Gln Ala Leu  Asn Asn Val Asn Asn  Ala Lys His
    3965                 3970                 3975

Ala Leu  Asn Gly Thr Gln Asn  Leu Asn Asn Ala Lys  Gln Ala Ala
    3980                 3985                 3990

Ile Thr  Ala Ile Asn Gly Ala  Ser Asp Leu Asn Gln  Lys Gln Lys
    3995                 4000                 4005

Asp Ala  Leu Lys Ala Gln Ala  Asn Gly Ala Gln Arg  Val Ser Asn
    4010                 4015                 4020

Ala Gln  Asp Val Gln His Asn  Ala Thr Glu Leu Asn  Thr Ala Met
    4025                 4030                 4035
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Gly Thr  Leu Lys His Ala Ile  Ala Asp Lys Thr Asn  Thr Leu Ala
    4040                4045                4050

Ser Ser  Lys Tyr Val Asn Ala  Asp Ser Thr Lys Gln  Asn Ala Tyr
    4055                4060                4065

Thr Thr  Lys Val Thr Asn Ala  Glu His Ile Ile Ser  Gly Thr Pro
    4070                4075                4080

Thr Val  Val Thr Thr Pro Ser  Glu Val Thr Ala Ala  Ala Asn Gln
    4085                4090                4095

Val Asn  Ser Ala Lys Gln Glu  Leu Asn Gly Asp Glu  Arg Leu Arg
    4100                4105                4110

Glu Ala  Lys Gln Asn Ala Asn  Thr Ala Ile Asp Ala  Leu Thr Gln
    4115                4120                4125

Leu Asn  Thr Pro Gln Lys Ala  Lys Leu Lys Glu Gln  Val Gly Gln
    4130                4135                4140

Ala Asn  Arg Leu Glu Asp Val  Gln Thr Val Gln Thr  Asn Gly Gln
    4145                4150                4155

Ala Leu  Asn Asn Ala Met Lys  Gly Leu Arg Asp Ser  Ile Ala Asn
    4160                4165                4170

Glu Thr  Thr Val Lys Thr Ser  Gln Asn Tyr Thr Asp  Ala Ser Pro
    4175                4180                4185

Asn Asn  Gln Ser Thr Tyr Asn  Ser Ala Val Ser Asn  Ala Lys Gly
    4190                4195                4200

Ile Ile  Asn Gln Thr Asn Asn  Pro Thr Met Asp Thr  Ser Ala Ile
    4205                4210                4215

Thr Gln  Ala Thr Thr Gln Val  Asn Asn Ala Lys Asn  Gly Leu Asn
    4220                4225                4230

Gly Ala  Glu Asn Leu Arg Asn  Ala Gln Asn Thr Ala  Lys Gln Asn
    4235                4240                4245

Leu Asn  Thr Leu Ser His Leu  Thr Asn Asn Gln Lys  Ser Ala Ile
    4250                4255                4260

Ser Ser  Gln Ile Asp Arg Ala  Gly His Val Ser Glu  Val Thr Ala
    4265                4270                4275

Thr Lys  Asn Ala Ala Thr Glu  Leu Asn Thr Gln Met  Gly Asn Leu
    4280                4285                4290

Glu Gln  Ala Ile His Asp Gln  Asn Thr Val Lys Gln  Ser Val Lys
    4295                4300                4305

Phe Thr  Asp Ala Asp Lys Ala  Lys Arg Asp Ala Tyr  Thr Asn Ala
    4310                4315                4320

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
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    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

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
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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
    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
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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

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
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    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

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
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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
    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
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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

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
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    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

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
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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
    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
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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

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
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    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

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
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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
    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
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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

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
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    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

Lys His  Leu Val Asp Asn Glu  Ile Pro Asn Ala Glu  Gln Thr Val
    9455                9460                9465

Asn Tyr  Gln Asn Ala Asp Asp  Asn Ala Lys Thr Asn  Phe Asp Asp
    9470                9475                9480

Ala Lys  Arg Leu Ala Asn Thr  Leu Leu Asn Ser Asp  Asn Thr Asn
    9485                9490                9495

Val Asn  Asp Ile Asn Gly Ala  Ile Gln Ala Val Asn  Asp Ala Ile
    9500                9505                9510

His Asn  Leu Asn Gly Asp Gln  Arg Leu Gln Asp Ala  Lys Asp Lys
    9515                9520                9525

Ala Ile  Gln Ser Ile Asn Gln  Ala Leu Ala Asn Lys  Leu Lys Glu
    9530                9535                9540

Ile Glu  Ala Ser Asn Ala Thr  Asp Gln Asp Lys Leu  Ile Ala Lys
    9545                9550                9555

Asn Lys  Ala Glu Glu Leu Ala  Asn Ser Ile Ile Asn  Asn Ile Asn
    9560                9565                9570
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Lys Ala  Thr Ser Asn Gln Ala  Val Ser Gln Val Gln  Thr Ala Gly
    9575                 9580                 9585

Asn His  Ala Ile Glu Gln Val  His Ala Asn Glu Ile  Pro Lys Ala
    9590                 9595                 9600

Lys Ile  Asp Ala Asn Lys Asp  Val Asp Lys Gln Val  Gln Ala Leu
    9605                 9610                 9615

Ile Asp  Glu Ile Asp Arg Asn  Pro Asn Leu Thr Asp  Lys Glu Lys
    9620                 9625                 9630

Gln Ala  Leu Lys Asp Arg Ile  Asn Gln Ile Leu Gln  Gln Gly His
    9635                 9640                 9645

Asn Gly  Ile Asn Asn Ala Met  Thr Lys Glu Glu Ile  Glu Gln Ala
    9650                 9655                 9660

Lys Ala  Gln Leu Ala Gln Ala  Leu Gln Asp Ile Lys  Asp Leu Val
    9665                 9670                 9675

Lys Ala  Lys Glu Asp Ala Lys  Gln Asp Val Asp Lys  Gln Val Gln
    9680                 9685                 9690

Ala Leu  Ile Asp Glu Ile Asp  Gln Asn Pro Asn Leu  Thr Asp Lys
    9695                 9700                 9705

Glu Lys  Gln Ala Leu Lys Tyr  Arg Ile Asn Gln Ile  Leu Gln Gln
    9710                 9715                 9720

Gly His  Asn Asp Ile Asn Asn  Ala Leu Thr Lys Glu  Glu Ile Glu
    9725                 9730                 9735

Gln Ala  Lys Ala Gln Leu Ala  Gln Ala Leu Gln Asp  Ile Lys Asp
    9740                 9745                 9750

Leu Val  Lys Ala Lys Glu Asp  Ala Lys Asn Ala Ile  Lys Ala Leu
    9755                 9760                 9765

Ala Asn  Ala Lys Arg Asp Gln  Ile Asn Ser Asn Pro  Asp Leu Thr
    9770                 9775                 9780

Pro Glu  Gln Lys Ala Lys Ala  Leu Lys Glu Ile Asp  Glu Ala Glu
    9785                 9790                 9795

Lys Arg  Ala Leu Gln Asn Val  Glu Asn Ala Gln Thr  Ile Asp Gln
    9800                 9805                 9810

Leu Asn  Arg Gly Leu Asn Leu  Gly Leu Asp Asp Ile  Arg Asn Thr
    9815                 9820                 9825

His Val  Trp Glu Val Asp Glu  Gln Pro Ala Val Asn  Glu Ile Phe
    9830                 9835                 9840

Glu Ala  Thr Pro Glu Gln Ile  Leu Val Asn Gly Glu  Leu Ile Val
    9845                 9850                 9855

His Arg  Asp Asp Ile Ile Thr  Glu Gln Asp Ile Leu  Ala His Ile
    9860                 9865                 9870

Asn Leu  Ile Asp Gln Leu Ser  Ala Glu Val Ile Asp  Thr Pro Ser
    9875                 9880                 9885

Thr Ala  Thr Ile Ser Asp Ser  Leu Thr Ala Lys Val  Glu Val Thr
    9890                 9895                 9900

Leu Leu  Asp Gly Ser Lys Val  Ile Val Asn Val Pro  Val Lys Val
    9905                 9910                 9915

Val Glu  Lys Glu Leu Ser Val  Val Lys Gln Gln Ala  Ile Glu Ser
    9920                 9925                 9930

Ile Glu  Asn Ala Ala Gln Gln  Lys Ile Asn Glu Ile  Asn Asn Ser
    9935                 9940                 9945

Val Thr  Leu Thr Leu Glu Gln  Lys Glu Ala Ala Ile  Ala Glu Val
    9950                 9955                 9960
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Asn Lys  Leu Lys Gln Gln Ala  Ile Asp His Val Asn  Asn Ala Pro
    9965                9970                9975

Asp Val  His Ser Val Glu Glu  Ile Gln Gln Gln Glu  Gln Ala His
    9980                9985                9990

Ile Glu  Gln Phe Asn Pro Glu   Gln Phe Thr Ile Glu   Gln Ala Lys
    9995                10000                10005

Ser Asn   Ala Ile Lys Ser Ile   Glu Asp Ala Ile Gln   His Met Ile
    10010                10015                10020

Asp Glu   Ile Lys Ala Arg Thr   Asp Leu Thr Asp Lys   Glu Lys Gln
    10025                10030                10035

Glu Ala   Ile Ala Lys Leu Asn   Gln Leu Lys Glu Gln   Ala Ile Gln
    10040                10045                10050

Ala Ile   Gln Arg Ala Gln Ser   Ile Asp Glu Ile Ser   Glu Gln Leu
    10055                10060                10065

Glu Gln   Phe Lys Ala Gln Met   Lys Ala Ala Asn Pro   Thr Ala Lys
    10070                10075                10080

Glu Leu   Ala Lys Arg Lys Gln   Glu Ala Ile Ser Arg   Ile Lys Asp
    10085                10090                10095

Phe Ser   Asn Glu Lys Ile Asn   Ser Ile Arg Asn Ser   Glu Ile Gly
    10100                10105                10110

Thr Ala   Asp Glu Lys Gln Ala   Ala Met Asn Gln Ile   Asn Glu Ile
    10115                10120                10125

Val Leu   Glu Thr Ile Arg Asp   Ile Asn Asn Ala His   Thr Leu Gln
    10130                10135                10140

Gln Val   Glu Ala Ala Leu Asn   Asn Gly Ile Ala Arg   Ile Ser Ala
    10145                10150                10155

Val Gln   Ile Val Thr Ser Asp   Arg Ala Lys Gln Ser   Ser Ser Thr
    10160                10165                10170

Gly Asn   Glu Ser Asn Ser His   Leu Thr Ile Gly Tyr   Gly Thr Ala
    10175                10180                10185

Asn His   Pro Phe Asn Ser Ser   Thr Ile Gly His Lys   Lys Lys Leu
    10190                10195                10200

Asp Glu   Asp Asp Asp Ile Asp   Pro Leu His Met Arg   His Phe Ser
    10205                10210                10215

Asn Asn   Phe Gly Asn Val Ile   Lys Asn Ala Ile Gly   Val Val Gly
    10220                10225                10230

Ile Ser   Gly Leu Leu Ala Ser   Phe Trp Phe Phe Ile   Ala Lys Arg
    10235                10240                10245

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
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    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
            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
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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
            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
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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                 185                 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

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
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               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
            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
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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

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
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                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
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                 320

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
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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                 400

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                 480

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                 560

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


<210> SEQ ID NO 30
<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
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```
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

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
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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
<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
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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

Asp Phe Thr Thr Thr His Gln Ser Pro Thr Thr Ser Asn His Thr His
                405                 410                 415

Asn Asn Val Val Glu Phe Glu Glu Thr Ser Ala Leu Pro Gly Arg Lys
            420                 425                 430

Ser Gly Ser Leu Val Gly Ile Ser Gln Ile Asp Ser Ser His Leu Thr
        435                 440                 445

Glu Arg Glu Lys Arg Val Ile Lys Arg Glu His Val Arg Glu Ala Gln
    450                 455                 460

Lys Leu Val Asp Asn Tyr Lys Asp Thr His Ser Tyr Lys Asp Arg Ile
465                 470                 475                 480

Asn Ala Gln Gln Lys Val Asn Thr Leu Ser Glu Gly His Gln Lys Arg
                485                 490                 495

Phe Asn Lys Gln Ile Asn Lys Val Tyr Asn Gly Lys
            500                 505
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-continued

<210> SEQ ID NO 33
<211> LENGTH: 520
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 33

Met Leu Thr Leu Gln Ile His Thr Gly Gly Ile Asn Leu Lys Lys Lys
1 5 10 15

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

Leu Gly Thr Leu Leu Ile Ser Gly Gly Val Thr Pro Ala Ala Asn Ala
35 40 45

Ala Gln His Asp Glu Ala Gln Gln Asn Ala Phe Tyr Gln Val Leu Asn
50 55 60

Met Pro Asn Leu Asn Ala Asp Gln Arg Asn Gly Phe Ile Gln Ser Leu
65 70 75 80

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

Leu Asn Asp Ser Gln Ala Pro Lys Ala Asp Ala Gln Gln Asn Asn Phe
100 105 110

Asn Lys Asp Gln Gln Ser Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn
115 120 125

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

Pro Ser Gln Ser Thr Asn Val Leu Gly Glu Ala Lys Lys Leu Asn Glu
145 150 155 160

Ser Gln Ala Pro Lys Ala Asp Asn Asn Phe Asn Lys Glu Gln Gln Asn
165 170 175

Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn Leu Asn Glu Glu Gln Arg
180 185 190

Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn
195 200 205

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

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

His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser
245 250 255

Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala Lys
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

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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

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
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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 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

aacatatgtt caacaaagat caacaaagc 29

<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 ccgccccaag ccaaagcact aac 43

<210> SEQ ID NO 41
<211> LENGTH: 43

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<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 41

gttagtgctt 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

<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

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<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 49

ggggaccact ttgtacaaga aagctgggtg acacctattg cacgattcg 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 ccctggtgta acaaatttat g 51

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

<400> SEQUENCE: 54

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 33

-continued

<210> SEQ ID NO 57
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 57

gaactgcagc gacaaactca ttatttgctt tgc 33

<210> SEQ ID NO 58
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 58

gaactcgagt ctagcttatt tacatgg 27

<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 tacaaac 27

<210> SEQ ID NO 61
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 61

gaactcgagg cattatgtgt atcacaaatt tggg 34

<210> SEQ ID NO 62
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Staphylococcus sp.

<400> SEQUENCE: 62

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: 61
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165

<400> SEQUENCE: 64

Ala Asp Ala Gln Gln Asn Gln His Asp Glu Ala Gln Gln Asn Ala Phe
1 5 10 15

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Tyr Gln Val Leu Asn Met Pro Asn Leu Asn Ala Asp Gln Arg Asn Gly
            20                  25                  30

Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Val Leu
        35                  40                  45

Gly Glu Ala Gln Lys Leu Asn Asp Ser Gln Ala Pro Lys
    50                  55                  60


<210> SEQ ID NO 65
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165

<400> SEQUENCE: 65

Ala Asp Ala Gln Gln Asn Asn Phe Asn Lys Asp Gln Gln Ser Ala Phe
1               5                   10                  15

Tyr Glu Ile Leu Asn Met Pro Asn Leu Asn Glu Ala Gln Arg Asn Gly
            20                  25                  30

Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Thr Asn Val Leu
        35                  40                  45

Gly Glu Ala Lys Lys Leu Asn Glu Ser Gln Ala Pro Lys
    50                  55                  60


<210> SEQ ID NO 66
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165

<400> SEQUENCE: 66

Ala Asp Asn Gln His Asp Glu Glu Gln Gln Asn Ala Phe Tyr Gln Ile
1               5                   10                  15

Leu Asn Met Pro Asn Leu Asn Ala Glu Gln Arg Asn Gly Phe Ile Gln
            20                  25                  30

Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ser Glu Ala
        35                  40                  45

Gln Lys Leu Asn Asp Ser Gln Ala Pro Lys
    50                  55


<210> SEQ ID NO 67
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165

<400> SEQUENCE: 67

Ala Asp Asn Lys His Asp Glu Glu Gln Gln Asn Ala Phe Tyr Gln Ile
1               5                   10                  15

Leu His Leu Pro Asn Leu Thr Ala Glu Gln Arg Asn Gly Phe Ile Gln
            20                  25                  30

Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala
        35                  40                  45

Gln Lys Leu Asn Asp Ala Gln Ala Pro Lys
    50                  55


<210> SEQ ID NO 68
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165

<400> SEQUENCE: 68

Ala Asp Asn Lys His Asp Glu Glu Gln Gln Asn Ala Phe Tyr Gln Ile
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-continued

```
1               5                   10                  15

Leu His Leu Pro Asn Leu Asn Ala Glu Gln Arg Asn Gly Phe Ile Gln
            20                  25                  30

Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Glu Leu Leu Ala Glu Ala
        35                  40                  45

Gln Lys Leu Asn Asp Ala Gln Ala Pro Lys
    50                  55


<210> SEQ ID NO 69
<211> LENGTH: 50
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165

<400> SEQUENCE: 69

Phe Asn Lys Asp Lys Lys Ser Ala Phe Tyr Glu Ile Leu Asn Met Pro
1               5                   10                  15

Asn Leu Asn Glu Ala Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Ala
            20                  25                  30

Ala Pro Ser Gln Ser Thr Asn Val Leu Gly Glu Ala Lys Lys Leu Asn
        35                  40                  45

Glu Ser
    50


<210> SEQ ID NO 70
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: X is either Gly or Ser

<400> SEQUENCE: 70

Tyr Ser Ile Arg Lys Xaa
1               5


<210> SEQ ID NO 71
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: X is any amino acid

<400> SEQUENCE: 71

Leu Pro Xaa Thr Gly
1               5


<210> SEQ ID NO 72
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165

<400> SEQUENCE: 72

Tyr Ser Ile Arg Lys
1               5


<210> SEQ ID NO 73
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165

<400> SEQUENCE: 73
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Ile Val Thr Lys
1

<210> SEQ ID NO 74
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus sp. NT165

<400> SEQUENCE: 74

Val Val Ser Gly
1

The invention claimed is:

1. An isolated polypeptide comprising a Staphylococcal Protein A (SpA) variant domain D segment comprising amino acid substitutions at amino acids corresponding to positions 9, 10, 36 and 37 of SEQ ID NO: 2, wherein the substitutions at amino acids corresponding to positions 9 and 10 are with a lysine residue and the substitutions at amino acids corresponding to positions 36 and 37 are with an alanine residue, and wherein the amino acid sequence is at least 80% identical to SEQ ID NO:2.

2. The polypeptide of claim **1**, wherein the amino acid sequence is at least 85% identical to SEQ ID NO: 2.

3. The isolated polypeptide of claim **1**, further comprising one or more immunogenic fragments of SpA E domain, SpA A domain, SpA B domain, or SpA C domain.

4. The isolated polypeptide of claim **1**, further comprising two or more of immunogenic SpA D domain fragments.

5. The isolated polypeptide of claim **1**, further comprising an immunogenic fragment of a second staphylococcal antigen.

6. The isolated polypeptide of claim **5**, wherein the second staphylococcal antigen is selected from the group consisting of Emp, EsxA, EsxB, EsaC, Eap, Ebh, EsaB, Coa, vWbp, vWh, Hla, SdrC, SdrD, SdrE, lsdA, lsdB, lsdC, ClfA, ClfB and SasF.

7. A composition comprising an isolated polypeptide comprising Staphylococcal Protein A (SpA) variant domain D segment comprising amino acid substitutions at amino acids corresponding to positions 9, 10, 36 and 37 of SEQ ID NO: 2, wherein the substitutions at amino acids corresponding to positions 9 and 10 are with a lysine residue and the substitutions at amino acids corresponding to positions 36 and 37 are with an alanine residue, and wherein the amino acid sequence is at least 80% identical to SEQ ID NO: 2, and optionally wherein the polypeptide further comprises one or more immunogenic fragments of SpA E domain, SpA A domain, SpA B domain, or SpA C domain.

8. The composition of claim **7**, further comprising at least one other staphylococcal antigen selected from the group consisting of Emp, EsxA, EsxB, EsaC, Eap, Ebh, EsaB, Coa, vWbp, vWh, Hla, SdrC, SdrD, SdrE, lsdA, lsdB, lsdC, ClfA, ClfB and SasF.

9. The composition of claim **7**, wherein the composition contains less than 1% by weight of staphylococcal bacterial components other than the polypeptide comprising the SpA variant domain D segment.

10. The composition of claim **7**, further comprising a staphylococcal PIA capsular polysaccharide or its oligosaccharide.

11. The composition of claim **7**, further comprising type V or type VIII capsular polysaccharide of *S. aureus* or its oligosaccharide.

12. The composition of claim **7**, further comprising a staphylococcal capsular polysaccharide conjugated to a protein carrier.

13. The isolated polypeptide of claim **1**, wherein the polypeptide further comprises one or more immunogenic variants of SpA E domain, SpA A domain, SpA B domain, or SpA C domain that have an amino acid sequence that is at least 80% identical to SEQ ID NOS: 3, 4, 6, and 5, respectively, and wherein the one or more domains comprise amino acid substitutions at amino acids corresponding to positions 9, 10, 36 and 37 of SEQ ID NO: 2, wherein the amino acid substitutions corresponding to positions 9 and 10 are with a lysine residue and the amino acid substitutions corresponding to positions 36 and 37 are with an alanine residue.

14. The composition of claim **7**, wherein the composition further comprises immunogenic fragments comprising one or more of SpA E domain, SpA A domain, SpA B domain, or SpA C domain that have an amino acid sequence that is at least 80% identical to SEQ ID NOS: 3, 4, 6, and 5, respectively, and wherein the one or more domains comprise amino acid substitutions at amino acids corresponding to positions 9, 10, 36 and 37 of SEQ ID NO: 2, wherein the amino acid substitutions corresponding to positions 9 and 10 are with a lysine residue and the amino acid substitutions corresponding to positions 36 and 37 are with an alanine residue.

* * * * *