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(54) **Title:** PSM PEPTIDES AS VACCINE TARGETS AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

(57) **Abstract:** This disclosure concerns compositions and methods for the treatment and inhibition of infectious disease, particularly Methicillin-resistant Stapholococcus aureus (MRSA). In certain embodiments, the disclosure concerns immunogenic peptides, for instance P SMa peptides, that can be used to induce protective immunity against MRSA. Also disclosed are methods of detecting MRSA in a sample, and methods of diagnosing MRSA in a subject.

# PSM PEPTIDES AS VACCINE TARGETS AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

## FIELD OF THE DISCLOSURE

This disclosure concerns compositions and methods for the treatment and inhibition of infectious disease, particularly Methicillin-resistant *Stapholococcus aureus* (MRSA). In certain embodiments, the disclosure concerns immunogenic peptides, for instance PSMα peptides, that can be used to induce protective immunity against MRSA.

## CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/933,573, filed June 6, 2007, and U.S. Provisional Application No. 60/983,141, filed October 26, 2007, the disclosures of which are both incorporated herein in their- entirety.

## BACKGROUND

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a dangerous human pathogen. Traditionally, MRSA infections occurred exclusively in hospitals and were limited to immunocompromised patients or individuals with predisposing risk factors. However, MRSA strains have recently emerged that can cause severe infections (such as necrotizing fasciitis) or death in otherwise healthy adults. These strains are increasingly community-acquired, and can be contracted outside of the health care settings. As is true for hospital-acquired MRSA, the incidence of these community-associated (CA)-MRSA infections is increasing. For example, the majority of infections in patients reporting to emergency departments in the United States is now due to CA-MRSA.

It is unclear what makes CA-MRSA strains more successful at causing human disease compared with their hospital-associated counterparts. Given the foregoing, it would be desirable to know the cause of the increased virulence of CA-MRSA, and to have methods of treating, ameliorating, and preventing MRSA.

## SUMMARY OF THE DISCLOSURE

Described herein is a class of secreted staphylococcal peptides with an extraordinary ability to recruit, activate, and subsequently lyse human neutrophils, thus eliminating the main cellular defense against *S. aureus* infection. These peptides are produced at especially high levels in CA-MRSA and to a large extent determine their aggressive behavior and ability to cause disease in animal models of infection. Thus, disclosed is a set of virulence factors of *S. aureus* that account for the enhanced virulence of CA-MRSA.

The peptides are encoded by the phenol-soluble modulin (PSM) gene cluster and include PSMα1, PSMα2, PSMα3, and PSMα4, most of which activate and subsequently lyse neutrophils. The identification of these peptides enables the production of vaccines and other preventative and/or therapeutic agents for use in subjects infected with MRSA.

One disclosed embodiment is an isolated immunogenic peptide that includes at least one antigenic phenol-soluble modulin alpha (PSMα) peptide. The peptide includes (a) the amino acid sequence set forth as SEQ ID NO: 8; (b) the amino acid sequence set forth as SEQ ID NO: 2; (c) the amino acid sequence set forth as SEQ ID NO: 3; (d) the amino acid sequence set forth as SEQ ID NO: 4; (e) the amino acid sequence set forth as SEQ ID NO: 1; (f) an amino acid sequence having at least 85% sequence identity with (b) or (e); or (g) an amino acid sequence having at least 90% sequence identity with (a), (c), or (d). In some embodiments, the amino acid sequence has at least 90% sequence identity with (b) or (e). In other embodiments, the amino acid sequence has at least 95% sequence identity with (a), (b), (c), (d), or (e).

Another embodiment is a method for eliciting an immune response in a subject. The method includes (a) selecting a subject in which an immune response to the immunogenic protein of claim 1 is desirable; and (b) administering to the subject a therapeutically effective amount of the immunogenic protein described above. In some embodiments, mixtures of PSMα peptides are administered to the subject, and in other embodiments, one or more PSMβ peptide is administered in combination with one or more PSMα peptide. The method is particularly useful in

stimulating an immune response against *S. aureus*, for example MRSA. Subjects at risk of developing such infections can therefore be selected and administered the immunogen to stimulate their immunity against such infection, thereby producing an immune response in the subject.

The foregoing and other features will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

## BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: includes three panels showing phenol-soluble modulins (PSMs) in *S. aureus*. FIG. 1A is an alignment of the PSM amino acid sequences. The PSMs are all formylated at the N-terminal methionine residue (designated by "f"). FIG. 1B shows the location of PSM genes in the genome of *S. aureus* MW24. FIG. 1C shows production of PSMs (by RP-HPLC/ESI-MS) in 8-hour stationary phase cultures and RNAIII (by quantitative RT-PCR, in 4-hour late exponential phase cultures, at maximal expression of *agr*) of standard CA- and HA-MRSA strains. *, *S. aureus* strain whose genome has been sequenced.

FIG. 2: includes four panels showing murine models of *S. aureus* infection. FIG. 2A shows a bacteremia model survival curve. CD1 Swiss female mice (n=7, wild-type, n=8, all others) were injected with $10^8$ CFUs of live *S. aureus* MW2 or isogenic PSM deletion mutant strains in 0.1 ml sterile saline via the tail vein. Control animals received sterile saline. Statistical analysis was performed using Fisher's exact test at each time point and the Kaplan-Meier test for survival curves (shown at the right). FIG. 2B is a graph showing the production of TNF-α in sera of mice in the bacteremia model at the end of the experiment. Sera of each group were pooled and TNF-α measurement was performed in triplicate. FIG. 2C is a graph showing results in a skin and soft tissue infection model. Crl: SKH1-hrBR mice (n=15 for all strains) were inoculated with 50 μl of $10^7$ live *S. aureus* LAC, isogenic PSM deletion mutant strains, or saline as control, in the right flank by subcutaneous injection. Skin lesion area dimensions were measured daily with a caliper. FIG. 2D

is a digital image showing representative lesions in mice infected with the LAC wild-type and isogenic PSMα deletion strains at day 4. In FIGS. 2A, 2C, *, P<0.05; **, P <0.01; ***, P <0.001, (*), significant difference in the opposite direction (for Δβ strain, P<0.05); all versus wild-type.

FIG. 3: includes three panels showing the interaction of PSMs with human neutrophils. FIG. 3A is a graph showing surface expression of gp91phox, the heme-containing subunit of NADPH oxidase, a major component of the respiratory burst, with synthetic PSM peptides. (+, lysis of neutrophils occurred). P values are versus buffer. FIG. 3B is a graph showing chemotaxis with synthetic PSM peptides. PSM peptides were used at different concentrations (PSMα1, 5 μg/ml; PSMα2, 5 μg/ml; PSMα3, 500 ng/ml; PSMα4, 5 μg/ml; PSMβ1, 10 μg/ml; PSMβ2, 10 μg/ml; δ-toxin 2 μg/ml). To compare results, values were calculated for a theoretical concentration of 5 μg/ml. P values are versus HBSS/HAS (0.05%) control. FIG. 3C is a graph showing secretion of IL-8 with synthetic PSM peptides at 10 μg/ml.

FIG. 4: includes several panels showing PSM-induced lysis of human neutrophils. FIG. 4A is a graph showing human neutrophil lysis, measured by release of lactate dehydrogenase (LDH) activity, with synthetic PSM peptides at 10 μg/ml. FIG. 4B is a graph showing human neutrophil lysis, measured by release of LDH activity, with culture filtrates of wild-type, PSM gene deletion and complemented CA- and HA-MRSA strains. Data represent means ± SEM of at least 3 independent measurements. FIG. 4C is a series of digital images showing *in vitro* lysis of human neutrophils with synthetic PSMα3. Neutrophils were incubated with PSMα3 (10 μg/ml) and observed by scanning electron microscopy at the time intervals indicated, in comparison to untreated controls. Arrows indicate structures interpreted as holes in the plasma membrane. FIG. 4D shows the circular dichroism spectra of synthetic PSM peptides with N-formyl-methionine taken in 50% trifluorethanol. The peak at ~ 190 nm is indicative of α-helicity. FIG. 4E shows the helical wheel computation for PSMα3. FIG. 4F is a pair of graphs showing the infiltration and killing of human leukocytes in a murine peritonitis model. Two hours after infection with the bacteria, neutrophils and monocytes were counted in peritoneal exudates, and dead and live cells were distinguished, using flow

cytometry. The number of mice was n=5 for all samples except n=4 for MW2 samples (the results from one mouse in the MW2 wild-type and PSMα deletion strain samples were not included because of extensive bleeding). FIG. 4G shows the interaction of *S. aureus* with mechanisms of innate host defenses (shown on the right). Enhanced development of bacterial sepsis and leukocyte lysis by PSMs (*), as shown herein, contributes to the exceptional virulence of CA-MRSA.

FIG. 5 is a series of graphs and digital images of gels showing detection of PSMs in *S. aureus* wild-type and PSM gene deletion strains. FIG. 5A shows RP-HPLC/ESI-MS of culture filtrates as total ion chromatograms. Chromatography was performed using a Pharmacia SOURCE 5μRPC ST 4.6/150 column and a water/acetonitrile gradient in 0.1% trifluoroacetic acid from 0 to 100% acetonitrile in 50 minutes at a flow rate of 1 ml/minute. FIG. 5B shows extracted ion chromatograms (EIC). Chromatography was performed as described in Example 3. In addition to verifying the absence of the respective deleted gene products, these analyses demonstrated that in the PSM gene deletion strains, the concentrations of the respective other PSM peptides were not significantly altered. EICs for the δ-Toxin of LAC wild-type and PSM gene deletion strains are shown as an example. For the very late eluting PSMα4, according to the HPLC results, the concentration in the Δ*hld* strain was lower than in the wild-type. However, as all α-type PSMs are encoded in a putative operon, this discrepancy is likely due to physico-chemical interaction with the column material rather than to different production levels. Accordingly, it has been previously observed that the later eluting peptides require the presence of δ-Toxin to elute completely. FIG. 5C shows SDS-PAGE of TCA-precipitated exoproteins of CA-MRSA wild-type strains and corresponding hid deletion strains. FIG. 5D shows Zymographic analysis of TCA-precipitated exoproteins of CA-MRSA wild-type strains and corresponding *hld* deletion strains. SDS-polyacrylamide gels (12%) were copolymerized with skim milk at a final concentration of 0.125 mg/ml. These analyses demonstrated that production of secreted proteins was not altered in the *hld* deletion strains.

FIG. 6 includes a series of graphs showing the interaction of PSMs with human neutrophils. FIG. 6A shows surface expression of CD11b, a β-integrin

located in neutrophil secretory vesicles and specific granules, on human neutrophils incubated with synthetic PSM peptides. The symbol "+" indicates that lysis of neutrophils occurred. P values are versus buffer. FIG. 6B shows calcium influx in human neutrophils with synthetic PSM peptides. PSM peptides were used at different concentrations (PSMαl, 1 μg/ml; PSMα2, 1 μg/ml; PSMα3, 100 ng/ml; PSMα4, 2.5 μg/ml; PSMβ1, 2.5 μg/ml; PSMβ2, 5 μg/ml; δ-Toxin 1 μg/ml). To compare results, values were calculated for a theoretical concentration of 1 μg/ml. P values are versus RPMI control. FIGS. 6 C and D show secretion of IL-8 with culture filtrates of wild-type in comparison to PSM deletion strains (FIG. 6C), and complemented CA- and HA-MRSA and corresponding wild-type and PSMα deletion control strains (FIG. 6D).

FIG. 7 includes a graph showing production levels of PSMα3 in PSMα- and PSMα3-complemented, and control strains. Concentrations of PSMα3 were determined by RP-HPLC/ESI-MS and calibration was performed with synthetic PSMα3. The relative production of the other α-type PSMs in the PSMα-complemented strains (complemented with the entire PSMα locus) was equivalent to the relations shown in FIG. 1 and similar to PSMα3 in absolute concentrations. All strains were grown with the addition of tetracycline (12.5μg/ml). Production levels of the PSMs encoded on the complementation plasmids were not decreased when strains were grown without tetracycline, indicating that the plasmids are stable under the used conditions (growth with shaking for 16 hours at 37°C).

FIG. 8 includes several graphs showing hemolytic activities of PSMs. FIG. 8A shows hemolysis with synthetic PSM peptides. FIG. 9B shows hemolysis with culture filtrates. In FIGS. 8A and 8B, the data are means ± SEM of 3 independent measurements. P values are vs. DMSO in FIG. 8A and vs. wild-type in FIG. 8B. Hemolytic activity was determined by incubating samples with a 2% (v/v) suspension of sheep red blood cells and incubation at 37°C for 1 hour. Synthetic PSM peptides or filtered bacterial overnight culture supernatants were diluted in PBS and added to sheep erythrocytes at a final concentration of 10 μg/ml for the peptides and 1:200 for culture filtrates.

FIG. 9 includes several panels showing flow cytometric analyses of

neutrophil recruitment and destruction by S. *aureus* infection. CD1 Swiss female mice were i.p. injected with 100 µl of sterile PBS or $10^7$ live S. *aureus* strains. Cells were harvested two hours after inoculation by rinsing the mouse abdominal cavity with RPMI medium plus 10% FBS, and stained with FITC-conjugated rat anti-mouse Gr1 as neutrophil surface marker. Propidium iodide was used to identify dead cells. FIG. 9A shows a forward/side light scatter gate for WBCs (R1). FIG. 9B shows a dot plot illustrating WBCs stained with FITC-conjugated rat $IgG_{2a}$ isotype control. FIGS. 9C and 9D show dot plots illustrating neutrophil recruitment ($FL1^+$) and destruction ($FL1^+$ / $FL2^+$) by i.p. injection of sterile PBS (FIG. 9C) or $10^7$ live S. *aureus* MW2 wild-type strain (FIG. 9D). Representative FACS dot plots gated on Gr1+ neutrophils are shown from three to five independent samples per treatment and from two separate experiments.

FIG. 10 includes two panels showing growth- and *agr*-dependent production of PSMs. FIG. 10A shows growth-dependent production of PSMs. PSM concentration during growth in TSB in a shaken flask culture was determined by RP-HPLC/ESI-MS. Data are means of 3 independent measurements. FIG. 10B shows control of PSM production by *agr*. CA-MRSA wild-type strains were grown to stationary phase (8 hours) with or without addition of 1 µM of *agr*-specific inhibitor (the cross-inhibiting *agr* signal of *S. epidermidis*), and compared to isogenic *agr* deletion strains (dashed curves). Culture filtrate samples were analyzed by RP-HPLC. The UV signal at 214 nm is shown.

FIG 11 is a graph showing that immunization with N-formylated synthetic PSM peptides triggers antibody production *in vivo*.

FIG 12 is a graph showing that immunization with non-N-formylated synthetic PSM peptides triggers antibody production *in vivo*.

FIG. 13 is a graph showing the neutralizing effect of PSM-specific antiserum on cytokine production from human PMNs challenged with MW2 bacterial culture supernatants.

FIG. 14 is a graph showing the neutralizing effect of PSM-specific antiserum on cytokine production from human PMNs challenged with LAC bacterial culture supernatants.

FIG. 15 is a graph showing that anti PSM-α-, -β-, and δ-toxin specific antisera mediated opsonophagocytosis and killing of *S. aureus* (MW2) by human PMNs.

FIG. 16 is a graph showing that anti PSM-α1-, α2-, α3-, β1-, β2-, and δ-toxin specific antisera mediated opsonophagocytosis and killing of *S. aureus* (MW2) by human PMNs.

FIG. 17 is a graph showing that anti PSM-α, -β, and δ-toxin specific antisera mediated opsonophagocytosis and killing of *S. aureus* (LAC) by human PMNs.

## SEQUENCE LISTING

The amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for amino acids, as defined in 37 C.F.R. 1.822. In the accompanying sequence listing:

**SEQ ID NO: 1** is the amino acid sequence of PSMα1.
MGIIAGIIKVIKSLIEQFTGK

**SEQ ID NO: 2** is the amino acid sequence of PSMα2.
MGIIAGIIKFIKGLIEKFTGK

**SEQ ID NO: 3** is the amino acid sequence of PSMα3.
MEFVAKLFKFFKDLLGKFLGNN

**SEQ ID NO: 4** is the amino acid sequence of PSMα4.
MAIVGTIIKIIKAIIDIFAK

**SEQ ID NO: 5** is the amino acid sequence δ-Toxin.
MAQDIISTISDLVKWIIDTVNKFTKK

**SEQ ID NO: 6** is the amino acid sequence of PSMβ1.
MEGLFNAIKDTVTAAINNDGAKLGTSIVSIVENGVGLLGKLFGF

**SEQ ID NO: 7** is the amino acid sequence of PSMβ2.
MTGLAEAIANTVQAAQQHDSVKLGTSIVDIVANGVGLLGKLFGF

**SEQ ID NO: 8** is a PSMα consensus sequence.
MGIIAGIIK(V/F)IK(S/G)LIE(Q/K)FTGK

## DETAILED DESCRIPTION

### *I. Overview of several embodiments*

The compositions and methods described herein take advantage of the surprising discovery of a class of secreted staphylococcal peptides that has an extraordinary ability to recruit, activate, and subsequently lyse human neutrophils, thus eliminating the main cellular defense against *S. aureus* infection. These peptides are produced at especially high levels in CA-MRSA and to a large extent determine their aggressive behavior and ability to cause disease in animal models of infection.

The peptides are encoded by the phenol-soluble modulin (PSM) gene cluster and include PSMα1, PSMα2, PSMα3, and PSMα4, most of which activate and subsequently lyse neutrophils. The identification of these peptides makes possible the production of vaccines and other preventative and/or therapeutic agents for use in subjects infected with MRSA.

Thus, disclosed herein is an isolated immunogenic peptide that includes at least one antigenic phenol-soluble modulin alpha (PSMα) peptide. This PSMα peptide includes: (a) the amino acid sequence set forth as SEQ ID NO: 8; (b) the amino acid sequence set forth as SEQ ID NO: 2; (c) the amino acid sequence set forth as SEQ ID NO: 3; (d) the amino acid sequence set forth as SEQ ID NO: 4; (e) the amino acid sequence set forth as SEQ ID NO: 1; (f) an amino acid sequence having at least 85% sequence identity with (b), or (e); or (g) an amino acid sequence having at least 90% sequence identity with (a), (c), or (d). In some embodiments, the PSMα peptide includes an amino acid sequence having at least 95% sequence identity with (a), (b), (c), (d), or (e), and in particular embodiments the PSMα peptide is (a) the amino acid sequence set forth as SEQ ID NO: 8; (b) the amino acid sequence set forth as SEQ ID NO: 2; (c) the amino acid sequence set forth as SEQ ID NO: 3; (d) the amino acid sequence set forth as SEQ ID NO: 4; (e) the amino acid sequence set forth as SEQ ID NO: 1; or (e) an amino acid sequence having at least 85% sequence identity with (b) or (e), or at least 90% sequence identity with (a), (c), or (d). The peptide can be immunogenic fragments or immunogenic fusion proteins that include heterologous proteins other than the PSMα peptides or variants thereof

and retain the immunogenicity of the peptides recited in SEQ ID NOS: 1, 2, 3, 4 or 8. Another embodiment includes an isolated polynucleotide that includes a nucleic acid sequence encoding the immunogenic PSMα peptide, or its immunogenic fragments or fusion proteins. In certain examples, the polynucleotide is operably linked to a promoter. Yet another embodiment is a vector that includes this polynucleotide. In some embodiments, the isolated immunogenic PSMα peptide provides protective immunity from MRSA when administered to a subject in a therapeutically effective amount, and in particular examples, the MRSA is community-associated MRSA (CA-MRSA).

Another embodiment is a pharmaceutical composition that includes the immunogenic PSMα peptide together with a pharmaceutically acceptable carrier, and, in some examples, a therapeutically effective amount of an adjuvant, such as IL-2, GM-CSF, TNF-α, IL-12, or IL-6. Certain examples of the pharmaceutical composition include mixtures of two or more PSMα peptides, or, optionally, a combination of one or more PSMα peptides and one or more PSMβ peptides.

Other embodiments are methods for eliciting an immune response in a subject. These methods include (a) selecting a subject in which an immune response to the immunogenic PSMα peptide is desirable; and (b) administering to the subject a therapeutically effective amount of the immunogenic PSMα peptide or a combination of PSMα peptides, thereby producing an immune response in the subject. Certain examples of the method also include administering one or more PSMβ peptide to the subject. In some examples, administration includes oral, topical, mucosal, or parenteral administration, and in certain examples, parenteral administration includes intravenous administration, intramuscular administration, or subcutaneous administration. The immunogenic PSMα peptide is administered, in some examples, in from about one to about six doses, for instance two doses. In certain examples, the method further includes administering a therapeutically effective amount of an adjuvant to the subject, for instance a therapeutically effective amount of IL-2, RANTES, GM-CSF, TNF-α, IFN-γ, G-CSF or a combination thereof.

Still other embodiments are methods for inhibiting MRSA infection in a

subject. These methods include (a) selecting a subject at risk for exposure to MRSA; and (b) administering to the subject a therapeutically effective amount of the immunogenic PSMα peptide or combination of PSMα peptides, thereby inhibiting MRSA infection in the subject. Some examples of the method further include administering one or more PSMβ peptides to the subject. In particular examples, the MRSA is community-associated MRSA (CA-MRSA).

Yet still other embodiments are antibodies that specifically bind to a *S. aureus* PSMα binding site, for instance a PSMα1, PSMα2, PSMα3, or PSMα4 binding site. Further embodiments are methods for diagnosing MRSA in a subject. These methods include (a) selecting a subject at risk for developing MRSA; (b) collecting a tissue or body fluid sample from the subject; and (c) determining whether an anti-PSMα antibody is present in the sample, wherein the presence of an anti-PSMα antibody in the sample indicates that the subject has MRSA. In some examples, the MRSA is community –associated MRSA (CA-MRSA), and in other examples, the anti-PSMα antibody is an anti-PSMα1 antibody, an anti-PSMα2 antibody, an anti-PSMα3 antibody, or an anti-PSMα4 antibody.

## *II. Abbreviations*

| | |
|---|---|
| **CA:** | community-associated |
| **CD:** | circular dichroism |
| **EIC:** | extracted ion chromatograms |
| **FITC:** | fluorescein isothiocyanate |
| **Fmlp;** | formyl-met-leu-phe |
| **HA:** | hospital-associated |
| **HSA:** | human serum albumin |
| **HBSS:** | Hank's Buffered Salt Solution |
| **LDH:** | lactate dehydrogenase |
| **MRSA:** | Methicillin-resistant *Staphylococcus aureus* |
| **PSM:** | phenol-soluble modulin |
| **PVL:** | Panton-Valentine leukocidin |
| **RPMI:** | Roswell Park Memorial Institute medium |
| **SDS-PAGE:** | sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| **TSB:** | tryptic soy broth |

## *III. Terms*

In order to facilitate review of the various embodiments of the disclosure, the

following explanations of specific terms are provided:

**Adjuvant:** An agent used to enhance antigenicity. Some adjuvants include a suspension of minerals (alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; or water-in-oil emulsion in which antigen solution is emulsified in mineral oil (Freund incomplete adjuvant), sometimes with the inclusion of killed mycobacteria (Freund's complete adjuvant) to further enhance antigenicity (inhibits degradation of antigen and/or causes influx of macrophages). Immunstimulatory oligonucleotides (such as those including a CpG motif) can also be used as adjuvants (for example see U.S. Patent No. 6,194,388; U.S. Patent No. 6,207,646; U.S. Patent No. 6,214,806; U.S. Patent No. 6,218,371; U.S. Patent No. 6,239,116; U.S. Patent No. 6,339,068; U.S. Patent No. 6,406,705; and U.S. Patent No. 6,429,199). Adjuvants also can include biological molecules, such as costimulatory molecules. Exemplary adjuvants include IL-2, RANTES, GM-CSF, TNF-α, IFN-γ, G-CSF, LFA-3, CD72, B7-1, B7-2, OX-40L and 41 BBL. Adjuvants also can include dsRNA.

**Antigen:** A compound, composition, or substance that can stimulate the production of antibodies or a T cell response in an animal, including compositions that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens. The term "antigen" includes all related antigenic epitopes. "Epitope" or "antigenic determinant" refers to a site on an antigen to which B and/or T cells respond. In one embodiment, T cells respond to the epitope, when the epitope is presented in conjunction with an MHC molecule. 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, about 9, or about 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.

**Antibody:** Immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, for instance, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen.

A naturally occurring antibody (for example, IgG, IgM, IgD) includes four polypeptide chains, two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. However, it has been shown that the antigen-binding function of an antibody can be performed by fragments of a naturally occurring antibody. Thus, these antigen-binding fragments are also intended to be designated by the term "antibody." Specific, non-limiting examples of binding fragments encompassed within the term antibody include (i) a Fab fragment consisting of the $V_L$, $V_H$, $C_L$ and $C_{H1}$ domains; (ii) an $F_d$ fragment consisting of the $V_H$ and $C_{H1}$ domains; (iii) an Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (iv) a dAb fragment (Ward et al., *Nature* 341:544-546, 1989) which consists of a $V_H$ domain; (v) an isolated complimentarity determining region (CDR); and (vi) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region.

Immunoglobulins and certain variants thereof are known and many have been prepared in recombinant cell culture (for instance, see U.S. Patent No. 4,745,055; U.S. Patent No. 4,444,487; WO 88/03565; EP 256,654; EP 120,694; EP 125,023; Faoulkner *et al.,* (1982) *Nature* 298:286; Morrison, (1979) *J. Immunol.* 123:793; Morrison *et al.*,(1984) *Ann Rev. Immunol* 2:239).

**Animal:** Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

**cDNA (complementary DNA):** A piece of DNA lacking internal, non-coding segments (introns) and regulatory sequences that determine transcription. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

**Conservative variants:** As used herein, the term "conservative variant," in the context of an immunogenic PSMα peptide, refers to a peptide or amino acid

sequence that deviates from another amino acid sequence only in the substitution of one or several amino acids for amino acids having similar biochemical properties (so-called conservative substitutions). Conservative amino acid substitutions are likely to have minimal impact on the activity of the resultant protein. Further information about conservative substitutions can be found, for instance, in Ben Bassat *et al.* (*J. Bacteriol.*, 169:751-757, 1987), O'Regan *et al.* (*Gene*, 77:237-251, 1989), Sahin-Toth *et al.* (*Protein Sci.*, 3:240-247, 1994), Hochuli *et al.* (*Bio/Technology*, 6:1321-1325, 1988) and in widely used textbooks of genetics and molecular biology. In some embodiments, conservative amino acid substitutions are those substitutions that do not substantially affect or decrease antigenicity of an immunogenic PSMα peptide. Specific, non-limiting examples of conservative substitutions include the following examples:

| Original Residue | Conservative Substitutions |
|---|---|
| Ala | Ser |
| Arg | Lys |
| Asn | Gln, His |
| Asp | Glu |
| Cys | Ser |
| Gln | Asn |
| Glu | Asp |
| His | Asn; Gln |
| Ile | Leu, Val |
| Leu | Ile; Val |
| Lys | Arg; Gln; Glu |
| Met | Leu; Ile |
| Phe | Met; Leu; Tyr |
| Ser | Thr |
| Thr | Ser |
| Trp | Tyr |
| Tyr | Trp; Phe |
| Val | Ile; Leu |

In some embodiments, a conservative substitution of a cysteine residue can also include Met, Gly, Glu, Asp, Val, Thr, Tyr, or Ala. The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid, provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. Non-conservative substitutions are those that reduce antigenicity.

**Epitope:** An antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic (that elicit a specific immune response). An antibody specifically binds a particular antigenic epitope on a polypeptide. 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, about 9, or 8 to 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, for instance, "Epitope Mapping Protocols" in *Methods in Molecular Biology*, Vol. 66, Glenn E. Morris, Ed (1996).

**Encode:** As used herein, the term "encode" refers to any process whereby the information in a polymeric macromolecule or sequence is used to direct the production of a second molecule or sequence that is different from the first molecule or sequence. As used herein, the term is construed broadly, and can have a variety of applications. In some aspects, the term "encode" describes the process of semi-conservative DNA replication, where one strand of a double-stranded DNA molecule is used as a template to encode a newly synthesized complementary sister strand by a DNA-dependent DNA polymerase.

In another aspect, the term "encode" refers to any process whereby the information in one molecule is used to direct the production of a second molecule that has a different chemical nature from the first molecule. For example, a DNA molecule can encode an RNA molecule (for instance, by the process of transcription incorporating a DNA-dependent RNA polymerase enzyme). Also, an RNA molecule can encode a peptide, as in the process of translation. When used to describe the process of translation, the term "encode" also extends to the triplet codon that encodes an amino acid. In some examples, an RNA molecule can encode a DNA molecule, for instance, by the process of reverse transcription incorporating an RNA-dependent DNA polymerase. In another example, a DNA molecule can encode a

peptide, where it is understood that "encode" as used in that case incorporates both the processes of transcription and translation.

**Expression Control Sequences:** Nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus, expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (for instance, ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements that are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see for instance, Bitter *et al.*, (1987) *Methods in Enzymology* 153:516-544). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda , plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like can be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (such as the metallothionein promoter) or from mammalian viruses (such as the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques can also be used to provide for transcription of the nucleic acid sequences.

**Gene expression:** The process by which the coded information of a nucleic acid transcriptional unit (including, for example, genomic DNA or cDNA) is

converted into an operational, non-operational, or structural part of a cell, often including the synthesis of a protein. Gene expression can be influenced by external signals; for instance, exposure of a cell, tissue or subject to an agent that increases or decreases gene expression. Expression of a gene also can be regulated anywhere in the pathway from DNA to RNA to protein. Regulation of gene expression occurs, for instance, through controls acting on transcription, translation, RNA transport and processing, degradation of intermediary molecules such as mRNA, or through activation, inactivation, compartmentalization or degradation of specific protein molecules after they have been made, or by combinations thereof. Gene expression can be measured at the RNA level or the protein level and by any method known in the art, including, without limitation, Northern blot, RT-PCR, Western blot, or *in vitro*, *in situ*, or *in vivo* protein activity assay(s).

**Hybridization:** Oligonucleotides and their analogs hybridize by hydrogen bonding, which includes Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary bases. Generally, nucleic acid consists of nitrogenous bases that are either pyrimidines (cytosine (C), uracil (U), and thymine (T)) or purines (adenine (A) and guanine (G)). These nitrogenous bases form hydrogen bonds between a pyrimidine and a purine, and the bonding of the pyrimidine to the purine is referred to as "base pairing." More specifically, A will hydrogen bond to T or U, and G will bond to C. "Complementary" refers to the base pairing that occurs between two distinct nucleic acid sequences or two distinct regions of the same nucleic acid sequence. For example, an oligonucleotide can be complementary to a PSMα peptide-encoding RNA, or a PSMα peptide-encoding DNA.

"Specifically hybridizable" and "specifically complementary" are terms that indicate a sufficient degree of complementarity such that stable and specific binding occurs between the oligonucleotide (or its analog) and the DNA or RNA target. The oligonucleotide or oligonucleotide analog need not be 100% complementary to its target sequence to be specifically hybridizable. An oligonucleotide or analog is specifically hybridizable when binding of the oligonucleotide or analog to the target DNA or RNA molecule interferes with the normal function of the target DNA or

RNA, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide or analog to non-target sequences under conditions where specific binding is desired, for example under physiological conditions in the case of *in vivo* assays or systems. Such binding is referred to as specific hybridization.

Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method of choice and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (especially the $Na^+$ and/or $Mg^{++}$ concentration) of the hybridization buffer will determine the stringency of hybridization, though wash times also influence stringency. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are discussed by Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, chapters 9 and 11.

**Immune response:** A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular antigen (an "antigen-specific response"). In one embodiment, an immune response is a T cell response, such as a CD4+ response or a CD8+ response. In another embodiment, the response is a B cell response, and results in the production of specific antibodies.

**Immunogenic protein or peptide:** A protein or peptide that includes an allele-specific motif or other sequence such that the peptide will bind an MHC molecule and induce a cytotoxic T lymphocyte ("CTL") response, or a B cell response (for instance, antibody production) against the antigen from which the immunogenic peptide is derived.

In one embodiment, immunogenic proteins and peptides are identified using sequence motifs or other methods, such as neural net or polynomial determinations, known in the art. Typically, algorithms are used to determine the "binding threshold" of peptides to select those with scores that give them a high probability of binding at a certain affinity and will be immunogenic. The algorithms are based

either on the effects on MHC binding of a particular amino acid at a particular position, the effects on antibody binding of a particular amino acid at a particular position, or the effects on binding of a particular substitution in a motif-containing protein. Within the context of an immunogenic protein or peptide, a "conserved residue" is one which appears in a significantly higher frequency than would be expected by random distribution at a particular position in a peptide. In one embodiment, a conserved residue is one where the MHC structure may provide a contact point with the immunogenic protein or peptide.

Immunogenic proteins and peptides also can be identified by measuring their binding to a specific MHC protein and by their ability to stimulate CD4 and/or CD8 when presented in the context of the MHC protein.

Generally, immunogenic PSMα peptides can be used to induce an immune response in a subject, such as a B cell response or a T cell response.

**Immunogenic composition:** A composition comprising an immunogenic PSMα peptide that induces a measurable B cell response (such as production of antibodies that specifically bind the PSMα, PSMβ, or δ-toxin antigens) against MRSA. For *in vitro* use, the immunogenic composition can consist of the immunogenic peptide alone. For *in vivo* use, the immunogenic composition will typically comprise the immunogenic polypeptide in a pharmaceutically acceptable carrier, and/or other agents. An immunogenic composition optionally can include an adjuvant, a costimulatory molecule, or a nucleic acid encoding a costimulatory molecule.

**Isolated:** An "isolated" biological component (such as a nucleic acid or protein or organelle) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, for instance, other chromosomal and extra-chromosomal DNA and RNA, proteins and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

**Mammal:** This term includes both human and non-human mammals.

Similarly, the term "subject" includes both human and veterinary subjects.

**MRSA:** Methicillin-resistant *Staphylococcus aureus* includes isolates of the bacterium *Staphylococcus aureus* that are characterized by antibiotic resistance to all penicillins, including methicillin and other narrow-spectrum β-lactamase-resistant penicillin antibiotics. MRSA was discovered for the first time in 1961 in the UK, but it is now widespread in hospital settings. MRSA is commonly termed a "superbug." MRSA may also be known as oxacillin-resistant *Staphylococcus aureus* (ORSA) and multiple-resistant *Staphylococcus aureus*. Strains of *S. aureus* that are non-resistant to methicillin are sometimes called methicillin-susceptible *Staphylococcus aureus* (MSSA) if an explicit distinction must be made.

Although MRSA has traditionally been seen as a hospital-associated infection, community-acquired MRSA strains have appeared in recent years, notably in the U.S. and Australia. The abbreviations CA-MRSA (community-acquired MRSA) and HA-MRSA (hospital-acquired MRSA) distinguish the two forms of the disease.

Approximately 10% of *S. aureus* isolates in the United States are susceptible to penicillin. However, many *S. aureus* strains, while resistant to penicillin, remain susceptible to penicillinase-stable penicillins, such as oxacillin and methicillin. Strains that are oxacillin and methicillin resistant, historically termed methicillin-resistant *S. aureus* (MRSA), are resistant to all ß-lactam agents, including cephalosporins and carbapenems. Hospital-associated MRSA isolates often are multiply resistant to other commonly used antimicrobial agents, including erythromycin, clindamycin, and tetracycline, while community-associated MRSA isolates are often resistant only to ß-lactam agents and erythromycin. Since 1996, MRSA strains with decreased susceptibility to vancomycin (minimum inhibitory concentration [MIC], 8-16 μg/ml) and strains fully resistant to vancomycin (MIC ≥ 32 μg/ml) have been reported.

MRSA have many virulence factors that enable them to cause disease in normal hosts. For example, MRSA are frequent causes of healthcare-associated bloodstream and catheter-related infections. MRSA are also an emerging cause of community-associated infections, especially skin and soft tissue infections and

necrotizing pneumonia. Vancomycin and two newer antimicrobial agents, linezolid and daptomycin, are among the drugs that are used for treatment of severe healthcare-associated MRSA infections. Although some strains remain susceptible to trimethoprim/sulfamethoxazole, gentamicin, or rifampin, these drugs are not typically used as first-line agents. Because of the rapid emergence of resistance to rifampin, this drug is unsuitable for use as a single agent to treat MRSA infections.

An MRSA outbreak can occur when one strain is transmitted to other patients or close contacts of the infected persons in the community. Often this occurs when a patient or health-care worker is colonized with an MRSA strain (for instance, carries the organism but shows no clinical signs or symptoms of infection) and, through contact, spreads the strain to another person. Handwashing and screening patients for MRSA should be performed to decrease transmission and reduce the number of patients infected with MRSA.

Several methods are used to identify MRSA. The National Committee for Clinical Laboratory Standards, now called the Clinical and Laboratory Standards Institute, recommends the cefoxitin disk screen test, the latex agglutination test for PBP2a, or a plate containing 6 μg/ml of oxacillin in Mueller-Hinton agar supplemented with NaCl (4% w/v; 0.68 mol/L) as suitable methods of testing for MRSA. For methods of inoculation, see the CLSI Approved Standard M100-S15 (CLSI. 2007. "Performance standards for antimicrobial susceptibility testing." *CLSI approved standard M100-S17*. Clinical and Laboratory Standards Institute, Wayne, PA).

Accurate detection of oxacillin/methicillin resistance can be difficult due to the presence of two subpopulations (one susceptible and the other resistant) that may coexist within a culture of staphylococci (Bannerman, 2003. "Staphylococcus, Micrococcus and other catalase-positive cocci that grow aerobically." In P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, R.H. Yolken [eds.], *Manual of Clinical Microbiology* 8th ed. ASM Press, Washington, D.C.). All cells in a culture may carry the genetic information for resistance, but only a small number may express the resistance in vitro. This phenomenon is termed heteroresistance and occurs in staphylococci resistant to penicillinase-stable penicillins, such as oxacillin.

Cells expressing heteroresistance grow more slowly than the oxacillin-susceptible population and may be missed at temperatures above 35°C. This is why CLSI recommends incubating isolates being tested against oxacillin, methicillin, or nafcillin at 33-35° C (maximum of 35°C) for a full 24 hours before reading.

When used correctly, broth-based and agar-based tests usually can detect MRSA. The cefoxitin disk diffusion method can be used in addition to routine susceptibility test methods or as a back-up method. In addition, nucleic acid amplification tests, such as the polymerase chain reaction (PCR), can be used to detect the mecA gene, which mediates oxacillin resistance in staphylococci. Staphylococcal resistance to oxacillin/methicillin occurs when an isolate carries an altered penicillin-binding protein, PBP2a, which is encoded by the mecA gene. The new penicillin-binding protein binds beta-lactams with lower avidity, which results in resistance to this class of antimicrobial agents.

The CLSI breakpoints for *S. aureus* are different than those for coagulase-negative staphylococci (CoNS):

**Interpretive Criteria (in µg/ml) for Oxacillin MIC Tests**

| | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| *S. aureus* | ≤ 2 µg/ml | N/A | ≥ 4 µg/ml |
| CoNS | ≤ 0.25 µg/ml | N/A | ≥ 0.5 µg/ml |

**Interpretive Criteria (in mm) for Oxacillin Disk Diffusion Tests**

| | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| *S. aureus* | ≥ 13 mm | 11-12 mm | ≤ 10 mm |
| CoNS | ≥ 18 mm | N/A | ≤ 17mm |

N/A = not applicable

**Interpretive Criteria (in mm) for Cefoxitin Disk Diffusion Test**

| | Susceptible* † | Resistant** |
|---|---|---|
| *S. aureus* | ≥ 22 mm | ≤ 21 mm |
| CoNS | ≥ 25 mm | ≤ 24 mm |

* Report as oxacillin susceptible
** Report as oxacillin resistant
†There is no intermediate category with the cefoxitin disk diffusion test

There are several reasons why oxacillin and cefoxitin are used for testing instead of Methicillin. First, methicillin is no longer commercially available in the United States. Second, oxacillin maintains its activity during storage better than methicillin and is more likely to detect heteroresistant strains. However, cefoxitin is an even better inducer of the mecA gene and disk diffusion tests using cefoxitin give clearer endpoints and are easier to read than tests with oxacillin.

**Nucleic acid molecule:** A polymeric form of nucleotides, which can include both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide, deoxynucleotide or a modified form of either type of nucleotide. A "nucleic acid molecule" as used herein is synonymous with "nucleic acid" and "polynucleotide." A nucleic acid molecule is usually at least 10 bases in length, unless otherwise specified. The term includes single- and double-stranded forms of DNA. A nucleic acid molecule can include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages.

Nucleic acid molecules can be modified chemically or biochemically or can contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications, such as uncharged linkages (for example, methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, *etc.*), charged linkages (for example, phosphorothioates, phosphorodithioates, *etc.*), pendent moieties (for example, peptides), intercalators (for example, acridine, psoralen, *etc.*), chelators, alkylators, and modified linkages (for example, alpha anomeric nucleic acids, *etc.*). The term "nucleic acid molecule" also includes any topological conformation, including single-stranded, double-stranded, partially duplexed, triplexed, hairpinned, circular and padlocked conformations.

**Operably linked:** A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is in a functional relationship with the second nucleic acid sequence. For instance, a promoter is

operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. When recombinantly produced, operably linked nucleic acid sequences are generally contiguous and, where necessary to join two protein-coding regions, in the same reading frame. However, nucleic acids need not be contiguous to be operably linked.

**Parenteral administration:** administration by injection or infusion. Specific, non-limiting examples of parenteral routes of administration include: intravenous, intramuscular, intrathecal, intraventricular, intraarterial, intracardiac, subcutaneous, intradermal, intraperitoneal, epidural, intravitreal, and intraosseous infusion.

**Pharmaceutically acceptable carriers:** The pharmaceutically acceptable carriers of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the PSMα peptides herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (such as powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

A "therapeutically effective amount" is a quantity of a composition or a cell to achieve a desired effect in a subject being treated. For instance, this can be the amount necessary to induce an immune response in a subject. When administered to a subject, a dosage will generally be used that will achieve target tissue concentrations (for example, in lymphocytes) that has been shown to achieve an *in*

*vitro* effect.

**Polynucleotide:** The term polynucleotide or nucleic acid sequence refers to a polymeric form of nucleotide at least 10 bases in length. A recombinant polynucleotide includes a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (for instance, a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single- and double-stranded forms of DNA.

**Peptide:** Any chain of amino acids, regardless of length or post-translational modification (for instance, glycosylation or phosphorylation). In one embodiment, the protein is a PSMα peptide. Longer peptides are often referred to as proteins. With regard to proteins, "comprises" indicates that additional amino acid sequence or other molecules can be included in the molecule, "consists essentially of" indicates that additional amino acid sequences are not included in the molecule, but that other agents (such as labels or chemical compounds) can be included, and "consists of" indicates that additional amino acid sequences and additional agents are not included in the molecule.

**Probes and primers:** A probe comprises an isolated nucleic acid attached to a detectable label or reporter molecule. Primers are short nucleic acids, preferably DNA oligonucleotides, of about 15 nucleotides or more in length. Primers may be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, for example by polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art. One of skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, for example, a primer comprising 20 consecutive

nucleotides will anneal to a target with a higher specificity than a corresponding primer of only 15 nucleotides. Thus, in order to obtain greater specificity, probes and primers can be selected that comprise about 20, 25, 30, 35, 40, 50 or more consecutive nucleotides.

**PSMα peptide:** Any of several peptides encoded by the phenol-soluble modulin (PSM) α gene cluster. The peptides disclosed herein were identified in *S. aureus*, and include PSMα1 (SEQ ID NO: 1), PSMα2 (SEQ ID NO: 2), PSMα3 (SEQ ID NO: 3), and PSMα4 (SEQ ID NO: 4), most of which activate and subsequently lyse neutrophils. In some embodiments, the PSMα peptides are formylated, for example, N-terminal formylation, whereas in other embodiments they are not formylated. In other embodiments, the PSMα peptides include variants that contain a substitution of one or several amino acids for amino acids having similar biochemical properties (so-called conservative substitutions). The peptide may also be an immunogenic fragment or a fusion with a heterologous peptide sequence. Conservative amino acid substitutions are likely to have minimal impact on the activity of the resultant protein. Further information about conservative substitutions can be found, for instance, in section IVB of the Detailed Description, below.

Without being bound by theory, the membrane-damaging activity of PSMαs most likely is due to their strong α-helicity and amphipathy, which are typical features of pore-forming peptides.

**PSMβ peptide:** Any of several peptides encoded by the phenol-soluble modulin (PSM) β gene cluster. The peptides disclosed herein were identified in *S. aureus*, and include PSMβ1 (SEQ ID NO: 6) and PSMβ2 (SEQ ID NO: 7). In some embodiments, the PSMβ peptides are formylated, for example, N-terminal formylation, whereas in other embodiments they are not formylated. In other embodiments, the PSMβ peptides include variants that contain a substitution of one or several amino acids for amino acids having similar biochemical properties (so-called conservative substitutions). The peptide may also be an immunogenic fragment or a fusion with a heterologous peptide sequence. Conservative amino acid substitutions are likely to have minimal impact on the activity of the resultant

protein. Further information about conservative substitutions can be found, for instance, in section IVB of the Detailed Description, below.

**Purified:** The PSM peptides disclosed herein can be purified (and/or synthesized) by any of the means known in the art (see, for instance, *Guide to Protein Purification*, ed. Deutscher, *Meth. Enzymol.* 185, Academic Press, San Diego, 1990; and Scopes, *Protein Purification: Principles and Practice*, Springer Verlag, New York, 1982). Substantial purification denotes purification from other proteins or cellular components. A substantially purified protein is at least about 60%, 70%, 80%, 90%, 95%, 98% or 99% pure. Thus, in one specific, non-limiting example, a substantially purified protein is 90% free of other proteins or cellular components.

**Risk of exposure to MRSA:** a subject is at "risk of exposure to MRSA" if there is an increased probability that the subject will be exposed to the bacterium relative to the general population. Accordingly, risk is a statistical concept based on empirical and/or actuarial data. Commonly, risk is correlated with one or more indicators, such as occupation, geographical location, living conditions, contact with potential MRSA carriers, or other occurrences, events or undertakings, of a subject. For example, indicators include but are not limited to close living or working conditions, and any condition or occupation that brings the subject in close contact with the public.

**Sequence identity:** The similarity between two nucleic acid sequences or between two amino acid sequences is expressed in terms of the level of sequence identity shared between the sequences. Sequence identity is typically expressed in terms of percentage identity; the higher the percentage, the more similar the two sequences. Methods for aligning sequences for comparison are described in detail below, in section IV B of the Detailed Description.

**Subcutaneous administration:** delivery, most often by injection, of an agent into the subcutis. The subcutis is the layer of tissue directly underlying the cutis, composed mainly of adipose tissue. Subcutaneous injections are given by injecting a fluid into the subcutis. Within the context of administering immunogenic PSMα peptides, subcutaneous administration most often will involve injection of a

PSMα peptide with an acceptable carrier into the subcutis of a subject at risk of exposure to MRSA.

**Therapeutically active polypeptide:** An agent, such as a PSMα or PSMβ peptide that causes induction of an immune response, as measured by clinical response (for example increase in a population of immune cells, increased cytolytic activity against cells that express a PSMα peptide, or protection from MRSA infection). In one embodiment, a therapeutically effective amount of PSMα peptide is an amount used to generate an immune response against MRSA. The immune response may be generated in a person at risk for, or thought to already be infected with, MRSA.

**Vector:** A nucleic acid molecule capable of transporting a non-vector nucleic acid sequence which has been introduced into the vector. One type of vector is a "plasmid," which refers to a circular double-stranded DNA into which non-plasmid DNA segments can be ligated. Other vectors include cosmids, bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC). Another type of vector is a viral vector, wherein additional DNA segments can be ligated into all or part of the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (for example, vectors having a bacterial origin of replication replicate in bacteria hosts). Other vectors can be integrated into the genome of a host cell upon introduction into the host cell and are replicated along with the host genome. Some vectors contain expression control sequences (such as promoters) and are capable of directing the transcription of an expressible nucleic acid sequence that has been introduced into the vector. Such vectors are referred to as "expression vectors." A vector can also include one or more selectable marker genes and/or genetic elements known in the art.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Definitions of common terms in molecular biology can be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular*

*Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. "Comprising" means "including." "Comprising A or B" means "including A," "including B" or "including A and B." It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or peptides are approximate, and are provided for description.

Suitable methods and materials for the practice or testing of the disclosure are described below. However, the provided materials, methods, and examples are illustrative only and are not intended to be limiting. Accordingly, except as otherwise noted, the methods and techniques of the present disclosure can be performed according to methods and materials similar or equivalent to those described and/or according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification (see, for instance, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, 1989; Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Press, 2001; Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates, 1992 (and Supplements to 2000); Ausubel *et al.*, Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, 4th ed., Wiley & Sons, 1999).

## IV. *PSMα Peptides as Vaccine Targets Against Methicillin-Resistant* Staphylococcus aureus

### A. Overview

Methicillin-resistant *Staphylococcus aureus* (MRSA) is bacterial infection that is resistant to certain antibiotics, such as methicillin, as well as other more common antibiotics such as oxacillin, penicillin and amoxicillin. *S. aureus* infections, including MRSA, occur most frequently among persons in hospitals and

healthcare facilities (such as nursing homes and dialysis centers) who have weakened immune systems.

MRSA infections that are acquired by persons who have not been recently (within the past year) hospitalized or had a medical procedure (such as dialysis, surgery, catheters) are known as CA-MRSA infections. MRSA infections in the community are usually manifested as skin infections, such as pimples and boils, or more serious conditions, such as necrotizing fasciitis or death, and occur in otherwise healthy people. Prior to this disclosure, it has been unclear what makes CA-MRSA strains more successful at causing human disease compared with their hospital-associated counterparts.

Disclosed herein is a class of secreted staphylococcal peptides with an extraordinary ability to recruit, activate, and subsequently lyse human neutrophils, thus eliminating the main cellular defense against *S. aureus* infection. These peptides are produced at high levels in standard CA-MRSA strains and contribute significantly to their ability to cause disease in animal models of infection. This novel set of *S. aureus* virulence factors accounts at least in part for the enhanced virulence of CA-MRSA. The present disclosure provides a treatment for persons at risk of an MRSA infection or who are thought to be infected with MRSA. Persons at risk of such an infection include persons living in institutions (such as nursing homes) or other crowded facilities, persons with a relative lack of immunity (such as the immunocompressed or elderly), and persons who come in contact with others who may have MRSA (such as athletes or personnel at medical facilities).

### B. PSM peptides

Disclosed herein are several peptides encoded by the phenol-soluble modulin (PSM) gene cluster that have a significant effect on the ability of MRSA strains to cause disease. The peptides include PSMα1 (SEQ ID NO: 1), PSMα2 (SEQ ID NO: 2), PSMα3 (SEQ ID NO: 3), and PSMα4 (SEQ ID NO: 4), most of which activate and subsequently lyse neutrophils. Also provided is a PSMα consensus sequence (SEQ ID NO: 8), and two PSMβ peptides (SEQ ID NOs: 6 and 7). PSMα1 and PSMα2 (SEQ ID NOs: 1 and 2) share about 85% sequence identity.

The identification of these peptides enables the production of vaccines and other preventative and/or therapeutic agents for use in subjects infected with MRSA. The PSMα peptides described herein were identified in *S. aureus*. As described herein, *S. aureus* was found to secrete 4 shorter (~ 20 amino acids, α-type) and 2 longer (~ 40 amino acids, β-type) PSM-like peptides, whose genes are arranged in two gene clusters. In addition, *S. aureus* produces δ-toxin, which is similar to the α-type PSMs. Although the specific PSMα peptides disclosed herein range from 20 amino acids to 22 amino acids in length, other PSMα peptides and PSMα variants can be longer or shorter. For instance, N-terminal or C-terminal additions of short amino acid sequences (for instance 1, 2, 3, or more amino acids) also yield active PSMα peptides, in certain embodiments

In some embodiments, the PSMα peptides have non-conservative substitutions. For instance, PSMα1 and PSMα2 differ at positions 10, 13, and 17. Thus, PSMα1 (SEQ ID NO: 1) could, in a specific, non-limiting example, have a phenylalanine in place of the valine at position 10, and/or a glycine in place of the serine at position 13, and/or a lysine in place of the glutamine at position 17. Conversely, PSMα2 (SEQ ID NO: 2) could have a valine in place of the phenylalanine at position 10, and/or a serine in place of the glycine at position 13, and/or a glutamine in place of the lysine at position 17. Similar substitutions can be made in the sequences of PSMα3 and PSMα4 (SEQ ID NOs: 3 and 4) at these and other positions where the PSMα sequences differ from one another.

The PSM peptides disclosed herein also include variants that contain a substitution of one or several amino acids for amino acids having similar biochemical properties (so-called conservative substitutions). Conservative amino acid substitutions are likely to have minimal impact on the activity of the resultant protein. Further information about conservative substitutions can be found, for instance, in Ben Bassat *et al.* (*J. Bacteriol.*, 169:751-757, 1987), O'Regan *et al.* (*Gene*, 77:237-251, 1989), Sahin-Toth *et al.* (*Protein Sci.*, 3:240-247, 1994), Hochuli *et al.* (*Bio/Technology*, 6:1321-1325, 1988) and in widely used textbooks of genetics and molecular biology. In some examples, PSMα peptide variants can have no more than 1, 2, 3, or 4 conservative amino acid changes. The following table

shows exemplary conservative amino acid substitutions that can be made to PSMα peptides:

| Original Residue | Conservative Substitutions |
|---|---|
| Ala | Ser |
| Arg | Lys |
| Asn | Gln; His |
| Asp | Glu |
| Cys | Ser |
| Gln | Asn |
| Glu | Asp |
| Gly | Pro |
| His | Asn; Gln |
| Ile | Leu; Val |
| Leu | Ile; Val |
| Lys | Arg; Gln; Glu |
| Met | Leu; Ile |
| Phe | Met; Leu; Tyr |
| Ser | Thr |
| Thr | Ser |
| Trp | Tyr |
| Tyr | Trp; Phe |
| Val | Ile; Leu |

In addition, the peptides disclosed herein can include a label or detectable compound or composition facilitating detection of the peptide. Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, and radioactive isotopes. For example, the label is, in certain embodiments, a detectable marker, such as a radiolabeled amino acid or a polypeptide of biotinyl moieties that can be detected by marked avidin (for example, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Various methods of labeling polypeptides and glycoproteins are known in the art and can be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionucleotides (such as $^{35}S$ or $^{131}I$), fluorescent labels (such as fluorescein isothiocyanate (FITC), rhodamine, lanthanide phosphors), enzymatic labels (such as horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups,

predetermined polypeptide epitopes recognized by a secondary reporter (such as a leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), or magnetic agents, such as gadolinium chelates. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

### C. Nucleic Acid Sequences and Variants

As any molecular biology textbook teaches, a peptide of interest is encoded by its corresponding nucleic acid sequence (for instance, an mRNA or genomic DNA). Accordingly, nucleic acid sequences encoding PSM peptides are contemplated herein, at least to make and use the PSM peptides of the disclosed compositions and methods.

In one example, *in vitro* nucleic acid amplification (such as polymerase chain reaction (PCR)) can be utilized as a method for producing nucleic acid sequences encoding PSMα peptides. PCR is a standard technique, which is described, for instance, in *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, San Diego, CA:Academic Press, 1990), or *PCR Protocols, Second Edition* (*Methods in Molecular Biology*, Vol. 22, ed. by Bartlett and Stirling, Humana Press, 2003).

A representative technique for producing a nucleic acid sequence encoding a PSMα peptide by PCR involves preparing a sample containing a target nucleic acid molecule that includes the PSMα peptide-encoding nucleic acid sequence. For example, DNA or RNA (such as mRNA or total RNA) can serve as a suitable target nucleic acid molecule for PCR reactions. Optionally, the target nucleic acid molecule can be extracted from cells by any one of a variety of methods well known to those of ordinary skill in the art (for instance, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 1989; Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publ. Assoc. and Wiley-Intersciences, 1992). PSMα peptides are, in some embodiments expressed in a variety of cell types; for example, prokaryotic and eukaryotic cells. In examples where RNA is the initial target, the RNA is reverse transcribed (using one of a myriad of reverse transcriptases commonly known in the art) to produce a

double-stranded template molecule for subsequent amplification. This particular method is known as reverse transcriptase (RT)-PCR. Representative methods and conditions for RT-PCR are described, for example, in Kawasaki *et al.* (In *PCR Protocols, A Guide to Methods and Applications*, Innis *et al.* (eds.), 21-27, Academic Press, Inc., San Diego, California, 1990).

The selection of amplification primers will be made according to the portion(s) of the target nucleic acid molecule that is to be amplified. In various embodiments, primers (typically, at least 10 consecutive nucleotides of PSMα peptide-encoding nucleic acid sequence) can be chosen to amplify all or part of a PSMα peptide-encoding nucleic acid sequence. Variations in amplification conditions may be required to accommodate primers and amplicons of differing lengths and composition; such considerations are well known in the art and are discussed for instance in Innis *et al.* (*PCR Protocols, A Guide to Methods and Applications*, San Diego, CA:Academic Press, 1990). From a provided PSMα peptide-encoding nucleic acid sequence, one skilled in the art can easily design many different primers that can successfully amplify all or part of a PSMα peptide-encoding sequence.

As described herein, disclosed are nucleic acid sequences encoding PSMα peptides. Though particular nucleic acid sequences are disclosed herein, one of skill in the art will appreciate that also provided are many related sequences with the functions described herein, for instance, nucleic acid molecules encoding conservative variants of a PSMα peptide are disclosed herein. One indication that two nucleic acid molecules are closely related (for instance, are variants of one another) is sequence identity, a measure of similarity between two nucleic acid sequences or between two amino acid sequences expressed in terms of the level of sequence identity shared between the sequences. Sequence identity is typically expressed in terms of percentage identity; the higher the percentage, the more similar the two sequences.

Methods for aligning sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970;

Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237-244, 1988; Higgins and Sharp, *CABIOS* 5:151-153, 1989; Corpet *et al.*, *Nucleic Acids Research* 16:10881-10890, 1988; Huang, *et al.*, *Computer Applications in the Biosciences* 8:155-165, 1992; Pearson *et al.*, *Methods in Molecular Biology* 24:307-331, 1994; Tatiana *et al.*, (1999), *FEMS Microbiol. Lett.*, 174:247-250, 1999. Altschul *et al.* present a detailed consideration of sequence-alignment methods and homology calculations (*J. Mol. Biol.* 215:403-410, 1990).

The National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST™, Altschul *et al.*, *J. Mol. Biol.* 215:403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence-analysis programs blastp, blastn, blastx, tblastn and tblastx. A description of how to determine sequence identity using this program is available on the internet under the help section for BLAST™.

For comparisons of amino acid sequences of greater than about 30 amino acids, the "Blast 2 sequences" function of the BLAST™ (Blastp) program is employed using the default BLOSUM62 matrix set to default parameters (cost to open a gap [default = 5]; cost to extend a gap [default = 2]; penalty for a mismatch [default = -3]; reward for a match [default = 1]; expectation value (E) [default = 10.0]; word size [default = 3]; number of one-line descriptions (V) [default = 100]; number of alignments to show (B) [default = 100]). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to the sequence of interest, for example the PSMα peptide of interest.

For comparisons of nucleic acid sequences, the "Blast 2 sequences" function of the BLAST™ (Blastn) program is employed using the default BLOSUM62 matrix

set to default parameters (cost to open a gap [default = 11]; cost to extend a gap [default = 1]; expectation value (E) [default = 10.0]; word size [default = 11]; number of one-line descriptions (V) [default = 100]; number of alignments to show (B) [default = 100]). Nucleic acid sequences with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, r at least 98%, or at least 99% sequence identity to the PSMα peptide-encoding nucleic acid of interest.

Another indication of sequence identity is hybridization. In certain embodiments, PSMα peptide-encoding nucleic acid variants hybridize to a disclosed (or otherwise known) PSMα peptide-encoding nucleic acid sequence, for example, under low stringency, high stringency, or very high stringency conditions. Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method of choice and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (especially the $Na^+$ concentration) of the hybridization buffer will determine the stringency of hybridization, although wash times also influence stringency. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are discussed by Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, chapters 9 and 11.

The following are representative hybridization conditions and are not meant to be limiting.

Very High Stringency (detects sequences that share at least 90% sequence identity)

| | |
|---|---|
| Hybridization: | 5x SSC at 65°C for 16 hours |
| Wash twice: | 2x SSC at room temperature (RT) for 15 minutes each |
| Wash twice: | 0.5x SSC at 65°C for 20 minutes each |

High Stringency (detects sequences that share at least 80% sequence identity)

| | |
|---|---|
| Hybridization: | 5x-6x SSC at 65°C-70°C for 16-20 hours |
| Wash twice: | 2x SSC at RT for 5-20 minutes each |
| Wash twice: | 1x SSC at 55°C-70°C for 30 minutes each |

Low Stringency (detects sequences that share at least 50% sequence identity)
Hybridization: 6x SSC at RT to 55°C for 16-20 hours
Wash at least twice: 2x-3x SSC at RT to 55°C for 20-30 minutes each.

One of ordinary skill in the art will appreciate that PSMα peptide-encoding nucleic acid sequences of various lengths are useful for a variety purposes, such as for use as PSMα peptide probes and primers. In some embodiments, an oligonucleotide can include at least 15, at least 20, at least 23, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50 or more consecutive nucleotides of a PSMα peptide-encoding nucleic acid sequence.

### D. Therapeutic Methods and Pharmaceutical Compositions

An immunogenic PSMα peptide, combination of two or more PSMα peptides, or a combination of PSMα and PSMβ peptides as disclosed herein can be administered to a subject in order to generate an immune response. In exemplary applications, compositions are administered to a subject who is at risk for exposure to MRSA, who has been exposed to MRSA, or who has a MRSA infection, in an amount sufficient to raise an immune response to *S. aureus* bacteria. Administration induces a sufficient immune response to inhibit infection with MRSA, slow the proliferation of the bacteria, inhibit their growth, or to reduce a sign or a symptom of a MRSA infection. Amounts effective for this use will depend upon the extent of exposure to MRSA bacteria, the route of entry of the bacteria into the body of the subject, the general state of the subject's health, and the robustness of the subject's immune system. A therapeutically effective amount of the compound is that which provides an objectively identifiable improvement in resistance to infection with MRSA.

An immunogenic PSMα peptide (or combination of PSM peptides) can be administered by any means known to one of skill in the art (see Banga, "Parenteral Controlled Delivery of Therapeutic Peptides and Proteins," in *Therapeutic Peptides and Proteins*, Technomic Publishing Co., Inc., Lancaster, PA, 1995) either locally or systemically, such as by intramuscular, subcutaneous, or intravenous injection, but even oral, nasal, or anal administration is contemplated. In one embodiment,

administration is by subcutaneous or intramuscular injection. To extend the time during which the protein is available to stimulate a response, the protein can be provided as an implant, an oily injection, or as a particulate system. The particulate system can be a microparticle, a microcapsule, a microsphere, a nanocapsule, or similar particle. (*see,* for instance, Banga, *supra).* A particulate carrier based on a synthetic polymer has been shown to act as an adjuvant to enhance the immune response, in addition to providing a controlled release. Aluminum salts can also be used as adjuvants to produce an immune response.

Optionally, one or more cytokines, such as interleukin (IL)-2, IL-6, IL-12, IL-15, RANTES, granulocyte macrophage colony stimulating factor (GM-CSF), tumor necrosis factor (TNF) -α, interferon (IFN)-α or IFN-γ, one or more growth factors, such as GM-CSF or G-CSF, one or more costimulatory molecules, such as ICAM-1, LFA-3, CD72, B7-1, B7-2, or other B7 related molecules; one or more molecules such as OX-40L or 41 BBL, or combinations of these molecules, can be used as biological adjuvants (see, for example, Salgaller *et al.*, (1998) *J. Surg. Oncol.* 68(2):122-38; Lotze *et al.,* (2000), *Cancer J Sci. Am.* 6(Suppl 1):S61-6; Cao *et al.*, (1998) *Stem Cells* 16(Suppl 1):251-60; Kuiper *et al.,* (2000) *Adv. Exp. Med. Biol.* 465:381-90). These molecules can be administered systemically (or locally) to the host.

Some embodiments are pharmaceutical compositions including an immunogenic PSMα peptide, combination of two or more PSMα peptides, or combination of PSMα and PSMβ peptides. In one specific embodiment, the immunogenic PSMα peptide (or combination) is mixed with an adjuvant containing two or more of a stabilizing detergent, a micelle-forming agent, and an oil. Suitable stabilizing detergents, micelle-forming agents, and oils are detailed in U.S. Patent No. 5,585,103; U.S. Patent No. 5,709,860; U.S. Patent No. 5,270,202; and U.S. Patent No. 5,695,770. A stabilizing detergent is any detergent that allows the components of the emulsion to remain as a stable emulsion. Such detergents include polysorbate, 80 (TWEEN) (Sorbitan-mono-9-octadecenoate-poly(oxy-1,2-ethanediyl; manufactured by ICI Americas, Wilmington, DE), TWEEN 40™, TWEEN 20™, TWEEN 60™, ZWITTERGENT™ 3-12, TEEPOL HB7™, and

SPAN 85™. These detergents are usually provided in an amount of approximately 0.05 to 0.5%, such as at about 0.2%. A micelle forming agent is an agent which is able to stabilize the emulsion formed with the other components such that a micelle-like structure is formed. Such agents generally cause some irritation at the site of injection in order to recruit macrophages to enhance the cellular response. Examples of such agents include polymer surfactants described by BASF Wyandotte publications, for instance, Schmolka, (1977) *J. Am. Oil. Chem. Soc.* 54:110, and Hunter *et al.*, (1981) *J. Immunol* 129:1244, PLURONIC™ L62LF, L101, and L64, PEG1000, and TETRONIC™ 1501, 150R1, 701, 901, 1301, and 130R1. The chemical structures of such agents are well known in the art. In one embodiment, the agent is chosen to have a hydrophile-lipophile balance (HLB) of between 0 and 2, as defined by Hunter and Bennett, (1984) *J. Immun.* 133:3167. The agent can be provided in an effective amount, for example between 0.5 and 10%, or in an amount between 1.25 and 5%.

The oil included in the composition is chosen to promote the retention of the antigen in oil-in-water emulsion, for example, to provide a vehicle for the desired antigen, and preferably has a melting temperature of less than 65°C such that emulsion is formed either at room temperature (about 20°C to 25°C), or once the temperature of the emulsion is brought down to room temperature. Examples of such oils include tetratetracontane and peanut oil or other vegetable oils. In one specific, non-limiting example, the oil is provided in an amount between 1 and 10%, or between 2.5 and 5%. The oil should be both biodegradable and biocompatible so that the body can break down the oil over time, and so that no adverse affects, such as granulomas, are evident upon use of the oil.

In one embodiment, the adjuvant is a mixture of stabilizing detergents, micelle-forming agent, and oil available under the name PROVAX® (IDEC Pharmaceuticals, San Diego, CA). An adjuvant can also be an immunostimulatory nucleic acid, such as a nucleic acid including a CpG motif, or a biological adjuvant (see above).

In one specific, non-limiting example, a pharmaceutical composition for intravenous administration would include about 0.1 ng to about 1000 mg of PSMα

peptide per dose, for instance 10 ng, 100 ng, 1 mg, 10 mg, or 100 mg. In another specific, non-limiting example, a pharmaceutical composition for intravenous administration would include a total of about 0.1 ng to about 1000 mg of a combination of PSMα peptides or a combination of PSMα and PSMβ peptides per dose, for instance 10 ng, 100 ng, 1 mg, 10 mg, or 100 mg. Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remingtons Pharmaceutical Sciences*, 19th Ed., Mack Publishing Company, Easton, Pennsylvania, 1995.

Single or multiple administrations of the compositions are administered depending on the dosage and frequency as required and tolerated by the subject. In one embodiment, the dosage is administered once as a bolus, but in another embodiment can be applied periodically until a therapeutic result is achieved. For instance, in one embodiment the vaccine is administered in at least two doses, for instance 3, 4, 5, or 6 or more, with the second and subsequent doses administered at least a week after the first dose, for instance, one month, two months, three months or six months or more after the fist dose. Generally, the dose is sufficient to inhibit infection with MRSA without producing unacceptable toxicity to the subject.

## EXAMPLES

### Example 1: PSMα Peptides as Vaccine Targets Against Methicillin-Resistant Staphylococcus Aureus

This Example demonstrates that PSMα peptides are major virulence determinants of *S. aureus,* and that their increased production in CA-MRSA contributes to the enhanced virulence of those strains compared to traditional HA-MRSA.

Among several putative determinants of CA-MRSA virulence, the Panton-Valentine leukocidin (PVL) has received most attention (Baba *et al.* (2002) *Lancet* 359, 1819-27; Diep *et al.* (2006) *Lancet* 367, 731-9; Vandenesch *et al.* (2003) *Emerg Infect Dis* 9, 978-84; Gillet *et al.* (2002) *Lancet* 359, 753-9). A role of this lytic toxin as a virulence factor in cutaneous infection was suggested early (Grojec &

Jeljaszewicz (1981) *Zentralbl Bakteriol Mikrobiol Hyg* [A] 250, 446-55; Cribier *et al.* (1992) *Dermatology* 185, 175-80) and results from a mouse infection model indicate that purified PVL or heterologous over-expression of PVL contributes to the development of necrotizing pneumonia in laboratory strains of *S. aureus* (Labandeira-Rey *et al.* (2007) *Science* 315(5815):1130-3). This type of disease is rare among CA-MRSA infections (less than 2%; Fridkin *et al.* (2005) *N Engl J Med* 352, 1436-44), and whether PVL contributes to necrotizing pneumonia caused by CA-MRSA remains to be demonstrated. In contrast, results obtained using CA-MRSA isogenic PVL deletion strains and the respective murine infection models indicate that PVL does not play a significant role in CA-MRSA skin and soft tissue infections, which represent the most frequent manifestations of CA-MRSA disease, or in bacteremia (Voyich *et al.* (2006) *J Infect Dis* 194, 1761-70). Thus, prior to this disclosure, the basis of virulence of CA-MRSA remained undefined.

As described herein, a group of peptides was identified in *S. aureus* using analytical reversed-phase HPLC/electrospray mass spectrometry and preparative reversed-phase chromatography with subsequent N-terminal peptide sequencing (FIG. 6), *S. aureus* was found to secrete 4 shorter (~ 20 amino acids, α-type) and 2 longer (~ 40 amino acids, β-type) PSM-like peptides (FIG. 1A), whose genes are arranged in 2 gene clusters (FIG. 1B). In addition, *S. aureus* produces δ-toxin, which is similar to the α-type PSMs. Of note, the PSMα genes have not been described previously, owing to the lack of similarity to PSM genes of *S. epidermidis* and the failure to exceed the threshold length for gene annotation.

To demonstrate that PSMs determine the virulence of CA-MRSA, production of PSMs was compared in representative hospital-associated (HA)- and CA-MRSA strains. While the PSM genes occur in all sequenced *S. aureus* strains, dramatically higher *in vitro* PSM production was detected in the most prominent CA-MRSA compared to HA-MRSA (FIG. 1C), indicating that PSMs contribute to the enhanced virulence of CA-MRSA (Voyich *et al.* (2005) *J Immunol* 175, 3907-19). Isogenic gene deletion strains of the PSMα and PSMβ gene loci were constructed, and of the *hld* gene coding for the δ-toxin, in the CA-MRSA strains MW2 (USA400; Baba *et al.* (2002) *Lancet* 359, 1819-27) and LAC (USA300; Diep

*et al.* (2006) *Lancet* 367, 731-9). MW2 was the first reported and sequenced CA-MRSA strain (Baba *et al.* (2002) *Lancet* 359, 1819-27) and USA300 currently accounts for most CA-MRSA infections in the US (Diep *et al.* (2006) *Lancet* 367, 731-9). RP-HPLC-ESI/MS analysis confirmed the specific absence of the particular PSM peptide(s) in the respective gene deletion strains (FIG. 6).

The virulence of the PSM deletion strains was compared to the wild-type strains in murine abscess and bacteremia models (Voyich *et al.* (2006) *J Infect Dis* 194, 1761-70). These models were selected based on the prevalence of CA-MRSA in skin and soft tissue infections (Moran *et al.* (2006) *N Engl J Med* 355, 666-74) and severe sepsis (Adem *et al.* (2005) *N Engl J Med 353,* 1245-51; Kravitz *et al.* (2005) *Clin Infect Dis* 40, 941-7). MW2 (USA400), which typically causes sepsis in humans (Adem *et al.* (2005) *N Engl J Med 353,* 1245-51; Kravitz *et al.* (2005) *Clin Infect Dis* 40, 941-7) was used for the bacteremia model. LAC (USA300), by far the most prominent cause of community-associated skin and soft tissue infection in the US (Moran *et al.* (2006) *N Engl J Med* 355, 666-74), was used in a skin and soft tissue infection model. In the bacteremia model, there was significantly reduced mortality in the mice infected with the PSMα deletion strain and to a lesser extent, the δ-toxin-negative strain (FIG. 2A). Consistent with the sepsis data, levels of the inflammatory cytokine TNF-α were significantly reduced in blood samples of mice infected with those mutant strains (FIG. 2B). Additionally, there was a significantly decreased ability of the LAC PSMα deletion strain, but not of the other PSM deletion strains, to cause skin lesions in mice (FIGS. 2C, 2D). Together, these data demonstrate that α-type PSMs have an essential role in the most important manifestations of CA-MRSA induced disease.

To demonstrate the mechanism by which PSM peptides promote virulence, the ability of these molecules to alter phagocyte function was tested, focusing on neutrophils as the most important cell type responsible for the elimination of invading bacteria. Synthetic PSMs primed neutrophils for activation (as determined by expression of gp91phox and CD11b, FIGS. 3A and FIG. 7), provoked neutrophil chemotaxis and $Ca^{2+}$ flux (FIGS. 3B and FIG. 7), and induced release of the cytokine IL-8 (FIG. 3C), but not TNF-α or IL-1β. PSMα peptides, particularly

PSMα3, generally showed the most pronounced pro-inflammatory activity. In contrast, PSMs failed to increase expression of IL-8, TNF-α or IL-1β in PBMCs (peripheral blood mononuclear cells), or Mono Mac 6 cells. Thus, the pro-inflammatory activity of the PSMs is very specific and the increased levels of TNF-α as a general indicator of inflammation that was observed in the bacteremia model likely are secondary effects of immune cell cross-activation. Changes in IL-8 production by neutrophils after interaction with the HA- and CA-MRSA wild-type, PSM deletion, and PSM complemented strains (for production levels of complemented strains see FIG. 8) were consistent with the results obtained using synthetic PSMs (FIG. 7C, FIG. 3C), indicating that α-type PSMs, particularly PSMα3, have a pronounced influence on the pro-inflammatory activity of CA-MRSA. Taken together, these results demonstrate that *S. aureus* PSMα peptides (i) efficiently activate and trigger an inflammatory response in human neutrophils, and (ii) contribute dramatically to staphylococcal virulence.

Inasmuch as enhanced virulence of CA-MRSA has been linked to leukolytic activity (Voyich *et al.* (2005) *J Immunol* 175, 3907-19), it was then shown that PSMαs lyse human neutrophils *in vitro* using synthetic PSMs. PSMs of the α-type caused significant lysis, particularly PSMα3 (FIG. 4A). Accordingly, clarified culture media from CA-MRSA PSMα deletion and HA-MRSA strains had dramatically reduced capacity to cause lysis of human neutrophils (FIG. 4B). Lytic activity of these strains was entirely restored by genetic complementation with a plasmid expressing all α-type PSMs, and almost completely restored with a plasmid expressing PSMα3 alone, indicating that most of the noted cytolytic activity of CA-MRSA is due to this peptide. In contrast, the PSMβ and δ-toxin-negative strains did not show significantly reduced lysis of human neutrophils. When monitored by scanning electron microscopy, neutrophils showed signs of priming (for instance, flattening) and structures indicating that the integrity of the plasma membrane was compromised within 5 minutes of exposure to PSMα3 (FIG. 4C). After 60 minutes, many neutrophils were completely destroyed. PSMs, mainly those of the α-type, also caused lysis of erythrocytes, which may contribute to the development of disease (FIG. 9).

Without being bound by theory, the membrane-damaging activity of PSMαs most likely is due to their strong α-helicity (FIG. 4D) and amphipathy (FIG. 4E), which are typical features of pore-forming peptides (Mellor *et al.* (1988) *Biochim Biophys Acta* 942, 280-94). Importantly, although *S. aureus* is known to produce factors that may cause neutrophil lysis *in vitro*, prior to this disclosure it was not understood which molecules are responsible for the elimination of neutrophils *in vivo*. In a murine peritonitis model, neutrophil and monocyte infiltration and lysis was significantly increased after infection with CA-MRSA wild-type strains compared to the isogenic PSMα deletion strains. In addition, significantly increased neutrophil and monocyte infiltration was observed with the PSMα-complemented HA-MRSA strain 252 compared to the parental strain (FIG. 4F, FIG. 10). These findings demonstrate that α-type PSMs contribute significantly to the lysis of leukocytes *in vitro* and *in vivo*, and to a large extent are responsible for the enhanced cytolytic activity of CA-MRSA strains.

Collectively, these results indicate that a primary role of PSMαs in pathogenesis is to destroy leukocytes and thus, PSMαs play a key role in the evasion of innate host defense by *S. aureus* (FIG. 5). However, as PSMαs also trigger the inflammatory response, the bacteria must be able to limit PSMα secretion to times at which these cells can be efficiently inactivated. Using isogenic *agr* deletion strains in CA-MRSA and *agr*-specific inhibitor peptides, we found that all *S. aureus* PSMs are under tight control of the *agr* quorum-sensing system (FIG. 11). This mechanism links gene expression to bacterial cell density via a secreted bacterial signal, limiting the production of target genes to a time when the signal molecules achieve a high concentration, such as by confinement in the neutrophil phagosome (Kong *et al.* (2006) *Int J Med Microbiol* 296, 133-9; Redfield (2002) *Trends Microbiol* 10, 365-70). Thus, it is presumably due to control by *agr* that PSMs are produced at the right time to fulfill their task in pathogenesis and are repressed when production would jeopardize bacterial survival. Furthermore, strain-to-strain differences in PSM production appear to be in part caused by differential *agr* activity. Production of RNAIII, the regulatory molecule of the *agr* system, was in general lower in HA-MRSA compared to CA-MRSA. However, the production of

PSMs, especially α-type PSMs, was not entirely correlated with RNAIII levels (FIG. 1C), indicating that *agr*-independent regulation also contributes to the low PSMα levels observed in HA-MRSA. Notably, these findings highlight the importance of gene expression and regulation in the endeavor to understand the basis of CA-MRSA virulence.

Taken together, PSMα peptides are major virulence determinants of *S. aureus* and their increased production in CA-MRSA contributes to the enhanced virulence of those strains compared to traditional HA-MRSA (Voyich *et al.* (2005) *J Immunol* 175, 3907-19). Importantly, the newly identified peptides encoded by the PSMα gene cluster represent the first molecules for which a significant effect has been demonstrated on the ability of CA-MRSA strains to cause disease in animal infection models.

## Example 2: Methods for Figures 1-5

This Example describes the methods used in generating the data shown in FIGS. 1-5.

*A. Bacterial strains*

HA- and CA-MRSA were standard strains whose genomes have been sequenced (Baba *et al.* (2002) *Lancet* 359, 1819-27; Diep *et al.* (2006) *Lancet* 367, 731-9; Holden *et al.* (2004) *Proc Natl Acad Sci U S A* 101, 9786-91; Gill *et al.* (2005) *J Bacteriol* 187, 2426-38; Kuroda *et al.* (2001) *Lancet* 357, 1225-40) or prototypical strains from a UCSF collection (Diep *et al.* (2006) *J Infect Dis* 193, 1495-503). Bacteria were grown in tryptic soy broth (TSB).

*B. Reversed-phase chromatography/mass spectrometry*

Bacterial strains were inoculated 1:100 from a pre-culture grown overnight, grown for 8 hours in TSB, and culture filtrates were obtained by centrifugation at 10,000 x g for 15 minutes. For preparative chromatography, samples were precipitated with trichloroacetic acid, the precipitate was dissolved in 8 M urea, and samples were injected onto a HR 16/10 column packed with SOURCE 15PHE

material (GE Healthcare). PSM-containing fractions were collected and injected on a Zorbax SB-C18 9.4 mm x 25 cm column (Agilent) for further purification. Columns were run with a water-acetonitrile gradient in 0.1% triflouroacetic acid as described (Yao *et al.* (2005) *J Infect Dis* 191, 289-98). After acid removal of the N-terminal formyl group (Shively *et al.* (1982) *Anal Biochem* 120, 312-2), purified PSMs were analyzed by Edman sequencing. For analytical chromatography, an Agilent 1100 system coupled to an Agilent TrapSL mass spectrometer and a Zorbax SB-C8 2.1 x 30 mm column (Agilent) were used as decribed (Vuong *et al.* (2004) *Cell Microbiol* 6, 753-9). For PSM quantification, culture filtrates were directly injected, and calibration was performed with synthetic PSMs. The two most abundant peaks of the electrospray mass spectra obtained were used for peak integration. Evaluation was performed with Agilent Quant Analysis software.

*C. Construction of PSM and agr gene deletion strains and complementation plasmids*

The PSMα and PSMβ deletion strains were constructed by allelic replacement with a spectinomycin resistance cassette as described (Vuong *et al.* (2000) *Infect Immun* 68, 1048-53). The *hld* deletion strains were constructed in a way so as not to interfere with the function of the regulatory RNA molecule (RNAIII), in whose encoding DNA it is embedded (Novick *et al.* (1993) *Embo J* 12, 3967-75). These strains were constructed by alteration of the start codon of the δ-toxin *hld* gene using the procedure of Bae & Schneewind ((2006) *Plasmid* 55, 58-63). The two PCR fragments used in that procedure were amplified using oligonucleotides that contained an MfeI site at the place of the *hld* start codon, resulting in a one base change from ATG to ATT and abolishing translation of *hld*. All PSM deletion strains were confirmed by analytical PCR with genomic DNA and RP-HPLC/ESI-MS of culture filtrates (FIG. 6). *In vitro* growth of the deletion strains was indistinguishable from the respective wild-type strain. In addition to measuring PSM production, the secreted protein protease profiles of the *hld* deletion strains were tested by SDS-PAGE and zymographic analysis (Vuong *et al.* (2000) *Infect Immun* 68, 1048-53) to ensure that the regulatory function of RNAIII was not

affected (FIG. 6). Deletion strains in *agr* were produced by phage transduction from strain RN6911.

The $pTX_{\Delta}$ plasmids were derived from plasmid pTX1530, by deletion of the 5' part of the *xylR* repressor gene via digestion with *NdeI* and *PstI* and re-ligation, to achieve high-level, constitutive expression of genes cloned under control of the *xyl* promoter (FIG. 8). The PSMα gene locus or the PSMα3 gene were PCR-amplified using chromosomal DNA of strain MW2 as template, digested with BamHI/MluI, ligated into BamHI/MluI digested $pTX_{\Delta}$ and transformed into *S. aureus* RN4220 and subsequently in the target strains. These plasmids confer resistance to tetracycline, which was added to cultures at 12.5 μg/ml. *In vitro* growth of PSM-expressing strains with $pTX_{\Delta}$ derivatives was indistinguishable from that of the respective control strains.

*D. Quantitative reverse-transcription (RT) polymerase chain reaction (PCR)*

Oligonucleotide primers and probes were designed with Primer Express software (version 2.0; Applied Biosystems) and synthesized by Applied Biosystems. The experiments were performed in triplicate as described (Yao *et al.* (2006) *J Infect Dis* 193, 841-848), with 16S rRNA as a control.

*E. Circular dichroism (CD) measurement*

The structures of synthetic PSM peptides were analyzed by CD spectroscopy on a Jasco spectropolarimeter model J-720 instrument. Solutions of PSM peptides, each at 1.0 mg/ml, were prepared in 50% trifluoroethanol. Measurements were performed in triplicate and the resulting scans were averaged, smoothed, and the buffer signal was subtracted.

*F. Human neutrophil isolation*

PMNs were isolated from venous blood of healthy volunteers in accordance with protocols approved by the Institutional Review Board for Human Subjects, NIAID, and the University of Tübingen, Germany, as described (Voyich *et al.*

(2006) *J Infect Dis* 194, 1761-70; de Haas *et al.* (2004) *J Exp Med* 199, 687-95).

*G. Neutrophil chemotaxis and calcium ion fluxes*

Neutrophils were subjected to a brief hypotonic shock with pyrogen-free water (Sigma), washed, and suspended at 5 x $10^6$ cells/ml in HBSS containing 0.05% human serum albumin (HAS; CLB). Chemotaxis of neutrophils was determined by using fluorescently-labeled neutrophils that migrated through a membrane fitted into an insert of a 24-well microtiter plate transwell system (Costar) containing a prewetted 3-μm-pore-size polycarbonate filter as described (de Haas *et al.* (2004) *J Exp Med* 199, 687-95). For measurement of calcium ion fluxes, 5 x $10^6$ neutrophils/ml were loaded with 2 μM Fluo-3-AM (Molecular Probes) in RPMI containing 0.05% HSA (RPMI-HSA) for 20 minutes at room temperature under agitation, washed twice with buffer, and resuspended in RPMI-HSA at $10^6$ cells/ml. Calcium fluxes were analyzed with a FACScalibur (Becton Dickinson).

*H. Priming of human neutrophils*

Priming of PMNs by synthetic PSMs was determined by increased surface expression of CD11b and gp91phox (granule exocytosis). PMNs were incubated with 10–10,000 ng/ml PSM, 10 μM fMLP, 10 ng/ml lipopolysaccharide, or 10 μg/ml lipoteichoic acid in 96-well tissue culture plates at 37°C with rotation for 60 minutes. The assay was terminated by centrifuging cells at 4°C for 8 minutes at 350 x g. Cells were washed twice in cold Dulbecco's phosphate-buffered saline and stained with and isotype control antibody (BD Biosciences) or those specific for CD11b (mAb 44, BD Biosciences) or gp91phox (mAb 7D533). Propidium iodide (0.5 μg/ml) was used to identify dead cells. PMNs were analyzed on a FACSCalibur flow cytometer (Becton Dickinson) and dead cells were excluded with a single gate. Percent positive neutrophils were determined with a marker defined by the boundary of the isotype-matched control antibody.

*I. Lysis of human neutrophils*

Lysis of PMNs by synthetic PSMs or clarified *S. aureus* culture media was

determined essentially as described (Voyich *et al.* (2006) *J Infect Dis* 194, 1761-70; Voyich *et al.* (2005) *J Immunol* 175, 3907-19). Synthetic PSMs (1 or 10 μg/ml) were added to wells of a 96-well tissue culture plate containing $10^6$ PMNs and plates were incubated at 37°C for up to 3 hours. At the desired times, PMN lysis was determined by release of lactate dehydrogenase (LDH; Cytotoxicity Detection Kit, Roche Applied Sciences). Alternatively, wild-type and isogenic mutant *S. aureus* strains were cultured for 18 hours at 37°C in 50 ml TSB with shaking using a 100 ml flask. Bacteria were removed by centrifugation and culture media were sterilized by filtration and stored at –80°C in aliquots until used. Culture medium was diluted 1:10 in RPMI/H, mixed with human PMNs ($10^6$) and tested for its ability to cause PMN lysis.

*J. Scanning electron microscopy*

For scanning electron microscopy, PMNs were fixed, washed, and mounted on stubs as described previously (Voyich *et al.* (2005) *J Immunol* 175, 3907-19). After mounting on stubs, samples were coated lightly with chromium using an ion beam sputterer (South Bay Technology, Inc.), and examined with a Hitachi S5200 field emission scanning electron microscope (Hitachi High Technologies America). Digital images were collected and adjusted for brightness and contrast with Photoshop CS (Adobe Systems).

*K. Measurement of cytokine production*

After isolation and washing, PMN or PBMC were resuspended in RPMI 1640 medium (Sigma) supplemented with 10% human serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM sodium pyruvate, and 10 mM HEPES. Cells were distributed to a 96-well culture plate at 200 μl and 5 x $10^5$ cells per well. Synthetic PSM peptides or filtered bacterial culture supernatants were diluted in fresh culture medium and added to the plate at 100 μl/well. Plates were incubated at 37°C in a 5.5% $CO^2$ incubator for 5 hours. Then, the plate was centrifuged at 1500 rpm for 10 minutes, and supernatant was harvested from each well. Mono Mac 6 cells were obtained from DSMZ (Germany), and were grown in

RPMI 1640 plus 10% heat inactivated fetal bovine serum (HyClone), 2 mM L-glutamine, 10.0 units/ml penicillin, 10.0 μg/ml streptomycin, 0.2 x non-essential amino acid solution, and OPI medium supplement (Sigma). Cells were grown in T75 culture flasks in a 37°C $CO^2$ incubator and harvested by centrifugation. After removal of supernatant, cells were resuspended in fresh culture medium, counted, and distributed into a 96-well cell culture plate at 100 μl and 2.5 x $10^5$ cells per well. Phorbol 12-myristate 13-acetate (Sigma) was added to a final concentration of 2.5 ng/ml for cell pre-stimulation. The plate was incubated in a 37°C $CO^2$ incubator for 3 hours. At the end of the pre-stimulation period, synthetic PSM peptides were diluted in culture medium and added to the cells at 100 μl/well. Cells were incubated in a 37°C $CO^2$ incubator for 20 – 22 hours, and the plate was centrifuged at 1500 rpm for 10 minutes. Human TNF-α, IL-1β, IL-8, and mouse TNF-α were measured in the culture supernatants with commercial ELISA assay kits (R&D systems) according to the manufacturer's instructions.

*L. Murine bacteremia, skin abscess, and peritonitis models*

CD1 Swiss female mice and Crl: SKH1-hrBR hairless mice (outbred, immunocompetent) were obtained from Charles River Laboratories and were between 4 and 6 weeks of age at the time of use. *S. aureus* strains were grown to mid-exponential phase, washed once with sterile PBS, then resuspended in PBS at 1 x $10^8$ CFUs/100 μl (bacteremia model) or 1 x $10^7$ CFUs / 50 μl (abscess model) as described (Voyich *et al.* (2006) *J Infect Dis* 194, 1761-70). For the bacteremia model, each mouse was injected with $10^8$ CFUs of live *S. aureus* in 0.1 ml sterile saline via the tail vein. Control animals received sterile saline only. After inoculation, animal health and disease advancement were monitored every 3 hours for the first 24 hours, then every 8 hours for up to 72 hours. Animals were euthanized immediately if showing signs of respiratory distress, mobility loss, or inability to eat and drink. All surviving animals were euthanized at 72 hours. At the time of death, serum samples were harvested from test animals for mouse TNF-α ELISA tests.

For the abscess model, Crl: SKH1-hrBR mice were anesthetized with

isoflurane, and inoculated with 50 μl of $10^7$ live *S. aureus* strains or saline in the right flank by subcutaneous injection. Test animals were examined at 24-hour intervals for a total of 14 days. Skin lesion dimensions were measured daily with a caliper. Length (L) and width (W) values were applied to calculate the area of lesions using the formula of L x W. All animals were euthanized after completion of the entire procedure.

For the peritonitis model, CD1 Swiss female mice were injected intraperitoneally with 0.1 ml of $10^7$ live *S. aureus*. Two hours after the inoculation, animals were euthanized by an isoflurane overdose, and 6.0 ml of RPMI medium containing 10% FBS was injected into the abdominal cavities. Mice were surgically opened and 4.0 ml of exudates were collected with 23G needles. The collected exudates were aliquoted to 0.4 ml per sample, centrifuged at 1500 rpm for 5 minutes, and cell pellets were resuspended in 100 ml of staining buffer (PBS containing 1% goat serum). Samples were stained with fluorescein isothiocyanate (FITC)-conjugated anti-mouse Ly-6G (clone 1A8, BD Biosciences) as neutrophil marker and allophycocyanin (APC)-conjugated anti-mouse CD14 (clone Sa2-8, eBioscience) as a marker for monocytes and macrophages, or with appropriate isotype control antibodies. Propidium iodide (0.5 μg/ml, BD Biosciences) was used to identify dead cells. Samples were analyzed on a FACSCalibur flow cytometer (Becton Dickinson) using CELLQUEST PRO software, collecting events of 20 seconds for each sample.

All animals were housed and maintained under pathogen–free conditions at the Rocky Mountain Laboratory animal facility.

*M. Statistical analysis*

Unless noted otherwise, unpaired t-tests were used to calculate 2-tailed P values using Graph Pad Prism 4 software.

## Example 3: Methods for Figures 5-10

This Example describes the methods used in generating the data shown in FIGS. 5-10.

*A. Quantitative reverse-transcription (RT) polymerase chain reaction (PCR)*

Oligonucleotide primers and probes were designed with Primer Express software (version 2.0; Applied Biosystems) and synthesized by Applied Biosystems. The protocols were performed in triplicate, with 16S rRNA as a control.

*B Human neutrophil isolation*

PMNs were isolated from venous blood of healthy volunteers in accordance with protocols approved by the Institutional Review Board for Human Subjects, NIAID, and the University of Tübingen, Germany.

*C. Neutrophil chemotaxis and calcium ion fluxes*

Neutrophils were subjected to a brief hypotonic shock with pyrogen-free water (Sigma), washed, and suspended at 5 x $10^6$ cells/ml in HBSS containing 0.05% human serum albumin (HAS; CLB). Chemotaxis of neutrophils was determined by using fluorescently-labeled neutrophils that migrated through a membrane fitted into an insert of a 24-well microtiter plate transwell system (Costar) containing a prewetted 3-μm-pore-size polycarbonate filter. For measurement of calcium ion fluxes, 5 x $10^6$ neutrophils/ml were loaded with 2 μM Fluo-3-AM (Molecular Probes) in RPMI containing 0.05% HSA (RPMI-HSA) for 20 minutes at room temperature under agitation, washed twice with buffer, and resuspended in RPMI-HSA at $10^6$ cells/ml. Calcium fluxes were analyzed with a FACScalibur (Becton Dickinson).

*D. Priming of human neutrophils*

Priming of PMNs by synthetic PSMs was determined by increased surface expression of CD11b and gp91*phox* (granule exocytosis). PMNs were incubated with 10–10000 ng/ml PSM, 10 μM fMLP, 10 ng/ml lipopolysaccharide, or 10 μg/ml

lipoteichoic acid in 96-well tissue culture plates at 37°C with rotation for 60 minutes. The assay was terminated by centrifuging cells at 4°C for 8 minutes at 350 x g. Cells were washed twice in cold Dulbecco's phosphate-buffered saline and stained with and isotype control antibody (BD Biosciences) or those specific for CD11b (mAb 44, BD Biosciences) or gp91*phox* (mAb 7D5[4]). Propidium iodide (0.5 μg/ml) was used to identify dead cells. PMNs were analyzed on a FACSCalibur flow cytometer (Becton Dickinson) and dead cells were excluded with a single gate. Percent positive neutrophils were determined with a marker defined by the boundary of the isotype-matched control antibody.

*E. Lysis of human neutrophils*

Lysis of PMNs by synthetic PSMs or clarified *S. aureus* culture media was determined essentially as described above in Example 2. Synthetic PSMs (1 or 10 μg/ml) were added to wells of a 96-well tissue culture plate containing $10^6$ PMNs and plates were incubated at 37°C for up to 3 hours. At the desired times, PMN lysis was determined by release of lactate dehydrogenase (LDH; Cytotoxicity Detection Kit, Roche Applied Sciences). Alternatively, wild-type and isogenic mutant *S. aureus* strains were cultured for 18 hours at 37°C in 50 ml TSB with shaking using a 100 ml flask. Bacteria were removed by centrifugation and culture media were sterilized by filtration and stored at –80°C in aliquots until used. Culture medium was diluted 1:10 in RPMI/H, mixed with human PMNs ($10^6$) and tested for its ability to cause PMN lysis.

*F. Measurement of cytokine production*

After isolation and washing, PMN or PBMC were resuspended in RPMI 1640 medium (Sigma) supplemented with 10% human serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM sodium pyruvate, and 10 mM HEPES. Cells were distributed to a 96-well culture plate at 200 μl and 5 x $10^5$ cells per well. Synthetic PSM peptides or filtered bacterial culture supernatants were diluted in fresh culture medium and added to the plate at 100 μl/well. Plates were incubated at 37°C in a 5.5% $CO_2$ incubator for 5 hours. Then, the plate was

centrifuged at 1500 rpm for 10 minutes, and supernatant was harvested from each well. Mono Mac 6 cells were obtained from DSMZ (Germany), and were grown in RPMI 1640 plus 10% heat inactivated fetal bovine serum (HyClone), 2 mM L-glutamine, 10.0 units/ml penicillin, 10.0 μg/ml streptomycin, 0.2 x non-essential amino acid solution, and OPI medium supplement (Sigma). Cells were grown in T75 culture flasks in a 37°C $CO_2$ incubator and harvested by centrifugation. After removal of supernatant, cells were resuspended in fresh culture medium, counted, and distributed into a 96-well cell culture plate at 100 μl and 2.5 x $10^5$ cells per well. Phorbol 12-myristate 13-acetate (Sigma) was added to a final concentration of 2.5 ng/ml for cell pre-stimulation. The plate was incubated in a 37°C $CO_2$ incubator for 3 hours. At the end of the pre-stimulation period, synthetic PSM peptides were diluted in culture medium and added to the cells at 100 μl/well. Cells were incubated in a 37°C $CO_2$ incubator for 20 – 22 hours, and the plate was centrifuged at 1500 rpm for 10 minutes. Human TNF-α, IL-1β, IL-8, and mouse TNF-α were measured in the culture supernatants with commercial ELISA assay kits (R&D systems) according to the manufacturer's instructions.

*G. Circular dichroism (CD) measurement*

The structures of synthetic PSM peptides were analyzed by CD spectroscopy on a Jasco spectropolarimeter model J-720 instrument. Solutions of PSM peptides, each at 1.0 mg/ml, were prepared in 50% trifluoroethanol. Measurements were performed in triplicate and the resulting scans were averaged, smoothed, and the buffer signal was subtracted.

*H. Scanning electron microscopy*

For scanning electron microscopy, PMNs were fixed, washed, and mounted on stubs as described above in Example 2. After mounting on stubs, samples were coated lightly with chromium using an ion beam sputterer (South Bay Technology, Inc.), and examined with a Hitachi S5200 field emission scanning electron microscope (Hitachi High Technologies America). Digital images were collected and adjusted for brightness and contrast with Photoshop CS (Adobe Systems).

*I. Statistical analysis*

Unless noted otherwise, unpaired t-tests were used to calculate 2-tailed *P* values using Graph Pad Prism 4 software.

**Example 4: Administration of PSMα peptides to a human subject**

This Example demonstrates a method of administering a PSMα peptide to a subject, for example, for the treatment, amelioration, or prevention of MRSA (for instance CA-MRSA) in the subject. A suitable subject for receiving the PSMα peptide vaccine is one who is at risk for exposure to MRSA bacteria, for instance a subject who deals closely with the public or who lives in close quarters with other people, such as a nursing home or other long-term care facility, particularly if the individuals residing there having chronic illnesses or impaired immunity. The PSMα peptide vaccine is, in one example, PSMα3 provided as a pharmaceutical composition, and is administered subcutaneously in a dose that includes about 0.1 μg to 10 mg of immunogenic PSMα peptide. A second dose is administered in the same fashion approximately three days to three months after the first dose, and the efficacy of protection against MRSA infection is assessed by measuring antibody titers using standard laboratory protocols.

**Example 5: Immunization with synthetic PSM peptides triggered specific antibody productions *in vivo*.**

This Example demonstrates the immunogenicity of specific synthetic PSM peptides. In order to determine the immunogenicity of synthetic PSM peptides, mice (BALB/c, female, 6 – 8 weeks) were injected subcutaneously with 50 μg (100 μL) of each PSM peptide in sterile PBS emulsified with complete Freund's adjuvant (CFA) for primary immunization. A booster injection containing incomplete Freund's adjuvant (IFA) instead of CFA was given to the animals 14 days after the primary immunization. The control group of animals received injections of sterile PBS and adjuvants only. Three blood withdrawals were performed on each mouse. The first two blood samples were collected via retro orbital route immediately before primary

injection and before booster injection, respectively. All animals were sacrificed 21 days after booster injection for the terminal blood samples.

PSM-specific antibodies in mouse serum were determined by ELISA assays. Microtiter plates (*Nunc* 96-well flat-bottom MaxiSorp plates) were coated with 20 μg/mL of each synthetic PSM peptides in PBS plus 0.05% $NaN_3$, and incubated overnight at 4°C. The plates were washed with PBS containing 0.05% Tween-20, and blocked for 1 hour at room temperature with 1% BSA (*Sigma*) and 0.05% $NaN_3$ in PBS. Serum samples were diluted in assay diluent (Tris-buffered saline containing 0.1% BSA and 0.05% Tween-20, pH 7.2), and added to the washed wells at 100 μL/well for an incubation of 2 hours at room temperature. Plates were washed again and HRP-labeled goat anti-mouse IgG (*R&D Systems*) and goat anti-mouse IgM (*Jackson ImmunoResearch Laboratory*) were diluted in assay diluent and added to the plates at 100 μL/well for an incubation of 1 hour at room temperature in order to detect PSM specific mouse IgG and IgM, respectively. Plates were washed again and a substrate solution containing equal volume of Tetramethylbenzidine and $H_2O_2$ was added to the plates at 100 μL/well for color development. The reaction was terminated by adding 50 μL of 1 M $H_2SO_4$ to each well, and optical density (O.D.) was measured at 450 nm using an ELISA plate reader.

As shown in FIGS. 11 and 12, all of the synthetic PSM peptides were immunogenic, triggering specific antibody productions *in vivo*. As shown in FIG. 11, of the N-formylated peptides, PSMα1, PSM α3, and PSMα4 generated the most robust antibody production. FIG. 12 shows that, of the non N-formylated PSM peptides, PSMα1, PSMα3, and PSMβ1 generated the strongest response.

**Example 6: Neutralizing effect of PSM-specific antiserum on cytokine production from human PMNs challenged with bacterial culture supernatants**

This Example demonstrates the neutralizing effect of PSM-specific antiserum on cytokine production from human PMNs challenged with bacterial culture supernatants. For measurement of the neutralizing activity of PSM-specific

antisera described above (see Example 5) against cytokine production from human PMNs challenged with bacterial supernatants *in vitro*, bacterial overnight culture supernatants (strains MW2 and LAC) were pre-incubated with 50% PSM-specific mouse antiserum or control serum at 37 $^{O}$C for 2 hours with gentle rotation in a 5.5% $CO_2$ incubator.

Human PMNs were isolated from venous blood of healthy volunteers. After isolation and washing, PMNs were resuspended in RPMI 1640 medium (*Sigma*) supplemented with 10% human serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM sodium pyruvate, and 10 mM HEPES. Cells were distributed to a 96-well cell culture plate (*Costar*$^{TM}$) at 200 μL and 1 x $10^6$ cells per well. Antiserum – pretreated bacterial culture supernatants were diluted in fresh cell culture medium and added to the plate at 100 μL/well to reach a final dilution factor of 1:100. Plates were incubated at 37 $^{O}$C in a 5.5% $CO_2$ incubator for 5 hours. The plates were then centrifuged at 1500 rpm for 10 min, and supernatants were harvested from each well.

Human IL-8 in the culture supernatant was measured with commercial ELISA assay kits (*R&D systems*) according to the manufacturer's instructions. Results were expressed as Mean ± SEM, and an unpaired two-tailed t-test was applied to determine the significance of the differences in IL-8 production between PSM antiserum pretreated and control serum treated groups.

As shown in FIG. 13 (MW2) and FIG. 14 (LAC), PSM-specific antiserum significantly inhibited IL-8 production from human PMNs challenged with community – acquired *S. aureus* culture supernatants.

**Example 7: PSM-specific antiserum mediated opsonophagocytosis and killing of *S. aureus* by human PMNs.**

This Example demonstrates that PSM-specific antiserum mediated opsonophagocytosis and killing of *S. aureus* by human PMNs. In order to demonstrate that PSM-specific antisera mediates phagocytosis and killing of *S. aureus in vitro*, human PMNs were isolated from venous blood of healthy

volunteers. After isolation and washing, PMNs were resuspended in Dulbecco's PBS containing 10 mM d-glucose (DPBS/G) and kept on ice. Cell concentration was adjusted to 1 x $10^7$ cells/mL.

*S. aureus* strains MW2, LAC, and 252 were grown to late exponential phase in TSB medium. Bacterial cells (5 x $10^7$cells/25μL/sample) were opsonized with 50% PSM-specific mouse antiserum or adjuvant control serum (25 μL/sample) for 30 minutes at 37°C with gentle rotation. Phagocytosis was then performed by mixing 5 x $10^6$ opsonized bacterial cells (5 μL) with $10^6$ freshly isolated human PMNs (100 μL), and gently rotating the mixture for 30 minutes at 37°C in a 5.5% $CO_2$ incubator. At the end of the incubation period, phagocytosis was terminated by transferring all samples onto ice.

Samples were diluted in TSB medium and plated 100 μL per TSB plate without antibiotics. Phagocytosis activity was determined and compared among groups by number of viable bacterial colonies on the plates. Results were expressed as Mean ± SEM, and an unpaired two-tailed t-test was applied to determine the significance of the differences in viable bacterial colony number among bacterial groups opsonized with PSM-specific antiserum or control serum.

As shown in FIGS. 15 and 16 (MW2) and FIG. 17 (LAC), viable colony number was significantly reduced from PSM specific antiserum pre – opsonized bacterial groups, indicating that PSM antiserum mediated phagocytosis *in vitro* by efficiently opsonizing bacterial cells. In FIGs. 15 and 17, although it appears that a combination of all of the PSMs was not effective (see the columns labeled "All"), this is an artifact that resulted from the dilution of the most effective PSMs by the less effective PSMs.

In view of the many possible embodiments to which the principles of the disclosure may be applied, it should be recognized that the illustrated embodiment is only a preferred example of the invention and should not be taken as a limitation on the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. An isolated immunogenic peptide comprising at least one antigenic phenol-soluble modulin alpha (PSMα) peptide, wherein the PSMα peptide comprises:

(a) an amino acid sequence set forth as SEQ ID NO: 8;

(b) an amino acid sequence set forth as SEQ ID NO: 2;

(c) an amino acid sequence set forth as SEQ ID NO: 3;

(d) an amino acid sequence set forth as SEQ ID NO: 4;

(e) an amino acid sequence set forth as SEQ ID NO: 1;

(f) an amino acid sequence having at least 85% sequence identity with (b) or (e); or

(g) an amino acid sequence having at least 90% sequence identity with (a), (c), or (d).

2. The isolated immunogenic peptide of claim 1, wherein the PSMα peptide comprises the amino acid sequence having at least 95% sequence identity with (a), (b), (c), (d), or (e).

3. The isolated immunogenic peptide of claim 1 or 2, wherein the PSMα peptide consists essentially of:

(a) the amino acid sequence set forth as SEQ ID NO: 8;

(b) the amino acid sequence set forth as SEQ ID NO: 2;

(c) the amino acid sequence set forth as SEQ ID NO: 3;

(d) the amino acid sequence set forth as SEQ ID NO: 4;

(e) the amino acid sequence set forth as SEQ ID NO: 1; or

(f) an amino acid sequence having at least 85% sequence identity with (b) or (e); or

(g) an amino acid sequence having at least 90% sequence identity with (a), (c), or (d).

4. The isolated immunogenic peptide of any of claims 1-3, wherein the PSMα peptide consists essentially of the amino acid sequence having at least 95% sequence identity with (a), (b), (c), (d), or (e).

5. The isolated immunogenic peptide of any of claims 1-4, wherein the PSMα peptide consists of:

(a) the amino acid sequence set forth as SEQ ID NO: 8;

(b) the amino acid sequence set forth as SEQ ID NO: 2;

(c) the amino acid sequence set forth as SEQ ID NO: 3;

(d) the amino acid sequence set forth as SEQ ID NO: 4;

(e) the amino acid sequence set forth as SEQ ID NO: 1; or

(f) an amino acid sequence having at least 85% sequence identity with (b) or (e); or

(g) an amino acid sequence having at least 90% sequence identity with (a), (c), or (d).

6. The isolated immunogenic peptide of any of claims 1-5, wherein the PSMα peptide consists of the amino acid sequence having at least 95% sequence identity with (a), (b), (c), (d), or (e).

7. An isolated polynucleotide comprising a nucleic acid sequence encoding the immunogenic peptide of any of claims 1-6.

8. The polynucleotide of claim 7, operably linked to a promoter.

9. A vector comprising the polynucleotide of claim 7 or 8.

10. The isolated immunogenic peptide of any of claims 1-6, wherein the PSMα peptide provides protective immunity from MRSA when administered to a subject in a therapeutically effective amount.

11. The isolated immunogenic peptide of claim 10, wherein the MRSA is community-associated MRSA (CA-MRSA).

12. A pharmaceutical composition comprising the immunogenic peptide of any of claims 1-6 or a combination thereof, and a pharmaceutically acceptable carrier.

13. The pharmaceutical composition of claim 12, further comprising an amino acid sequence set forth as SEQ ID NO: 6 or SEQ ID NO: 7, or an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 6 or SEQ ID NO: 7.

14. The composition of claim 12 or 13, further comprising a therapeutically effective amount of an adjuvant.

15. The composition of claim 14, wherein the adjuvant comprises IL-2, GM-CSF, TNF-α, IL-12, and IL-6.

16. A method for eliciting an immune response in a subject, comprising:

(a) selecting a subject in which an immune response to the immunogenic peptide of claim 1 is desirable; and

(b) administering to the subject a therapeutically effective amount of the immunogenic peptide of any of claims 1-6 or a combination thereof, thereby producing an immune response in the subject.

17. The method of claim 16, wherein the immunogenic peptide is administered in combination with a therapeutically effective amount of a PSMβ peptide comprising the amino acid sequence set forth as SEQ ID NO: 6 or SEQ ID NO: 7, or an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 6 or SEQ ID NO: 7.

18. The method of claim 16 or 17, wherein administration comprises oral, topical, mucosal, or parenteral administration.

19. The method of claim 18, wherein parenteral administration comprises intravenous administration, intramuscular administration, or subcutaneous administration.

20. The method of any of claims 16-19, wherein administration comprises from about one to about six doses.

21. The method of any of claims 16-20, wherein administration comprises two doses.

22. The method of any of claims 16-21, further comprising administering a therapeutically effective amount of an adjuvant to the subject.

23. The method of any of claims 16-22, further comprising administering to the subject a therapeutically effective amount of IL-2, RANTES, GM-CSF, TNF-α, IFN-γ, G-CSF or a combination thereof.

24. A method of inhibiting MRSA infection in a subject, the method comprising:

(a) selecting a subject at risk for exposure to MRSA; and

(b) administering to the subject a therapeutically effective amount of the immunogenic peptide of any of claims 1-6, thereby inhibiting MRSA infection in the subject.

25. The method of claim 24, further comprising administering to the subject a therapeutically effective amount of a PSMβ peptide comprising the amino acid sequence set forth as SEQ ID NO: 6 or SEQ ID NO: 7, or an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 6 or SEQ ID NO:

7.

26. The method of claim 24 or 25, wherein the MRSA is community-associated MRSA (CA-MRSA).

27. An antibody that specifically binds to a *S. aureus* PSMα binding site.

28. The antibody of claim 27, wherein the *S. aureus* PSMα binding site is a PSMα1, PSMα2, PSMα3, or PSMα4 binding site.

29. A method for diagnosing MRSA in a subject, wherein the method comprises:

(a) selecting a subject at risk for developing MRSA;

(b) collecting a tissue or body fluid sample from the subject; and

(c) determining whether an anti-PSMα antibody is present in the sample, wherein the presence of an anti-PSMα antibody in the sample indicates that the subject has MRSA.

30. The method of claim 29, wherein the MRSA is community – associated MRSA (CA-MRSA).

31. The method of claim 29 or 30, wherein the anti-PSMα antibody is an anti-PSMα1 antibody, an anti-PSMα2 antibody, an anti-PSMα3 antibody, or an anti-PSMα4 antibody.

**Fig. 1A**

```
                1        10        20        30        40
PSMα1 fMGIIAGIIKVIKSLIEQFTGK
PSMα2 fMGIIAGIIKFIKGLIEKFTGK
PSMα3 fMEFVAKLFKFFKDLLGKFLGNN
PSMα4 fMAIVGTIIKIIKAIIDIFAK
δ-Toxin fMAQDIISTISDLVKWIIDTVNKFTKK
PSMβ1 fMEGLFNAIKDTVTAAINNDGAKLGTSIVSIVENGVGLLGKLFGF
PSMβ2 fMTGLAEAIANTVQAAQQHDSVKLGTSIVDIVANGVGLLGKLFGF
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**Fig. 1B**

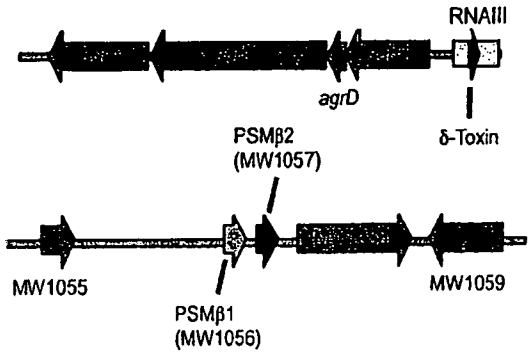



**Fig. 1C**

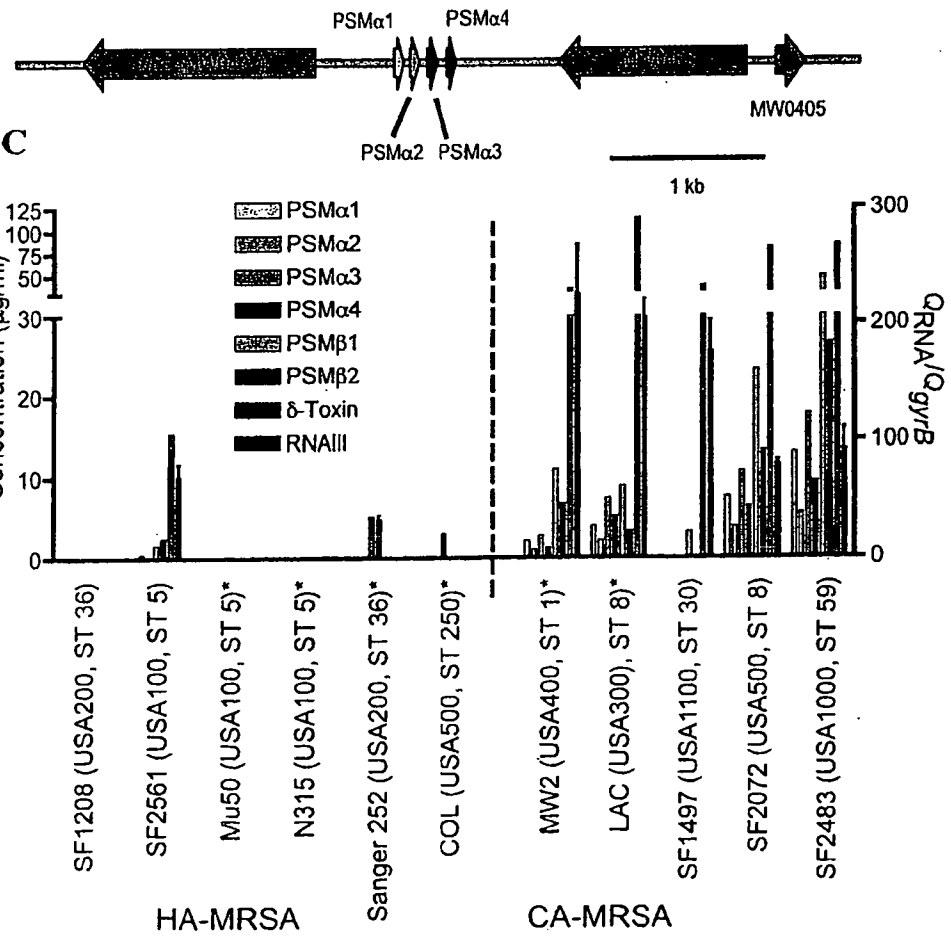


Fig. 2A

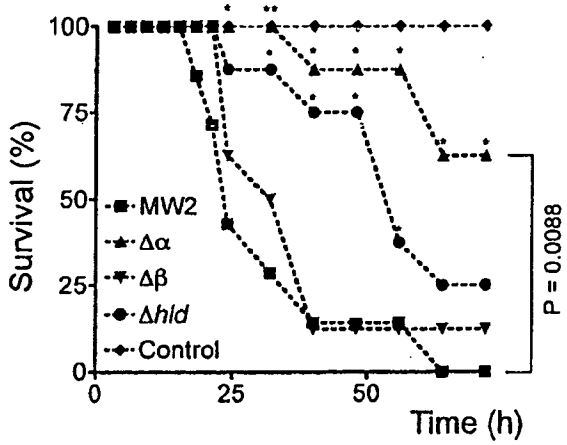



Fig. 2B

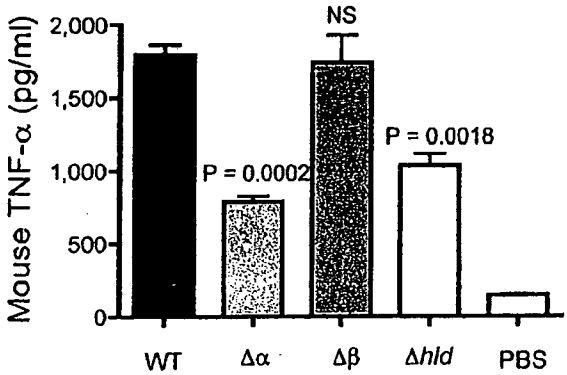



Fig. 2C

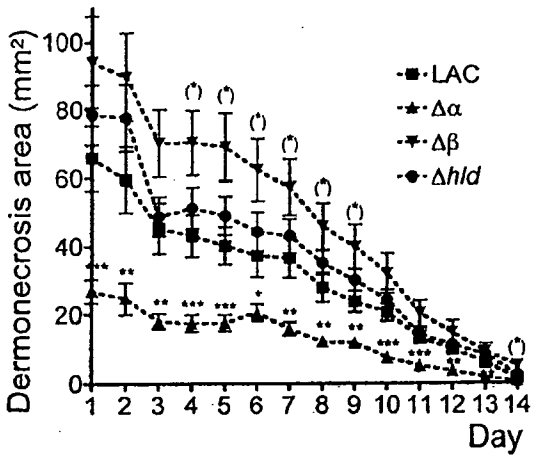



Fig. 2D

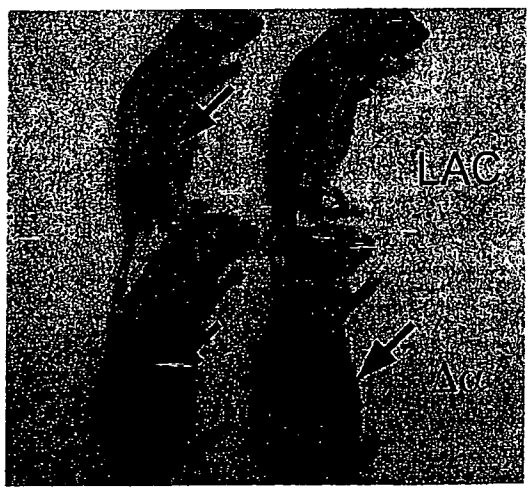


Fig. 3A

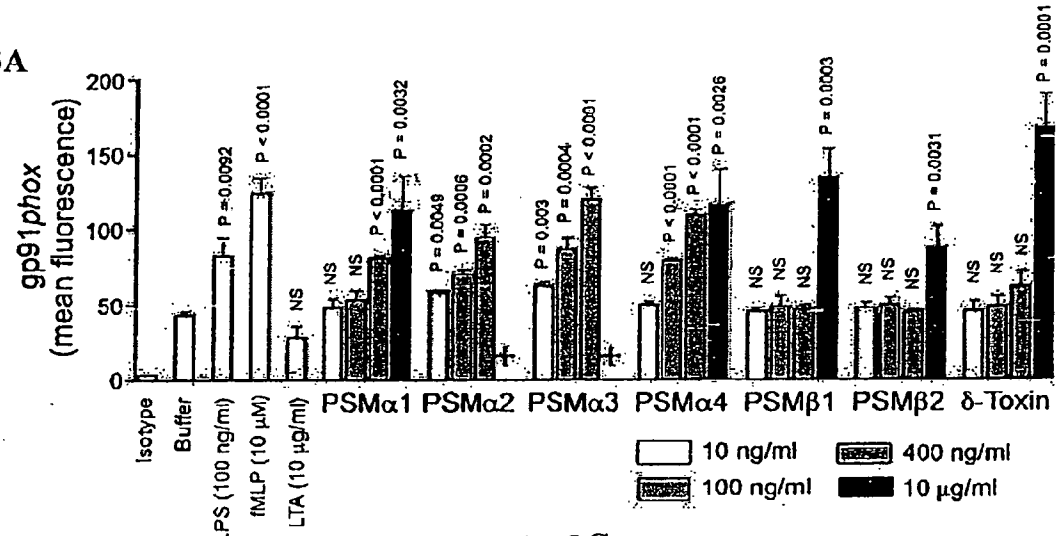



Fig. 3C

Fig. 3B

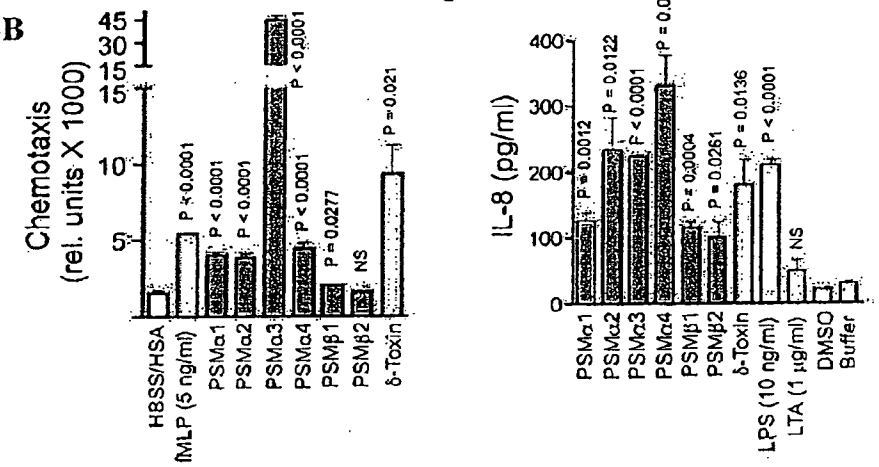


Fig. 4F

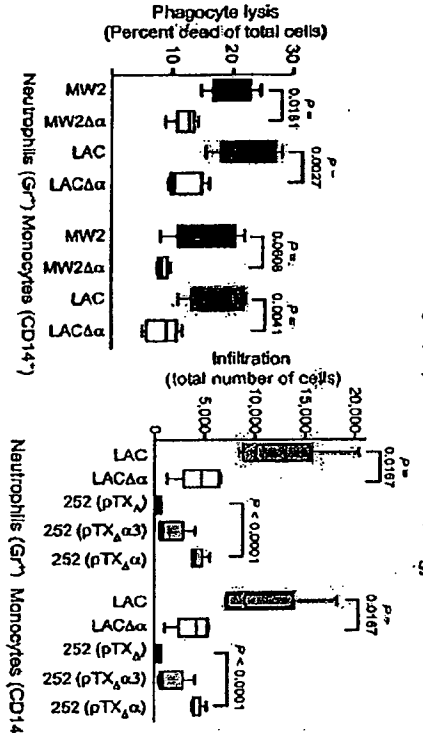



Fig. 4D

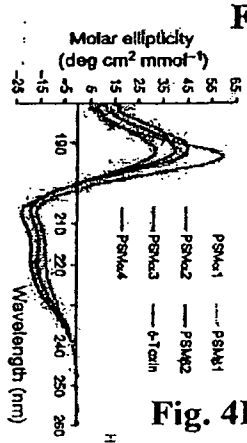



Fig. 4E

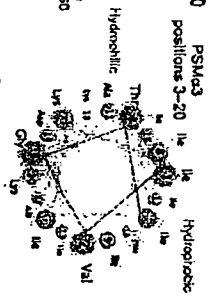



Fig. 4A

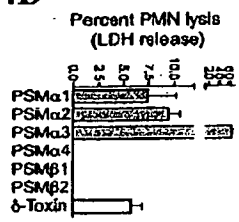



Fig. 4B

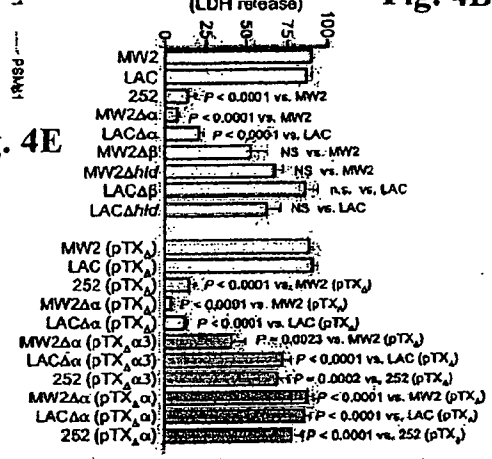



Fig. 4G

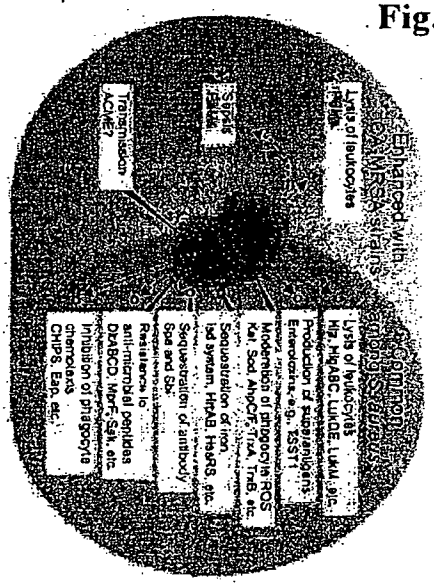
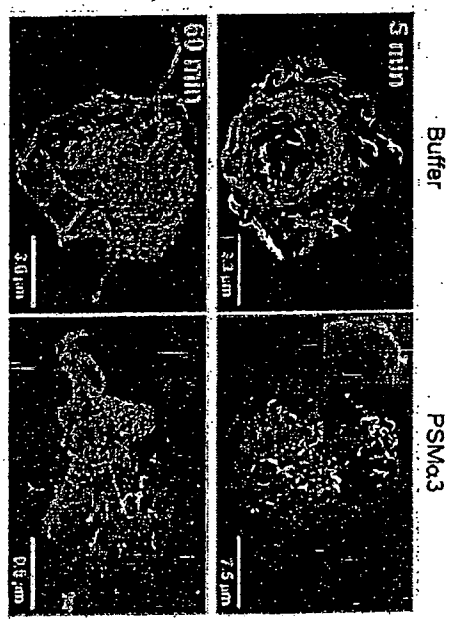



Fig. 4C



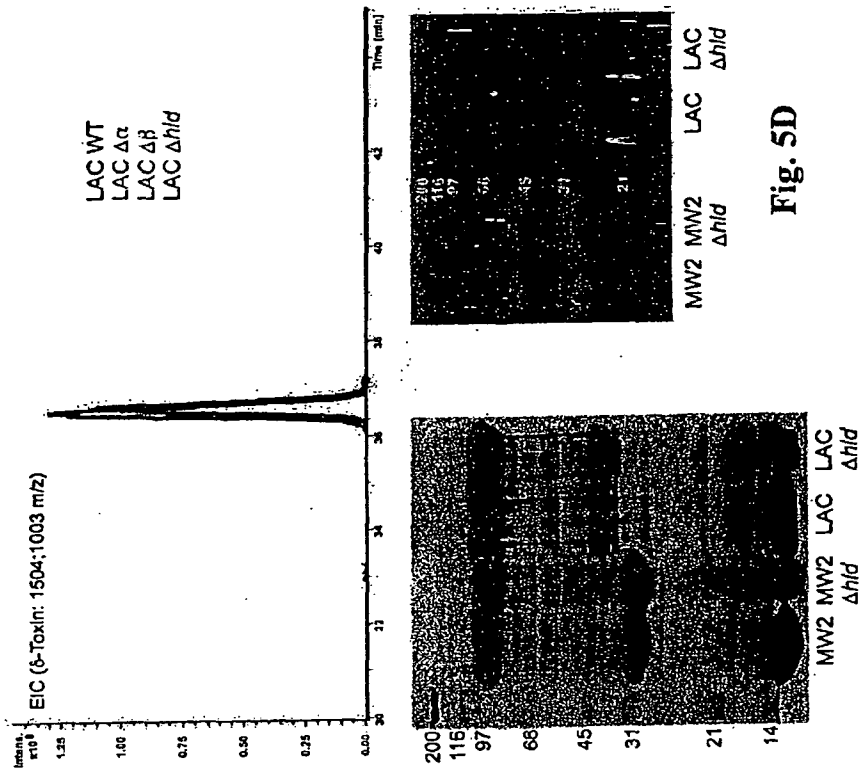



Fig. 5C

Fig. 5D

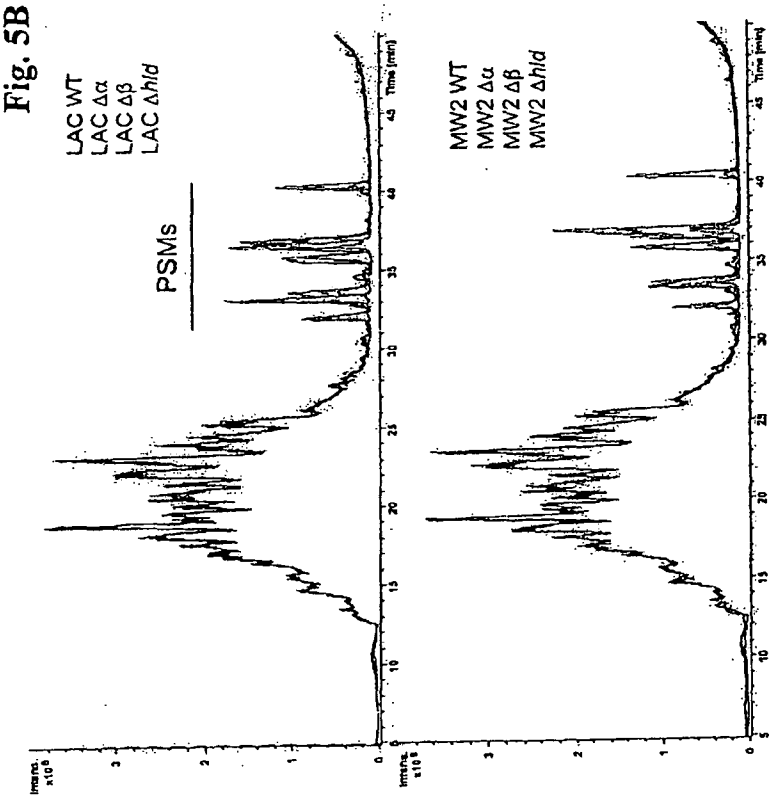



Fig. 5A

Fig. 5B

Fig. 6A

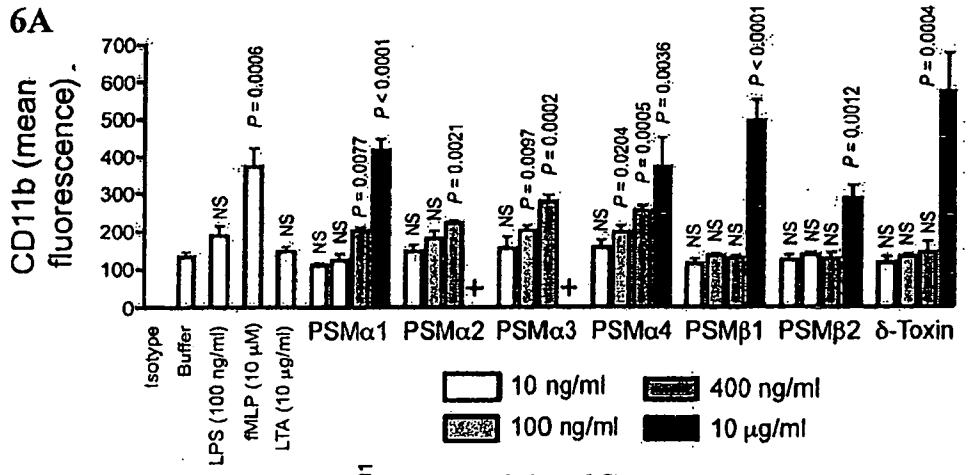



Fig. 6B

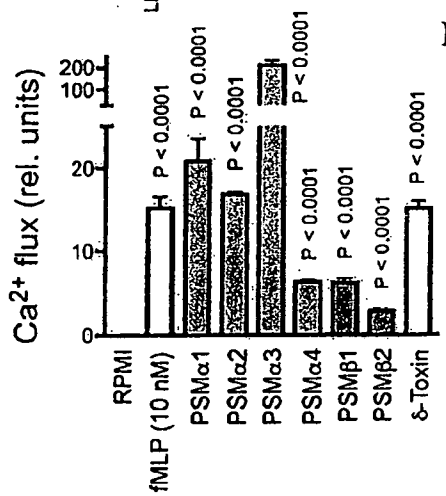



Fig. 6C

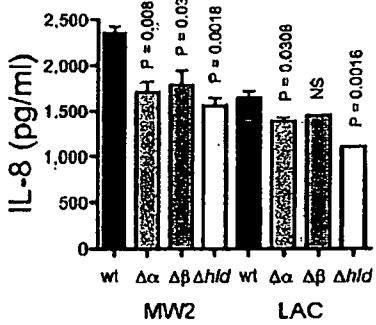



Fig. 6D

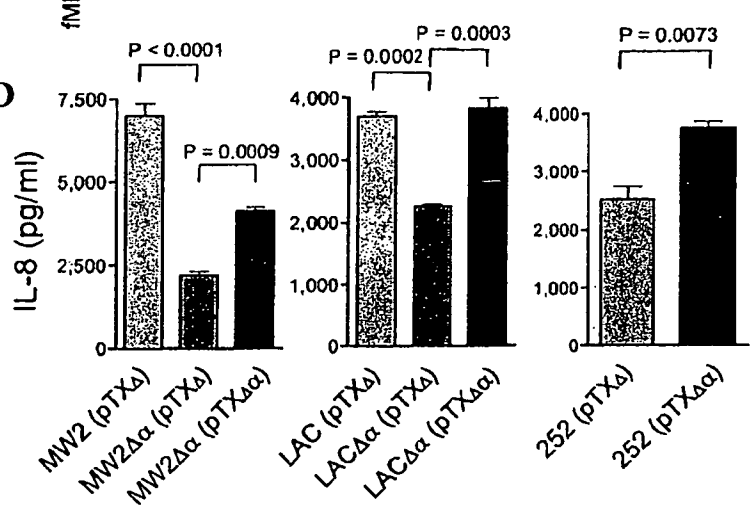
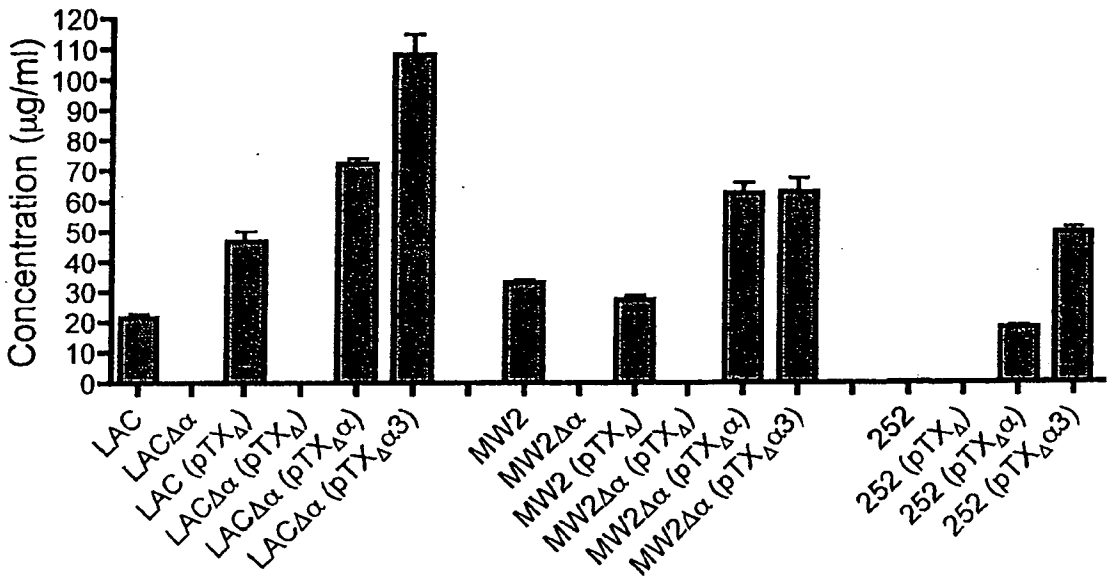


**Fig. 7A**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110
100
90
80
70
60
50
40
30
20
10
0
LAC
LACΔα
LAC (pTXΔ)
LACΔα (pTXΔ)
LACΔα (pTXΔα)
LACΔα (pTXΔα3)
MW2
MW2Δα
MW2 (pTXΔ)
MW2Δα (pTXΔ)
MW2Δα (pTXΔα)
MW2Δα (pTXΔα3)
252
252 (pTXΔ)
252 (pTXΔα)
252 (pTXΔα3)

**Fig. 8A**

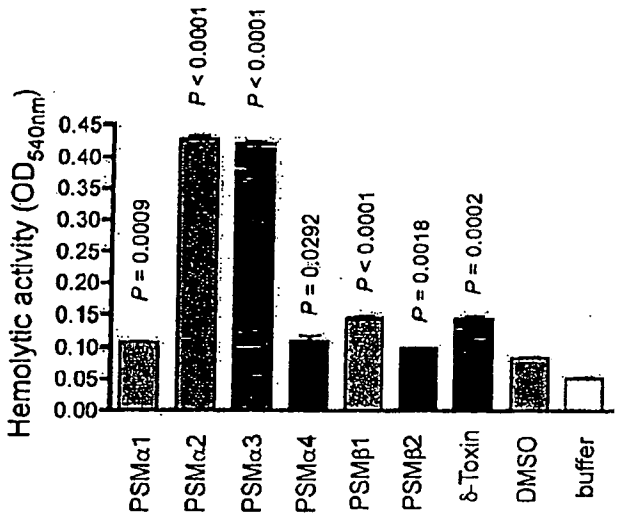
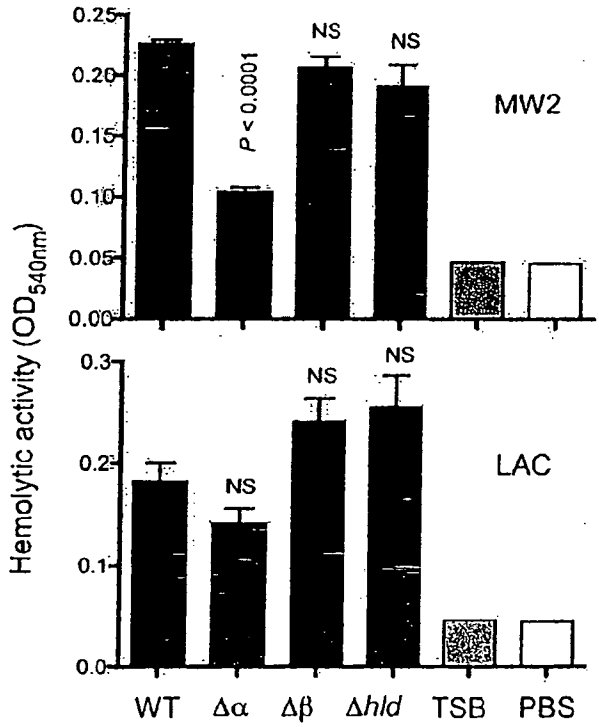

Hemolytic activity ($OD_{540nm}$)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
P = 0.0009
P < 0.0001
P < 0.0001
P = 0.0292
P < 0.0001
P = 0.0018
P = 0.0002
PSMα1
PSMα2
PSMα3
PSMα4
PSMβ1
PSMβ2
δ-Toxin
DMSO
buffer

**Fig. 8B**


Hemolytic activity ($OD_{540nm}$)
0.25
0.20
0.15
0.10
0.05
0.00
P < 0.0001
NS
NS
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0.0
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NS
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LAC
WT
Δα
Δβ
Δhld
TSB
PBS

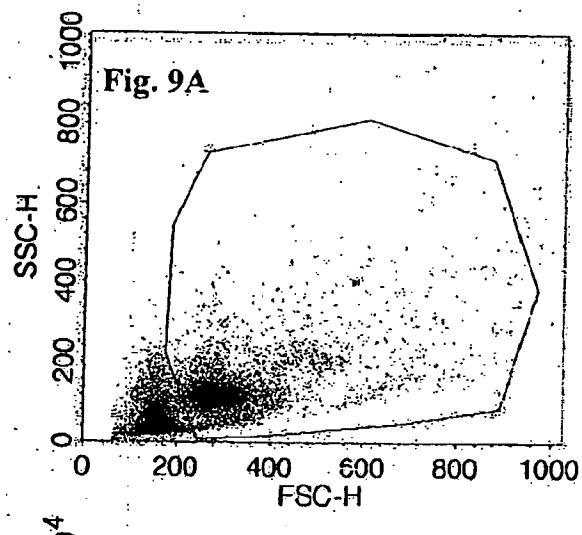



Fig. 9A

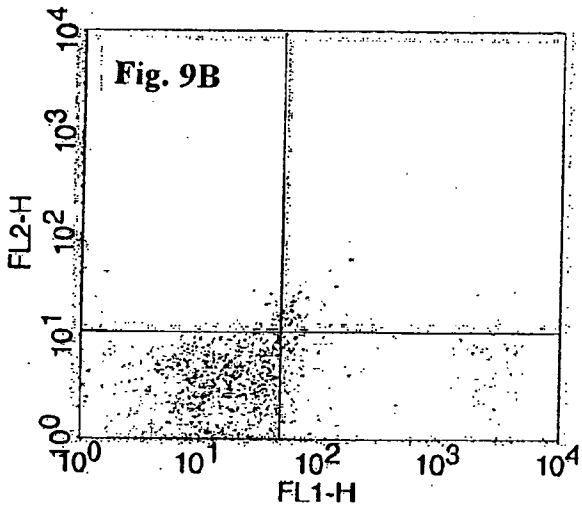



Fig. 9B

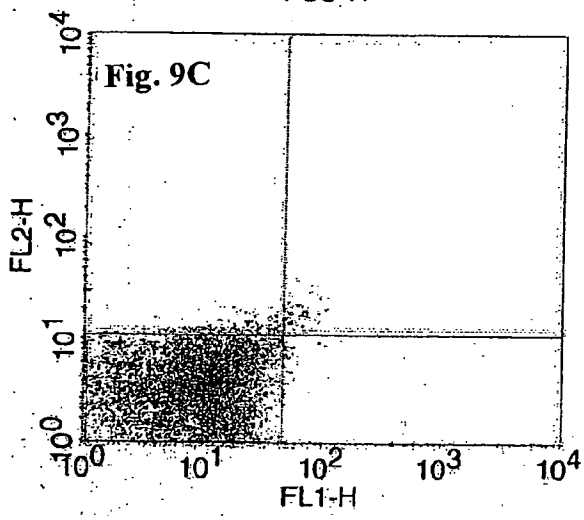



Fig. 9C

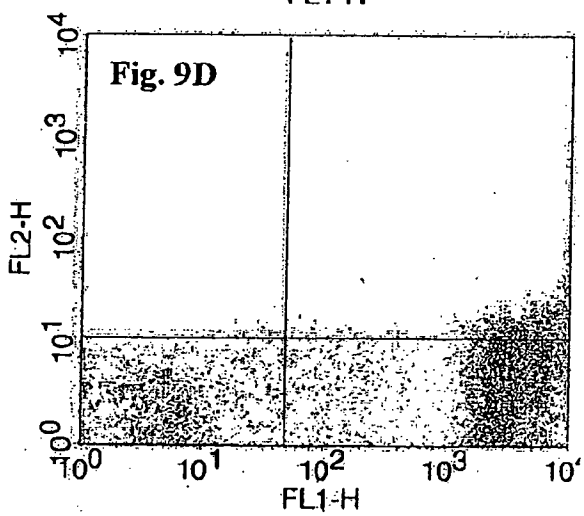



Fig. 9D

Fig. 10A

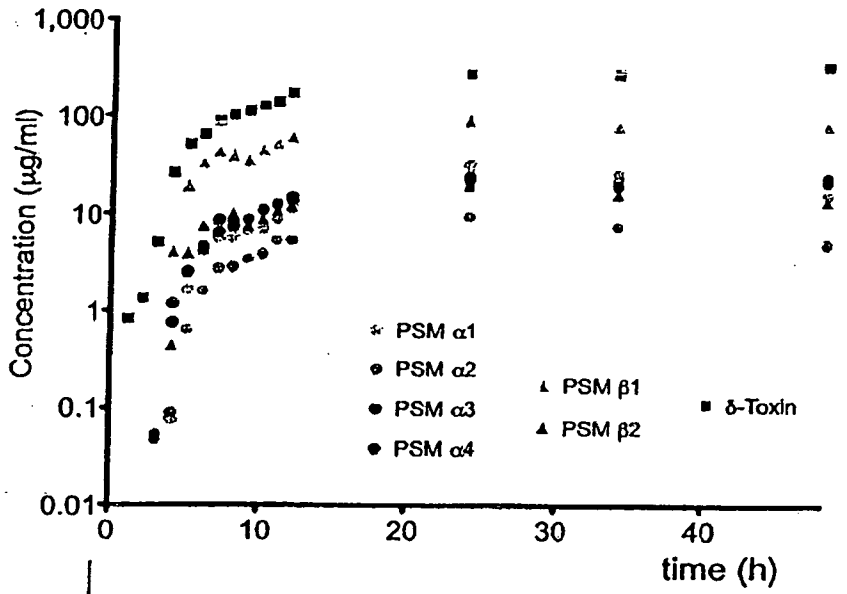
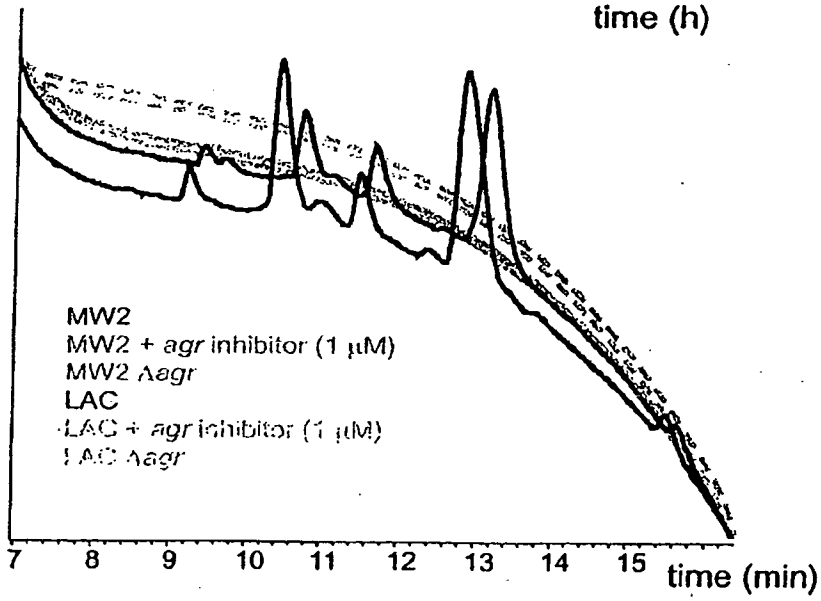



Fig. 10B



# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/063119

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/31 A61K38/00 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DURR MANUELA C ET AL: "Neutrophil chemotaxis by pathogen-associated molecular patterns - formylated peptides are crucial but not the sole neutrophil attractants produced by Staphylococcus aureus" CELLULAR MICROBIOLOGY, vol. 8, no. 2, February 2006 (2006-02), pages 207-217, XP002494944 ISSN: 1462-5814 page 213, left-hand column<br>-----<br>-/-- | 1-31 |

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 9 September 2008 | 26/09/2008 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Cervigni, S |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/063119

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | GILL STEVEN R ET AL: "Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant Staphylococcus aureus strain and a biofilm-producing methicillin-resistant Staphylococcus epidermidis strain" JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 187, no. 7, 1 April 2005 (2005-04-01), pages 2426-2438, XP002463146 ISSN: 0021-9193 the whole document page 2431, left-hand column; tables 1,2 page 2435, right-hand column, paragraph 1 | 1-31 |
| X | VELDKAMP KARIN ELLEN ET AL: "Staphylococcal culture supernates stimulate human phagocytes" INFLAMMATION, vol. 21, no. 5, 1997, pages 541-551, XP002494943 ISSN: 0360-3997 page 542 | 1-31 |
| X | OTTO MICHAEL ET AL: "Activity of Staphylococcus epidermidis phenol-soluble modulin peptides expressed in Staphylococcus carnosus" JOURNAL OF INFECTIOUS DISEASES, vol. 190, no. 4, 15 August 2004 (2004-08-15), pages 748-755, XP002494945 ISSN: 0022-1899 page 754, right-hand column | 1-31 |
| E | WO 2008/103751 A (UNIV CALIFORNIA [US]; GALLO RICHARD [US]; NIZET VICTOR [US]; COGEN ANN) 28 August 2008 (2008-08-28) the whole document | 1-31 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/063119

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16-26 and 29-31 are directed - at least in part - to a method of treatment or to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-31 (all in part)

An isolated immunogenic peptide as characterised in claim 1, paragraph (a). Related nucleotides, vectors and antibodies, pharmaceutical compositions and methods.

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2. claims: 1-31 (all in part)

An isolated immunogenic peptide as characterised in claim 1, paragraph (b). Related nucleotides, vectors and antibodies, pharmaceutical compositions and methods.

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3. claims: 1-31 (all in part)

An isolated immunogenic peptide as characterised in claim 1, paragraph (c). Related nucleotides, vectors and antibodies, pharmaceutical compositions and methods.

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An isolated immunogenic peptide as characterised in claim 1, paragraph (d). Related nucleotides, vectors and antibodies, pharmaceutical compositions and methods.

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5. claims: 1-31 (all in part)

An isolated immunogenic peptide as characterised in claim 1, paragraph (e). Related nucleotides, vectors and antibodies, pharmaceutical compositions and methods.

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6. claims: 1-31 (all in part)

An isolated immunogenic peptide as characterised in claim 1, paragraph (f). Related nucleotides, vectors and antibodies, pharmaceutical compositions and methods.

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7. claims: 1-31 (all in part)

An isolated immunogenic peptide as characterised in claim 1, paragraph (g). Related nucleotides, vectors and antibodies, pharmaceutical compositions and methods.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/063119

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2008103751 A | 28-08-2008 | NONE | |