(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 September 2002 (19.09.2002)

PCT

(10) International Publication Number
WO 02/072600 A2

(51) International Patent Classification[7]: C07K

(21) International Application Number: PCT/US02/02296

(22) International Filing Date: 28 January 2002 (28.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/264,072	26 January 2001 (26.01.2001)	US
60/274,611	12 March 2001 (12.03.2001)	US
60/298,413	18 June 2001 (18.06.2001)	US
60/308,116	30 July 2001 (30.07.2001)	US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/072600 A2

(54) Title: MONOCLONAL ANTIBODIES TO THE CLFA PROTEIN AND METHOD OF USE IN TREATING OR PREVENTING INFECTIONS

(57) Abstract: Monoclonal antibodies which can bind to the ClfA protein and which are generated from binding subdomains or active fragments of the ClfA protein from *Staphylococcus aureus*, including the active fragments proteins from its fibrinogen binding domain such as Clf40 protein, the Clf33 protein, or ClfA N3, are provided which can be useful in the treatment and protection against infection from staphylococcal bacteria such as *Staphylococcus aureus*. In addition, medical instruments can be treated using the monoclonal antibodies of the invention in order to reduce or eliminate the possibility of their becoming infected or further spreading the infection. In particular, the antibodies of the present invention are advantageous because they can prevent adherence of the bacteria to host cells by impairing or inhibiting the ability of *S. aureus* ClfA to bind to fibrinogen or fibrin, and thus can be utilized in methods or treating or preventing staphylococcal inventions.

MONOCLONAL ANTIBODIES TO THE CLFA PROTEIN AND METHOD OF USE IN TREATING OR PREVENTING INFECTIONS

Cross Reference to Related Applications

The present application claims the benefit of U.S. provisional applications Ser. No. 60/308,116, filed July 30, 2001, Ser. No. 60/298,413, filed June 18, 2001, Ser. No. 60/274,611, filed March 12, 2001, and Ser. No. 60/264,072, filed January 26, 2001.

Field of the Invention

The present invention relates in general to antibodies that have been generated against clumping factor A (or ClfA), a surface localized protein expressed in *Staphylococcus aureus* and other staphylococcus bacteria, and in particular to monoclonal antibodies against the ClfA protein and its active fragments or proteins from its fibrinogen binding domain such as Clf40, Clf33, or ClfA N3, and their use in inhibiting the binding of the ClfA protein to fibrinogen or fibrin and treating or preventing *S. aureus* infections.

Background of the Invention

The successful colonization of the host is a process required for most microorganisms to cause infections in animals and humans. Microbial adhesion is the first crucial step in a series of events that can eventually lead to disease. Pathogenic microorganisms colonize the host by attaching to host tissues or serum conditioned implanted biomaterials, such as catheters, artificial joints, and vascular grafts, through specific adhesins present on the surface of the bacteria. MSCRAMM™s (**M**icrobial **S**urface **C**omponents **R**ecognizing **A**dhesive **M**atrix **M**olecules) are a family of cell surface adhesins that recognize and specifically bind to distinct components in the host's extracellular matrix. Once the bacteria have successfully adhered and colonized host tissues, their physiology is dramatically altered and damaging components such as toxins and proteolytic enzymes are secreted. Moreover, adherent bacteria often produce a biofilm and quickly become more resistant to the killing effect of most antibiotics.

S. aureus causes a spectrum of infections that range from cutaneous lesions such as wound infections, impetigo, and furuncles to life-threatening conditions that include pneumonia, septic arthritis, sepsis, endocarditis, and biomaterial related infections. *S. aureus* is known to express a repertoire of different MSCRAMMs that can act individually or in concert to facilitate microbial adhesion to specific host tissue components. MSCRAMMs provide an excellent target for immunological attack by antibodies, in particular monoclonal antibodies. The presence of the appropriate anti-MSCRAMM high affinity antibodies can have a double-edged attack, first the antibodies can prevent microbial adherence and second the increased levels of MSCRAMM antibodies facilitate a rapid clearance of the organism from the body through opsonophagocytic killing.

However, it has still remained a problem to identify and utilize the information concerning MSCRAMM™s from *S. aureus* such as the ClfA protein to generate effective monoclonal antibodies because of the variability in the binding properties of the different MSCRAMM™s and their role in infectivity and spread of bacterial infections. In particular, it has been a problem to develop monoclonal antibodies which can bind to ClfA and which can be use to inhibit or impair the binding of staphylococcal ClfA to fibrinogen or fibrin and thus be useful in methods of preventing or treating staphylococcal infections. It has thus remained a highly desirable goal in the field of infectious diseases to develop monoclonal antibodies and other compositions which are successful in treating and preventing a wide variety of staph infections, particularly by inhibiting or impairing the bacteria's ability to bind to fibrinogen or fibrin.

Summary of the Invention

Accordingly, it is an object of the present invention to provide monoclonal antibodies that can bind to the *S. aureus* ClfA protein and thus be useful in methods to treat or prevent staphylococcal infections.

It is also an object of the present invention to provide monoclonal antibodies which are able to bind ClfA, and which are generated from the binding subdomains of the *S. aureus* ClfA protein, including the Clf40, Clf33 and ClfA N3 proteins, or active portions thereof, to be utilized in methods of treating or protecting against staphylococcal infections.

It is also an object of the present invention to provide a monoclonal antibodies to the Clf40, Clf33 and ClfA N3 proteins which can be useful in preventing adherence of Staphylococcal bacteria by inhibiting or impairing the binding of the ClfA protein to fibrinogen or fibrin.

It is a further object of the present invention to provide antibodies and antisera which can recognize the fibrinogen binding A domain of the ClfA protein and which can thus be useful in methods of treating, preventing, identifying or diagnosing staphylococcal infections.

It is a further object of the invention to provide amino acid sequences and the nucleic acid sequences which code for the variable light sequence and the variable heavy sequences of the monoclonal antibodies of the present invention.

It is still further an object of the present invention to provide a monoclonal antibody to ClfA which is protective against infection from *S. aureus*, and which can achieve cross-reactivity against other types of staph infection.

These and other objects are provided by virtue of the present invention which comprises the isolation and use of monoclonal antibodies to the ClfA protein and/or its binding subdomains, including the proteins Clf40, Clf33, and ClfA N3, for the prevention and treatment of *Staphylococcus* infections. The present application thus describes the discovery, production, characterization, and in vivo evaluation of monoclonal antibodies against ClfA, a surface localized protein expressed by virtually every *S. aureus* strain. Data presented here clearly demonstrate that monoclonal antibodies against ClfA and its active subdomains such as Clf40, Clf33 and N3 can be used to treat or protect against *S. aureus* infections.

The discovery and isolation of anti-ClfA monoclonal antibodies in accordance with the present invention can thus be used to impair or inhibit binding of the ClfA

protein to fibrinogen or fibrin and thus be useful in methods or treating or preventing staph infections. In accordance with the invention, suitable compositions and vaccines based on the isolated ClfA protein subdomains and antibodies raised thereto, as well as methods for their use, are also contemplated.

These embodiments and other alternatives and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the present specification and/or the references cited herein, all of which are incorporated by reference.

Brief Description of the Drawing Figures

Figure 1 is a graph of a biacore analysis used to measure ClfA binding and subsequent binding/inhibition of fibrinogen when monoclonal antibodies 13-1 or 13-2 in accordance with the present invention are bound to a chip using rabbit anti-mouse Fc (RAM-Fc) antibody.

Figure 2 is a graph of a biacore analysis of the Chimeric monoclonal antibody 12-9 in accordance with the present invention.

Figure 3 is a graph of a flow cytometric analysis of monoclonal antibody Chimeric 12-9 showing binding to *S. aureus* (Strain Newman).

Figure 4 is a graph showing binding affinity to ClfA of Chimeric and Humanized monoclonal antibody 12-9 in accordance with the invention.

Figure 5 is a graph showing the protection against Staphylococcus aureus murine lethal challenge model.

Figure 6 is a graph showing the whole cell inhibition of S. aureus adherence to immobilized fibrinogen using the monoclonal antibodies of the present invention.

Figure 7 is a graph showing the comparative binding of S. aureus using the 12-9 murine, 12-9 chimeric, and 12-9 humanized monoclonal antibodies in accordance with the present invention.

Figure 8 is a depiction of the variable heavy chain and variable light sequences of the monoclonal antibodies of the present invention showing the conserved sequences in the CDR1, CDR2 and CDR3 regions.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the present invention, there are provided monoclonal antibodies which can bind to the ClfA protein of *S. aureus*, and these monoclonal antibodies have been raised against active binding subdomain proteins including Clf40, Clf33, and the ClfA N3 regions which have been isolated and purified by the present inventors. The monoclonal antibodies in accordance with the invention have been shown to treat or protect against *S. aureus* infections.

Previously, McDevitt et al (McDevitt et al, 1994, Mol. Microbiol. 11, 237-248) identified a 92 kDa surface protein, from *S. aureus* strain Newman, demonstrated to be responsible for the fibrinogen-dependent clumping of bacteria, and this is now disclosed in U.S. Pat. No. 6,177,084, incorporated herein by reference. The gene, designated *ClfA*, was cloned and sequenced, and this is disclosed in U.S. Pat. No. 6,008,341, also incorporated by reference, and this region, representing a 896 amino acid protein as predicted from the DNA sequence, mediates adherence of bacteria to fibrinogen-coated surfaces, thereby identifying ClfA as a MSCRAMM™. The *ClfA* gene consists of a cytoplasmic domain, a transmembrane domain, an anchoring domain to the cell wall and a region (designated R) that connects the cell anchoring domains with the NH_2-terminal region A (composed of a unique 520 residue segment). The fibrinogen-binding domain of this MSCRAMM has been localized to a 218-residue segment within region A. McDevitt et al (McDevitt et al, 1995, Mol. Microbiol. 16, 895-907) has shown that region A of ClfA is sufficient for the clumping phenotype.

However, previously, no one has been able to generate monoclonal antibodies to the *S. aureus* ClfA protein. Accordingly, the present invention relates to an isolated and/or purified monoclonal antibody which can bind to the ClfA protein or its binding subdomains, including the Clf40, Clf33 and ClfA N3 proteins,

and which thus can be useful in methods of preventing and treating staphylococcal infection when used in amounts effective to prevent or treat such infections. These monoclonal antibodies may be produced using, e.g., the method of Kohler and Milstein, Nature 256:495-497 (1975), or other suitable ways known in the field, and in addition can be prepared as chimeric, humanized, or human monoclonal antibodies in ways that would be well known in this field. Still further, monoclonal antibodies may be prepared from a single chain, such as the light or heavy chains, and in addition may be prepared from active fragments of an antibody which retain the binding characteristics (e.g., specificity and/or affinity) of the whole antibody. By active fragments is meant an antibody fragment which has the same binding specificity as a complete antibody which binds to the ClfA protein, and the term "antibody" as used herein is meant to include said fragments. Additionally, antisera prepared using monoclonal or polyclonal antibodies in accordance with the invention are also contemplated and may be prepared in a number of suitable ways as would be recognized by one skilled in the art.

As indicated above, antibodies to ClfA may be prepared in a number of suitable ways that would be well known in the art, such as the well-established Kohler and Milstein method described above which can be utilized to generate monoclonal antibodies. In one such method, mice are injected intraperitoneally once a week for a prolonged period with a purified recombinant ClfA protein, or isolated subdomain protein such as Clf40, Clf33, or ClfA N3, or an active portion thereof, followed by a test of blood obtained from the immunized mice to determine reactivity to the purified ClfA. Following identification of mice reactive to ClfA, lymphocytes isolated from mouse spleens are fused to mouse myeloma cells to produce hybridomas positive for the antibodies against ClfA which are then isolated and cultured, following by purification and isotyping.

In order to generate monoclonal antibodies in accordance with the invention, it is thus preferred that these be generated using recombinantly prepared ClfA, Clf40, Clf33 or N3 proteins using conventional methods well known in the art. For

example, one such method employs the use of *E. coli* expression vector pQE-30 as an expression vector for cloning and expressing recombinant proteins and peptides.

Using PCR, the A domain of *ClfA* (Clf40 representing AA 40-559 or Clf33 representing AA 221-550) was amplified from *S. aureus* Newman genomic DNA and subcloned into the *E. coli* expression vector PQE-30 (Qiagen), which allows for the expression of a recombinant fusion protein containing six histidine residues. This vector was subsequently transformed into the *E. coli* strain ATCC 55151, grown in a 15-liter fermentor to an optical density (OD_{600}) of 0.7 and induced with 0.2 mM isopropyl-1-beta-D galactoside (IPTG) for 4 hours. The cells were harvested using an AG Technologies hollow-fiber assembly (pore size of 0.45 μm) and the cell paste frozen at –80° C. Cells were lysed in 1X PBS (10mL of buffer/1 g of cell paste) using 2 passes through the French Press @ 1100psi. Lysed cells were spun down at 17,000rpm for 30 minutes to remove cell debris. Supernatant was passed over a 5-mL HiTrap Chelating (Pharmacia) column charged with 0.1M $NiCl_2$. After loading, the column was washed with 5 column volumes of 10mM Tris, pH 8.0, 100mM NaCl (Buffer A). Protein was eluted using a 0-100% gradient of 10mM Tris, pH 8.0, 100mM NaCl, 200mM imidazole (Buffer B) over 30 column volumes. Clf40 or Clf33 eluted at ~13% Buffer B (~26mM imidazole). Absorbance at 280nm was monitored. Fractions containing Clf40 or Clf33 were dialyzed in 1x PBS.

The protein was then put through an endotoxin removal protocol. Buffers used during this protocol were made endotoxin free by passing over a 5-mL Mono-Q sepharose (Pharmacia) column. Protein was divided evenly between 4x 15mL tubes. The volume of each tube was brought to 9mL with Buffer A. 1mL of 10% Triton X-114 was added to each tube and incubated with rotation for 1 hour at 4°C. Tubes were placed in a 37°C water bath to separate phases. Tubes were spun down at 2,000rpm for 10 minutes and the upper aqueous phase from each tube was collected and the detergent extraction repeated. Aqueous phases from the 2nd extraction were combined and passed over a 5-mL IDA chelating (Sigma) column, charged with 0.1M $NiCl_2$ to remove remaining detergent. The column was washed with 9 column volumes of Buffer A before the protein was eluted with 3 column

volumes of Buffer B. The eluant was passed over a 5-mL Detoxigel (Sigma) column and the flow-through collected and reapplied to the column. The flow-through from the second pass was collected and dialyzed in 1x PBS. The purified product was analyzed for concentration, purity and endotoxin level before administration into the mice.

The amino acid sequence for Clf40 obtained in this manner is shown herein as SEQ ID NO:2, and is encoded by nucleic acids having the sequence as set forth in SEQ ID NO:1, or degenerates thereof. In addition, the amino acid sequence for Clf33 obtained in this manner is shown herein as SEQ ID NO:4, and is encoded by nucleic acids having the sequence as set forth in SEQ ID NO:3, or degenerates thereof.

In accordance with the invention, following isolation of the ClfA protein or its active subdomains such as Clf40, Clf33 or ClfA N3, monoclonal antibodies to these proteins can be produced by a number of suitable ways. For example, in one preferred method, the purified Clf40 and Clf33 proteins were used to generate a panel of murine monoclonal antibodies. Briefly, a group of Balb/C mice received a series of subcutaneous immunizations of 50 g of Clf40 or Clf33 protein in solution or mixed with adjuvant as described below:

Injection	Day	Amount (μg)	Route	Adjuvant
Primary	0	50	Subcutaneous	Freund's Complete
Boost #1	14	5(Clf40) 10(Clf33)	Intravenous	PBS

Three days after the final boost, the spleens were removed, teased into a single cell suspension and the lymphocytes harvested. The lymphocytes were then fused to a SP2/0-Ag14 myeloma cell line (ATCC #1581). Cell fusion, subsequent plating and feeding were performed according to the Production of Monoclonal Antibodies protocol from Current Protocols in Immunology (Chapter 2, Unit 2.).

Any clones that were generated from the fusion were then screened for specific anti-Clf40 antibody production using a standard ELISA assay. Positive

clones were expanded and tested further. Fifteen positive clones were originally identified and cloned by limiting dilution for further characterization. Single cell clones were tested for activity in a direct binding ELISA, a modified ELISA to measure inhibition of fibrinogen binding to CLF40, whole bacterial cell binding by flow cytometry and affinity for Clf40 binding by Biacore analysis.

S. aureus bacterial samples (strains Barnett, 67-0, ATCC#25923 and ATCC#49230) were collected, washed and incubated with Mab 13-2, 12-9, 13-1 or PBS alone (control) at a concentration of 2 mg/ml after blocking protein A sites with rabbit IgG (50 mg/ml). Following incubation with antibody, bacterial cells were incubated with Goat-$F_{(ab')2}$-Anti-Mouse-$F_{(ab')2}$-FITC which served as the detection antibody. After antibody labeling, bacterial cells were aspirated through the FACScaliber flow cytometer to analyze fluorescence emission (excitation: 488, emission: 570). For each bacterial strain, 10,000 events were collected and measured.

High binding 96 well plates were coated with 1 mg/ml solution of Clf40 in PBS (pH 7.4), covered, and incubated at room temperature for 2 hours. Plates were then washed with PBS, 0.05% Tween 20 and blocked with 1% BSA solution for 1 hour at room temperature. Following washing, monoclonal antibody supernatant was added and plates were incubated for 1 hour at room temperature. Plates were then washed and 0.1 mg/ml human fibrinogen solution was added to each well. Plates were incubated for 1 hour at room temperature and washed. Sheep anti-fibrinogen AP conjugate was added at a 1:750 dilution in PBS, 0.05% Tween 20, 0.1% BSA and allowed to incubate for 1 hour at room temperature. Plates were then washed and pNPP (developing solution) was added at a final concentration of 1 mg/ml. Plates were incubated 15-30 minutes at 37 ^{0} C and results were read at 405 nm and analyzed using Perkin Elmer HTS 7000 Bio-Assay reader.

Kinetic analysis was performed on a Biacore 3000 using the Ligand capture method included in the software. A rabbit anti-mouse-Fc antibody (Biacore) was amine coupled to a CM5 chip. The monoclonal antibody being analyzed was then

passed over the chip, allowing binding to the Fc portion. Varying concentrations of the Clf40 or Clf33 protein were then passed over the chip surface and data collected. Using the Biacore provided Evaluation software (Version 3.1), k_{on} and k_{off} were measured and K_A and K_D were calculated.

As shown in data below, immunizations to generate monoclonal antibodies in accordance with the present invention directed to Clf40 or active portions of Clf40 (N2N3 or N3 regions) have yielded monoclonal antibodies with different and diverse reactivity and cross-reactivity profiles.

Although production of antibodies using recombinant forms of the ClfA protein is preferred, antibodies may be generated from natural isolated and purified ClfA proteins or regions as well, and monoclonal or polyclonal antibodies can be generated using the natural ClfA proteins or active regions in the same manner as described above to obtain such antibodies. Still other conventional ways are available to generate the ClfA antibodies of the present invention using recombinant or natural purified ClfA proteins or its active regions, as would be recognized by one skilled in the art.

As would be recognized by one skilled in the art, the antibodies of the present invention may also be formed into suitable pharmaceutical compositions for administration to a human or animal patient in order to treat or prevent an infection caused by staphylococcal bacteria. Pharmaceutical compositions containing the antibodies of the present invention, or effective fragments thereof, may be formulated in combination with any suitable pharmaceutical vehicle, excipient or carrier that would commonly be used in this art, including such as saline, dextrose, water, glycerol, ethanol, other therapeutic compounds, and combinations thereof. As one skilled in this art would recognize, the particular vehicle, excipient or carrier used will vary depending on the patient and the patient's condition, and a variety of modes of administration would be suitable for the compositions of the invention, as would be recognized by one of ordinary skill in this art. Suitable methods of administration of any pharmaceutical composition disclosed in this application include, but are not limited to, topical, oral, anal, vaginal, intravenous,

intraperitoneal, intramuscular, subcutaneous, intranasal and intradermal administration.

For topical administration, the composition is formulated in the form of an ointment, cream, gel, lotion, drops (such as eye drops and ear drops), or solution (such as mouthwash). Wound or surgical dressings, sutures and aerosols may be impregnated with the composition. The composition may contain conventional additives, such as preservatives, solvents to promote penetration, and emollients. Topical formulations may also contain conventional carriers such as cream or ointment bases, ethanol, or oleyl alcohol.

Additional forms of antibody compositions, and other information concerning compositions, methods and applications with regard to other MSCRAMM™s will generally also be applicable to the present invention involving antibodies to the ClfA MSCRAMM™ and are disclosed, for example, in U.S. Patent 6,288,214 (Hook et al.), incorporated herein by reference.

The antibody compositions of the present invention which are generated against the ClfA protein or its effective subdomains such as Clf40, Clf33 or N3 may also be administered with a suitable adjuvant in an amount effective to enhance the immunogenic response against the conjugate. For example, suitable adjuvants may include alum (aluminum phosphate or aluminum hydroxide), which is used widely in humans, and other adjuvants such as saponin and its purified component Quil A, Freund's complete adjuvant, RIBBI adjuvant, and other adjuvants used in research and veterinary applications. Still other chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff *et al. J. Immunol.* 147:410-415 (1991) and incorporated by reference herein, encapsulation of the conjugate within a proteoliposome as described by Miller *et al.*, *J. Exp. Med.* 176:1739-1744 (1992) and incorporated by reference herein, and encapsulation of the protein in lipid vesicles such as Novasome™ lipid vesicles (Micro Vescular Systems, Inc., Nashua, NH) may also be useful.

In any event, the antibody compositions of the present invention will thus be useful for interfering with, modulating, inhibiting binding interactions between ClfA on staphylococcal bacteria and fibrinogen on host cells and tissues, or in displacing staphylococcal bacteria which has become bound to fibrinogen associated with host cells and tissues. Accordingly, the present invention will have particular applicability in developing compositions and methods of preventing or treating staphylococcal infection, and in inhibiting binding of staphylococcal bacteria to host tissue and/or cells.

In accordance with the present invention, methods are provided for preventing or treating a staphylococcal infection which comprise administering an effective amount of an antibody to the ClfA protein or its active subregions such as Clf40, Clf33 or N3 as described above in amounts effective to treat or prevent the infection. In addition, these monoclonal antibodies have been shown to be useful in impairing the binding of staphylococcal bacteria to fibrinogen or fibrin, and have thus proved effective in treating or preventing infection from staph bacteria such as *S. aureus*. Even further, the antibodies in accordance with the invention are doubly effective in that they have been shown to be cross-reactive across a wide variety of *S. aureus* strains which will thus improve the effectiveness and efficiency of compositions based on the monoclonals of the present invention.

Accordingly, in accordance with the invention, administration of the antibodies of the present invention in any of the conventional ways described above (e.g., topical, parenteral, intramuscular, etc.), and will thus provide an extremely useful method of treating or preventing staphylococcal infections in human or animal patients. By effective amount is meant that level of use, such as of an antibody titer, that will be sufficient to either prevent adherence of the bacteria, to inhibit binding of staph bacteria to host cells and thus be useful in the treatment or prevention of a staph infection. As would be recognized by one of ordinary skill in this art, the level of antibody titer needed to be effective in treating or preventing staphylococcal infection will vary depending on the nature and condition of the patient, and/or the severity of the pre-existing staphylococcal infection.

In addition to the use of antibodies to the ClfA protein and the regions in the A domain of that protein to treat or prevent *S. aureus* infection as described above, the present invention contemplates the use of these antibodies in a variety of ways, including the detection of the presence of *S. aureus* to diagnose a staph infection, whether in a patient or on medical equipment which may also become infected. In accordance with the invention, a preferred method of detecting the presence of staph infections involves the steps of obtaining a sample suspected of being infected by one or more staphylococcal bacteria species or strains, such as a sample taken from an individual, for example, from one's blood, saliva, tissues, bone, muscle, cartilage, or skin. The cells can then be lysed, and the DNA extracted, precipitated and amplified. Following isolation of the sample, diagnostic assays utilizing the antibodies of the present invention may be carried out to detect the presence of *S. aureus*, and such assay techniques for determining such presence in a sample are well known to those skilled in the art and include methods such as radioimmunoasssay, Western blot analysis and ELISA assays. In general, in accordance with the invention, a method of diagnosing an *S. aureus* infection is contemplated wherein a sample suspected of being infected with *S. aureus* infection has added to it a ClfA protein antibody in accordance with the present invention, and *S. aureus* is indicated by antibody binding to the ClfA proteins in the sample.

Accordingly, antibodies in accordance with the invention may be used for the specific detection of staphylococcal map proteins, for the prevention of infection from staph bacteria, for the treatment of an ongoing infection, or for use as research tools. The term "antibodies" as used herein includes monoclonal, polyclonal, chimeric, single chain, bispecific, simianized, and humanized or primatized antibodies as well as Fab fragments, such as those fragments which maintain the binding specificity of the antibodies to the ClfA proteins, including the products of an Fab immunoglobulin expression library. Accordingly, the invention contemplates the use of single chains such as the variable heavy and light chains of the antibodies as will be set forth below. Generation of any of these types of antibodies

or antibody fragments is well known to those skilled in the art. In the present case, monoclonal antibodies to ClfA proteins have been generated and isolated and shown to protect against staphylococcal infection.

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

Alternatively, the antibody may be labeled indirectly by reaction with labeled substances that have an affinity for immunoglobulin. The antibody may be conjugated with a second substance and detected with a labeled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibody may be conjugated to biotin and the antibody-biotin conjugate detected using labeled avidin or streptavidin. Similarly, the antibody may be conjugated to a hapten and the antibody-hapten conjugate detected using labeled anti-hapten antibody. These and other methods of labeling antibodies and assay conjugates are well known to those skilled in the art.

Antibodies to ClfA as described above may also be used in production facilities or laboratories to isolate additional quantities of the proteins, such as by affinity chromatography. For example, the antibodies of the invention may also be utilized to isolate additional amounts of the ClfA protein or its active fragments.

The isolated antibodies of the present invention, or active fragments thereof, may also be utilized in the development of vaccines for passive immunization against staph infections. Further, when administered as pharmaceutical composition to a wound or used to coat medical devices or polymeric biomaterials *in vitro* and *in vivo*, the antibodies of the present invention, may be useful in those cases where there is a previous staph infection because of the ability of this antibody to further restrict and inhibit *S. aureus* binding to fibrinogen or fibrin and thus limit the extent and spread of the infection. In addition, the antibody may be

modified as necessary so that, in certain instances, it is less immunogenic in the patient to whom it is administered. For example, if the patient is a human, the antibody may be "humanized" by transplanting the complimentarity determining regions of the hybridoma-derived antibody into a human monoclonal antibody as described, e.g., by Jones *et al.*, *Nature* 321:522-525 (1986) or Tempest *et al. Biotechnology* 9:266-273 (1991) or "veneered" by changing the surface exposed murine framework residues in the immunoglobulin variable regions to mimic a homologous human framework counterpart as described, e.g., by Padlan, Molecular Imm. 28:489-498 (1991), these references incorporated herein by reference. Even further, when so desired, the monoclonal antibodies of the present invention may be administered in conjunction with a suitable antibiotic to further enhance the ability of the present compositions to fight bacterial infections.

Medical devices or polymeric biomaterials to be coated with the antibodies, proteins and active fragments described herein include, but are not limited to, staples, sutures, replacement heart valves, cardiac assist devices, hard and soft contact lenses, intraocular lens implants (anterior chamber or posterior chamber), other implants such as corneal inlays, kerato-prostheses, vascular stents, epikeratophalia devices, glaucoma shunts, retinal staples, scleral buckles, dental prostheses, thyroplastic devices, laryngoplastic devices, vascular grafts, soft and hard tissue prostheses including, but not limited to, pumps, electrical devices including stimulators and recorders, auditory prostheses, pacemakers, artificial larynx, dental implants, mammary implants, penile implants, cranio/facial tendons, artificial joints, tendons, ligaments, menisci, and disks, artificial bones, artificial organs including artificial pancreas, artificial hearts, artificial limbs, and heart valves; stents, wires, guide wires, intravenous and central venous catheters, laser and balloon angioplasty devices, vascular and heart devices (tubes, catheters, balloons), ventricular assists, blood dialysis components, blood oxygenators, urethral/ureteral/urinary devices (Foley catheters, stents, tubes and balloons), airway catheters (endotracheal and tracheostomy tubes and cuffs), enteral feeding tubes (including nasogastric, intragastric and jejunal tubes), wound drainage tubes,

tubes used to drain the body cavities such as the pleural, peritoneal, cranial, and pericardial cavities, blood bags, test tubes, blood collection tubes, vacutainers, syringes, needles, pipettes, pipette tips, and blood tubing.

It will be understood by those skilled in the art that the term "coated" or "coating", as used herein, means to apply the antibody or active fragment, or pharmaceutical composition derived therefrom, to a surface of the device, preferably an outer surface that would be exposed to streptococcal bacterial infection. The surface of the device need not be entirely covered by the protein, antibody or active fragment.

In a preferred embodiment, the antibodies may also be used as a passive vaccine which will be useful in providing suitable antibodies to treat or prevent a staphylococcal infection. As would be recognized by one skilled in this art, a vaccine may be packaged for administration in a number of suitable ways, such as by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or nasopharyngeal (i.e., intranasal) administration. One such mode is where the vaccine is injected intramuscularly, e.g., into the deltoid muscle, however, the particular mode of administration will depend on the nature of the bacterial infection to be dealt with and the condition of the patient. The vaccine is preferably combined with a pharmaceutically acceptable carrier to facilitate administration, and the carrier is usually water or a buffered saline, with or without a preservative. The vaccine may be lyophilized for resuspension at the time of administration or in solution.

The preferred dose for administration of an antibody composition in accordance with the present invention is that amount will be effective in preventing of treating a staphylococcal infection, and one would readily recognize that this amount will vary greatly depending on the nature of the infection and the condition of a patient. As indicated above, an "effective amount" of antibody or pharmaceutical agent to be used in accordance with the invention is intended to mean a nontoxic but sufficient amount of the agent, such that the desired prophylactic or therapeutic effect is produced. As will be pointed out below, the

exact amount of the antibody or a particular agent that is required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, the particular carrier or adjuvant being used and its mode of administration, and the like. Accordingly, the "effective amount" of any particular antibody composition will vary based on the particular circumstances, and an appropriate effective amount may be determined in each case of application by one of ordinary skill in the art using only routine experimentation. The dose should be adjusted to suit the individual to whom the composition is administered and will vary with age, weight and metabolism of the individual. The compositions may additionally contain stabilizers or pharmaceutically acceptable preservatives, such as thimerosal (ethyl(2-mercaptobenzoate-S)mercury sodium salt) (Sigma Chemical Company, St. Louis, MO).

When used with suitable labels or other appropriate detectable biomolecule or chemicals, the monoclonal antibodies described herein are useful for purposes such as *in vivo* and *in vitro* diagnosis of staphylococcal infections or detection of staphylococcal bacteria. Laboratory research may also be facilitated through use of such antibodies. Various types of labels and methods of conjugating the labels to the antibodies of the invention are well known to those skilled in the art, such as the ones set forth below.

For example, the antibody can be conjugated (directly or via chelation) to a radiolabel such as, but not restricted to, ^{32}P, ^{3}H, ^{14}C, ^{35}S, ^{125}I, or ^{131}I. Detection of a label can be by methods such as scintillation counting, gamma ray spectrometry or autoradiography. Bioluminescent labels, such as derivatives of firefly luciferin, are also useful. The bioluminescent substance is covalently bound to the protein by conventional methods, and the labeled protein is detected when an enzyme, such as luciferase, catalyzes a reaction with ATP causing the bioluminescent molecule to emit photons of light. Fluorogens may also be used to label proteins. Examples of fluorogens include fluorescein and derivatives, phycoerythrin, allo-phycocyanin,

phycocyanin, rhodamine, and Texas Red. The fluorogens are generally detected by a fluorescence detector.

The location of a ligand in cells can be determined by labeling an antibody as described above and detecting the label in accordance with methods well known to those skilled in the art, such as immunofluorescence microscopy using procedures such as those described by Warren and Nelson (*Mol. Cell. Biol.,* 7: 1326-1337, 1987).

As indicated above, the monoclonal antibodies of the present invention, or active portions or fragments thereof, are particularly useful for interfering with the initial physical interaction between a staphylococcal pathogen responsible for infection and a mammalian host, such as the adhesion of the bacteria to mammalian extracellular matrix proteins such as fibrinogen, and this interference with the physical interaction may be useful both in treating patients and in preventing or reducing bacteria infection on in-dwelling medical devices to make them safer for use.

In another embodiment of the present invention, a kit which may be useful in isolating and identifying staphylococcal bacteria and infection is provided which comprises the antibodies of the present invention in a suitable form, such as lyophilized in a single vessel which then becomes active by addition of an aqueous sample suspected of containing the staphylococcal bacteria. Such a kit will typically include a suitable container for housing the antibodies in a suitable form along with a suitable immunodetection reagent which will allow identification of complexes binding to the ClfA antibodies of the invention. For example, the immunodetection reagent may comprise a suitable detectable signal or label, such as a biotin or enzyme that produces a detectable color, etc., which normally may be linked to the antibody or which can be utilized in other suitable ways so as to provide a detectable result when the antibody binds to the antigen.

In short, the antibodies of the present invention which bind to the ClfA protein or active fragments thereof are thus extremely useful in treating or preventing staphylococcal infections in human and animal patients and in medical or other in-

dwelling devices. Accordingly, the present invention relates to methods of identifying and isolating antibodies which can bind to ClfA and which can be used in methods of treatment of staph infections which involve opsonophagocytic killing of the bacteria. Antibodies which are identified and/or isolated using the present method, such as the ClfA antibody which can bind the ClfA protein and which can prevent or treat a staph infection thus is part of the present invention

EXAMPLES

The following examples are provided which exemplify aspects of the preferred embodiments of the present invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1. Isolation and Sequencing of Clf40 and Clf33

Using PCR, the A domain of *ClfA* (Clf40 representing AA 40-559 or Clf33 representing AA 221-550) was amplified from *S. aureus* Newman genomic DNA and subcloned into the *E. coli* expression vector PQE-30 (Qiagen), which allows for the expression of a recombinant fusion protein containing six histidine residues. This vector was subsequently transformed into the *E. coli* strain ATCC 55151, grown in a 15-liter fermentor to an optical density (OD_{600}) of 0.7 and induced with 0.2 mM isopropyl-1-beta-D galactoside (IPTG) for 4 hours. The cells were harvested using an AG Technologies hollow-fiber assembly (pore size of 0.45 μm) and the cell paste frozen at –80° C. Cells were lysed in 1X PBS (10mL of buffer/1 g of cell paste) using 2 passes through the French Press @ 1100psi. Lysed cells were spun down at 17,000rpm for 30 minutes to remove cell debris. Supernatant was passed

over a 5-mL HiTrap Chelating (Pharmacia) column charged with 0.1M $NiCl_2$. After loading, the column was washed with 5 column volumes of 10mM Tris, pH 8.0, 100mM NaCl (Buffer A). Protein was eluted using a 0-100% gradient of 10mM Tris, pH 8.0, 100mM NaCl, 200mM imidazole (Buffer B) over 30 column volumes. Clf40 or Clf33 eluted at ~13% Buffer B (~26mM imidazole). Absorbance at 280nm was monitored. Fractions containing Clf40 or Clf33 were dialyzed in 1x PBS.

The protein was then put through an endotoxin removal protocol. Buffers used during this protocol were made endotoxin free by passing over a 5-mL Mono-Q sepharose (Pharmacia) column. Protein was divided evenly between 4x 15mL tubes. The volume of each tube was brought to 9mL with Buffer A. 1mL of 10% Triton X-114 was added to each tube and incubated with rotation for 1 hour at 4°C. Tubes were placed in a 37°C water bath to separate phases. Tubes were spun down at 2,000rpm for 10 minutes and the upper aqueous phase from each tube was collected and the detergent extraction repeated. Aqueous phases from the 2nd extraction were combined and passed over a 5-mL IDA chelating (Sigma) column, charged with 0.1M $NiCl_2$ to remove remaining detergent. The column was washed with 9 column volumes of Buffer A before the protein was eluted with 3 column volumes of Buffer B. The eluant was passed over a 5-mL Detoxigel (Sigma) column and the flow-through collected and reapplied to the column. The flow-through from the second pass was collected and dialyzed in 1x PBS. The purified product was analyzed for concentration, purity and endotoxin level before administration into the mice.

The protein and nucleic acid sequences are included below. The Clf40 amino acid sequence is included below as SEQ ID NO:2, and this is coded for by the nucleic acid sequence SEQ ID NO:1, and would also be coded by degenerates thereto. The Clf33 amino acid sequence is included below as SEQ ID NO:4, and this is coded for by the nucleic acid sequence SEQ ID NO:3, and would also be coded by degenerates thereto.

Example 2. Monoclonal Antibody Production Using Clf40 and Clf33

The purified Clf40 or Clf33 protein was used to generate a panel of murine monoclonal antibodies. Briefly, a group of Balb/C mice received a series of subcutaneous immunizations of 50 μg of Clf40 or Clf33 protein in solution or mixed with adjuvant as described below in Table I:

Table I:

Injection	Day	Amount (μg)	Route	Adjuvant
Primary	0	50	Subcutaneous	Freund's Complete
Boost #1	14	5(Clf40) 10(Clf33)	Intravenous	PBS

Three days after the final boost, the spleens were removed, teased into a single cell suspension and the lymphocytes harvested. The lymphocytes were then fused to a SP2/0-Ag14 myeloma cell line (ATCC #1581). Cell fusion, subsequent plating and feeding were performed according to the Production of Monoclonal Antibodies protocol from Current Protocols in Immunology (Chapter 2, Unit 2.).

Any clones that were generated from the fusion were then screened for specific anti-Clf40 antibody production using a standard ELISA assay. Positive clones were expanded and tested further. Fifteen positive clones were originally identified and cloned by limiting dilution for further characterization. Single cell clones were tested for activity in a direct binding ELISA, a modified ELISA to measure inhibition of fibrinogen binding to CLF40, whole bacterial cell binding by flow cytometry and affinity for Clf40 binding by Biacore analysis. Test results are include in Table II below:

Table II:

ClfA Mono-clonal Antibody	Binding Kinetics	Inhibition of Fbg binding	Binding to *S. aureus* Barnett	Binding to *S. aureus* 67-0	Binding to *S. aureus* ATCC #25923	Binding to *S. aureus* ATCC #49230
F12-9	k_{on} 7.74 x 10^{5} k_{off} 4.46 x 10^{-4} K_D 5.76 x 10^{-10}	50-70%	72%	62%	60%	94%
F13-1	k_{on} 1.11 x 10^{5} k_{off} 6.13 x 10^{-3} K_D 5.51 x 10^{-8}	0-15%	-	-	-	9%
F13-2	k_{on} 1.19 x 10^{5} k_{off} 2.81 x 10^{-4} K_D 2.35 x 10^{-9}	40-60%	59%	65%	55%	93%

Binding to Whole Bacteria

S. aureus bacterial samples (strains Barnett, 67-0, ATCC#25923 and ATCC#49230) were collected, washed and incubated with Mab 13-2, 12-9, 13-1 or PBS alone (control) at a concentration of 2 mg/ml after blocking protein A sites with rabbit IgG (50 mg/ml). Following incubation with antibody, bacterial cells were incubated with Goat-$F_{(ab')2}$-Anti-Mouse-$F_{(ab')2}$-FITC which served as the detection antibody. After antibody labeling, bacterial cells were aspirated through the FACScaliber flow cytometer to analyze fluorescence emission (excitation: 488, emission: 570). For each bacterial strain, 10,000 events were collected and measured.

Inhibition (ELISA)

High binding 96 well plates were coated with 1 μg/ml solution of Clf40 in PBS (pH 7.4), covered, and incubated at room temperature for 2 hours. Plates were then washed with PBS, 0.05% Tween 20 and blocked with 1% BSA solution for 1 hour at room temperature. Following washing, monoclonal antibody supernatant

was added and plates were incubated for 1 hour at room temperature. Plates were then washed and 0.1 mg/ml human fibrinogen solution was added to each well. Plates were incubated for 1 hour at room temperature and washed. Sheep anti-fibrinogen AP conjugate was added at a 1:750 dilution in PBS, 0.05% Tween 20, 0.1% BSA and allowed to incubate for 1 hour at room temperature. Plates were then washed and pNPP (developing solution) was added at a final concentration of 1 mg/ml. Plates were incubated 15-30 minutes at 37 ^{0} C and results were read at 405 nm and analyzed using Perkin Elmer HTS 7000 Bio-Assay reader.

Kinetic Analysis

Kinetic analysis was performed on a Biacore 3000 using the Ligand capture method included in the software. A rabbit anti-mouse-Fc antibody (Biacore) was amine coupled to a CM5 chip. The monoclonal antibody being analyzed was then passed over the chip, allowing binding to the Fc portion. Varying concentrations of the Clf40 or Clf33 protein were then passed over the chip surface and data collected. Using the Biacore provided Evaluation software (Version 3.1), k_{on} and k_{off} were measured and K_A and K_D were calculated.

Example 3. Additional Studies of Clf40 and Clf33

Using PCR, the A domain of *ClfA* (Clf40 representing AA 40-559, Clf33-N2N3 domain representing AA 221-550 or Clf-N3 domain representing AA370-559) was amplified from *S. aureus* Newman genomic DNA and subcloned into the *E. coli* expression vector PQE-30 (Qiagen), which allows for the expression of a recombinant fusion protein containing six histidine residues. This vector was subsequently transformed into the *E. coli* strain ATCC 55151, grown in a 15-liter fermentor to an optical density (OD_{600}) of 0.7 and induced with 0.2 mM isopropyl-1-beta-D galactoside (IPTG) for 4 hours. The cells were harvested using an AG Technologies hollow-fiber assembly (pore size of 0.45 mm) and the cell paste frozen at –80° C. Cells were lysed in 1X PBS (10mL of buffer/1 g of cell paste) using 2 passes through the French Press @ 1100psi. Lysed cells were spun down at

17,000rpm for 30 minutes to remove cell debris. Supernatant was passed over a 5-mL HiTrap Chelating (Pharmacia) column charged with 0.1M $NiCl_2$. After loading, the column was washed with 5 column volumes of 10mM Tris, pH 8.0, 100mM NaCl (Buffer A). Protein was eluted using a 0-100% gradient of 10mM Tris, pH 8.0, 100mM NaCl, 200mM imidazole (Buffer B) over 30 column volumes. Clf protein was eluted at ~13% Buffer B (~26mM imidazole). Absorbance at 280nm was monitored. Fractions containing Clf40 or Clf33 were dialyzed in 1x PBS.

The protein was then put through an endotoxin removal protocol. Buffers used during this protocol were made endotoxin free by passing over a 5-mL Mono-Q sepharose (Pharmacia) column. Protein was divided evenly between 4x 15mL tubes. The volume of each tube was brought to 9mL with Buffer A. 1mL of 10% Triton X-114 was added to each tube and incubated with rotation for 1 hour at 4°C. Tubes were placed in a 37°C water bath to separate phases. Tubes were spun down at 2,000rpm for 10 minutes and the upper aqueous phase from each tube was collected and the detergent extraction repeated. Aqueous phases from the 2nd extraction were combined and passed over a 5-mL IDA chelating (Sigma) column, charged with 0.1M $NiCl_2$ to remove remaining detergent. The column was washed with 9 column volumes of Buffer A before the protein was eluted with 3 column volumes of Buffer B. The eluant was passed over a 5-mL Detoxigel (Sigma) column and the flow-through collected and reapplied to the column. The flow-through from the second pass was collected and dialyzed in 1x PBS. The purified product was analyzed for concentration, purity and endotoxin level before administration into the mice.

Monoclonal Antibody Production

The purified Clf40, Clf33 or N3 protein was used to generate a panel of murine monoclonal antibodies. Briefly, a group of Balb/C or SJL mice received a series of subcutaneous immunizations of 1-10 mg of protein in solution or mixed with adjuvant as described below in Table III:

Table III:

RIMMS

Injection	Day	Amount (mg)	Route	Adjuvant
#1	0	5	Subcutaneous	FCA/RIBI
#2	2	1	Subcutaneous	FCA/RIBI
#3	4	1	Subcutaneous	FCA/RIBI
#4	7	1	Subcutaneous	FCA/RIBI
#5	9	1	Subcutaneous	FCA/RIBI

Conventional

Injection	Day	Amount (mg)	Route	Adjuvant
Primary	0	5	Subcutaneous	FCA
Boost #1	14	1	Intraperitoneal	RIBI
Boost #2	28	1	Intraperitoneal	RIBI
Boost #3	42	1	Intraperitoneal	RIBI

At the time of sacrifice (RIMMS) or seven days after a boost (conventional) serum was collected and titered in ELISA assays against MSCRAMMs or on whole cells (S. *aureus* and S. *epidermidis*). Three days after the final boost, the spleens or lymph nodes were removed, teased into a single cell suspension and the lymphocytes harvested. The lymphocytes were then fused to a SP2/0-Ag14 myeloma cell line (ATCC #1581). Cell fusion, subsequent plating and feeding were performed according to the Production of Monoclonal Antibodies protocol from Current Protocols in Immunology (Chapter 2, Unit 2.).

Any clones that were generated from the fusion were then screened for specific anti-Clf40, SdrG or FnbpA antibody production using a standard ELISA assay. Positive clones were expanded and tested further. Candidates were further tested for activity in a direct binding ELISA, a modified ELISA to measure inhibition of fibrinogen binding to CLF40, whole bacterial cell binding by flow cytometry and Clf40 binding / inhibition of fibrinogen-Clf40 binding by Biacore analysis.

Biacore Analysis

Throughout the analysis, the flow rate remained constant at 10 ml/min. Prior to the ClfA 40 injection, test antibody was adsorbed to the chip via RAM-Fc binding.

At time 0, ClfA 40 at a concentration of 30 mg/ml was injected over the chip for 3 min followed by 2 minutes of dissociation. This phase of the analysis measured the relative association and disassociation kinetics of the Mab / ClfA interaction. In the second phase of the analysis, the ability of the Mab bound ClfA to interact and bind fibrinogen was measured. Fibrinogen at a concentration of 100 mg/ml was injected over the chip and after 3 minutes a report point is taken.

Binding to Whole Bacteria

Bacterial samples (Newman) were collected, washed and incubated with Mab or PBS alone (control) at a concentration of 2 mg/ml after blocking protein A sites with rabbit IgG (50 mg/ml). Following incubation with antibody, bacterial cells were incubated with Goat-$F_{(ab')2}$-Anti-Mouse-$F_{(ab')2}$-FITC which served as the detection antibody. After antibody labeling, bacterial cells were aspirated through the FACScaliber flow cytometer to analyze fluorescence emission (excitation: 488, emission: 570). For each bacterial strain, 10,000 events were collected and measured.

Inhibition (ELISA)

High binding 96 well plates were coated with 1 ug/ml solution of Clf40 in PBS (pH 7.4), covered, and incubated at room temperature for 2 hours. Plates were then washed with PBS, 0.05% Tween 20 and blocked with 1% BSA solution for 1 hour at room temperature. Following washing, monoclonal antibody supernatant was added and plates were incubated for 1 hour at room temperature. Plates were then washed and 0.1 mg/ml human fibrinogen solution was added to each well. Plates were incubated for 1 hour at room temperature and washed. Sheep anti-fibrinogen AP conjugate was added at a 1:750 dilution in PBS, 0.05% Tween 20, 0.1% BSA and allowed to incubate for 1 hour at room temperature. Plates were then washed and pNPP (developing solution) was added at a final concentration of 1 mg/ml. Plates were incubated 15-30 minutes at 37 ^{0} C and results were read at 405 nm and analyzed using Perkin Elmer HTS 7000 Bio-Assay reader.

Example 4. Immunization with all or portions of Clf40 generate monoclonal antibodies with different reactivity patterns.

Table IV below shows the results of immunization tests with the active regions of the present invention, including Clf40, Clf33 (which constitutes the N2N3 region of the ClfA A domain), and the ClfA N3 region alone.

Table IV

ntigen	Fusion	Monoclonal	Reactivity							
			ELISA			Biacore		Flow Cytometry	Inhibition	FYI
			Clf40	SdrG	FnbpA	Binding	Inhibition			
:lfA N3	RIMMS	F29-19	Y	N	N	N	N	nt	nt	
ıcludes following:		F29-71	Y	N	N	N	N	nt	nt	
29 F30 F31 F32		F29-92	Y	N	N	N	N	nt	nt	
34 F36		F31-20	Y	N	N	N	N	nt	nt	
		F31-36	Y	N	N	N	N	nt	nt	
		F31-100	Y	N	N	N	N	nt	nt	
		F31-195	Y	N	N	N	N	nt	nt	
		F32-22	Y	N	N	N	N	nt	nt	
		F34-15	Y	N	N	N	N	nt	nt	
		F36-77	Y	N	N	N	N	nt	nt	
		F36-197	Y	N	N	N	N	nt	nt	
:lfA N2N3 (Clf33)	Conventional	INH-M010001	Y	N	N	Y	N	Y	Y	12-9
ıcludes following:		F12-3	Y	nt	nt	Y	nt	Y	N	
11 F12 F17 F18		F12-1	Y	nt	nt	Y	nt	Y	N	
		F12-5	Y	nt	nt	Y	nt	Y	N	
		F12-10	Y	nt	nt	Y	nt	Y	Y	
:lfA N2N3 (Clf33)	RIMMS	F33-7	Y	N	N	Y	Y	Y	nt	
ıcludes following:		F35-279	Y	N	N	Y	Y	N	nt	
33 F35 F38 F40		F35-177	Y	N	N	Y	N	Y	nt	
		F40-7	Y	N	N	Y	Y	Y	nt	
		F38-300	Y	N	N	Y	Y	N	nt	
		F35-129	Y	N	N	N	N	nt	nt	
:lf40	Conventional	INH-M000030	Y	nt	nt	Y	N	Y	Y	13-2
ıcludes following:		INH-M010004	Y	nt	nt	Y	N	Y	N	15-EC6
13 F14 F15 F16		INH-M010003	Y	nt	nt	Y	N	N	N	13-1
		F13-6	Y	nt	nt	Y	nt	Y	Y	

Y = a positive result N = a negative result Nt = not tested

The results displayed in this table show that immunizations to generate monoclonal antibodies with Clf40 or portions of Clf40 (N2N3 or N3) yield monoclonal antibodies with broad and diverse reactivity profiles and which exhibit substantial cross-reactivity across a wide variety of staphylococcal strains.

Example 5: Use of the Biacore to select high affinity Mabs that block ClfA binding to Fibrinogen.

Biacore Analysis

Throughout the experiment represented in Figure 1, the flow rate remained constant at 10 ml/min. Prior to the ClfA 40 injection, 946 RU of Mab 13-1 and 768 RU of Mab 13-2 were adsorbed to the chip via RAM-Fc binding. At time 0 on the graph, ClfA 40 at a concentration of 30 mg/ml was injected over the chip for 3 min followed by 2 minutes of dissociation. The 13-1 Mab bound 58 RU of ClfA and the 13-2 Mab bound 168 RU of ClfA at the end of the ClfA injection time. This phase of the experiment measured the relative association and disassociation kinetics of the Mab / ClfA interaction. In the second phase of the experiment measures the ability of the Mab bound ClfA to interact and bind fibrinogen. Fibrinogen at a concentration of 100 mg/ml was injected over the chip and after 3 minutes 64 RU of fibrinogen bound to the ClfA bound to Mab 13-1 but 0 RUs of fibrinogen bound to the ClfA bound to Mab 13-2.

Example 6. Comparison of Mab 13.2 against *S. aureus* strain Barnett and *S. aureus* ATCC 25923

Antibody Scale-up and Purification

Hybridoma cells were grown in RPMI/DMEM, 1X Nutridoma-SP media containing 2mM sodium pyruvate, 4mM L-glutamine and 2X penicillin-streptomycin to 2-3 liter culture volumes. Hybridoma supernatants were then harvested by centrifugation. The supernatants were filtered through 0.45 μM filters and the IgG was affinity purified using protein G chromatography. The monoclonal antibodies

were eluted using 0.1M glycine, pH 2.7 and immediately neutralized with one-tenth volume of 2M Tris, pH 8.0. The purified IgG was then dialyzed against 1X D-phosphate buffered saline, pH 7.4. If needed, the purified antibody was concentrated and aliquots frozen.

Staphylococcus aureus strains

S. aureus cells were taken from a frozen glycerol stock and were inoculated onto a single blood agar plate and grown for 24 hours at 37°C. Single colonies were then transferred to new blood agar plates. Eighty plates were inoculated to prepare 50 mls of final frozen stock. The plates were then incubated for 24 hours at 37°C. Following incubation, the colonies were scraped off the surface of each plate into four 50 ml tubes containing 10 mls of 1X PBS (20 plates per tube) while gently vortexing to remove the bacteria from the scraper. An additional 10 mls of 1X PBS was then added to the 10 mls of bacterial suspension, while vigorously vortexing to facilitate separation of any agar debris from the bacteria. The suspension was pelleted by centrifugation, 3500xg at 4°C for 10 minutes. The bacteria was washed in D-PBS and resuspended in 50 mls of freezing media. The bacterial stock was placed into 1 ml aliquots by snap freezing in an ethanol/dry ice bath and placed in a -80°C freezer. The concentration (CFU/ml) of the frozen stock was determined by thawing 1 ml aliquot of stock, and preparing serial dilutions from 10^{-5} to 10^{-11}. Dilutions were plated in duplicate on blood agar plates and incubated for 37°C for 16-18 hours. The CFU/ml was determined (CFU/ml=(average # colonies X dilution factor)/0.050 mls) and averaged for each dilution to determine the average CFU/ml. On the day of injection, aliquots of each strain were thawed, combined into one tube per strain, and vortexed.

Animal, Sex, Species, Number, Age and Source

Female Balb/C mice (5-6 weeks of age) were purchased from Taconic Quality Laboratory Animals and Services for Research (Germantown, NY). Animals were allowed to acclimate for at least 14 days prior to initiation of treatment. Upon

arrival, the mice were examined, group housed (5 / cage) in polycarbonate shoe box cages with absorbent bedding. All mice were placed on a 12 hour light-dark cycle under the required husbandry standards found in the NIH Guide for the Care and Use of Laboratory Animals.

Identification and Randomization

All animals were uniquely identified using tail tattoos prior to dosing. Prior to initiation of treatment, the animals were individually weighed and their health was evaluated. Mice were randomized and assigned to treatment groups using stratified body weights.

ClfA Specific Monoclonal Antibodies (Mab), Isotype

ClfA specific murine monoclonal antibodies were isotyped using Becton Dickenson Cytometric Bead Array for Murine Isotyping. Isotype was determined using flow cytometry according to the manufacturers protocol.

13.1 Clf40 Mab, IgG_1

13.2 Clf40 Mab, IgG_1

12.9 Clf33 Mab, IgG_1

Controls

ATTC 1771, IgG_1

Phosphate Buffered Saline, pH 7.4 (PBS) was purchased from Life Technologies, Inc. (Cat. No. 10010-023; Lot No. 1078749).

Experimental Design

Table V.

		TREATMENT					CHALLENGE		
Group #	No. of	Anti-body	Dose	Route	Fre-quency	Time Point	Bacteria	Stock Dilu-	Vol-ume/

	Mice							tion.	Route
1	12	13-2	36 mg/kg	i.p.	Once	-18 hr.	ATCC 25923	1:20	0.1 ml/ IV
2	15	CRL1771	36 mg/kg				ATCC 25923	1:20	
3	15	D-PBS	N/A				ATCC 25923	1:20	
4	12	13-2	36 mg/kg				Barnett	1:20	
5	15	CRL1771	36 mg/kg				Barnett	1:20	
6	15	D-PBS	N/A				Barnett	1:20	

In vivo animal data

Mice were treated by intraperitoneal (IP; 0.5ml) injection with 0.5 mg of monoclonal antibody 13-2, isotype control monoclonal antibody CRL-1771, or PBS. Eighteen hours after IgG administration, the mice were challenged with a single intravenous (IV) injection of *S. aureus* strain Barnett or *S. aureus* ATCC 25923. The mice were followed for 12 days at which point all remaining mice were sacrificed. Significant differences in the relative survival times between treatment groups were detected. Eighty-three percent (10/12) of the mice that received Mab 13-2, 13% (2/15) of the animals receiving CRL-1771, and 0% (0/15) that received PBS survived the bacterial challenge with *S. aureus* Barnett (13-2 vs. PBS, $p<0.0001$; 13-2 vs. CRL-1771, $p = 0.0009$). Statistical analysis of the animal data was conducted using Kaplan-Meier Survival Analysis with a Mantel-Cox (logrank) test. In the experiment where *S. aureus* ATCC 25923 was the bacterial challenge, 67% (8/12) of the mice that were administered Mab 13-2 survived, 27% (4/12) survived in the CRL-1771 treated group, and only 7% (1/15) survived in the PBS group (13-2 vs. CRL-1771, $p=0.02$; 13-2 vs. PBS, 0.0002). These results clearly

indicate that MSCRAMM specific monoclonal antibodies provide a significant level of protection against lethal infection with *S. aureus* strains.

Example 7. Isolation and Sequencing of Variable Region Sequences.

A. Monoclonal Antibody 13-2.

Messenger RNA was isolated from ClfA 13-2 hybridoma cells using the Fast Track 2.0 kit (Invitrogen; cat #K4500). Briefly, $1.4x10^8$ hybridoma cells cultured in DMEM-10 medium with 10 % FBS were washed with PBS, pelleted by centrifugation then lysed in detergent containing Protein/RNase Degrader. PolyA$^+$ mRNA was isolated by affinity purification on oligo-dT cellulose. Synthesis of first strand cDNA was accomplished using 5μg of mRNA and reverse transcriptase in a cDNA synthesis kit (Novagen; cat #69001-3) containing 20 pmol of 3' oligonucleotide mouse-specific primers (Novagen; cat# 69796 and 69812) for each variable heavy and variable light chain. A portion (5 to 50 ng) of the cDNA was amplified by the polymerase chain reaction (PCR) using the PCR Reagent System (Life Technologies; cat#10198-018) and a mouse variable heavy and light chain specific primer set (Novagen; cat# 70081-3, 5 pmol each) for 30 cycles (94 C hot start then cycles of 94 C for 1 min, 50 C for 1min and 72 C for 1min). PCR products were fractionated electrophoretically in a 1% ultra pure agarose gel in sodium acetate buffer and visualized by ethidium bromide staining. PCR fragments matching the predicted size were excised from the gel and purified using BIO 101 Geneclean spin columns (cat #1101-400) for ligation into the pCR2.1-TOPO (Invitrogen) plasmid, followed by transformation into competent TOP10 E. coli. (Invitrogen; cat# K4500). After isolating plasmid DNA using QIAprep Spin Miniprep Kit (QIAGEN; cat# 27106), positive clones with inserts were identified by restriction endonuclease digestion and agarose gel electrophoresis, followed by sequencing on an ABI automated sequencer using M13 Forward and M13 Reverse primers.

The resulting sequences were as follows:

13-2VLA-1 (variable light sequence)

AACATTATGATGACACAGTCGCCATCATCTCTGGCTGTGTCTGCAGGAGAAAA
GGTCACTATGAGCTGTAAGTCCAGTCAAAGTGTTTTATACAGTTCAAATCAGAA
GAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTACTGA
TCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTGATCGCTTCACAGGCAGT
GGATCTGGGACAGATTTTACTCTTACCATCAACAGTGTACAAGCTGAAGACCTG
GCAGTTTATTACTGTCATCAATACCTCTCCTCGCACACGTTCGGAGGGGGGAC
CAAGCTGGAAATAAAA

NIMMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLLIY
WASTRESGVPDRFTGSGSGTDFTLTINSVQAEDLAVYYCHQYLSSHTFGGGTKLE
IK

- Amino acids representing a CDR are underlined

13-2VHC-3 (variable heavy sequence)

CAGGTGCATCTGAAGGAGTCAGGACCTGGCCTGGTGGCACCCTCACAGAGCC
TGTCCATCACATGCACTGTCTCTGGATTCTCATTATCCAGATATAATATACACTG
GGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGCTGGGAATGATATGGGGT
GGTGAAAACACAGACTATAATTCAGCTCTCAAATCCAGACTGAGCATCAGCAA
GGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGA
CACAGCCATGTACTACTGTGCCAGCGCCTACTATGGTAACTCCTGGTTTGCTTA
CTGGGGCCAGGGGACTCTGGTCACTGTCTCTGCA

QVHLKESGPGLVAPSQSLSITCTVSGFSLSRYNIHWVRQPPGKGLEWLGMIWGGE
NTDYNSALKSRLSISKDNSKSQVFLKMNSLQTDDTAMYYCASAYYGNSWFAYWG
QGTLVTVSA

- Amino acids representing a CDR are underlined

B. Monoclonal Antibody 12-9.

Messenger RNA was isolated from ClfA 12-9 hybridoma cells using the Fast Track 2.0 kit (Invitrogen; cat #K4500). Briefly, $1.4x10^8$ hybridoma cells cultured in DMEM-10 medium with 10 % FBS were washed with PBS, pelleted by centrifugation then lysed in detergent containing Protein/RNase Degrader. PolyA$^+$ mRNA was isolated by affinity purification on oligo-dT cellulose. Synthesis of first strand cDNA was accomplished using 5μg of mRNA and reverse transcriptase in a cDNA synthesis kit (Novagen; cat #69001-3) containing 20 pmol of 3'

oligonucleotide mouse-specific primers (Novagen; cat# 69796 and 69812) for each variable heavy and variable light chain. A portion (5 to 50 ng) of the cDNA was amplified by the polymerase chain reaction (PCR) using the PCR Reagent System (Life Technologies; cat#10198-018) and a mouse variable heavy and light chain specific primer set (Novagen; cat# 70081-3, 5 pmol each) for 30 cycles (94 C hot start then cycles of 94 C for 1 min, 50 C for 1min and 72 C for 1min). PCR products were fractionated electrophoretically in a 1% ultra pure agarose gel in sodium acetate buffer and visualized by ethidium bromide staining. PCR fragments matching the predicted size were excised from the gel and purified using BIO 101 Geneclean spin columns (cat #1101-400) for ligation into the pCR2.1-TOPO (Invitrogen) plasmid, followed by transformation into competent TOP10 E. coli. (Invitrogen; cat# K4500). After isolating plasmid DNA using QIAprep Spin Miniprep Kit (QIAGEN; cat# 27106), positive clones with inserts were identified by restriction endonuclease digestion and agarose gel electrophoresis, followed by sequencing on an ABI automated sequencer using M13 Forward and M13 Reverse primers.

The resulting sequences were as follows:

12-9VLA-1 (variable light sequence)

AACATTATGATGACACAGTCGCCATCATCTCTGGCTGTGTCTGCAGGAGAAAA
GGTCACTATGAGCTGTAAGTCCAGTCAAAGTGTTTTATACAGTTCAAATCAGAA
GAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCTGA
TCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTGATCGCTTCACAGGCAGT
GGATCTGGGACAGATTTTACTCTTACCATCAGCAGTGTACAAGCTGAAGACCT
GGCAGTTTATTACTGTCATCAATACCTCTCCTCGTACACGTTCGGAGGGGGGA
CCAAGCTGGAAATAAAA

NIMMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLLIY
WASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSYTFGGGTKLEI
K

- Amino acids representing a CDR are underlined

12-9VHC-1 (variable heavy sequence)

CAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCACCCTCACAGAGCC
TGTCCATCACATGCGCTATCTCTGGGTTCTCATTATCCAGATATAGTGTACACT

GGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGCTGGGAATGATATGGGG
TGGTGGAAACACAGACTATAATTCAGCTCTCAAATCCAGACTGAGCATCAGCAA
GGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGA
CACAGCCATGTATTACTGTGCCAGAAAAGGGGAATTCTACTATGGTTACGACG
GGTTTGTTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA

QVQLKESGPGLVAPSQSLSITCAISGFSLSRYSVHWVRQPPGKGLEWLGMIWGG
GNTDYNSALKSRLSISKDNSKSQVFLKMNSLQTDDTAMYYCARKGEFYYGYDGFV
YWGQGTLVTVSA

- Amino acids representing a CDR are underlined

C. Monoclonal Antibody 35-220.

Isolation and Sequencing of Variable Region Sequences:

Messenger RNA was isolated from ClfA 35-220 hybridoma cells using the Fast Track 2.0 kit (Invitrogen; cat #K4500). Briefly, $1.4x10^8$ hybridoma cells cultured in DMEM-10 medium with 10 % FBS were washed with PBS, pelleted by centrifugation then lysed in detergent containing Protein/RNase Degrader. $PolyA^+$ mRNA was isolated by affinity purification on oligo-dT cellulose. Synthesis of first strand cDNA was accomplished using 5mg of mRNA and reverse transcriptase in a cDNA synthesis kit (Novagen; cat #69001-3) containing 20 pmol of 3' oligonucleotide mouse-specific primers (Novagen; cat# 69796 and 69812) for each variable heavy and variable light chain. A portion (5 to 50 ng) of the cDNA was amplified by the polymerase chain reaction (PCR) using the PCR Reagent System (Life Technologies; cat#10198-018) and a mouse variable heavy and light chain specific primer set (Novagen; cat# 70081-3, 5 pmol each) for 30 cycles (94 C hot start then cycles of 94 C for 1 min, 50 C for 1min and 72 C for 1min). PCR products were fractionated electrophoretically in a 1% ultra pure agarose gel in sodium acetate buffer and visualized by ethidium bromide staining. PCR fragments matching the predicted size were excised from the gel and purified using BIO 101 Geneclean spin columns (cat #1101-400) for ligation into the pCR2.1-TOPO (Invitrogen) plasmid, followed by transformation into competent TOP10 E.coli. (Invitrogen; cat# K4500). After isolating plasmid DNA using QIAprep Spin Miniprep

Kit (QIAGEN; cat# 27106), positive clones with inserts were identified by restriction endonuclease digestion and agarose gel electrophoresis, followed by sequencing on an ABI automated sequencer using M13 Forward and M13 Reverse primers.

The resulting sequences were as follows:

35-220VLD-4 (variable light sequence DNA)

AACATTATGATGACACAGTCGCCATCATCTCTGGCTGTGTCTGCAGGAGAAAA
GGTCACTATGAGCTGTAGGTCCAGTCAAAGTGTTTTATACAGTTCAAATCAGAA
GAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTACACTGCTGA
TCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTGATCGCTTCACAGGCAGT
GGATCTGGGACAGATTTTACTCTTACCATCAGCAGTGTACAAGCTGAAGACCT
GGCAGTTTATTACTGTCATCAATACCTCTCCTCGTACACGTTCGGAGGGGGGA
CCAAGCTGGAAATAAAA

35-220VLD-4 (variable light sequence)

N I M M T Q S P S S L A V S A G E K V T M S C R S S Q S V L
Y S S N Q K N Y L A W Y Q Q K P G Q S P T L L I Y W A S T R
E S G V P D R F T G S G S G T D F T L T I S S V Q A E D L A
V Y Y C H Q Y L S S Y T F G G G T K L E I K

Amino acids representing a CDR are underlined

35-220VHC-1 (variable heavy sequence DNA)

CAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCACCCTCACAGAGCC
TGTCCATCACATGCACTGTCTCTGGGTTCTCATTATCCAGATATAGTGTACACT
GGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGCTGGGAATGATATGGGG
TGGTGGAAACACAGACTATAATTCAGCTCTCAAATCCAGACTGAGCATCACCAA
GGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGA
CACAGCCATGTACTACTGTGCCACCGCCTACTATGGTAACTCCTGGTTTGCTTA
CTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA

35-220VHC-1 (variable heavy sequence)

Q V Q L K E S G P G L V A P S Q S L S I T C T V S G F S L S R
Y S V H W V R Q P P G K G L E W L G M I W G G G N T D Y N
S A L K S R L S I T K D N S K S Q V F L K M N S L Q T D D T A
M Y Y C A T A Y Y G N S W F A Y W G Q G T L V T V S A

Amino acids representing a CDR are underlined

D. Monoclonal Antibody 35-006.

Isolation and Sequencing of Variable Region Sequences:

Messenger RNA was isolated from ClfA 35-006 hybridoma cells using the Fast Track 2.0 kit (Invitrogen; cat #K4500). Briefly, $1.4x10^8$ hybridoma cells cultured in DMEM-10 medium with 10 % FBS were washed with PBS, pelleted by centrifugation then lysed in detergent containing Protein/RNase Degrader. PolyA$^+$ mRNA was isolated by affinity purification on oligo-dT cellulose. Synthesis of first strand cDNA was accomplished using 5mg of mRNA and reverse transcriptase in a cDNA synthesis kit (Novagen; cat #69001-3) containing 20 pmol of 3' oligonucleotide mouse-specific primers (Novagen; cat# 69796 and 69812) for each variable heavy and variable light chain. A portion (5 to 50 ng) of the cDNA was amplified by the polymerase chain reaction (PCR) using the PCR Reagent System (Life Technologies; cat#10198-018) and a mouse variable heavy and light chain specific primer set (Novagen; cat# 70081-3, 5 pmol each) for 30 cycles (94 C hot start then cycles of 94 C for 1 min, 50 C for 1min and 72 C for 1min). PCR products were fractionated electrophoretically in a 1% ultra pure agarose gel in sodium acetate buffer and visualized by ethidium bromide staining. PCR fragments matching the predicted size were excised from the gel and purified using BIO 101 Geneclean spin columns (cat #1101-400) for ligation into the pCR2.1-TOPO (Invitrogen) plasmid, followed by transformation into competent TOP10 E.coli. (Invitrogen; cat# K4500). After isolating plasmid DNA using QIAprep Spin Miniprep Kit (QIAGEN; cat# 27106), positive clones with inserts were identified by restriction endonuclease digestion and agarose gel electrophoresis, followed by sequencing on an ABI automated sequencer using M13 Forward and M13 Reverse primers.

The resulting sequences were as follows:

35-006VLD-1 (variable light sequence DNA)

AACATTATGATGACACAGTCGCCATCATCTCTGGCTGTGTCTGCAGGAGAAAA
GGTCACTATGAGCTGTAAGTCCAGTCAAAGTGTTTTATACAGTTCAAATCAGAA
GAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCTGA
TCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTGATCGCTTCACAGGCAGT
GGATCTGGGACAGATTTTACTCTTACCATCAGCAGTGTACAAGCTGAAGACCT
GGCAGTTTATTGCTGTCATCAATACCTCTCCTCGTACACGTTCGGAGGGGGGA
CCGAGCTGGAAATAAAA

35-006VLD-1 (variable light sequence)

N I M M T Q S P S S L A V S A G E K V T M S C K S S Q S V L
Y S S N Q K N Y L A W Y Q Q K P G Q S P K L L I Y W A S T R
E S G V P D R F T G S G S G T D F T L T I S S V Q A E D L A
V Y C C H Q Y L S S Y T F G G G T E L E I K

Amino acids representing a CDR are underlined

35-006VHC-1 (variable heavy sequence DNA)

CAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCACCCTCACAGAGCC
TGTCCATCACATGCACTGTCTCTGGGTTCTCATTATCCAGATATAGTGTACACT
GGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGCTGGGAATGATATGGGG
TGGTGGAAGCACAGACTATAATTCAGCTCTCAAATCCAGACTGAACATCAGCAA
GGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACTGATGA
CACAGCCATGTACTACTGTGCCAGAAGGCTCTGGTACTTCGATGTCTGGGGCG
CAGGGACCACGGTCACCGTCTCCTCA

35-006VHC-1 (variable heavy sequence)

Q V Q L K E S G P G L V A P S Q S L S I T C T V S G F S L S R
Y S V H W V R Q P P G K G L E W L G M I W G G G S T D Y N
S A L K S R L N I S K D N S K S Q V F L K M N S L Q T D D T A
M Y Y C A R R L W Y F D V W G A G T T V T V S S

Amino acids representing a CDR are underlined

Example 8. Generation of Chimeric 12-9 With Equivalence in Binding Kinetics and Whole Cell Reactivity to Murine 12-9.

Chimeric 12-9 was generated using human constant regions (light chain: kappa; heavy chain: G1, 3 or 4) isolated from whole blood of human volunteers (selection of Poly A RNA and PCR amplification of first strand cDNA). For

expression in mammalian cells, a unique restriction site Bsm 1 was added to the 5' end of both the heavy and light chain variable region sequences. At the 3' end (the splice junction to the respective constant region) a Bsiw1 site was added to the light chain variable region and an Apa1 site was added to the heavy chain variable region. This was accomplished through the design of oligonucleotide primers and PCR amplification of the appropriate 12-9 DNA template followed by confirmatory DNA sequencing.

Expression of chimeric versions of 12-9 protein was accomplished using the pCEP4 (Invitrogen, cat# V044-50) mammalian expression vector containing a human immunoglobulin leader secretion sequence (Bsm1 as the cloning site) with a kappa constant region for light chain expression or gamma (1,3 or 4) constant region for heavy chain expression. The mammalian expression plasmid was designed for expression of both heavy and light chains with separate hCMV promoters on the same plasmid or the expression of the light and heavy chains on separate pCEP4 plasmids via co-transfection. Functional chimeric 12-9 was expressed after transfection of plasmid DNA containing the heavy and light chains of 12-9 into HEK293 EBNA cells with Fugene (Roche Diagnostic, cat# 1814443) under hygromycin selection (300 μg/ml). Supernatants were harvested and analyzed by Biacore for binding kinetics and flow cytometry for binding to *S. aureus* cells.

The results represented in Figures 2 and 3 with recombinant chimeric 12-9 confirm that the sequence of the heavy and light chains of 12-9 replicates the binding kinetics and specificity of the original 12-9 characterized as a hybridoma supernatant.

Example 9. Humanization of the Heavy and Light Chain Variable Regions of 12-9

This process of humanization focuses on changing only the solvent exposed residues of the mouse variable regions that are not involved in the molecule's specificity and affinity for the ClfA target antigen. The information for these

determinations utilized solvent availability determinations published by Padlan (A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand binding properties. Molecular Immunology, 28(4); 489-498, 1991), and molecular modeling in silico or algorithms to determine T-cell epitopes were not used to make these determinations.

The approach represents a process by which the mouse variable region residues of the light and heavy chain are changed by site directed mutagenesis to reflect the surface exposed architecture of the most homologous human variable region from the public database. Specifically, the amino acids defining the variable heavy and light chains were assigned a Kabot position number and "exposure" designation based on Padlan, allowing the alignment of the amino acids from each human framework sub-group (I-III for the heavy chain and I-IV for the light chain). To support this analysis, a BLAST search was carried out on the human immunoglobulin database as well as the entire protein database where the variable region with the highest homology to the mouse sequence (both germ-line and mature) were chosen and aliened with the murine sequence of interest. Once aliened, the human subgroup with the highest homology to the mouse sequence was identified. The exposed mouse amino acid residues were mutated to mimic the most homologous human subgroup. In cases were there was more than one amino acid found in the subgroup at that position, the amino acid represented in the human germ line sequence with the highest homology to the 12-9 was used. These changes were accomplished with mutagenic oligonucleotides via PCR followed by conformational DNA sequencing.

12-9VL-Hu (humanized variable light sequence DNA)

GACATTGTGATGACACAGTCGCCAGACTCTCTGGCTGTGTCTCTGGGAGAAAG
GGTCACTATGAACTGTAAGTCCAGTCAAAGTGTTTTATACAGTTCAAATCAGAA
GAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCTGA
TCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTGATCGCTTCAGCGGCAGT
GGATCTGGGACAGATTTTACTCTTACCATCAGCAGTGTACAAGCTGAAGACCT
GGCAGTTTATTACTGTCATCAATACCTCTCCTCGTACACGTTCGGAGGGGGGA
CCAAGCTGGAAATAAAA

12-9VL-Hu (humanized variable light sequence)

DIVMTQSP**D**SLAVS**L**GE**R**VTM**N**C<u>KSSQSVLYSSNQKNYLA</u>WYQQKPGQSPKLLIY <u>WASTRES</u>GVPDRF**S**GSGSGTDFTLTISSVQAEDLAVYYC<u>HQYLSSYT</u>FGGGTKLE IK

Amino acids representing a CDR are underlined, amino acids in bold represent humanization changes

12-9VH-Hu (humanized variable heavy sequence DNA)

CAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGAAGCCCTCACAGACCC TGTCCATCACATGCACCATCTCTGGGTTCTCATTATCCAGATATAGTGTACACT GGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGCTGGGAATGATATGGGG TGGTGGAAACACAGACTATAATTCAGCTCTCAAATCCAGACTGAGCATCAGCAA AGACAACTCCAAGAACCAAGTTTTCTTAAAAATGAACAGTCTGACCGCCGCTGA CACAGCCGTGTATTACTGTGCCAGAAAAGGGGAATTCTACTATGGTTACGACG GGTTTGTTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTTCC

12-9VH-Hu (humanized variable heavy sequence)

QVQLKESGPGLV**K**PSQ**T**LSITC**T**ISGFSLS<u>RYSVH</u>WVRQPPGKGLEWLG<u>MIWGG GNTDYNSALKS</u>RLSISKDNSK**N**QVFLKMNSL**TAA**DTA**V**YYCAR<u>KGEFYYGYDGFV YWGQGTLVTVSS</u>

Amino acids representing a CDR are underlined, amino acids in bold represent humanization changes

Example 10. Comparison of the ClfA monoclonal antibodies, 12-9A (INH-M010001) and 35-052.1 (INH-M01016), with the isotype matched control CRL1771 antibody, INH-M000029, in a mouse sepsis model using Methicillin Resistant *S. aureus* Strain 67-0 (MRSA).

The purpose of this example is to characterize the protective effects of the ClfA monoclonal antibodies, 12-9A (INH-M010001) and 35-052.1 (INH-M01016) compared with the isotype-matched control CRL1771 antibody (INH-M000029) using a 0.3 mg dose of antibody and *S. aureus* strain 67-0 in a mouse sepsis model.

Species	Strain	Sex	Number	Age*	Weight*	Source
Mice	Balb/C	Female	90	4-5 weeks	12-16 grams	Taconic Farms, Inc. (Germantown, NY)

*Estimated range at initiation of study.

Dosing was performed by the administration of an intraperitoneal (i.p.) injection of monoclonal antibody to the appropriate animals (see below). Administration of the antibody was performed approximately 18 hours prior to the intravenous (i.v.) injection of *S. aureus*. Systemic infection was measured using a single parameter (mortality).

		TREATMENT					CHALLENGE		
Group #	No. of Mice	Antibody	Dose	Route	Frequency	Time Point*	Bacteria	CFU	Volume/ Route
1	30	12-9A	0.3 mg.	i.p.	Once	-18 hr.	*S. aureus* 67-0	$\sim 10^8$	0.1 ml./ i.v.
2	30	35-052	0.3 mg.						
3	30	CRL1771	0.3 mg.						

*Time points reflect hours post bacterial challenge.

Preparation, Storage and Handling:

Staphylococcus aureus

MRSA strain 67-0 cells were taken from a frozen glycerol stock and were inoculated onto a single blood agar plate and grown for 24 hours at 37°C. Single colonies were then transferred to new blood agar plates. Eighty plates were inoculated to prepare 50 mls of final frozen stock. The plates were then incubated for 24 hours at 37°C. Following incubation, the colonies were scraped off the surface of each plate into four 50 ml tubes containing 10 mls of 1X PBS (20 plates per tube) while gently vortexing to remove the bacteria from the scraper. An additional 10 mls of 1X PBS was then added to the 10 mls of bacterial suspension, while vigorously vortexing to facilitate separation of any agar debris from the bacteria. The suspension was pelleted by centrifugation, 3500xg at 4°C for 10 minutes. The bacteria was

washed in D-PBS and resuspended in 50 mls of freezing media. The bacterial stock was placed into 1 ml aliquots by snap freezing in an ethanol/dry ice bath and placed in an -80°C freezer. The concentration (CFU/ml) of the frozen stock was determined by thawing 1 ml aliquot of stock, and preparing serial dilutions from 10^{-5} to 10^{-11}. Dilutions were plated in duplicate on blood agar plates and incubated for 37°C for 16-18 hours. The CFU/ml was determined (CFU/ml=(average # colonies X dilution factor)/0.050 mls) and averaged for each dilution to determine the average CFU/ml. On the day of injection, aliquots of each strain will be thawed, combined into one tube and vortexed. Dilutions of each stock will then be prepared.

ClfA 12-9A Monoclonal Antibody, INH-M010001 (LN: IAA2E1354)

The 12-9A monoclonal antibody (IgG_1 subtype) was purified from serum free hybridoma culture medium using protein G affinity chromatography. The material was reported to be at a concentration of 7.0 mg/ml with an endotoxin concentration of 1.0 EU/mg of protein. The material was stored refrigerated at 4°C. On the day of injection, the material will be diluted to 0.6 mg/ml and 0.5 ml will be administered via an intraperitoneal injection to the appropriate group of animals. The final dose that will be administered will be 0.3 mg of IgG.

ClfA 35-052.1 Monoclonal Antibody, INH-M01016 (LN: IAA2H1422)

The 35-052 monoclonal antibody (IgG_1 subtype) was purified from serum free hybridoma culture medium using protein G affinity chromatography. The material was reported to be at a concentration of 4.2 mg/ml with an endotoxin concentration of 1.0 EU/mg of protein. The material was stored refrigerated at 4°C. On the day of injection, the material will be diluted to 0.6 mg/ml and 0.5 ml will be administered via an intraperitoneal injection to the

appropriate group of animals. The final dose that will be administered will be 0.3 mg of IgG.

Control CRL 1771 Monoclonal Antibody (INH-M000029, LN: IAA2E1337)

The CRL 1771 monoclonal antibody (IgG_1 subtype) was purified from serum free hybridoma culture medium using protein G affinity chromatography. The material was reported to be at a concentration of 5.0 mg/ml with an endotoxin concentration of 0.2 EU/mg of protein. The material was stored refrigerated at 4°C. On the day of injection, the material will be diluted 0.6 mg/ml and 0.5 ml will be administered via an intraperitoneal injection. The final dose that will be administered will be 0.3 mg of IgG.

Housing, Food, Water and Environment:

Upon receipt, all animals were examined and group housed (5/cage) in polycarbonate shoebox style cages with absorbent bed-o-cobb bedding. All animals have free access to feed (Harlan /Teklad Mouse Pelleted Diet #7012) and tap water with a 12-hour light-dark cycle. All aspects of the animal care and the required husbandry conditions will be in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Identification and Randomization:

All mice were uniquely identified by tail tattoo before treatment. Prior to treatment, the mice were individually weighed and their health re-evaluated. Mice were assigned to treatment groups based on randomization by stratified body weights.

The data demonstrate the therapeutic value of an anti-ClfA antibody such as 12-9 that interferes with ClfA – fibrinogen adhesion compared with a non ClfA specific isotype control (CRL 1771) as well as a specific control (35-052) that recognizes ClfA at a site independent of ClfA – fibrinogen binding.

Example 11. *S. aureus* Strain recognition of 12-9 and 35-052 Compared to Isotype Control (CRL 1771)

S. aureus bacterial samples (strains Newman -WT, 67-0, 560 Sal 1, 203 Sal 2, 451 Sal 4, 206 Sal5, 397 Sal 6, 49, 189, 203 and 4046) were collected at 3 hr and overnight, washed and incubated with Mab 12-9, 35-52 or 1771 alone (control) at a concentration of 2 mg/ml after blocking protein A sites with rabbit IgG (50 mg/ml). The *S. aureus* strains containing a Sal designation represent 5 distinct lineages accounting for 65.68% of all clinical isolates (Booth, et al., *Infect. Immun.* 69, 345-353, 2001). As well, Newman ClfA::emr (ClfA knockout) and NewmanSpa::kan (Protein A knockout) were analyzed in the same manner as specificity controls. Following incubation with antibody, bacterial cells were incubated with Goat-$F_{(ab')2}$-Anti-Mouse-$F_{(ab')2}$-FITC which served as the detection antibody. After antibody labeling, bacterial cells were aspirated through the FACScaliber flow cytometer to analyze fluorescence emission (excitation: 488, emission: 570). For each bacterial strain, 10,000 events were collected and measured.

Table VI. *S. aureus* Strain Reactivity

		Fluorescence Intensity (Geometric Mean)		
S. aureus Strain	Culture Time	12-9	35-052	CRL 1771
NewmanWT	3hr	30.8	11.1	0.5
	overnight	44.3	30	0.9
67-0	3hr	11.2	4.2	2
	overnight	27.6	1.9	1.1
560 SAL 1	3hr	28.8	8.4	3.9
	overnight	36.1	6.2	1.2
203 SAL2	3hr	16.1	0.6	2.2
	overnight	40.4	1.9	1.4
451 SAL4	3hr	1.1	0	0
	overnight	12.9	0	0
206 SAL5	3hr	8.8	1.3	1
	overnight	33.5	7.7	0.9
397 SAL6	3hr	28.9	7.9	0.3
	overnight	62.1	40.0	1.0
49 Europe	3hr	7.3	1.2	0
	overnight	11.3	5.7	0
189 Japan	3hr	11.0	0	0
	overnight	15.7	0	0
203 Singapore	3hr	22.1	3.3	0.1
	overnight	15.4	2.5	0.2
4046 USA	3hr	27.7	2.5	1.3
	overnight	23.5	1.2	0.3
Newman ClfA::emr	3hr	0.2	0.3	0.2
	overnight	1.4	0.8	0.9
Newman Spa::kan	3hr	18.6	4.9	0
	Overnight	23.9	9.2	0

☐ Indicates positive activity

This data highlights the importance of selecting an anti-ClfA antibody (such as 12-9) that is capable of recognizing a functional epitope on the ClfA molecule: i.e. the binding site for fibrinogen.

Another set of *S. aureus* isolates, a representation of 11 different clonal genotype complexes identified as disproportionately common as causes of disease, derived via multi-locus sequence typing (Day, et.al. 2001. A link between virulence and ecological abundance in natural populations of *Staphylococcus aureus*. *Science*, 292:114-116). Each strain was tested for reactivity against 12-9 by flow cytometry as described above.

Table VII. 12-9 Reactivity with *S. aureus* isolates.

***S. aureus* Strain Designation**	**Sequence Type**	**Clonal Complex**	**12-9 Reactivity**
16	25	8	+
96	47	10	+
117	12	4	+
138	30	9	+
150	9	14	+
160	34	7	+
207	15	5	+
252	36	9	+
315	8	3	+
364	39	9	+
396	30	9	+
433	5	2	+
434	8	3	+
451	5	2	+
456	45	10	+
458	15	5	+
476	1	1	+
481	47	10	+
512	1	1	+
597	25	8	+
720	22	7	+
730	45	10	+
837	12	4	+
863	20	11	+
888	39	9	+
959	34	9	+

This reactivity further demonstrates the conservation of the 12-9 epitope on ClfA across isolated strains of *S. aureus*, suggesting that ClfA – fibrinogen binding is functionally conserved.

Example 12. Variable Region Homology in Anti-ClfA Antibodies That Inhibit Whole Cell *S. aureus* Binding.

An unexpected result from the selection of anti-ClfA antibodies based on there ability to inhibit ClfA binding to fibrinogen was the similarity in the complementary determining region (CDR) amino acid sequences of the light and heavy variable chain regions. To profile this, anti-ClfA antibodies were selected on the basis of whole cell *S. aureus* inhibition of binding to fibrinogen-coated plates using the following procedure: Antibodies of interest were diluted serially starting at 4µg/ml in assay buffer. Concurrently, an overnight culture of *S. aureus* (Newman spa::kan)was washed, blocked with rabbit IgG then stained with Syto 13 cell permeable fluorescent DNA stain and incubated for 10 min. Equal volumes of stained cells and diluted antibody were mixed and incubated at 4°C for 30min then each sample added to duplicate wells of a human fibrinogen coated/blocked microtiter plate. Plates were incubated at 4°C for one hour, washed, buffer added to each well and read in a fluorescent plate reader.

The variable light and heavy chains of the anti-ClfA monoclonals, 12-9, 13-2, 35-006 and 35-220 as well as CRL 1771 (non-specific control) were cloned and sequenced to derive a predicted amino acid sequence in the following manner: Briefly, $1.4x10^8$ hybridoma cells cultured in DMEM-10 medium with 10 % FBS were washed with PBS, pelleted by centrifugation then lysed in detergent containing Protein/RNase Degrader. PolyA$^+$ mRNA was isolated by affinity purification on oligo-dT cellulose. Synthesis of first strand cDNA was accomplished using 5mg of mRNA and reverse transcriptase in a cDNA synthesis kit (Novagen; cat #69001-3) containing 20 pmol of 3' oligonucleotide mouse-specific primers (Novagen; cat# 69796 and 69812) for each variable heavy and variable light chain. A portion (5 to 50 ng) of the cDNA was amplified by the polymerase chain reaction (PCR) using the

PCR Reagent System (Life Technologies; cat#10198-018) and a mouse variable heavy and light chain specific primer set (Novagen; cat# 70081-3, 5 pmol each) for 30 cycles (94 C hot start then cycles of 94 C for 1 min, 50 C for 1min and 72 C for 1min). PCR products were fractionated electrophoretically in a 1% ultra pure agarose gel in sodium acetate buffer and visualized by ethidium bromide staining. PCR fragments matching the predicted size were excised from the gel and purified using BIO 101 Geneclean spin columns (cat #1101-400) for ligation into the pCR2.1-TOPO (Invitrogen) plasmid, followed by transformation into competent TOP10 E. coli. (Invitrogen;cat# K4500). After isolating plasmid DNA using QIAprep Spin Miniprep Kit (QIAGEN; cat# 27106), positive clones with inserts were identified by restriction endonuclease digestion and agarose gel electrophoresis, followed by sequencing on an ABI automated sequencer using M13 Forward and M13 Reverse primers.

As shown in Figure 7, the data shows that there is considerable conservation in the most variable portion of the immunoglobulin chains that define the binding specificity for anti-ClfA monoclonals with inhibition of S. aureus binding to fibrinogen. This homology is represented from three different hybridoma-generating fusions (12,13 and 35); under variable conditions such as the make-up of the Clf-A antigen, the method and sequence of the immunizations prior to fusion. In particular, this data revealed consensus conserved regions in the CDR1 and CDR2 regions of the variable heavy chain of monoclonal antibodies binding to ClfA as well as conserved regions in the CDR1, CDR2, and CDR3 regions of the variable light chains of the antibodies of the present invention. This data thus shows that preparation of antibodies with the conserved sequences should have the same binding properties and thus will fall within the scope of the present invention.

Accordingly, in accordance with the present invention, antibodies which will bind to ClfA can be prepared using variable light or heavy chains which have the same key CDR regions as indicated in the consensus of Figure 8. In particular, these antibodies will include those which have a variable heavy chain wherein the CDR1 region includes the sequence RYSVH, and/or a CDR2 region that includes the

sequence MIWGGGNTDYNSALKS, and a variable light chain that has a CDR1 region that includes the sequence KSSQSVLYSSNQKNYLA, a CDR2 region that includes the sequence WASTRES, and/or a CDR3 region that includes the sequence HQYLSSYT.

Example 13. Expression of humanized 12-9 For Pre-clinical and Clinical Use.

For simultaneous expression of the heavy and light immunoglobulin polypeptide chains, the two genes were cloned into a single plasmid with each gene under the control of a separate hCMV-MIE promoter. This double gene vector holds a single copy of the GS selectable marker (Lonza; Slough, UK) for introduction into the host cell in a single transfection event. Cells were transfected using Fugene-6 (Roche) under conditions suggested by the manufacturer. Supernatants were tested from transient or stably derived cell lines and compared with murine and chimeric derived 12-9.

This example demonstrates that humanized 12-9 can be humanized, cloned and expressed a single expression cassette capable of yields to support commercial scale quality and purity.

What Is Claimed Is:

1. A monoclonal antibody which binds to the ClfA protein from *S. aureus*

2. An antibody according to Claim 1 wherein the monoclonal antibody is raised against a protein selected from the group consisting of *S. aureus* Clf40 protein, the *S. aureus* Clf33 protein, and the *S. aureus* ClfA N3 protein.

3. An antibody according to Claim 1, wherein said antibody treats or prevents *S. aureus* infection in a human or animal.

4. An antibody according to Claim 1, wherein said antibody inhibits binding of staphylococcal bacteria to fibrinogen or fibrin.

5. An antibody according to Claim 1, wherein said antibody is suitable for parenteral, oral, intranasal, subcutaneous, aerosolized or intravenous administration in a human or animal.

6. An antibody according to Claim 1 wherein the monoclonal antibody is of a type selected from the group consisting of murine, chimeric, humanized and human monoclonal antibodies.

7. An antibody according to Claim 1 wherein the antibody is a single chain monoclonal antibody.

8. An antibody according to Claim 1 which comprises an antibody fragment having the same binding specificity of an antibody which binds to the *S. aureus* ClfA protein.

9. An antibody according to Claim 1 that is raised against a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.

10. An antibody according to Claim 9 wherein the protein has an amino acid sequence encoded by a nucleic acid sequence according to SEQ ID NO:1 or SEQ ID NO:3, or degenerates thereof.

11. An antibody according to Claim 1 having a variable light chain having an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14 and SEQ ID NO:18.

12. An antibody according to Claim 1 having a variable light sequence encoded by a nucleic acid sequence according to a sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:17, and degenerates thereof.

13. An antibody according to Claim 1 having a variable heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:16, and SEQ ID NO:20.

14. An antibody according to Claim 1 having a variable heavy chain encoded by a nucleic acid having a sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:19, and degenerates thereof.

15. Isolated antisera containing an antibody according to Claim 1.

16. A diagnostic kit comprising an antibody according to Claim 1 and means for detecting binding by that antibody.

17. A diagnostic kit according to Claim 16 wherein said means for detecting binding comprises a detectable label that is linked to said antibody.

18. A method of diagnosing an infection of *S. aureus* comprising adding an antibody according to Claim 1 to a sample suspected of being infected with *S. aureus*, and determining if antibodies have bound to the sample.

19. A pharmaceutical composition for treating or preventing an infection of *S. aureus* comprising an effective amount of the antibody of Claim 1 and a pharmaceutically acceptable vehicle, carrier or excipient.

20. A method of treating or preventing an infection of *S. aureus* comprising administering to a human or animal patient an effective amount of an antibody according to Claim 1.

21. A method of inducing an immunological response comprising administering to a human or animal an immunogenic amount of an isolated ClfA protein from *S. aureus* selected from the group consisting of the *S. aureus* Clf40 protein, *S. aureus* Clf33 protein, and the *S. aureus* N3 protein.

22. A method of identifying monoclonal antibodies to the ClfA protein comprising adding an isolated protein selected from the group consisting of *S. aureus* Clf40, Clf33 and ClfA N3 to a sample suspected of containing anti-ClfA antibodies, and determining if antibodies have bound to the isolated protein added to the sample.

23. An isolated antibody according to Claim 1 that has the ability to bind to the amino acid sequence of SEQ ID NO:2.

24. An isolated antibody according to Claim 1 that has the ability to bind to an amino acid sequence coded by the nucleic acid sequence of SEQ ID NO:1 or degenerates thereof.

25. An isolated active fragment from the A domain of the ClfA protein from *S. aureus* selected from the group consisting of the Clf40 protein, the Clf33 protein and the ClfA N3 region.

26. An isolated antibody according to Claim 1 further comprising a physiologically acceptable antibiotic.

27. An antibody according to Claim 1 wherein the variable heavy chain has a CDR1 region that includes the sequence RYSVH.

28. An antibody according to Claim 1 wherein the variable heavy chain has a CDR2 region that includes the sequence MIWGGGNTDYNSALKS.

29. An antibody according to Claim 1 wherein the variable heavy chain has a CDR1 region that includes the sequence KSSQSVLYSSNQKNYLA.

30. An antibody according to Claim 1 wherein the variable light chain has a CDR2 region that includes the sequence WASTRES.

31. An antibody according to Claim 1 wherein the variable light chain has a CDR3 region that includes the sequence HQYLSSYT.

32. An isolated antibody according to Claim 1 that is cross-reactive to multiple strains of *S. aureus*.

33. A humanized antibody according to Claim 6 wherein the variable light chain has the amino acid sequence according to SEQ ID NO:18.

34. A humanized antibody according to Claim 6 wherein the variable light chain is encoded by a nucleic acid having the sequence according to SEQ ID NO:17 or degenerates thereof.

35. A humanized antibody according to Claim 6 wherein the variable heavy chain has the amino acid sequence according to SEQ ID NO:20.

36. A humanized antibody according to Claim 6 wherein the variable heavy chain is encoded by a nucleic acid having the sequence according to SEQ ID NO:19 or degenerates thereof.

37. A monoclonal antibody according to Claim 1 which recognizes the A domain of *S. aureus* ClfA protein.

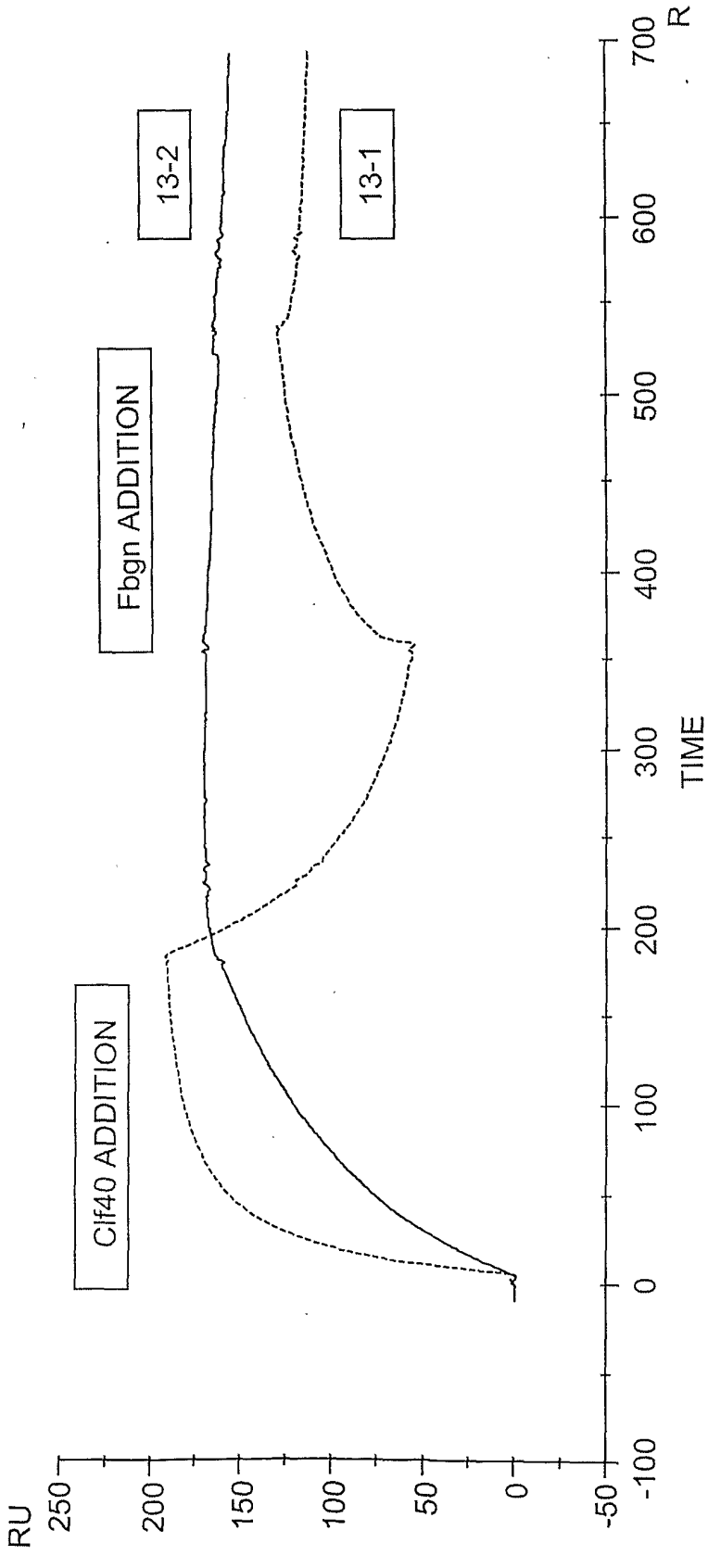

RU
250
200
150
100
50
0
-50
-100
0
100
200
300
400
500
600
700
TIME
R
Clf40 ADDITION
Fbgn ADDITION
13-2
13-1

FIG. 1

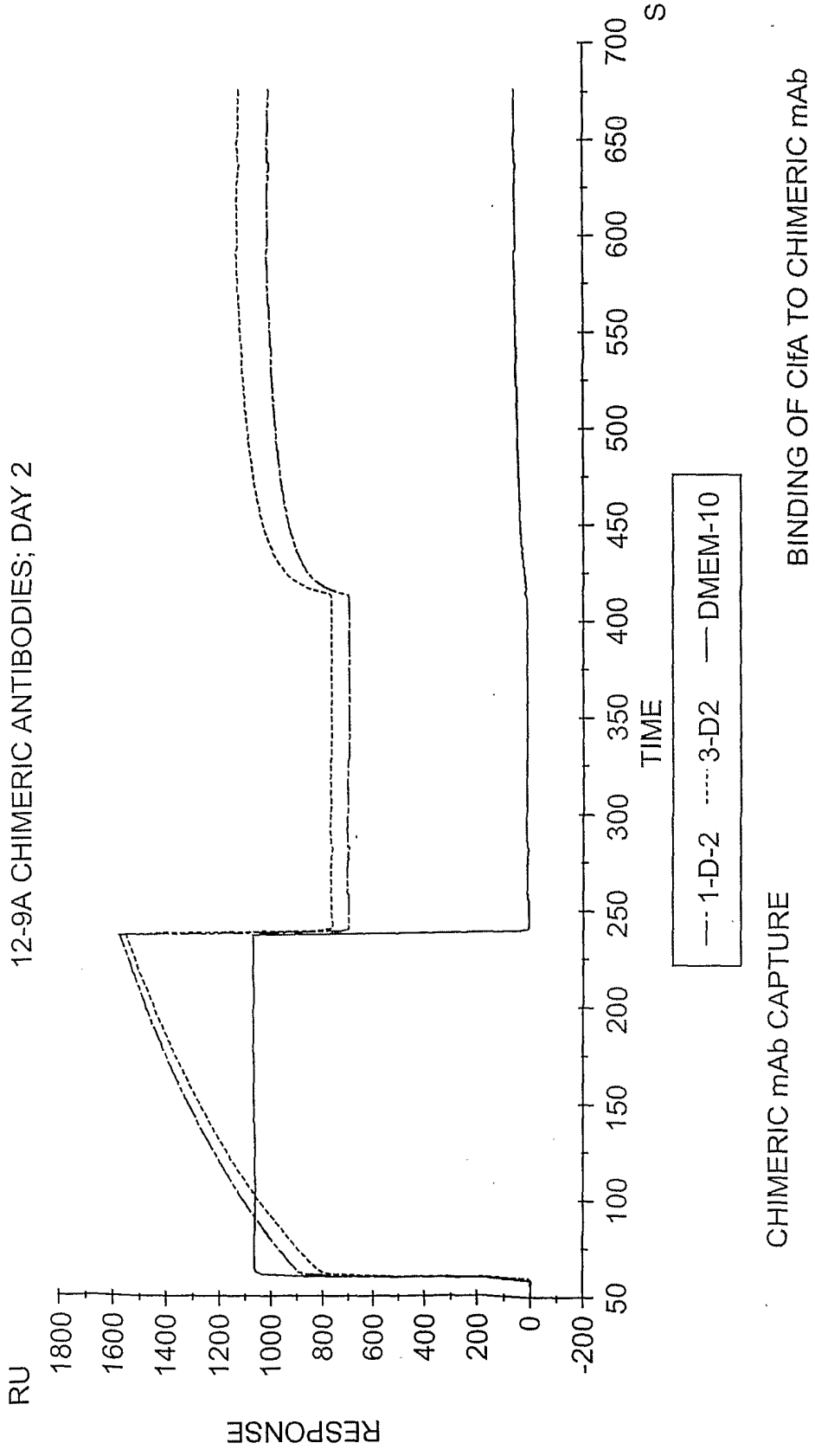

12-9A CHIMERIC ANTIBODIES; DAY 2
RU
RESPONSE
1800
1600
1400
1200
1000
800
600
400
200
0
-200
50
100
150
200
250
300
350
400
450
500
550
600
650
700
S
TIME
1-D-2
3-D2
DMEM-10
CHIMERIC mAb CAPTURE
BINDING OF ClfA TO CHIMERIC mAb

FIG. 2

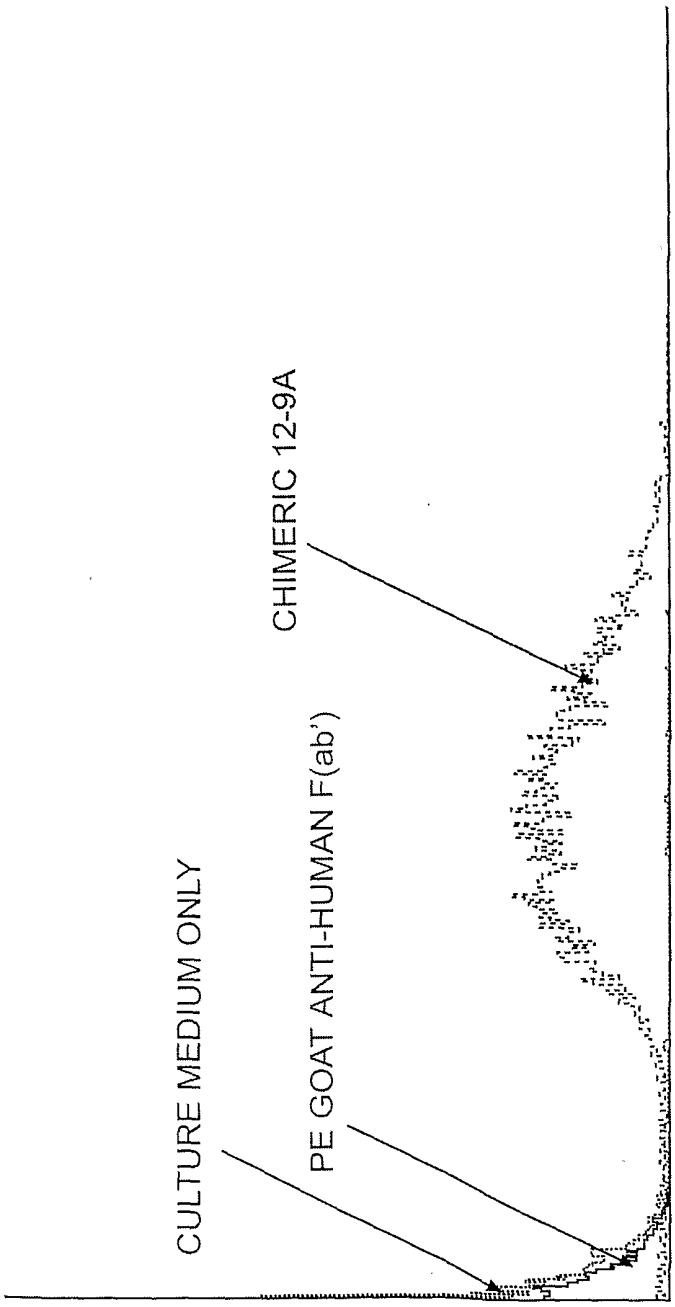


FIG. 3

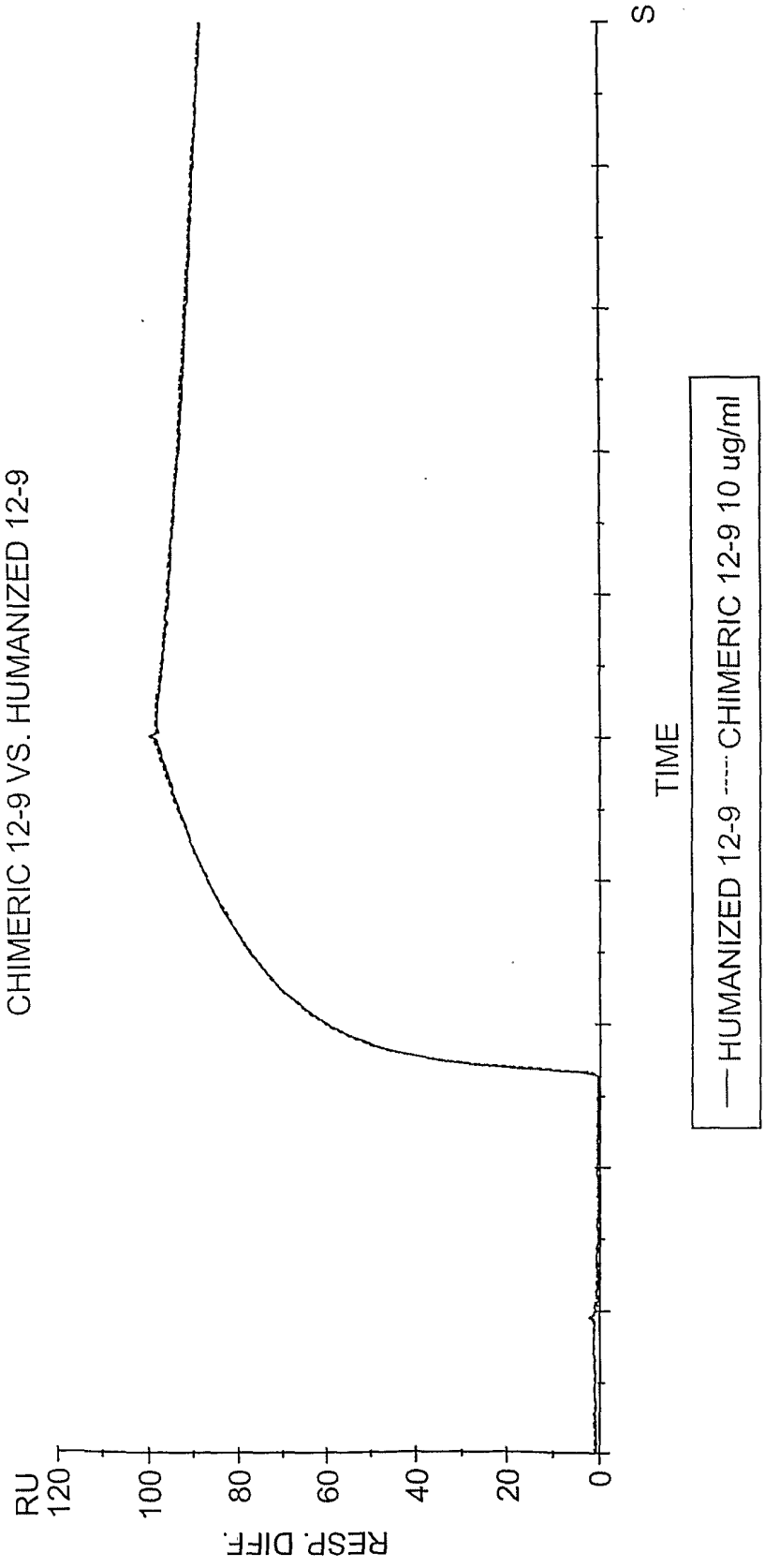

CHIMERIC 12-9 VS. HUMANIZED 12-9
RESP. DIFF.
RU
120
100
80
60
40
20
0
TIME
S
HUMANIZED 12-9
CHIMERIC 12-9 10 ug/ml

FIG. 4

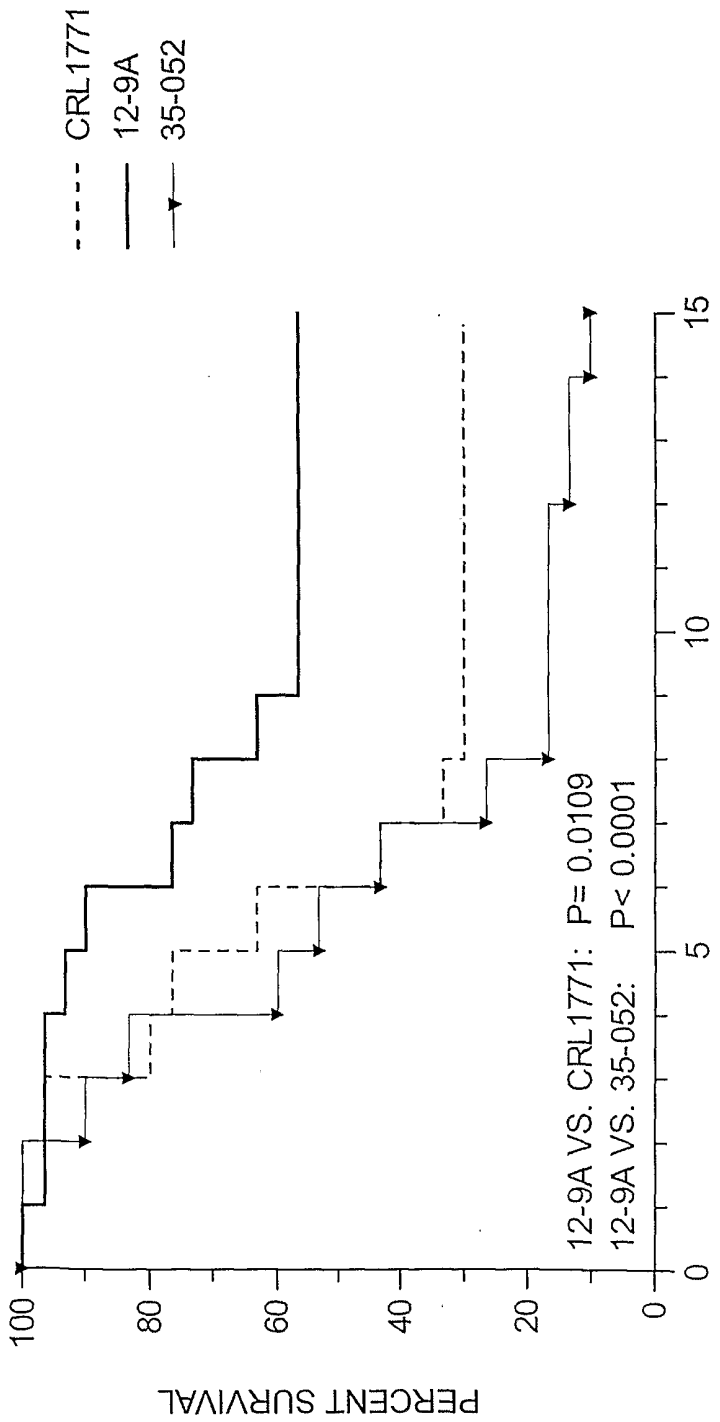

CRL1771
12-9A
35-052
PERCENT SURVIVAL
100
80
60
40
20
0
0
5
10
15
DAY
12-9A VS. CRL1771: P= 0.0109
12-9A VS. 35-052: P< 0.0001

FIG. 5

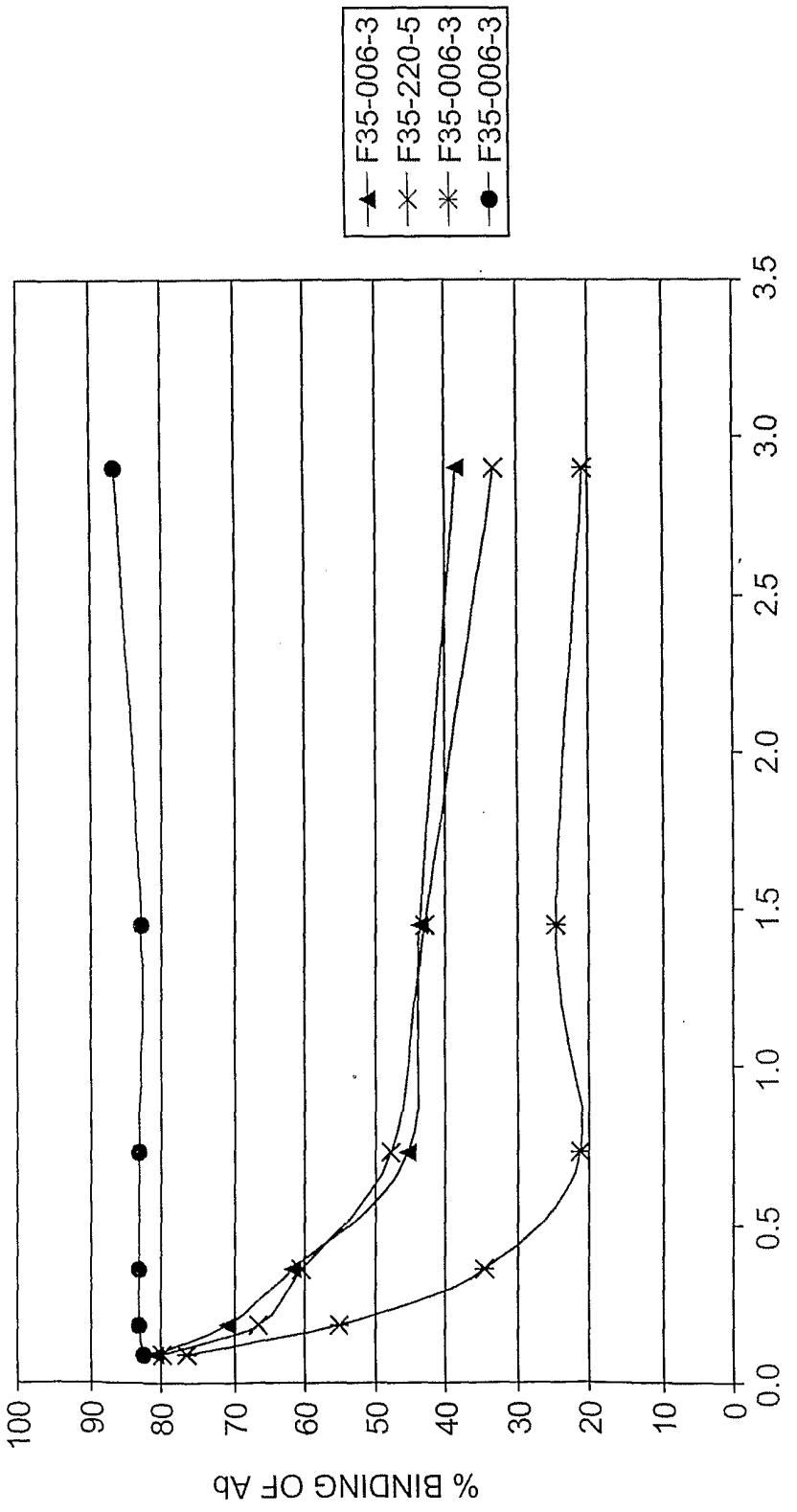

F35-006-3
F35-220-5
F35-006-3
F35-006-3
% BINDING OF Ab
100
90
80
70
60
50
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
ug/ml Ab

FIG. 6

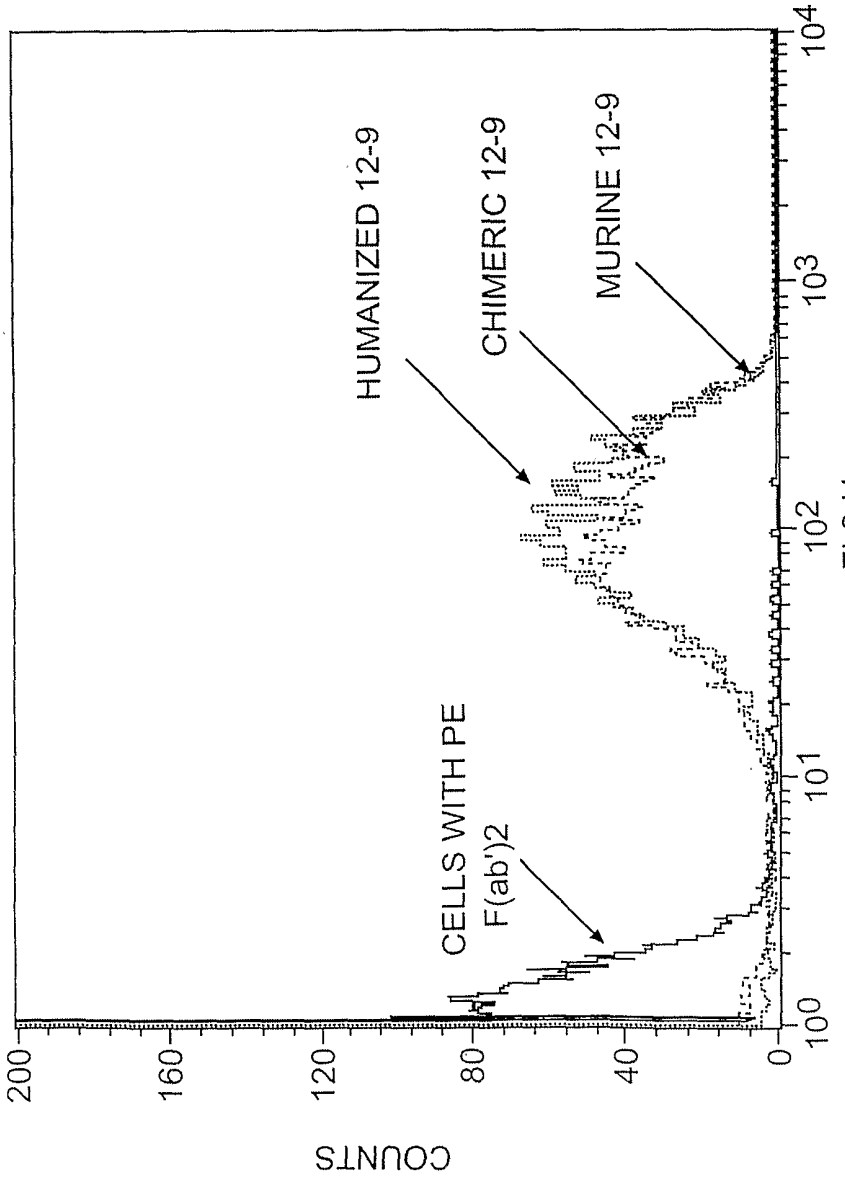

HUMANIZED 12-9
CHIMERIC 12-9
MURINE 12-9
CELLS WITH PE F(ab')2
COUNTS
FL2-H
0
40
80
120
160
200
100
101
102
103
104

FIG. 7

VARIABLE LIGHT CHAIN

ANTIBODY	CDR 1	CDR 2	CDR 3
1771	L S S Q S L L D S D G K T F L N	L V S K L D S	W Q G T H F P Y T
12-9 (ClfA)	K S S Q S V L Y S S N Q K N Y L A	W A S T R E S	H Q Y L S S Y T
13-2	K S S Q S V L Y S S N Q K N Y L A	W A S T R E S	H Q Y L S S H T
35-006	K S S Q S V L Y S S N Q K N Y L A	W A S T R E S	H Q Y L S S Y T
35-220	R S S Q S V L Y S S N Q K N Y L A	W A S T R E S	H Q Y L S S Y T
CONSENSUS	K S S Q S V L Y S S N Q K N Y L A R	W A S T R E S	H Q Y L S S Y T H

VARIABLE LIGHT CHAIN

ANTIBODY	CDR1	CDR2	CDR3
1771	S G F S W H	Y I H Y S G S T D C N P S L K S	M P D S
12-9 (ClfA)	R Y S V H	M I W G G G N T D Y N S A L K S	K G E F Y Y G Y D G F V Y
13-2	R Y N I H	M I W G G E N T D Y N S A L K S	A Y Y G N S W F A Y
35-006	R Y S V H	M I W G G G S T D Y N S A L K S	R L W Y F D V
35-220	R Y S V H	M I W G G G N T D Y N S A L K S	A Y Y G N S W F A Y
CONSENSUS	R Y S V H N I	M I W G G G N T D Y N S A L K S E S	A Y Y G N S W F A * * * Y K G E F Y Y G Y D R L W Y F D V

FIG. 8

SUBSTITUTE SHEET (RULE 26)

SEQUENCE LISTING

<110> PATTI, Joseph M
HUTCHINS, Jeff T
DOMANSKI, Paul
PATEL, Pratiksha
HALL, Andrea

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<130> P07069US04/BAS

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tcaaacacta ataatggcga aacgagtgtg gcgcaaaatc cagcacaaca ggaaacgaca 180

caatcatcat caacaaatgc aactacggaa gaaacgccgg taactggtga agctactact 240

acgacaacga atcaagctaa tacaccggca acaactcaat caagcaatac aaatgcggag 300

gaattagtga atcaaacaag taatgaaacg acttttaatg atactaatac agtatcatct 360

gtaaattcac ctcaaaattc tacaaatgcg gaaaatgttt caacaacgca agatacttca 420

actgaagcaa caccttcaaa caatgaatca gctccacaga gtacagatgc aagtaataaa 480

gatgtagtta atcaagcggt taatacaagt gcgcctagaa tgagagcatt tagtttagcg 540

gcagtagctg cagatgcacc ggcagctggc acagatatta cgaatcagtt gacgaatgtg 600

acagttggta ttgactctgg tacgactgtg tatccgcacc aagcaggtta tgtcaaactg 660

aattatggtt tttcagtgcc taattctgct gttaaaggtg acacattcaa aataactgta 720

cctaaagaat taaacttaaa tggtgtaact tcaactgcta aagtgccacc aattatggct 780

ggagatcaag tattggcaaa tggtgtaatc gatagtgatg gtaatgttat ttatacattt 840

acagactatg taaatactaa agatgatgta aaagcaactt tgaccatgcc cgcttatatt 900

gaccctgaaa atgttaaaaa gacaggtaat gtgacattgg ctactggcat aggtagtaca 960

acagcaaaca aaacagtatt agtagattat gaaaaatatg gtaagtttta taacttatct 1020

attaaaggta caattgacca aatcgataaa acaaataata cgtatcgtca gacaatttat 1080

gtcaatccaa gtggagataa cgttattgcg ccggttttaa caggtaattt aaaaccaaat 1140

acggatagta atgcattaat agatcagcaa aatacaagta ttaaagtata taaagtagat 1200

aatgcagctg atttatctga aagttacttt gtgaatccag aaaactttga ggatgtcact 1260

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tctatgtcat gggacaacga agtagcattt aataacggat caggttctgg tgacggtatc 1500

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20 25 30

Asn Val Ser Asp Thr Lys Thr Ser Ser Asn Thr Asn Asn Gly Glu Thr
35 40 45

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

Thr Asn Ala Thr Thr Glu Glu Thr Pro Val Thr Gly Glu Ala Thr Thr
65 70 75 80

```
Thr Thr Thr Asn Gln Ala Asn Thr Pro Ala Thr Thr Gln Ser Ser Asn
                85                  90                  95

Thr Asn Ala Glu Glu Leu Val Asn Gln Thr Ser Asn Glu Thr Thr Phe
            100                 105                 110

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

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

Pro Ser Asn Asn Glu Ser Ala Pro Gln Ser Thr Asp Ala Ser Asn Lys
145                 150                 155                 160

Asp Val Val Asn Gln Ala Val Asn Thr Ser Ala Pro Arg Met Arg Ala
                165                 170                 175

Phe Ser Leu Ala Ala Val Ala Ala Asp Ala Pro Ala Ala Gly Thr Asp
            180                 185                 190

Ile Thr Asn Gln Leu Thr Asn Val Thr Val Gly Ile Asp Ser Gly Thr
        195                 200                 205

Thr Val Tyr Pro His Gln Ala Gly Tyr Val Lys Leu Asn Tyr Gly Phe
    210                 215                 220

Ser Val Pro Asn Ser Ala Val Lys Gly Asp Thr Phe Lys Ile Thr Val
225                 230                 235                 240

Pro Lys Glu Leu Asn Leu Asn Gly Val Thr Ser Thr Ala Lys Val Pro
                245                 250                 255

Pro Ile Met Ala Gly Asp Gln Val Leu Ala Asn Gly Val Ile Asp Ser
            260                 265                 270

Asp Gly Asn Val Ile Tyr Thr Phe Thr Asp Tyr Val Asn Thr Lys Asp
        275                 280                 285

Asp Val Lys Ala Thr Leu Thr Met Pro Ala Tyr Ile Asp Pro Glu Asn
    290                 295                 300
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```
Val Lys Lys Thr Gly Asn Val Thr Leu Ala Thr Gly Ile Gly Ser Thr
305                 310                 315                 320

Thr Ala Asn Lys Thr Val Leu Val Asp Tyr Glu Lys Tyr Gly Lys Phe
                325                 330                 335

Tyr Asn Leu Ser Ile Lys Gly Thr Ile Asp Gln Ile Asp Lys Thr Asn
            340                 345                 350

Asn Thr Tyr Arg Gln Thr Ile Tyr Val Asn Pro Ser Gly Asp Asn Val
        355                 360                 365

Ile Ala Pro Val Leu Thr Gly Asn Leu Lys Pro Asn Thr Asp Ser Asn
    370                 375                 380

Ala Leu Ile Asp Gln Gln Asn Thr Ser Ile Lys Val Tyr Lys Val Asp
385                 390                 395                 400

Asn Ala Ala Asp Leu Ser Glu Ser Tyr Phe Val Asn Pro Glu Asn Phe
                405                 410                 415

Glu Asp Val Thr Asn Ser Val Asn Ile Thr Phe Pro Asn Pro Asn Gln
            420                 425                 430

Tyr Lys Val Glu Phe Asn Thr Pro Asp Asp Gln Ile Thr Thr Pro Tyr
        435                 440                 445

Ile Val Val Val Asn Gly His Ile Asp Pro Asn Ser Lys Gly Asp Leu
    450                 455                 460

Ala Leu Arg Ser Thr Leu Tyr Gly Tyr Asn Ser Asn Ile Ile Trp Arg
465                 470                 475                 480

Ser Met Ser Trp Asp Asn Glu Val Ala Phe Asn Asn Gly Ser Gly Ser
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Gly Glu Ile Glu Pro Ile Pro Glu
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tatggttttt cagtgcctaa ttctgctgtt aaaggtgaca cattcaaaat aactgtacct 180

aaagaattaa acttaaatgg tgtaacttca actgctaaag tgccaccaat tatggctgga 240

gatcaagtat tggcaaatgg tgtaatcgat agtgatggta atgttattta tacatttaca 300

gactatgtaa atactaaaga tgatgtaaaa gcaactttga ccatgcccgc ttatattgac 360

cctgaaaatg ttaaaaagac aggtaatgtg acattggcta ctggcatagg tagtacaaca 420

gcaaacaaaa cagtattagt agattatgaa aaatatggta agttttataa cttatctatt 480

aaaggtacaa ttgaccaaat cgataaaaca aataatacgt atcgtcagac aatttatgtc 540

aatccaagtg gagataacgt tattgcgccg gttttaacag gtaatttaaa accaaatacg 600

gatagtaatg cattaataga tcagcaaaat acaagtatta aagtatataa agtagataat 660

gcagctgatt tatctgaaag ttactttgtg aatccagaaa actttgagga tgtcactaat 720

agtgtgaata ttacattccc aaatccaaat caatataaag tagagtttaa tacgcctgat 780

gatcaaatta caacaccgta tatagtagtt gttaatggtc atattgatcc gaatagcaaa 840

ggtgatttag ctttacgttc aactttatat gggtataact cgaatataat ttggcgctct 900

atgtcatggg acaacgaagt agcatttaat aacggatcag gttctggtga cggtatcgat 960

aaaccagttg ttcctgaaca acctgatgag 990

<210> 4
<211> 331
<212> PRT
<213> Staphylococcus aureus

<400> 4

Met Val Ala Ala Asp Ala Pro Ala Ala Gly Thr Asp Ile Thr Asn Gln
1 5 10 15

Leu Thr Asn Val Thr Val Gly Ile Asp Ser Gly Thr Thr Val Tyr Pro
20 25 30

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His Gln Ala Gly Tyr Val Lys Leu Asn Tyr Gly Phe Ser Val Pro Asn
        35                  40                  45

Ser Ala Val Lys Gly Asp Thr Phe Lys Ile Thr Val Pro Lys Glu Leu
    50                  55                  60

Asn Leu Asn Gly Val Thr Ser Thr Ala Lys Val Pro Pro Ile Met Ala
65                  70                  75                  80

Gly Asp Gln Val Leu Ala Asn Gly Val Ile Asp Ser Asp Gly Asn Val
                85                  90                  95

Ile Tyr Thr Phe Thr Asp Tyr Val Asn Thr Lys Asp Asp Val Lys Ala
            100                 105                 110

Thr Leu Thr Met Pro Ala Tyr Ile Asp Pro Glu Asn Val Lys Lys Thr
        115                 120                 125

Gly Asn Val Thr Leu Ala Thr Gly Ile Gly Ser Thr Thr Ala Asn Lys
    130                 135                 140

Thr Val Leu Val Asp Tyr Glu Lys Tyr Gly Lys Phe Tyr Asn Leu Ser
145                 150                 155                 160

Ile Lys Gly Thr Ile Asp Gln Ile Asp Lys Thr Asn Asn Thr Tyr Arg
                165                 170                 175

Gln Thr Ile Tyr Val Asn Pro Ser Gly Asp Asn Val Ile Ala Pro Val
            180                 185                 190

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

Gln Gln Asn Thr Ser Ile Lys Val Tyr Lys Val Asp Asn Ala Ala Asp
    210                 215                 220

Leu Ser Glu Ser Tyr Phe Val Asn Pro Glu Asn Phe Glu Asp Val Thr
225                 230                 235                 240

Asn Ser Val Asn Ile Thr Phe Pro Asn Pro Asn Gln Tyr Lys Val Glu
                245                 250                 255
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Phe Asn Thr Pro Asp Asp Gln Ile Thr Thr Pro Tyr Ile Val Val Val
            260                 265                 270

Asn Gly His Ile Asp Pro Asn Ser Lys Gly Asp Leu Ala Leu Arg Ser
        275                 280                 285

Thr Leu Tyr Gly Tyr Asn Ser Asn Ile Ile Trp Arg Ser Met Ser Trp
    290                 295                 300

Asp Asn Glu Val Ala Phe Asn Asn Gly Ser Gly Ser Gly Asp Gly Ile
305                 310                 315                 320

Asp Lys Pro Val Val Pro Glu Gln Pro Asp Glu
                325                 330
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<210> 5
<211> 336
<212> DNA
<213> Staphylococcus aureus

<400> 5
aacattatga tgacacagtc gccatcatct ctggctgtgt ctgcaggaga aaaggtcact 60

atgagctgta agtccagtca aagtgtttta tacagttcaa atcagaagaa ctacttggcc 120

tggtaccagc agaaaccagg gcagtctcct aaactactga tctactgggc atccactagg 180

gaatctggtg tccctgatcg cttcacaggc agtggatctg ggacagattt tactcttacc 240

atcaacagtg tacaagctga agacctggca gtttattact gtcatcaata cctctcctcg 300

cacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 6
<211> 112
<212> PRT
<213> Staphylococcus aureus

<400> 6

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Asn Ile Met Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1               5                   10                  15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
            20                  25                  30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
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35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Asn Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys His Gln
85 90 95

Tyr Leu Ser Ser His Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 7
<211> 354
<212> DNA
<213> Staphylococcus aureus

<400> 7
caggtgcatc tgaaggagtc aggacctggc ctggtggcac cctcacagag cctgtccatc 60

acatgcactg tctctggatt ctcattatcc agatataata tacactgggt tcgccagcct 120

ccaggaaagg gtctggagtg gctgggaatg atatggggtg gtgaaaacac agactataat 180

tcagctctca aatccagact gagcatcagc aaggacaact ccaagagcca agttttctta 240

aaaatgaaca gtctgcaaac tgatgacaca gccatgtact actgtgccag cgcctactat 300

ggtaactcct ggtttgctta ctggggccag gggactctgg tcactgtctc tgca 354

<210> 8
<211> 118
<212> PRT
<213> Staphylococcus aureus

<400> 8

Gln Val His Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15

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

Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Met Ile Trp Gly Gly Glu Asn Thr Asp Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65 70 75 80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Ser Ala Tyr Tyr Gly Asn Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 9
<211> 336
<212> DNA
<213> Staphylococcus aureus

<400> 9
aacattatga tgacacagtc gccatcatct ctggctgtgt ctgcaggaga aaaggtcact 60

atgagctgta agtccagtca aagtgtttta tacagttcaa atcagaagaa ctacttggcc 120

tggtaccagc agaaaccagg gcagtctcct aaactgctga tctactgggc atccactagg 180

gaatctggtg tccctgatcg cttcacaggc agtggatctg ggacagattt tactcttacc 240

atcagcagtg tacaagctga agacctggca gtttattact gtcatcaata cctctcctcg 300

tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 10
<211> 112
<212> PRT
<213> Staphylococcus aureus

<400> 10

Asn Ile Met Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln

35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys His Gln
85 90 95

Tyr Leu Ser Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 11
<211> 363
<212> DNA
<213> Staphylococcus aureus

<400> 11
caggtgcagc tgaaggagtc aggacctggc ctggtggcac cctcacagag cctgtccatc 60

acatgcgcta tctctgggtt ctcattatcc agatatagtg tacactgggt tcgccagcct 120

ccaggaaagg gtctggagtg gctgggaatg atatggggtg gtggaaacac agactataat 180

tcagctctca aatccagact gagcatcagc aaggacaact ccaagagcca agttttctta 240

aaaatgaaca gtctgcaaac tgatgacaca gccatgtatt actgtgccag aaaaggggaa 300

ttctactatg gttacgacgg gtttgtttac tggggccaag ggactctggt cactgtctct 360

gca 363

<210> 12
<211> 121
<212> PRT
<213> Staphylococcus aureus

<400> 12

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Thr Cys Ala Ile Ser Gly Phe Ser Leu Ser Arg Tyr
20 25 30

Ser Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Met Ile Trp Gly Gly Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65 70 75 80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Arg Lys Gly Glu Phe Tyr Tyr Gly Tyr Asp Gly Phe Val Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> 13
<211> 336
<212> DNA
<213> Staphylococcus aureus

<400> 13
aacattatga tgacacagtc gccatcatct ctggctgtgt ctgcaggaga aaaggtcact 60

atgagctgta ggtccagtca aagtgtttta tacagttcaa atcagaagaa ctacttggcc 120

tggtaccagc agaaaccagg gcagtctcct acactgctga tctactgggc atccactagg 180

gaatctggtg tccctgatcg cttcacaggc agtggatctg ggacagattt tactcttacc 240

atcagcagtg tacaagctga agacctggca gtttattact gtcatcaata cctctcctcg 300

tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 14
<211> 112
<212> PRT
<213> Staphylococcus aureus

<400> 14

Asn Ile Met Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Arg Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Thr Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys His Gln
85 90 95

Tyr Leu Ser Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 15
<211> 354
<212> DNA
<213> Staphylococcus aureus

<400> 15
caggtgcagc tgaaggagtc aggacctggc ctggtggcac cctcacagag cctgtccatc 60

acatgcactg tctctgggtt ctcattatcc agatatagtg tacactgggt tcgccagcct 120

ccaggaaagg gtctggagtg gctgggaatg atatggggtg gtggaaacac agactataat 180

tcagctctca aatccagact gagcatcacc aaggacaact ccaagagcca agttttctta 240

aaaatgaaca gtctgcaaac tgatgacaca gccatgtact actgtgccac cgcctactat 300

ggtaactcct ggtttgctta ctggggccaa gggactctgg tcactgtctc tgca 354

<210> 16
<211> 118
<212> PRT
<213> Staphylococcus aureus

<400> 16

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln
1 5 10 15

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

Ser Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Met Ile Trp Gly Gly Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys
50 55 60

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

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Thr Ala Tyr Tyr Gly Asn Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 17
<211> 336
<212> DNA
<213> Staphylococcus aureus

<400> 17
gacattgtga tgacacagtc gccagactct ctggctgtgt ctctgggaga aagggtcact 60

atgaactgta agtccagtca aagtgtttta tacagttcaa atcagaagaa ctacttggcc 120

tggtaccagc agaaaccagg gcagtctcct aaactgctga tctactgggc atccactagg 180

gaatctggtg tccctgatcg cttcagcggc agtggatctg ggacagattt tactcttacc 240

atcagcagtg tacaagctga agacctggca gtttattact gtcatcaata cctctcctcg 300

tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 18
<211> 112
<212> PRT
<213> Staphylococcus aureus

<400> 18

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

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Glu Arg Val Thr Met Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
            20                  25                  30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys His Gln
                85                  90                  95

Tyr Leu Ser Ser Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
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<210> 19
<211> 363
<212> DNA
<213> Staphylococcus aureus

<400> 19
caggtgcagc tgaaggagtc aggacctggc ctggtgaagc cctcacagac cctgtccatc 60

acatgcacca tctctgggtt ctcattatcc agatatagtg tacactgggt tcgccagcct 120

ccaggaaagg gtctggagtg gctgggaatg atatggggtg gtggaaacac agactataat 180

tcagctctca aatccagact gagcatcagc aaagacaact ccaagaacca agttttctta 240

aaaatgaaca gtctgaccgc cgctgacaca gccgtgtatt actgtgccag aaaaggggaa 300

ttctactatg gttacgacgg gtttgtttac tggggccaag ggactctggt cactgtctct 360

tcc 363

<210> 20
<211> 121
<212> PRT
<213> Staphylococcus aureus

<400> 20

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Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1               5                   10                  15
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```
Thr Leu Ser Ile Thr Cys Thr Ile Ser Gly Phe Ser Leu Ser Arg Tyr
            20                  25                  30

Ser Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45

Gly Met Ile Trp Gly Gly Gly Asn Thr Asp Tyr Asn Ser Ala Leu Lys
    50                  55                  60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
65                  70                  75                  80

Lys Met Asn Ser Leu Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95

Arg Lys Gly Glu Phe Tyr Tyr Gly Tyr Asp Gly Phe Val Tyr Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
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