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(71) Applicant: **KYOWA HAKKO KIRIN CO., LTD.**
[JP/JP]; 1-6-1, Ohtemachi, Chiyoda-ku, Tokyo, 1008185
(JP).

(72) Inventors: **TAHARA, Tomoyuki**; c/o Kyowa Hakko Kirin
California, Inc., 9420, Athena Circle, La Jolla, California,
92037 (US). **KRISTIAN, Sascha**; c/o Kyowa Hakko Kirin
California, Inc., 9420, Athena Circle, La Jolla, California,
92037 (US). **KANDA, Yutaka**; c/o Kyowa Hakko Kirin
California, Inc., 9420, Athena Circle, La Jolla, California,
92037 (US).

(74) Agents: **HAMADA, Yuriko** et al.; Eikoh Patent Firm,
Toranomon East Bldg. 10F, 7-13, Nishi-Shimbashi 1-
chome, Minato-ku, Tokyo, 1050003 (JP).

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(54) Title: ANTI-STAPHYLOCOCCUS AUREUS CAPSULAR POLYSACCHARIDE MONOCLONAL ANTIBODY

(57) Abstract: The invention relates to an antibody and antigen-binding fragment thereof which specifically recognizes an epitope comprising mannosaminuronic acid residue of capsular polysaccharide (hereinafter, described as CP) and binds to CP expressed on *Staphylococcus aureus* (hereinafter, described as *S. aureus*), a nucleotide encoding amino acid sequence of the antibody, a production method of the antibody and a method for treating a infectious diseases caused by *S. aureus*.



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DESCRIPTION

Anti-Staphylococcus aureus capsular polysaccharide monoclonal antibody

5 Technical Field

The present invention relates to an antibody and antigen-binding fragment thereof which specifically recognizes an epitope comprising mannosaminuronic acid residue of capsular polysaccharide (hereinafter, described as CP) and binds to CP expressed on *Staphylococcus aureus* (hereinafter, described as *S. aureus* or SA), a
10 nucleotide encoding amino acid sequence of the antibody, a production method of the antibody and a method for treating a infectious diseases caused by *S. aureus*.

Background Art

SA is a principal source of community- and hospital-acquired infections,
15 including life-threatening conditions such as sepsis, bacteremia, endocarditis and pneumonia. Since no vaccines or passive immunization drugs are available, the treatment of SA infections relies on conventional antibiotics. SA is a leading cause of hospital-acquired infections. In the US 2005, hospital-acquired infections caused around 90,000 deaths and more than \$5 billion in excess healthcare costs. In summary, there is an urgent
20 unmet need for the development of novel anti-SA therapeutics. It is expected that an anti-SA mAb drug could be used as adjunctive therapy with first-line standard-of-care (SOC) antibiotic vancomycin or in combination with second-line SOC, linezolid and daptomycin, in vancomycin treatment failures.

Staphylococcus aureus causes disease by different pathogenic mechanisms.
25 First, extracellular "superantigen" toxins of *S. aureus* cause food poisoning and toxic shock syndrome; these diseases occur mostly in otherwise healthy hosts. Second, common and serious, bacteremia and its complications occur in individuals with decreased resistance; in this clinical setting, *S. aureus* is an opportunistic pathogen. *S. aureus* may be considered the most virulent of the opportunistic pathogens because it is the first or second most
30 common cause of bacteremia and its complications in newborns, cases of postoperative infection, and primary or secondary immunodeficiencies such as severe burns. In the United States and throughout the world, *S. aureus* accounts for approximately one third of blood isolates from hospitalized patients. Similarly, in a study of the therapeutic effect of activated Protein C (APC), *S. aureus* was found to be the second most common cause of
35 bacteremia in patients with severe sepsis. *S. aureus*-associated hospitalizations have twice as high a death rate, length of stay, and cost as hospitalizations because of other causes. Patients with end-stage renal disease (ESRD) undergoing hemodialysis are

unusually susceptible to *S. aureus* bacteremia. Compounding the difficulty of treating *S. aureus* infections is the rise in multidrug resistant isolates, including methicillin and vancomycin (NPL1).

Staphylococcus aureus is considered an opportunistic bacterium and is isolated from the skin and nasopharynx of approximately 25% of healthy humans (NPL2). Although *S. aureus* strains are noninvasive bacteria, they cause several diseases in humans by different pathogenic mechanisms. The most frequent and serious diseases include bacteremia in hospitalized and immunocompromised patients and its complications such as endocarditis, septic arthritis, and osteomyelitis (NPL3). In several studies, increased secretion of tumor necrosis factor-alpha (TNF-alpha) and interleukin-1beta (IL-1beta) by monocytes subsequent to stimulation with *S. aureus* endotoxins, peptidoglycan, and membrane component has been found (NPL4).

Approximately 90% of *S. aureus* isolates produce capsular polysaccharide (CP). Although 11 capsular serotypes have been described, over 80% of the human clinical isolates belong to serotypes 5 (CP5) and 8 (CP8), and there is evidence that these CPs are virulence factors of *S. aureus*, causing bacteremia and associated diseases (NPL5). Strains of serotypes 1 and 2 are heavily encapsulated, easily distinguished by their mucoid colonial morphology, and yet rarely encountered clinically. *S. aureus* strains belonging to the remaining serotypes from nonmucoid colonies on agar plates and are referred to as microencapsulated (NPL6). Naturally occurring antibodies to these capsular polysaccharides are detectable in normal human serum (NPL7), but the role that these antibodies play in immunity to staphylococcal infections has not been demonstrated, and any passive immunotherapy including monoclonal antibody drugs against *S. aureus* has not been approved by Food and Drug Administration (FDA).

Opsonization of *S. aureus* is critical for uptake and killing of the bacterium by professional phagocytic cells. Complement and antibody are the principal serum opsonins and have been shown to play an important role in opsonophagocytic killing of the staphylococcus. Complement component C3b and its degradation fragment iC3b are the principal complement opsonins and represent important components of the host innate immune system. C3b can potentially be generated by three activation pathways—the classical, alternative, and lectin pathways. The classical pathway is most commonly activated by the interaction of antibody (IgG or IgM) with an antigenic bacterial surface. The alternative pathway, activated by a repeating polysaccharide or similar polymeric structure, is probably most important in nonimmune serum lacking antibodies specific to the bacterium. The lectin pathway is mediated through a mannan-binding lectin (MBL) found in serum. Cunnion et al. challenged control and C3-depleted mice intravenously with CP5-positive *S. aureus* strain. Whereas only 8% of the control mice succumbed to

the infection, 64% of the complement-depleted animals died. The investigators also evaluated the *in vitro* parameters of C3 binding to serotype 5 and 8 isolates of *S. aureus*. At a high serum concentration (20%, similar to whole blood), the alternative pathway was active, contributing 90% of the total C3 binding to *S. aureus*. At a lower serum concentration (2%) that might be present in tissues, the classical complement pathway predominated. In a comparison of bacterial strains grown on solid medium, a CP5 strain bound 42% fewer C3 molecules than its isogenic capsular polysaccharide-negative mutant. Both C3b and iC3b fragments of C3 bound to *S. aureus* cells, and about one third of the bound C3b was shed from the bacterial surface as iC3b regardless of the capsular polysaccharide phenotype of the strain. CP5 was shown to mask C3 fragments deposited on the organism from binding to complement receptor 1. The observation that encapsulated *S. aureus* organisms interfere with opsonization by complement is consistent with the fact that these cells are poorly phagocytosed in normal human serum (NPL8).

S. aureus infections are controlled by host neutrophils, which kill this pathogen via opsonophagocytosis (OPA or OPH), a process that requires opsonization of bacteria by the complement system. Activation of this system results in the covalent deposition of complement component 3 (C3, C3b, C3d) onto bacterial surfaces. On gram-negative bacteria, this can lead to direct complement-mediated lysis of cells, while gram-positives are resistant to lysis due to their thick peptidoglycan layer. However, C3, C3b and C3d can also interact with complement receptors on neutrophils such as Mac-1 (CD11b/CD18) to promote phagocytosis. Deposition of C3 onto pneumococci can result from activation of either the classical or alternative pathways. Activation of the classical pathway can be directed to bacterial surfaces with the aid of antibodies, while the alternative pathway stochastically activates complement on bacterial surfaces. In addition, members of the leukocyte immunoglobulin (Ig) G Fc receptor (FcγR) family play a key role in antibody-mediated phagocytosis and can either enhance antigen presentation or down-modulate immune responses (NPL9). Once opsonized, bacteria can be recognized by surface receptors on neutrophils, monocytes or macrophages and ingested by phagocytosis. Once internalized, *S. aureus* is exposed to a variety of potent microbicidal substances including reactive oxygen and nitrogen intermediates, antimicrobial peptides and proteins such as lysozyme, alpha-defensins, cathepsins and leukocyte proteinase 3 (myeloblastin), and then efficiently killed in the phagolysosome (NPL10).

Staphylococcus aureus, the first or second most common pathogen isolated from patients, is capsulated; there are at least 12 capsular types, and types 5 and 8 comprise approximately 85% of blood. Types 5 and 8, composed of a trisaccharide repeat unit including a mannose uronic acid and 2 fucoses, are non-immunogenic (NPL11).

The *Staphylococcus aureus* type 5 capsular polysaccharide is composed of 2-acetamido-2-deoxy-l-fucose (1 part), 2-acetamido-2-deoxy-d-fucose (1 part), and 2-acetamido-2-deoxy-d-mannuronic acid (1 part). On the basis of methylation analysis, optical rotation, high-field one- and two-dimensional ¹H- and ¹³C-N.M.R. experiments, and selective cleavage with 70% aqueous hydrogen fluoride, the polysaccharide was found to be a partially O-acetylated (50%) polymer of the repeating trisaccharide unit, [→4)-3-O-Ac-β-D-ManpNAcA-(1→4)-α-L-FucpNAc-(1→3)-β-D-FucpNAc-(1→]n. The chemical formula of CP8 is, [→3)-4-O-Ac-β-D-ManpNAcA-(1→3)-α-L-FucpNAc-(1→3)-β-D-FucpNAc-(1→]n. Type 5 and 8 polysaccharides differ only in the linkages between the sugars and in the sites of O-acetylation of the mannosaminuronic acid residues, yet they are serologically distinct (NPL12).

With respect to the biological functions of *Staphylococcus aureus* capsular polysaccharide, Kampen et al. reported that the expression of a CP5 or CP8 capsule protected the bacteria from being killed by neutrophils *in vitro*, and that the capsule-expressing SA strains did not stimulate respiratory burst activity in whole blood. Their study demonstrated that the expression of CP5 or CP8 confers resistance to opsonophagocytic killing and prevents the bacteria from inducing respiratory burst of neutrophils (NPL13).

Soell et al. tested the immunomodulatory effects of CP5 and CP8 on human epithelial cells, endothelial cells, and monocytes. Biotinylated CP5 and CP8 bind to those cells in a dose- and calcium-dependent manner through specific interactions. CP5 and CP8 trigger epithelial cells to produce interleukin-8 (IL-8); endothelial cells to produce IL-8 and IL-6; and monocytes to produce IL-8, IL-6, IL-1 beta, and tumor necrosis factor alpha (TNF alpha). The production of the proinflammatory cytokines including TNF alpha, IL-1 beta, and IL-6, has been associated with inflammatory diseases and septic shock in human (NPL14).

Howden et al. investigated on the molecular mechanisms of low-level vancomycin resistance in *Staphylococcus aureus* (vancomycin-intermediate *S. aureus*; VISA). Enhanced production of capsular polysaccharide (CP8) in VISA strains were confirmed by immunological bioassays and fully supported the transcriptome data. Recently up-regulation of capsule gene expression has been documented as part of the global transcriptional response that contributes to innate immune system evasion. It is worth considering if the increased capsule production could directly contribute to the low-level vancomycin resistance in VISA strains (NPL15, NPL16).

Nelles et al. reported that monoclonal antibodies were raised against CP5 and CP8 of *Staphylococcus aureus* by hyperimmunizing BALB/c mice with formalin-fixed *Staphylococcus aureus* strain Reynolds and Becker. They established 19 clones of mouse

anti-CP5 monoclonal antibodies (clone 17-1, etc.) and 9 clones of mouse anti-CP8 monoclonal antibodies (clone 18-1, etc.). All of the monoclonal antibodies possessed either mouse immunoglobulin M (IgM) or immunoglobulin G3 (IgG3) isotype. They showed agglutination of *Staphylococcus aureus* strains *in vitro* (NPL17).

5 Karakawa et al. reported that monoclonal antibodies were raised against CP8 of *Staphylococcus aureus* by hyperimmunizing BALB/c mice with formalin-fixed *Staphylococcus aureus* strain. They established three mouse anti-CP8 monoclonal antibodies including clone 1716, 1725 and 2512. They were mouse IgM isotype and showed human polymorphonuclear leukocyte (PMN)-mediated phagocytosis against CP8-
10 positive *Staphylococcus aureus* strains *in vitro* (NPL18).

Poutrel et al. reported that monoclonal antibodies were raised against CP5 of *Staphylococcus aureus* by hyperimmunizing BALB/c mice with formalin-fixed *Staphylococcus aureus* Reynolds strain. They established a mouse anti-CP5 monoclonal antibodies 120D3. It was mouse IgM isotype and showed specific binding to CP5-
15 positive *Staphylococcus aureus* strains *in vitro* (NPL19).

Cook et al. established two mouse anti-CP8 monoclonal antibodies 8E8 (IgG2b) and 1C10 (IgG3) by immunizing BALB/c mice with a CP8-conjugated vaccine. The clone 8E8 showed opsonophagocytic killing (OPK) activity against a *Staphylococcus aureus* strain Becker *in vitro* depending on the presence of human neutrophils and rabbit
20 complement, however, the clone 8E8 was not efficacious in the lethal challenge mouse model, in which antibodies were passively transferred to the peritoneum and the animals were infected via the tail vein 18–24 h later (NPL20).

The number of the reports demonstrating *in vivo* efficacy of anti-capsular polysaccharide is limited. Lee et al. showed protective efficacy of anti-CP5 polyclonal
25 antibodies (rabbit IgG fraction) in a model of *Staphylococcus aureus*-mediated endocarditis in rats. The polyclonal antibodies were prepared by immunizing rabbit with CP5-conjugate vaccine and purified from rabbit serum by using Protein G column. Rupp et al. reported the result of phase II, randomized, multicenter, double-blind, placebo-controlled trial of Altastaph (a polyclonal human IgG with high level of antibody to CP5
30 and CP8) in treatment of *Staphylococcus aureus*-mediated bacteremia. Five of 21 (23%) subjects in the Altastaph group died, whereas 2 of 18 (11%) subjects in the placebo group died. Compared to the control patients, the Altastaph recipients had a shorter median time to the resolution of fever and a shorter length of hospital stay. The study was not powered to show efficacy (NPL21).

35 For the all of circumstances stated above, any therapeutic anti-CP monoclonal antibodies, which is highly effective to cure *S. aureus*-mediated infection has not been fully determined yet. Also, none of prior art to establish anti-CP monoclonal antibody

having high binding activity to CP in various status such as expressed on cell surface, solubilized in biofluid, and immobilized on solid surface has ever been found in publication.

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Summary of Invention

30 Technical Problem

For the background of the art, an antibodies which specifically recognize an epitope comprising mannosaminuronic acid residues of capsular polysaccharide (CP) and bind to CP expressed on *S. aureus*, a method for producing antibodies and therapeutic method for infectious diseases caused by SA, are desired.

35

Solution to Problem

The present invention provides monoclonal antibodies and antigen-binding fragments which specifically recognize an epitope comprising mannosaminuronic acid residue of CP and bind to CP expressed on *Staphylococcus aureus*. In the present invention, *Staphylococcus aureus* capsular polysaccharide (hereinafter, might be described as SACP in case) is exemplified as CP type 5(CP5) or CP type 8(CP8). Optionally, the antibodies of the present invention are established from a hybridoma generated from an animal arose with CP or *S. aureus* or a B cell from a human donor exposed with CP or *S. aureus*.

The monoclonal antibodies of the present invention are exemplified as antibodies which recognize an epitope comprising mannosaminuronic acid residue of CP5 and bind to CP5 expressed on *Staphylococcus aureus*, antibodies which recognize an epitope comprising mannosaminuronic acid residue of CP8 and bind to CP8 expressed on SA, and so on.

In the present invention, anti-CP5 mouse monoclonal antibody 137G18A, anti-CP8 monoclonal antibody 138P2 and these variants described herein, can be preferably included. Alternatively, an antibody competes with at least one antibody of these, and an antibody which binds to the same (or identical) epitope or overlapped epitope as bound by at least one antibody of these, are included in the antibodies of the present invention. The antibodies respectively referred to herein are anti-CP antibodies. The anti-CP antibodies have one or more of the following mechanism(s): a) specifically recognize the epitope comprising mannosaminuronic acid residue of CP, b) specifically bind to CP (ex. CP5 or CP8) expressed on a *Staphylococcus aureus* strain; c) bind to *Staphylococcus aureus* cells; d) show a complement-deposition activity against *Staphylococcus aureus* cells, e) show an opsonophagocytosis or opsonophagocytic killing activity against *S. aureus* cells.

In the present invention, a monoclonal antibody which comprises complementarity determining regions (hereinafter, described as CDR) 1 to 3 of a heavy chain variable region (hereinafter, described as VH) comprising amino acid sequences of SEQ ID NOs:1 to 3, respectively and CDRs 1 to 3 of a light chain variable region (hereinafter, described as VL) comprising amino acid sequences of SEQ ID NOs: 4 to 6, respectively and binds to CP5, and a monoclonal antibody which comprises CDRs 1 to 3 of VH comprising amino acid sequences of SEQ ID NOs:7 to 9, respectively, and CDRs 1 to 3 of VL comprising amino acid sequences of SEQ ID NOs:10 to 12, respectively and binds to CP8, are further included.

Furthermore, monoclonal antibodies of the present invention also includes antibodies which compete with any one antibody identified in the above and antibodies which bind to a same epitope as bound by any one antibody identified in the above.

Alternatively, or in addition, an isolated anti-CP antibody, or antigen-binding fragment thereof, comprising a heavy chain variable domain (VH) and a light chain variable domain (VL), wherein the three heavy chain CDRs include an amino acid sequence at least 90%, 92%, 95%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NOs:1, 2, 3, and the three light chain CDRs include an amino acid sequence at least 90%, 92%, 95%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NOs:4, 5, 6. The antibody or antigen-binding fragment binds to *Staphylococcus aureus* capsular polysaccharide CP5.

Alternatively, or in addition, an anti-CP antibody, or antigen-binding fragment thereof, comprising a heavy chain variable domain (VH) and a light chain variable domain (VL), wherein in the VH and the VL each complementarity-determining region (CDR) includes the following amino acid sequences: VH CDR1: SEQ ID NO:7; VH CDR2: SEQ ID NO:8; VH CDR3: SEQ ID NO:9; VL CDR1: SEQ ID NO:10; VL CDR2: SEQ ID NO:11; VL CDR3: SEQ ID NO:12. The antibody or antigen-binding fragment binds to *Staphylococcus aureus* capsular polysaccharide CP8.

Alternatively, or in addition, an isolated anti-CP antibody, or antigen-binding fragment thereof, comprising a heavy chain variable domain (VH) and a light chain variable domain (VL), wherein the three heavy chain CDRs include an amino acid sequence at least 90%, 92%, 95%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NOs:7, 8, 9, and three light chain CDRs that include an amino acid sequence at least 90%, 92%, 95%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NOs:10, 11, 12. The antibody or antigen-binding fragment binds to *Staphylococcus aureus* capsular polysaccharide CP8.

In another aspect, the invention provides a composition including an anti-CP antibody according to the invention. The composition is optionally a pharmaceutical composition including any one of the anti-CP antibodies or antigen-binding fragments described herein and a pharmaceutical carrier. In various aspects, the composition further includes an anti-bacterial drug, a bacterial growth inhibitor, a bacterial toxin inhibitor, a bacterial phagocytosis mediator, or a bacterial attachment inhibitor. The anti-bacterial drug is for example an enzymatic inhibitor or a modulator of the bacterial membrane. The enzymatic inhibitor is for example cephem, etc. In a further aspect, the composition further includes a second anti-bacterial antibody.

In another aspect, the invention provides methods of administering the anti-CP antibodies or antigen-binding fragments described herein to a subject prior to, and/or after exposure to a *Staphylococcus aureus* strain. In other words, the anti-CP antibody of the invention is used to prevent or treat the infection with *Staphylococcus aureus*. The anti-CP antibody is administered at a dose sufficient to promote bacterial clearance.

Also included in the invention is a method for determining the presence of a *Staphylococcus aureus* infection in a patient, by contacting a biological sample obtained from the patient with an anti-SACP antibody or antigen-binding fragment described herein; detecting an amount of the antibody that binds to the biological sample; and comparing the amount of antibody that binds to the biological sample to a control value. The invention further provides a diagnostic kit comprising an anti-CP antibody or antigen-binding fragment described herein.

Advantageous Effects of Invention

Other features and advantages of the invention will be apparent from and are encompassed by the following detailed description and claims.

Brief Description of Drawings

FIG. 1 shows comparison of mouse anti-CP5 mAbs binding to immobilized CP5 by ELISA. 96-well plates were coated overnight at 4°C with purified CP5, then washed, and blocked. Mouse anti-CP5 mAbs were added at the indicated concentrations to the CP5 coated wells for 1 h. Isotype antibodies served as negative controls. Antibodies bound to the immobilized CP5 were detected with anti-mouse antibody light chain specific horseradish peroxidase (HRP)-conjugated secondary antibody. After 1 h, TMB substrate was added. Reaction was stopped by addition of 2 N sulfuric acid and the optical density read at 450 nm (OD_{450nm}). Samples were run in triplicate and average OD_{450nm} values + SD are shown. “****” shows; $p < 0.0005$, mouse 137G18A vs. three other mouse anti-CP5 mAbs.

FIGs. 2A to 2D show comparison of mouse anti-CP5 mAb binding to immobilized CP5 by BIAcore system. The sensorgrams from surface plasmon resonance analysis for the four anti-CP5 monoclonal antibodies including 137G10, 137G18A, 137L2AA, and 137F8 towards immobilized CP5 by using BIAcore 3000. In each sensorgram, Y-axis indicates the response unit and X-axis indicates the time of each binding experiment.

FIGs. 3A and 3B show competition ELISA with purified CP5 and two mouse anti-CP5 antibodies. 96-well plates were coated overnight at 4°C with purified CP5, then washed, and blocked. Subsequently, 10 µg/mL of 137G18A or 137L2AA mouse anti-CP5 mAbs (= primary antibodies) were added to the CP5 coated wells. Mouse IgG1 and or IgG2a isotype antibodies at 10 µg/mL served as negative controls. After 30-min incubation at 37°C, the wells were washed 3x and then either vehicle (PBS) or 10 µg/mL of the indicated secondary isotype or anti-CP5 mAbs were added to the wells for 30 min at 37°C. Subsequently, the wells were washed three times and then HRP-conjugated tertiary

anti-mouse IgG1 or anti-mouse IgG2a antibodies were added to respective wells to detect residually bound primary antibodies. After 1 h incubation at room temperature, TMB substrate was added. Reaction was stopped with 2N sulfuric acid and the optical density read at 450 nm (OD450nm). Samples were run in triplicate and average OD450nm values + SD are shown. “***” shows; $p < 0.005$; n.s., not significant, unpaired two-tailed t-test.

FIGs. 4A and 4B shows capturing of soluble CP5 antigens by mouse anti-CP5 mAbs. 96-well plates were coated overnight at 4°C with 100 μ L PBS containing 10 μ g/mL of the indicated mouse isotype control or anti-CP5 mAbs (= primary antibodies). Vehicle (PBS) alone served as negative control. Subsequently, the wells were washed three times with 200 μ L wash buffer (PBS, 0.05% Tween-20). Then, 200 μ L PBS, 1% casein was added to each well and the plate incubated for 2 h at 37°C to block unspecific binding sites. Subsequently, the wells were washed as above and then 100 μ L of 1 μ g/mL CP5 in PBS was added to each well. The plate was incubated for 1 h at 37°C and then washed as above. Subsequently, to determine whether soluble CP5 was captured by the primary antibodies or unspecifically binds to vehicle treated wells, 100 μ L of 1 μ g/mL biotinylated mouse anti-CP5 mAb 137G18A (= detection antibody) in PBS, 0.9% casein was added to each well. After 1 h incubation at 37°C, wells were washed as above. Then, 50 μ L of streptavidin-conjugated Horse radish peroxidase (HRP) was added to each well and the plate incubated for 1 h at 37°C. Then, the wells were washed as above and 100 μ L TMB substrate was added to each well. The reaction was stopped after 5 min by addition of 50 μ L 2N sulfuric acid and the optical density of each well read at 450 nm (OD450nm). Samples were run in quadruplicate and average OD450nm values + SD are shown. The higher the OD450nm values, the more CP5 was captured by the respective antibodies. “****” shows; $p < 0.0001$, isotype vs. anti-CP5 mAb, unpaired two-tailed t-test.

FIG. 5 shows detection of soluble CP5 antigens in plasma taken from *S. aureus* infected animals. Three C57BL/6 mice were infected intraperitoneally with 1.25×10^8 colony forming units (CFU) of agar-grown CP5 expressing *S. aureus* Reynolds in 200 μ L PBS. 2 days after infection, mice were euthanized and left kidneys and heparinized cardiac blood collected. Undiluted blood and serial blood dilutions were plated onto sheep agar plates to determine whether bacteria were present in the blood samples. Kidneys were homogenized and homogenates plated onto THY agar plates to enumerate the total bacterial burden. The CFU numbers in blood and kidneys for individual mice are shown in the table on the left (Detection limit in blood: 100 CFU/mL). Plasma was isolated from remaining blood using standard procedures, then sterile filtered (0.22 μ m), heat-inactivated and stored in aliquots at -80 degree centigrade until use. In parallel, plasma from cardiac blood from three uninfected mice was prepared as above. CP5 in the

plasma samples was detected by sandwich ELISA: A NUNC Maxisorp 96-well plate was coated overnight with 0.1 $\mu\text{g}/\text{well}$ capture antibody (Mouse anti-CP5 mAb 137G10; 100 $\mu\text{L}/\text{well}$) at 4 degree centigrade in 50 mM sodium carbonate/bicarbonate buffer. Then, wells were washed 3x with 200 μL TBST (PBS, 0.5% Tween-20). To block unspecific binding sites, 200 μL of a 1% casein solution was added to each well and the plate incubated for 2 hours at 37 degree centigrade. After washing wells as above, 100 μL of test plasma samples diluted in PBS (25% plasma from individual infected mice or 25% pooled plasma from three uninfected mice without (negative control plasma) or with addition of 10 ng/mL purified CP5 (positive control)) were added to the wells and the plate was incubated for 1 hour at 37 degree centigrade. Wells were then washed as above and 50 μL of 1 $\mu\text{g}/\text{mL}$ biotinylated mouse anti-CP5 mAb 137G18A in 1% casein (detection antibody) was added to each well. After 1-hour incubation at 37 degree centigrade, wells were washed 3x as above. For detection of bound biotinylated 137G18A, 50 μL of 0.2 $\mu\text{g}/\text{mL}$ streptavidin conjugated horseradish peroxidase (Jackson Immunoresearch) in PBS was added to the wells. After 1-hour incubation at 37 degree centigrade, wells were washed 3x again and 100 $\mu\text{L}/\text{well}$ TMB substrate was added. After 20 min, 50 μL 2 N sulfuric acid was added to stop the reaction. The absorbance of the wells was read at 450 nm (OD450nm) using a 96-well plate reader. Samples from infected mice and negative control plasma were run in duplicate and mean OD450nm values + SD data of one representative experiment of two performed are plotted in the graph on the right. A single well was run for the positive control plasma sample (= negative control plasma + 10 ng/mL purified CP5 added to the sample). The higher the OD450nm value for a given sample, the higher the degree of CP5 detection.

FIGs. 6A and 6B shows comparison of mouse anti-CP5 mAb binding to live antibiotic-resistant *S. aureus* cells (VRSA COLVA and MRSA BAA-41) by flow cytometry. 5×10^8 CFU/mL of live agar-grown CP5 expressing strains VRSA COLVA (in FIG. 6A) or MRSA BAA-41 (in FIG. 6B) were incubated with the indicated concentrations of mouse isotype or anti-CP5 mAbs 137G18A, 137G10, 137L2AA, or 137F8. Bound antibody was detected by standard methods using fluorescently labeled anti-mouse kappa chain specific secondary antibody and flow cytometry. Samples were run in triplicate and the averages of the mean fluorescent intensity (MFI) + SD of the bacterial particles are shown in the graphs. Representative results of one experiment of two performed for each bacterial strain are shown. “*” shows $p < 0.05$; “****” shows $p < 0.0005$, 137G18A vs. three other anti-CP5 mAbs; “#” shows $p < 0.05$; “##” shows $p < 0.005$; “###” shows $p < 0.0005$, 137G18A vs. 137G10 and 137F8.

FIG. 7 shows activity of mouse anti-CP5 mAbs in opsonophagocytosis assays with mouse macrophages and CP5 expressing MSSA strain Reynolds. $3.7\text{--}6.1 \times 10^5$ agar

grown *S. aureus* Reynolds cells (High CP5 expression) were preopsonized with the indicated amounts of control or anti-CP5 mAbs per reaction and then co-incubated with $\sim 2 \times 10^5$ J774 cells for 1 h. Subsequently, extracellular bacteria were killed by gentamicin. After washing, the J774 cells were lysed and intracellular *S. aureus* CFU enumerated.

- 5 Samples were run in triplicate and the data are expressed as percentages of phagocytosed CFU relative to the initial inoculum; average values + SD of one representative experiment of at least two performed are shown.

FIG. 8 shows activity of mouse anti-CP5 mAbs in opsonophagocytosis assays with mouse macrophages and CP5 expressing MSSA strain Reynolds. 3.7-6.1x10⁵ agar grown *S. aureus* Reynolds cells (High CP5 expression) were preopsonized with the indicated amounts of control or anti-CP5 mAbs per reaction and then co-incubated with $\sim 2 \times 10^5$ J774 cells for 1 h. Subsequently, extracellular bacteria were killed by gentamicin. After washing, the J774 cells were lysed and intracellular *S. aureus* CFU enumerated. Samples were run in triplicate and the data are expressed as percentages of phagocytosed CFU relative to the initial inoculum; average values + SD of one representative experiment of at least two performed are shown.

FIGs. 9A and 9B show mouse whole blood killing assays with mouse anti-CP5 antibodies. $\sim 5 \times 10^4$ CFU/mL of agar grown *S. aureus* Reynolds were incubated for 120 min in 50% heparinized mouse whole blood in the presence or absence of (in FIG. 9A) 5 μ g or (in FIG. 9B) 750 ng of the indicated mAbs. Then, CFU numbers in the samples were quantified. Samples were run in triplicate and data are expressed as percentages of CFU found after 2 h relative to the initial inoculum; average values + SD of one representative experiment of performed at least twice are shown (* shows $p < 0.05$; ** shows $p < 0.005$, anti-CP5 mAb vs. isotype control by unpaired t-test).

FIGs. 10A to 10H show *in vivo* efficacy evaluation of mouse anti-CP5 mAbs in mouse systemic infection models of passive immunization. 24 hours before intraperitoneal (i.p.) infection with $3.7-4 \times 10^7$ CFU of *S. aureus* Reynolds (FIGs. 10A-10E) or $3.1-3.7 \times 10^7$ CFU of *S. aureus* Lowenstein (FIGs. 10F-10H) in PBS, 5% mucin, CD-1 mice were pre-treated i.p. with the indicated amounts of mouse isotype or anti-CP5 mAbs. In most experiments, 24 hours after infection, tail vein blood was collected from each live mouse and CFU numbers determined. The survival of the mice was monitored for 9-10 days after infection. Activity comparison of four mouse anti-CP5 mAbs (FIGs. 10A-10C) and dose titration of 137G18A (FIGs. 10D-10E) in Reynolds model. In FIGs. 10F-10H, activity of 137G18A and 137G10, and 137L2AA in *S. aureus* Lowenstein model; the CFU detection limit in the blood was 200 CFU/mL (indicated by dotted bars) and values for individual mice are shown along with the median values which are shown as bars (* shows $p < 0.05$; ** shows $p < 0.005$; *** shows $p < 0.0005$; Survival curve comparisons of anti-CP5

mAbs vs. vehicle or isotype antibody with Mantel-Cox test or blood CFU comparison with unpaired t-test).

FIGs. 11A and 11B show activity of mouse anti-CP5 mAbs in mouse kidney infection model of passive immunization. 24 hours before intraperitoneal (i.p.) infection with 1.2×10^8 CFU of the CP5 strain *S. aureus* Reynolds in PBS, CD-1 mice were pre-treated either (FIG. 11A) i.p. or (FIG. 11B) subcutaneously (s.c.) with the indicated amounts of mouse isotype or anti-CP5 mAbs. 48 hours after infection, mice were euthanized, left kidneys excised, homogenized and bacterial CFU numbers determined. The results of one representative experiment of two are shown in the graph; CFU numbers for each mouse and median values are plotted; the CFU detection limit in the kidneys was 25 CFU/kidney as indicated by the dotted line (* shows $p < 0.05$, ** shows $p < 0.005$; CFU comparison of isotype antibody vs. 137G18A with unpaired t-test).

FIGs. 12A and 12B show binding of chimeric anti-CP5 antibody 137G18A to immobilized CP5 and capturing of soluble CP5 by the antibody. Fig. 12A shows binding of mouse/human chimeric anti-CP5 antibody 137G18A to immobilized CP5. 96-well plates were coated overnight with purified CP5, then washed, and blocked. Chimeric anti-CP5 antibody was added at the indicated concentrations to the CP5 coated wells for 1 h. An isotype antibody served as negative control. Bound antibodies were detected with anti-human IgG gamma chain specific horseradish peroxidase (HRP)-conjugated secondary antibody. After 1 h, TMB substrate was added. Reaction was stopped with sulfuric acid and the optical density (OD) read at 450 nm. Samples were run in duplicate and average OD values $\pm$ SD are shown in the graph (Fig. 12B shows capturing of soluble CP5. 96-well plates were coated overnight at 4°C with 100 μ L PBS containing 10 μ g/mL of the indicated human isotype control or chimeric anti-CP5 antibodies (= primary antibodies). Vehicle (PBS) alone served as negative control. Subsequently, the wells were washed three times with 200 μ L wash buffer (PBS, 0.05% Tween-20). Then, 200 μ L PBS, 1% casein was added to each well and the plate incubated for 2 h at 37°C to block unspecific binding sites. Subsequently, the wells were washed as above and then 100 μ L of 1 μ g/mL CP5 in PBS was added to each well. The plate was incubated for 1 h at 37°C and then washed as above. Subsequently, to determine whether soluble CP5 was captured by the primary antibodies or unspecifically binds to vehicle treated wells, 100 μ L of 1 μ g/mL biotinylated mouse anti-CP5 mAb 137G18A (= detection antibody) in PBS, 0.9% casein was added to each well. After 1 h incubation at 37°C, wells were washed as above. Then, 50 μ L of streptavidin-conjugated HRP was added to each well and the plate incubated for 1 h at 37°C. Then, the wells were washed as above and 100 μ L TMB substrate was added to each well. The reaction was stopped after 5 min by addition of 50 μ L 2N sulfuric acid and the optical density of each well read at 450 nm (OD_{450nm}).

Samples were run in quadruplicate and average OD_{450nm} values $\pm$ SD are shown. The higher the OD_{450nm} values, the more CP5 was captured by the respective antibodies. *** shows $p < 0.0001$, isotype vs. anti-CP5 antibody, unpaired two-tailed t-test.

FIG. 13 shows the sensorgrams from surface plasmon resonance analysis for the two anti-CP5 monoclonal antibodies including mouse/human chimeric 137G18A and 137G18A(N107D) towards immobilized CP5 by using BIAcore 3000. In each sensorgram, Y-axis indicates the response unit and X-axis indicates the time of each binding experiment.

FIGs. 14A and 14B show antigen-binding comparison between chimeric 137G18A and anti-LTA antibody Pagibaximab in human serum using *S. haemolyticus*. SH strain ATCC-43252 was grown overnight in complex media, then harvested and washed. In 96-well plates, $\sim 9 \times 10^7$ bacterial particles/well were incubated in 100 μ L of 10 mg/mL purified rabbit IgG at 4°C for 30 min to block unspecific antibody binding sites. Cells were then washed 3x in ice-cold PBS and resuspended in 100 μ L biotinylated primary antibody at 0.5 μ g/mL (137G18A) or 2 μ g/mL (Pagibaximab) or 2 μ g/mL isotype antibody in buffer alone (0% serum control) or the indicated percentages of human serum (individual serums from 5 healthy donors were tested) and incubated at 4°C for 30 min. Cells were then washed 3x in ice-cold PBS, resuspended in 50 μ L of 2.5 μ g/mL phycoerythrin-labeled streptavidin and incubated at 4°C for 30 min to detect antibodies bound to SH cells. After washing, cells were fixed and the fluorescence intensities of $\sim 20,000$ bacterial particles per sample measured by flow cytometry. Samples were run in duplicate. In FIG. 14A, representative FL-2 histograms for isotype antibody (light grey tinted), 137G18A (black line) and Pagibaximab (dark grey tinted) binding to SH cells in the absence of serum (left histogram) or presence of 50% serum of one donor serum (right histogram) are shown. In FIG. 14B, for each sample, the mean fluorescence intensity (MFI) was determined. The MFI values for binding to SH cells in the absence of serum (0% serum control) were set as 100%. Then, the relative binding for the MFI average of the duplicates run for each tested condition in the presence of serum was calculated as percentage relative to the 0% serum control MFI value and plotted. In the graph the values for 5 individually tested donor serum samples and the mean $\pm$ standard error of the mean (SEM) are shown. “****” shows $p < 0.0005$, chimeric 137G18A vs. Pagibaximab as compared by two-tailed paired t-test.

FIG. 15 shows antigen-binding comparison between chimeric 137G18A and anti-LTA antibody Pagibaximab in human serum using MRSA BAA-41. *S. aureus* BAA-41 was grown overnight on Columbia CNA agar plates supplemented with 2.5% NaCl (w/v) and 0.1% glucose (w/v) at 37°C, washed and heat-killed. In 96-well plates, $\sim 1.4 \times 10^7$ bacterial particles/well were incubated in 100 μ L heat-inactivated rabbit serum at

4°C for 30 min to block unspecific antibody binding sites. Cells were then washed 3x in ice-cold PBS and resuspended in 100 µL biotinylated primary antibody at 0.1 µg/mL chimeric 137G18A or 1-10 µg/mL Pagibaximab in buffer alone (0% serum control) or 10% of human serum (individual serums from 5 healthy donors were tested) and incubated at 4°C for 30 min. Cells were then washed 3x in ice-cold PBS, resuspended in 100 µL of 2.5 µg/mL phycoerythrin-labeled streptavidin and incubated at 4°C for 30 min to detect antibodies bound to the BAA-41 cells. After washing, cells were fixed and the fluorescence intensities of ~20,000 bacterial particles per sample measured by flow cytometry. Samples were run in duplicate. For each sample, the mean fluorescence intensity (MFI) was determined. The MFI values for binding to MRSA cells in the absence of serum (0% serum control) were set as 100%. Then, the relative binding for the MFI average of the duplicates run for each tested condition in the presence of serum was calculated as percentage relative to the 0% serum control MFI value and plotted. In the graph the values for 5 individually tested donor serum samples and the mean ± standard error of the mean (SEM) are shown. “*” shows $p=0.008$, chimeric 137G18A vs. Pagibaximab as compared by two-tailed paired t-test. Results from two separately conducted experiments were combined for the data shown in the graph.

FIG. 16 shows activity comparison of chimeric 137G18A, anti-LTA antibody Pagibaximab, and anti-PNAG antibody F598 in opsonophagocytosis assays with mouse macrophages and MSSA Reynolds. 3.7×10^5 CFU of agar-grown *S. aureus* Reynolds cells were pre-opsonized with the indicated concentrations of either isotype antibody, anti-PNAG mAb F598, anti-LTA antibody Pagibaximab or chimeric anti-CP5 antibody 137G18A. The pre-opsonized bacteria were then co-incubated for 1 h with $\sim 2 \times 10^5$ J774 cells (mouse macrophage cell line). Subsequently, extracellular bacteria were killed by gentamycin, macrophages washed and lysed to release intracellular staphylococci. Bacteria were enumerated by plating serial dilutions on agar plates. Samples were run in duplicate and average ± SD values are shown in the graphs. Two experiments with similar results were conducted.

FIG. 17 shows activity comparison of chimeric 137G18A, anti-LTA antibody Pagibaximab, and anti-PNAG antibody F598 in opsonophagocytosis assays with mouse macrophages and MSSA Reynolds. 4.4×10^5 CFU of exponential phase *S. aureus* Reynolds cells were pre-opsonized with the indicated concentrations of either isotype antibody, anti-PNAG mAb F598, anti-LTA antibody Pagibaximab or chimeric anti-CP5 antibody 137G18A. The pre-opsonized bacteria were then co-incubated for 1 h with $\sim 2 \times 10^5$ J774 cells (mouse macrophage cell line). Subsequently, extracellular bacteria were killed by gentamycin, macrophages washed and lysed to release intracellular staphylococci. Bacteria were enumerated by plating serial dilutions on agar plates.

Samples were run in duplicate and average $\pm$ SD values are shown in the graphs. Two experiments with similar results were conducted.

FIG. 18 shows activity comparison of chimeric 137G18A, anti-LTA antibody Pagibaximab, and anti-PNAG antibody F598 in opsonophagocytosis assays with human PMN and VRSA COLVA. Fluorescein-labeled VRSA COLVA cells (Low CP5 expression) were incubated with PBS (vehicle) or 10 μ g/mL of either isotype human IgG₁ (hIgG₁) antibody, anti-LTA antibody Pagibaximab, or chimeric anti-CP5 antibody 137G18A in presence of peripheral mononuclear cell (PMN). After 15 min, ice-cold buffer was added to the mixture to stop phagocytosis. Then, PMN were washed twice and the percentage of PMN with intracellular SA was quantified using fluorescence microscopy. Ethidium bromide was added to the samples to distinguish between intra- and extracellular bacteria. Samples were run in triplicate and average $\pm$ SD data of one experiment of two performed which yielded similar results are shown. “***” shows $p < 0.0001$, anti-CP5 mAbs vs. vehicle, isotype mAb, and F598; “#” shows $p < 0.01$, 137G18A vs. Pagibaximab, unpaired two-tailed t-test.

FIG. 19 shows activity of chimeric anti-CP5 antibody 137G18A in opsonophagocytic killing assays with human PMN. $\sim 3.1 \times 10^7$ CFU/mL of agar-grown CP5 expressing *S. aureus* strain Lowenstein (MSSA) were co-incubated with 2.5×10^6 /mL purified human blood PMN (Ratio PMN to bacteria = 1:10) and 2.5 μ g/mL of the indicated human IgG₁ isotype control antibody or the chimeric anti-CP5 antibody 137G18A. As control, samples were run in parallel with addition of buffer instead of human neutrophils. After 90 min, the bacterial numbers were quantified by plating serial dilutions of the samples onto agar plates. Samples were run in quadruplicate and average CFU/mL values $\pm$ are shown in the graph. The dotted line indicates the CFU concentration at the 0 min time point ($\sim 3.1 \times 10^7$ CFU/mL). “*” shows $p < 0.01$, two-tailed unpaired t-test.

FIGs. 20A and 20B show *in vivo* efficacy comparison of chimeric 137G18A, anti-LTA antibody Pagibaximab, and anti-PNAG antibody F598 in mouse passive immunization experiments with MSSA strain Reynolds. ~ 4.5 h before intraperitoneal (i.p.) infection with (in FIG. 20A) 2.85×10^7 CFU or (in FIG. 20B) 4×10^7 CFU of agar-grown *S. aureus* Reynolds (High CP5 expression), female CD-1 mice were pre-treated i.p. with 200 μ L PBS containing (in FIG. 20A) 100 μ g or (in FIG. 20B) 1.8 mg of the indicated antibodies. The survival of the animals was monitored for 10-21 days after experimental infection. “*” shows $p < 0.05$; “***” shows survival curve comparisons of anti-CP5 antibody 137G18A vs. isotype antibody, Pagibaximab, and F598 with Mantel-Cox test.

FIGs. 21A and 21B show *in vivo* efficacy of chimeric 137G18A in two rat sepsis models. 24 hours before intraperitoneal infection with (in FIG. 21A) $2.9-3.5 \times 10^9$

or (in FIG. 21B) $1.4-1.6 \times 10^9$ CFU of the CP5 expressing *S. aureus* strain Reynolds, groups of 5-6 (in FIG. 21A) Sprague Dawley or (in FIG. 21B) Fischer F344 rats were pre-treated subcutaneously with 1 ml PBS containing the indicated amounts of control isotype human IgG1 (open circles) or chimeric anti-CP5 antibody 137G18A (closed circles). The survival of the rats was monitored for 7-10 days after infection. The results of two independent experiments were combined for each graph. “***” shows $p < 0.005$; Survival curve comparisons of isotype antibody vs. chimeric 137G18A with Mantel-Cox test.

FIGs. 22A and 22B show the sensorgrams from surface plasmon resonance analysis for the two anti-CP5 monoclonal antibodies including mouse/human chimeric 137G18A and the fucosylated Fc-engineered version (IgG1/IgG3) towards immobilized recombinant human Fc gamma receptor IIIa by using BIAcore 3000. In each sensorgram, Y-axis indicates the response unit and X-axis indicates the time of each binding experiment.

FIGs. 23 to 26, 27A and 27B show activity comparison of chimeric 137G18A and its Potelligent (registered trademark), Complegent (registered trademark), and AccretaMab (registered trademark) versions in complement deposition assays with human or rat serum and MSSA strain Lowenstein.

Specifically, FIG. 23 shows activity comparison of chimeric 137G18A and its Potelligent, Complegent, and AccretaMab versions in complement deposition assays with human or rat serum and MSSA strain Lowenstein.

FIG. 24 shows activity comparison of chimeric 137G18A and its Potelligent, Complegent, and AccretaMab versions in complement deposition assays with human or rat serum and MSSA strain Lowenstein; C1q dependence.

FIG. 25 shows activity comparison of chimeric 137G18A and its Potelligent, Complegent, and AccretaMab versions in complement deposition assays with human or rat serum and MSSA strain Lowenstein; C1q dependence.

FIG. 26 shows activity comparison of chimeric 137G18A and its Potelligent, Complegent, and AccretaMab versions in complement deposition assays with human or rat serum and MSSA strain Lowenstein; C1q dependence.

FIGs. 27A and 27B show activity comparison of chimeric 137G18A and its Potelligent, Complegent, and AccretaMab versions in complement deposition assays with human or rat serum and MSSA strain Lowenstein; C1q dependence.

In FIGs. 23 to 26, 27A and 27B, 5×10^8 CFU/mL of the CP5 strain *S. aureus* Lowenstein were incubated for 1-30 min at 37°C with shaking in 2.5% normal human serum, C1q-depleted human serum $\pm$ C1q, or rat serum in the presence or absence of 2.5 μ g/mL or 1.25 μ g/mL of the indicated negative control or chimeric anti-CP5 antibodies. Subsequently, the deposition of C3b which is indicative of complement deposition on the

bacteria was detected with FITC-labeled anti-human or anti-rat C3 antibodies. The mean fluorescence intensity of the cells was measured by flow cytometry. Samples were run in triplicate or hexuplicate and mean MFI values $\pm$ SD are shown in the graphs. “*” shows $p < 0.05$; “**” shows $p < 0.005$; “***” shows $p < 0.0005$, two-tailed unpaired t-test.

FIG. 28 shows activity comparison of chimeric 137G18A and its Potelligent, Comlegant, and AccretaMab versions in opsonophagocytosis assays with human PMN and MSSA Lowenstein. In 96-well plates, 5×10^6 fluorescein-labeled *S. aureus* Lowenstein particles were incubated for 5 min with 5×10^5 purified human blood PMN in the presence of 100 ng of the indicated negative control or chimeric anti-CP5 antibodies. Subsequently, the samples were washed and 100 live PMN/sample analyzed by fluorescence microscopy for phagocytosis of staphylococci. Ethidium bromide at an endconcentration of 0.25 mg/mL was added to the samples to be able to differentiate between intra- and extracellular bacteria. Samples were run in quadruplicate and average values $\pm$ SD of one representative experiment of three performed are shown in the graphs. “*” $p < 0.02$; “**” $p < 0.005$ vs. chimeric IgG1 version of c137G18A, unpaired Student's t-test.

FIG. 29 shows activity comparison of chimeric 137G18A and its Potelligent, Comlegant, and AccretaMab versions in opsonophagocytosis assays with human PMN and VRSA COLVA. In 96-well plates, 5×10^6 fluorescein-labeled *S. aureus* Lowenstein particles were incubated for 15 min with 5×10^5 purified human blood PMN in the presence of 100 ng of the indicated negative control or chimeric anti-CP5 antibodies. Subsequently, the samples were washed and 100 live PMN/sample analyzed by fluorescence microscopy for phagocytosis of staphylococci. Ethidium bromide at an endconcentration of 0.25 mg/mL was added to the samples to be able to differentiate between intra- and extracellular bacteria. Samples were run in triplicate and average values $\pm$ SD of one representative experiment of three performed are shown in the graphs. “*” shows $p < 0.02$; “***” shows $p < 0.005$ vs. chimeric IgG1 version of c137G18A, unpaired Student's t-test.

FIG. 30 shows activity comparison of chimeric 137G18A and AccretaMab 137G18A in opsonophagocytic killing assays with human PMN and MSSA Lowenstein. 3×10^7 CFU/mL SA Löwenstein were co-incubated with 2.5×10^6 /mL purified human blood neutrophils (Ratio PMN:SA= 1:10) and 1 μ g/mL of the indicated versions of chimeric anti-CP5 antibody 137G18A or an isotype negative control mAb. As control, samples were run in parallel with addition of buffer instead of human neutrophils. After 270 min, the bacterial numbers were quantified by plating serial dilutions of the samples on agar plates. Samples were run in quadruplicate and average CFU/mL values $\pm$ SD are shown in the

graph. The dotted line indicates the CFU concentration at the 0 min time point ($\sim 3 \times 10^7$ CFU/mL). “***” shows $p < 0.001$, “****” shows $p < 0.0001$, unpaired t-test.

FIG. 31 shows activity of chimeric Fuc+ hIgG1 and Fuc- hIgG1/IgG3 versions of chimeric anti-CP5 antibody 137G18A in RNU rat sepsis model. $6.8-7.2 \times 10^9$ CFU/mL of agar-grown *S. aureus* Reynolds were incubated for 30 min in 6 mL ice-cold PBS $\pm$ 1 μ g/mL of the indicated control or chimeric anti-CP5 antibodies. Then, 1 ml of the suspensions were injected intraperitoneally into groups of 3-5 immunodeficient RNU rats. The survival of the rats was monitored for 7 days after infection. The results of two independent experiments were combined for the graph.

FIG. 32 shows comparison of mouse anti-CP8 mAb binding to immobilized CP8 by ELISA. 96-well plates were coated overnight with purified CP8, then washed, and blocked. Mouse anti-CP8 mAbs were added at the indicated concentrations to the CP8 coated wells for 1 h. Isotype antibodies served as negative controls. Bound antibodies were detected with anti-mouse IgG gamma chain specific HRP-conjugated secondary antibody. After 1 h, TMB substrate was added. Reaction was stopped with sulfuric acid and the optical density (OD) read at 450 nm. Samples were run in triplicate and average OD values $\pm$ SD are shown.

FIG. 33 shows comparison of mouse anti-CP8 mAb binding to immobilized CP8 by FCM with F-182 strain. 5×10^8 CFU/mL of live agar-grown CP8 expressing strain F-182 incubated with the indicated concentrations of mouse isotype or anti-CP8 mAbs. Bound antibody was detected by standard methods using fluorescently labeled anti-mouse IgG subclass specific secondary antibody and flow cytometry.

FIGs. 34A and 34B show the sensorgrams from surface plasmon resonance analysis for the two anti-CP5 monoclonal antibodies including 138P2 and 138P10 towards immobilized CP8 by using BIAcore 3000. In each sensorgram, Y-axis indicates the response unit and X-axis indicates the time of each binding experiment.

FIGs. 35A and 35B show capturing of soluble CP8 antigens by mouse anti-CP8 mAbs. 96-well plates were coated overnight at 4°C with 100 μ L PBS containing 10 μ g/mL of the indicated mouse isotype control or anti-CP8 mAbs (= primary antibodies). Vehicle (PBS) alone served as negative control. Subsequently, the wells were washed three times with 200 μ L wash buffer (PBS, 0.05% Tween-20). Then, 200 μ L PBS, 1% casein was added to each well and the plate incubated for 2 h at 37°C to block unspecific binding sites. Subsequently, the wells were washed as above and then 100 μ L of 100 ng/mL CP8 in PBS was added to each well. The plate was incubated for 1 h at 37°C and then washed as above. Subsequently, to determine whether soluble CP8 was captured by the primary antibodies or unspecifically binds to vehicle treated wells, 100 μ L of 1 μ g/mL biotinylated mouse anti-CP8 mAb 138P2 (= detection antibody) in PBS, 0.9% casein was

added to each well. After 1 h incubation at 37°C, wells were washed as above. Then, 50 µL of streptavidin-conjugated Horse radish peroxidase (HRP) was added to each well and the plate incubated for 1 h at 37°C. Then, the wells were washed as above and 100 µL TMB substrate was added to each well. The reaction was stopped after 5 min by addition of 50 µL 2N sulfuric acid and the optical density of each well read at 450 nm (OD_{450nm}). Samples were run in quadruplicate and average OD_{450nm} values $\pm$ SD are shown. The higher the OD_{450nm} values, the more CP8 was captured by the respective antibodies. “****” shows $p < 0.0001$, mouse isotype vs. anti-CP8 mAb, unpaired two-tailed t-test.

FIG. 36 shows detection of soluble CP8 antigens in plasma taken from *S. aureus* infected animals. Five C57BL/6 mice were infected intraperitoneally with 1.1×10^8 colony forming units (CFU) of agar-grown CP8 expressing *S. aureus* Wright in 200 µL PBS. 2 days after infection, mice were euthanized and left kidneys and heparinized cardiac blood collected. Undiluted blood and serial blood dilutions were plated onto sheep agar plates to determine whether bacteria were present in the blood samples. Kidneys were homogenized and homogenates plated onto THY agar plates to enumerate the total bacterial burden. The CFU numbers in blood and kidneys for individual mice are shown in the table on the left (Detection limit in kidneys and blood: 100 CFU/mL). Plasma was isolated from remaining blood using standard procedures, then sterile filtered (0.22 µm), heat-inactivated and stored in aliquots at -80°C until use. In parallel, plasma from cardiac blood from three uninfected mice was prepared as above. CP8 in the plasma samples was detected by sandwich ELISA: A NUNC Maxisorp 96-well plate was coated overnight with 0.1 µg/well capture antibody (Mouse anti-CP8 mAb 138P2; 100 µL/well) at 4°C in 50 mM sodium carbonate/bicarbonate buffer. Then, wells were washed 3x with 200 µL TBST (PBS, 0.5% Tween-20). To block unspecific binding sites, 200 µL of a 1% casein solution was added to each well and the plate incubated for 2 hours at 37°C. After washing wells as above, 100 µL of test plasma samples diluted in PBS (25% plasma from individual infected mice or 25% pooled plasma from three uninfected mice without (negative control plasma) or with addition of 1 ng/mL purified CP8 (positive control plasma)) were added to the wells and the plate was incubated for 1 hour at 37°C. Wells were then washed as above and 50 µL of 1 µg/mL biotinylated mouse anti-CP8 mAb 138P2 in 1% casein (detection antibody) was added to each well. After 1-hour incubation at 37°C, wells were washed 3x as above. For detection of bound biotinylated 138P2, 50 µL of 0.2 µg/mL streptavidin conjugated horseradish peroxidase (Jackson ImmunoResearch) in PBS was added to the wells. After 1-hour incubation at 37°C, wells were washed 3x again and 100 µL/well TMB substrate was added. After 20 min, 50 µL 2 N sulfuric acid was added to stop the reaction. The absorbance of the wells

was read at 450 nm (OD_{450nm}) using a 96-well plate reader. Samples from infected mice and negative control plasma samples were run in duplicate and mean OD_{450nm} values $\pm$ SD data of one representative experiment of two performed are plotted in the graph on the right. A single well was run for the negative control sample as well as the positive control plasma sample (= negative control plasma + 1 ng/mL purified CP8 added to the sample). The higher the OD_{450nm} value for a given sample, the higher the degree of CP8 detection.

FIGs. 37A and 37B show activity of mouse anti-CP8 mAbs in opsonophagocytosis assays with mouse macrophages and CP8 expressing MSSA strain Wright. Activity of mouse anti-CP8 mAbs in opsonophagocytosis assays (OPAs) with the mouse macrophage cell line J774. 3.4×10^5 (in FIG. 37A) or 2.1×10^5 (in FIG. 37B) CFU of agar grown *S. aureus* Wright cells (High CP8 expression) were preopsonized with the indicated amounts of mouse negative control or anti-CP8 mAbs 138P2 (IgG₁) or 138P10 (IgG_{2b}) per reaction and then co-incubated with $\sim 2 \times 10^5$ J774 cells for 1 h. Subsequently, extracellular bacteria were killed by gentamicin. After washing, the J774 cells were lysed and intracellular *S. aureus* CFU enumerated. Samples were run in duplicate and the data are expressed as percentages of phagocytosed CFU relative to the initial inoculum; average values $\pm$ SD of one representative experiment are shown.

FIGs. 38A, 38B, 39A and 39B show *in vivo* efficacy evaluation of mouse anti-CP8 mAbs in mouse passive immunization model of systemic infection with MSSA strain Wright. 24 hours before intraperitoneal (i.p.) infection with either 7.4×10^7 CFU of *S. aureus* Wright, 5.1×10^8 CFU of *S. aureus* Becker or 6.15×10^7 CFU of MRSA strain F-182 in PBS, 5% mucin, CD-1 mice were pre-treated subcutaneously with 200 μ L PBS (vehicle) $\pm$ the indicated amounts of mouse isotype or anti-CP8 mAbs. The survival of the mice was monitored for 10 days after infection. “*” shows $p < 0.01$; “***” shows $p < 0.003$; “*****” shows $p < 0.0005$; Survival curve comparisons of anti-CP8 mAbs vs. isotype antibody or vehicle control with Mantel-Cox test.

FIG. 40 shows activity of mouse anti-CP8 mAb 138P10 in mouse kidney infection model of passive immunization. 24 hours before intraperitoneal infection with $\sim 1.5 \times 10^8$ CFU of the CP8 strain Wright 200 μ L PBS, C57BL/6 mice were pre-treated subcutaneously with the indicated amounts of mouse IgG2b isotype or anti-CP8 mAb 138P10. 48 hours after infection, remaining mice were euthanized, left kidneys excised, homogenized and bacterial CFU numbers determined. The results of two independent experiments were combined for the data shown in the graph; CFU numbers for each mouse and median values are plotted; the CFU detection limit in the kidneys was 100 CFU/kidney as indicated by the dotted line (“*****” shows $p < 0.0001$; CFU comparison of isotype antibody vs. 138P10 with Mann-two-tailed Whitney test). Please note, that a total of six

isotype-treated and one 138P2-treated mice succumbed to infection before the 48-h time point at which kidneys were collected.

FIGs. 41A and 41B show binding of chimeric anti-CP8 antibody 138P2 and 138P10 to immobilized CP8 and capturing of soluble CP8 by the antibodies. 96-well plates were coated overnight with purified CP8, then washed, and blocked. Mouse/human chimeric anti-CP8 mAb 138P2 was added at the indicated concentrations to the CP8 coated wells for 1 h. An isotype antibody served as negative controls. Bound antibodies were detected with anti-human IgG gamma chain specific HRP-conjugated secondary antibody. After 1 h, TMB substrate was added. Reaction was stopped with sulfuric acid and the optical density (OD) read at 450 nm. Samples were run in triplicate and average OD values $\pm$ SD are shown. FIG. 41B shows capturing of soluble CP8. 96-well plates were coated overnight at 4°C with 100 μ L PBS containing 10 μ g/mL of the indicated human isotype control or chimeric anti-CP8 antibodies (= primary antibodies). Vehicle (PBS) alone served as negative control. Subsequently, the wells were washed three times with 200 μ L wash buffer (PBS, 0.05% Tween-20). Then, 200 μ L PBS, 1% casein was added to each well and the plate incubated for 2 h at 37°C to block unspecific binding sites. Subsequently, the wells were washed as above and then 100 μ L of 100 ng/mL CP8 in PBS was added to each well. The plate was incubated for 1 h at 37°C and then washed as above. Subsequently, to determine whether soluble CP8 was captured by the primary antibodies or unspecifically binds to vehicle treated wells, 100 μ L of 1 μ g/mL biotinylated mouse anti-CP8 mAb 138P2 (= detection antibody) in PBS, 0.9% casein was added to each well. After 1 h incubation at 37°C, wells were washed as above. Then, 50 μ L of streptavidin-conjugated HRP was added to each well and the plate incubated for 1 h at 37°C. Then, the wells were washed as above and 100 μ L TMB substrate was added to each well. The reaction was stopped after 5 min by addition of 50 μ L 2N sulfuric acid and the optical density of each well read at 450 nm (OD_{450nm}). Samples were run in quadruplicate and average OD_{450nm} values $\pm$ SD are shown. The higher the OD_{450nm} values, the more CP8 was captured by the respective antibodies. “****” shows $p < 0.0001$, isotype vs. anti-CP8 antibody, unpaired two-tailed t-test.

FIGs. 42A and 42B show comparison of chimeric anti-CP8 antibody binding to live *S. aureus* cells by flow cytometry (MSSA ATCC-25923 and MRSA F-182). 5×10^8 CFU/mL of stationary phase CP8 expressing *S. aureus* strains ATCC-25923 (in FIG. 42A) or F-182 (in FIG. 42B) were incubated with the indicated concentrations of purified mouse/human chimeric anti-CP8 antibodies 138P2 or 138P10 or a human IgG₁ isotype control antibody. Bound antibody was detected by standard methods using fluorescently labeled anti-human IgG gamma chain specific secondary antibody and flow cytometry. Samples were run in duplicate and the averages of the mean fluorescent intensity (MFI) $\pm$

SD are shown. Representative results of one experiment of two performed with similar results are shown.

FIG. 43 shows activity comparison of chimeric anti-CP8 antibodies 138P2 and 138P10, and anti-LTA antibody Pagibaximab in opsonophagocytosis assays with mouse macrophages and MSSA Becker. 4.7×10^5 CFU of agar-grown *S. aureus* Becker cells (High CP8 expression) were pre-opsonized with the indicated concentrations of either isotype antibody, anti-LTA antibody Pagibaximab or chimeric anti-CP8 antibodies 138P2 or 138P10. The pre-opsonized bacteria were then co-incubated for 1 h with $\sim 2 \times 10^5$ J774 cells (mouse macrophage cell line). Subsequently, extracellular bacteria were killed by gentamycin, macrophages washed and lysed to release intracellular staphylococci. Bacteria were enumerated by plating serial dilutions on agar plates. Samples were run in duplicate and average $\pm$ SD values are shown in the graphs. Two experiments with similar results were conducted.

FIGs. 44 and 45 show activity comparison of chimeric anti-CP8 antibodies 138P2 and 138P10, anti-LTA antibody Pagibaximab, and anti-PNAG antibody F598 in opsonophagocytosis assays with human PMN and MSSA Becker and MRSA F-182, respectively. MSSA Becker cells (High CP8 expression) or MRSA F-182 cells (Low CP8 expression) were incubated with PBS (vehicle) or 10 μ g/mL of either isotype human IgG₁ (hIgG₁) antibody, anti-PNAG mAb F598, chimeric anti-LTA antibody Pagibaximab, or chimeric anti-CP8 antibodies 138P2 or 138P10 and freshly purified human blood neutrophils at a ratio of 10:1 in the absence of a complement source. After 15 min, ice-cold buffer was added to the mixture to stop phagocytosis. Then, PMN were washed twice and the percentage of PMN with intracellular SA was quantified using fluorescence microscopy. Ethidium bromide was added to the samples to distinguish between intra- and extracellular bacteria. Samples were run in triplicate and average $\pm$ SD data of one experiment of two performed which yielded similar results are shown. “*” shows $p < 0.02$; “***” shows $p < 0.005$, anti-CP5 antibodies vs. vehicle, isotype mAb, F598, and Pagibaximab, unpaired two-tailed t-test.

Description of Embodiments

The present invention relates to the following (1) to (14):

- (1) A monoclonal antibody and antigen-binding fragment thereof, which recognizes an epitope comprising mannosaminuronic acid residue of CP and binds to CP expressed on *S. aureus*.
- (2) The monoclonal antibody and antigen-binding fragment thereof according to (1), wherein the antibody binds to at least one CP selected from CP type 5 (CP5) and CP type 8 (CP8).

(3) The monoclonal antibody and antigen-binding fragment thereof according to (1), wherein the antibody has a binding affinity for CP5 less than 10 nM.

(4) The monoclonal antibody and antigen-binding fragment thereof according to (1), wherein the antibody has a binding affinity for CP8 less than 20 nM.

5 (5) The monoclonal antibody and antigen-binding fragment thereof according to (1), wherein the antibody is any one of the antibody selected from (i) to (vi):

(i) a monoclonal antibody comprising CDRs 1 to 3 of a VH comprising amino acid sequences of SEQ ID NOs:1 to 3, respectively, and CDRs 1 to 3 of a VL comprising amino acid sequences of SEQ ID NOs:4 to 6, respectively;

10 (ii) a monoclonal antibody comprising a VH comprising the amino acid sequence of SEQ ID NO.13 and a VL comprising the amino acid sequence of SEQ ID NO:14;

(iii) a monoclonal antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:15 and a VL comprising the amino acid sequence of SEQ ID
15 NO:14;

(iv) an antibody which competes with the monoclonal antibody according to (i);

(v) an antibody which comprises an epitope bound by the monoclonal antibody according to (i);

20 (vi) an antibody which binds to the same epitope bound by the monoclonal antibody according to (i).

(6) The monoclonal antibody and antigen-binding fragment thereof according to (1), which is any one of the antibody selected from (i) to (v):

25 (i) a monoclonal antibody comprising CDRs 1 to 3 of VH comprising amino acid sequences of SEQ ID NOs:7 to 9, respectively, and CDRs 1 to 3 of VL comprising amino acid sequences of SEQ ID NOs:10 to 12, respectively;

(ii) a monoclonal antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:16 and a VL comprising the amino acid sequence of SEQ ID
30 NO:17;

(iii) an antibody which competes with the monoclonal antibody according to (i);

(iv) an antibody which comprises an epitope bound by the monoclonal antibody according to (i);

35 (v) an antibody which binds to the same epitope bound by the monoclonal antibody according to (i).

(7) The monoclonal antibody and antigen-binding fragment thereof according to (1), which is a recombinant antibody and antigen-binding fragment thereof.

(8) The recombinant antibody and antigen-binding fragment thereof according to (7), which is at least one antibody selected from a chimeric antibody, a humanized antibody and human antibody.

(9) A DNA encoding amino acid sequence of the monoclonal antibody and antigen-binding fragment thereof according to (1).

(10) A method for producing the monoclonal antibody and antigen-binding fragment thereof according to (1).

(11) A method for treating an infectious disease caused by *S. aureus* which comprises administration of the monoclonal antibody or antigen-binding fragment thereof according to (1).

(12) The method for treating an infectious disease caused by *S. aureus* according to (11), wherein the infectious disease is at least one disease selected from sepsis, septic shock, bacteremia, pneumonia, skin ulcer, atopic dermatitis, meningitis, enteritis, cholangiolitis and endocarditis.

(13) The method for treating an infectious disease caused by *S. aureus* according to (11), wherein *S. aureus* is resistant to at least one antibiotic selected from methicillin and vancomycin.

(14) The method for treating an infectious disease caused by *S. aureus* according to (11), wherein the method is combined with administration of at least one antibiotic.

(15) A recombinant vector which comprises the DNA according to (9).

(16) A transformant obtainable by introducing the recombinant vector according to (15) into a host cell.

(17) A process for producing the monoclonal antibody or the antigen-binding fragment thereof according to (1), which comprises culturing the transformant according to (16) to form and accumulate the monoclonal antibody or the antigen-binding fragment thereof according to (1) in the culture, and collecting the antibody or the antigen-binding fragment thereof from the culture.

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The present invention can provide a monoclonal antibody or an antigen-binding fragment thereof, which specifically recognizes the epitope comprising mannosaminuronic acid residue of CP and binds to *Staphylococcus aureus* capsular polysaccharide (SACP) [ex. type 5 (CP5) or type 8 (CP8)], a monoclonal antibody binds to the antigen with a high affinity (less than 20 nM, in dissociation constant, K_D), and these antibodies also exhibit a high phagocytotic activity and/or a high complement-deposition activity against *Staphylococcus aureus*; a hybridoma which produces the antibody; a DNA

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which encodes the antibody; a vector which comprises the DNA; a transformant obtainable by introducing the vector; a process for producing the antibody or the antigen-binding fragment thereof using the hybridoma or the transformant; a method for treating infectious diseases caused by *S. aureus*; a therapeutic agent and a diagnostic agent using the antibody or the antigen-binding fragment thereof.

In the present invention, *Staphylococcus aureus* includes any kind of *Staphylococcus aureus* and any phenotype of *Staphylococcus aureus* as long as capsular polysaccharide expressed on cell surface. Furthermore *Staphylococcus aureus* resistant to at least one antibiotic can be included in the target of the antibodies of the present invention. For the antibiotics, methicillin and vancomycin can be included.

Staphylococcus aureus capsular polysaccharide type 5 or type 8 of the present invention includes a carbohydrate comprising the chemical formula represented by $[-\rightarrow 4)-3-O-Ac-\beta-D-ManpNAcA-(1\rightarrow 4)-\alpha-L-FucpNAc-(1\rightarrow 3)-\beta-D-FucpNAc-(1\rightarrow)n$ and $[-\rightarrow 3)-4-O-Ac-\beta-D-ManpNAcA-(1\rightarrow 3)-\alpha-L-FucpNAc-(1\rightarrow 3)-\beta-D-FucpNAc-(1\rightarrow)n$, respectively. The nucleotide sequences of both the cap5 and cap8 gene clusters relating to expression of the capsular polysaccharide type 5 and type 8 have been determined (GenBank accession numbers U81973 and U73374, respectively). The cap5 and cap8 loci are allelic and comprise a 17.5-kb region of the chromosome; each contains 16 closely linked genes, cap5A through cap5P and cap8A through cap8P, transcribed in one orientation [*CLINICAL MICROBIOLOGY REVIEWS*, 2004, 218–234]. Both CP5 and CP8 are composed of the same three sugar residues, ManNAcA, L-FucNAc, and D-FucNAc. Therefore, it is not surprising that 12 of the 16 genes in the two gene clusters are nearly identical. The type-specific genes are located in the central region of the loci (comprising cap5H, cap5I, cap5J, and cap5K and the same genes for cap8), and they showed little homology between the two gene clusters. Wann et al. transduced cap5HIJK into the type 8 strain P1; the genes were integrated into the chromosome by homologous recombination, with the reciprocal loss of cap8HIJK. The resultant strain produced CP5, indicating that cap5HIJK was responsible for CP5 serotype specificity [*FEMS Microbiol. Lett* 1999, 170:97–103].

The regulatory loci agr and staphylococcal accessory regulator (sarA) control the expression of many *S. aureus* adhesins and exoproteins. The agr locus is a complex multigene system that responds to bacterial cell density. At high cell densities, a secreted octapeptide binds to the membrane receptor AgrC, activating the AgrA regulator by a phosphorylation mechanism. Phosphorylated AgrA upregulates promoters within the agr locus to produce the regulatory effector molecule RNA III. The sarA gene product activates the agr promoter as well as the promoters of certain other virulence genes, independently of agr. SarA binds to a consensus motif upstream of the -35 sequences of

those promoters that it regulates. It was demonstrated that agr positively regulated CP5 production by *S. aureus*. Subsequently, The agr and sarA single mutants and an agr-sarA double mutant from the serotype 8 strain Becker were constructed. The agr mutant showed minimal CP8 production and minimal cap8 gene transcription, confirming that agr is a major regulator of CP8 expression. The results of gene fusion studies indicated that regulation by agr was exerted at the transcriptional level. The sarA gene was also shown to activate cap8 gene expression at the transcriptional level, but the effect was only minor compared to that of agr. Likewise, it was reported that agr positively regulated cap5 expression both *in vitro* and *in vivo* in a rabbit endocarditis model. In comparison, sarA exerted a lesser positive impact on cap5 expression. A novel global regulator called mgr is reported that bears homology to the MarR family of transcriptional regulators. mgr upregulated CP8 and nuclease expression but repressed alpha toxin, coagulase, protease, and protein A expression [*FEMS Microbiol. Lett* 1999, 170:97-103].

The polypeptide comprising an amino acid sequence in which one or more amino acid residue(s) is/are deleted, substituted and/or added in the amino acid sequence represented by GenBank accession numbers U81973 and U73374 can be obtained, for example, by introducing a site-specific mutation into DNA encoding a polypeptide comprising the amino acid sequence represented by GenBank accession numbers U81973 and U73374 by site-specific mutagenesis [*Molecular Cloning, A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press (1989), *Current Protocols in Molecular Biology*, John Wiley & Sons (1987-1997), *Nucleic Acids Research*, 10, 6487 (1982), *Proc. Natl. Acad. Sci. USA*, 79, 6409 (1982), *Gene*, 34, 315 (1985), *Nucleic Acids Research*, 13, 4431 (1985), or *Proc. Natl. Acad. Sci. USA*, 82, 488 (1985)] or the like. The number of amino acid residues which are deleted, substituted or added is not particularly limited, and the number is preferably, 1 to dozens, such as 1 to 20, and more preferably 1 to several, such as 1 to 5.

As a gene encoding cap5 and cap8 clusters, the nucleotide sequence represented by GenBank accession numbers U81973 and U73374 may be included. As the gene encoding cap5 and cap8 clusters, the gene encoding cap5 and cap8 clusters of the present invention also included a gene containing a DNA comprising a nucleotide sequence having deletion(s), substitution(s) or addition(s) of one or more nucleotides in the nucleotide sequence represented by GenBank accession numbers U81973 and U73374 and also encoding cap5 and cap8 clusters; a gene containing a DNA consisting of a nucleotide sequence having at least 60% or higher homology, preferably 80% or higher homology, and more preferably 95% or higher homology, with the nucleotide sequence represented by GenBank accession numbers U81973 and U73374, and also encoding polypeptides having the function of cap5 and cap8 clusters; a gene consisting of a DNA which hybridizes with a

DNA having the nucleotide sequence represented by GenBank accession numbers U81973 and U73374 under stringent conditions and also containing a DNA that encodes a polypeptide having the partial function of cap5 and cap8 clusters; and the like.

In the present invention, the DNA which hybridizes under stringent conditions refers to a DNA which is obtained by colony hybridization, plaque hybridization, Southern blot hybridization, DNA microarray or the like using a DNA having the nucleotide sequence represented by GenBank accession numbers U81973 and U73374 as a probe. A specific example of such DNA is a hybridized colony- or plaque derived DNA which can be identified by performing hybridization at 65°C in the presence of 0.7 to 1.0 mol/L sodium chloride using a filter or slide glass with the PCR product or oligo DNA having immobilized thereon, and then washing the filter or slide glass at 65°C with a 0.1 to 2-fold concentration SSC solution (1-fold concentration SSC solution: 150 mmol/L sodium chloride and 15 mmol/L sodium citrate). Hybridization can be carried out according to the methods [*Molecular Cloning, A Laboratory Manual*, Second Edition, Cold Spring Harbor Lab. Press (1989), *Current Protocols in Molecular Biology*, John Wiley & Sons (1987-1997); *DNA Cloning 1: Core Techniques, A Practical Approach*, Second Edition, Oxford University (1995)] and the like. Specifically, the DNA capable of hybridization includes DNA having at least 60% or more homology, preferably 80% or more homology, more preferably 90% or more homology, and most preferably 95% or more homology to the nucleotide sequence represented by GenBank accession numbers U81973 and U73374.

In the nucleotide sequence of the gene encoding a protein of a prokaryote, genetic polymorphism is often recognized. The cap5 and cap8 cluster gene used in the present invention also includes a gene in which small modification is generated in the nucleotide sequence by such polymorphism as the gene used in the present invention.

The number of the homology in the present invention may be a number calculated by using a homology search program known by the skilled person, unless otherwise indicated. Regarding the nucleotide sequence, the number may be calculated by using BLAST [*J. Mol. Biol.*, 215, 403 (1990)] with a default parameter or the like, and regarding the amino acid sequence, the number may be calculated by using BLAST2 [*Nucleic Acids Res.*, 25, 3389 (1997); *Genome Res.*, 7, 649 (1997) with a default parameter; <http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/information3.html>] or the like.

As the default parameter, G (cost to open gap) is 5 for the nucleotide sequence and 11 for the amino acid sequence; -E (cost to extend gap) is 2 for the nucleotide sequence and 1 for the amino acid sequence; -q (penalty for nucleotide mismatch) is -3; -r (reward for nucleotide match) is 1; -e (expect value) is 10; -W (wordsize) is 11 residues for the nucleotide sequence and 3 residues for the amino acid sequence; -y [dropoff (X) for

blast extensions in bits] is 20 for blastn and 7 for a program other than blastn; -X (X dropoff value for gapped alignment in bits) is 15; and -Z (final X dropoff value for gapped alignment in bits) is 50 for blastn and 25 for a program other than blastn (<http://www.ncbi.nlm.nih.gov/blast/html/blastcgihelp.html>).

5 The polypeptide comprising a partial sequence of the amino acid sequence represented by GenBank accession numbers U81973 and U73374 can be prepared according to a method known by the skilled person. For example, it can be prepared by deleting a part of DNA encoding the amino acid sequence represented by GenBank
10 accession numbers U81973 and U73374 and culturing a transformant into which an expression vector containing the DNA is introduced. Also, based on the polypeptide or DNA prepared by using the above method, a polypeptide comprising an amino acid sequence in which one or more amino acid(s) is/are deleted, substituted or added in a partial sequence of the amino acid sequence represented by GenBank accession numbers
15 U81973 and U73374 can be prepared in the same manner as described above. In addition, the polypeptide comprising a partial sequence of the amino acid sequence represented by GenBank accession numbers U81973 and U73374; or a polypeptide comprising an amino acid sequence in which one or more amino acid(s) is/are deleted, substituted or added in a partial sequence of the amino acid sequence represented by NCBI
20 reference sequence GenBank accession numbers U81973 and U73374 can be produced by a chemical synthesis method such as fluorenylmethoxycarbonyl (Fmoc) method or t-butyloxycarbonyl (tBoc) method.

 The function of capsular polysaccharide in the present invention means that capsular polysaccharide is one of the important cell wall-anchored virulence factors of *S. aureus*. The CP5 strain was shown to be more virulent than an isogenic, acapsular mutant
25 in mouse models including renal abscess formation model, subcutaneous abscess formation model, and septic arthritis model. *S. aureus* capsular polysaccharide masks complement C3 fragment deposited on *S. aureus* cells from binding to complement receptor 1 expressed on phagocytes, and inhibits complement activation and deposition, and inhibits phagocytosis by host immune cells, and facilitates intraabdominal abscess formation in
30 animals. Structural studies of CP8 revealed that it has a zwitterionic charge motif conferred by the negatively charged carboxyl group of N-acethylmannosaminuronic acid and free amino groups available on partially N-acethylated fucosamine residues. Chemical modifications that neutralized the charged groups on CP8 abrogated its ability to provoke abscess *in vivo*, indicating that this charge motif was critical for the CP8
35 biological activity [*FEMS Microbiol. Lett* 1999, 170:97–103]. Interestingly, once phagocytosed, the CP5-positive strain was less efficiently killed than the acapsule mutant strain [*Infect. Immun.* 1999, 65:4216–4221].

Binding of the antibody or antigen-binding fragment of the present invention to *S. aureus* capsular polysaccharide type 5 or type 8 can be confirmed by a method in which the binding ability of a cell expressing a specified antigen and an antibody for the specific antigen can be examined, for example, by a radioimmunoassay using a solid phase sandwich method or the like, or a conventionally known immunological detecting method for a cell expressing CP using an enzyme immuno assay (ELISA) method, preferably a fluorescent cell staining method or the like. Examples include a fluorescent antibody staining method using the FMAT8100HTS system (manufactured by Applied Biosystem), [Cancer Immunol. Immunother., 36, 373 (1993)], a fluorescent cell staining method using a flow cytometry, a surface plasmon resonance using the Biacore system (manufactured by GE Healthcare) and the like. In addition, it can also be confirmed by a combination of conventionally known immunological detecting methods [Monoclonal Antibodies-Principles and Practice, Third edition, Academic Press (1996), Antibodies-A Laboratory Manual, Cold Spring Harbor Laboratory (1988), Monoclonal Antibody Experiment Manual, Kodansha Scientific (1987)] and the like.

In the present invention, as a cell expressing CP5 and/or CP8 includes any cells which so long as express CP5 or CP8. Examples include a cell of *Staphylococcus aureus*, and a recombinant cell which is produced by using gene recombinant techniques.

The cell which infects in the human body includes a cell expressing CP5 and/or CP8 in the body of a patient suffering from infectious disease, at-a-risk patient in ventilation, surgical operation, organ transplantation, trauma, burn, and immunocompromised conditions.

Specific examples of the cell obtained by using gene recombination technique includes a cell expressing CP, which is prepared by introducing an expression vector comprising cDNA encoding cap5 and cap8 gene clusters into a CP-deficient bacterial cell, etc., and the like.

Examples of the antibody of the present invention include a monoclonal antibody or an antigen-binding fragment thereof against *S. aureus* CP5 or CP8 which has a dissociation constant (hereinafter, referred to as " K_D ") less than 10×10^{-9} M of the antibody to CP5 or less than 20×10^{-9} M of the antibody to CP8, binds to various CP forms including cell surface CP, soluble CP, and immobilized CP with high affinity and has high antibody-dependent phagocytotic activity and a high complement-deposition activity.

The monoclonal antibody of the present invention includes an antibody produced by a hybridoma and a recombinant antibody produced by a transformant transformed with an expression vector containing a gene encoding the antibody.

The monoclonal antibody is an antibody secreted by a single clone antibody-producing cell, and recognizes only one epitope (also called antigen determinant) and has uniform amino acid sequence (primary structure).

In the present invention, the monoclonal antibody has a structure comprising a heterotetramer consisting of two H chains and two L chains. A H chain comprises a H chain variable region (VH) and a H chain constant region (CH); and a L chain comprises a L chain variable region (VL) and a L chain constant region (CL). In addition, CH comprises four domains: CH1 domain, hinge domain, CH2 domain and CH3 domain. Furthermore, a domain consisting of CH2 domain and CH3 domain together is defined as “Fc region”, “Fc domain” or simply “Fc” of an antibody.

Examples of the epitope include a single amino acid sequence, a three-dimensional structure comprising the amino acid sequence, a sugar chain-bound amino acid sequence, a three-dimensional structure comprising a sugar chain-bound amino acid sequence, and the like, recognized and bound by a monoclonal antibody. Examples of the epitope of the monoclonal antibody of the present invention include the epitope comprising mannosaminuronic acid residue of CP, preferably the epitope comprising mannosaminuronic acid residue of *S. aureus* capsular polysaccharide type 5 (SACP5) or type 8 (SACP8). Furthermore, the epitopes of the present invention include the epitope as bound by at least one antibody selected from an anti-CP5 mouse monoclonal antibody, which is any one of the antibody selected from

(i) an antibody which is the monoclonal antibody comprising CDRs 1 to 3 of VH comprising amino acid sequences of SEQ ID NOs:1 to 3, respectively, and CDRs 1 to 3 of VL comprising amino acid sequences of SEQ ID NOs:4 to 6, respectively,

(ii) an antibody which is the monoclonal antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:13 and a VL comprising the amino acid sequence of SEQ ID NO:14,

(iii) an antibody which is the monoclonal antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:15 and a VL comprising the amino acid sequence of SEQ ID NO:14,

(iv) an antibody which competes with the antibody thereof according to (i), and

(v) an antibody which comprises an epitope as bound by the antibody thereof according to (i) and (vi) an antibody which binds to the same epitope as bound by the antibody thereof according to (i), or (B) anti-CP8 mouse monoclonal antibody which is any one of the antibody selected from

(i) an antibody which is the monoclonal antibody comprising CDRs 1 to 3 of VH comprising amino acid sequences of SEQ ID NOs:7 to 9, respectively, and CDRs 1 to 3 of VL comprising amino acid sequences of SEQ ID NOs:10 to 12, respectively,

(ii) an antibody which is the monoclonal antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:16 and a VL comprising the amino acid sequence of SEQ ID NO:17,

(iii) an antibody which competes with the antibody thereof according to (i),

5 (iv) an antibody which comprises an epitope as bound by the antibody thereof according to (i), and

(v) an antibody which binds to the same epitope as bound by the antibody thereof according to (i).

10 The antibodies of the present invention can recognize a distinct conformational structure between CP5 and CP8 based on the bond of O-acetylation of the mannosaminuronic acid of CP, and specifically bind to each CP expressed on *S. aureus*, although the other polysaccharide structures are identical between CP5 and CP8. As a result of antibody binding to the epitope, therapeutic effect of the antibody is available.

15 In the present invention, an antibody which binds to *S. aureus* CP with high affinity is an antibody which has enough affinity for a therapeutic antibody, preferably an antibody which binds to CP of *Staphylococcus aureus* strain Reynolds or Wright, with dissociation constant K_D value less than 20×10^{-9} M.

20 Affinity is measured by kinetic analysis, and for example, can be measured by using a Biacore T100 and Biacore 3000 (manufactured by GE Healthcare Bio-Sciences), Octet (manufactured by ForteBio) or the like.

In the present invention, the term "dissociation is slow" means that a value of a dissociation rate constant k_d of an antibody calculated by Biacore T100, Biacore 3000 or Octet has a smaller value. The smaller dissociation rate constant represents that an antibody does not easily dissociate from an antigen-expressing cell. By increasing an amount of an antibody binding to the cell surface to thereby extend the effective time of the antibody, then a high medicinal efficacy can be expected. A dissociation rate constant k_d is measured, for example, using a Biacore T100, Biacore 3000, or Octet and can be calculated by software attached to the apparatus, Biacore T100 evaluation software (manufactured by Biacore), or the like.

30 In the present invention, the term "antibody-dependent phagocytotic activity" refers to an activity which leads to the phagocytosis to a target bacterial cell by such a manner that an antibody bound to an antigen on the target cell binds to an Fc receptor of an immune cell through an Fc region of the antibody, consequently resulting in the activation of the immune cell (neutrophil, macrophage, etc.).

35 A Fc receptor (hereinafter referred to as "FcR") is a receptor which binds to Fc region of an antibody and leads to various effector activities by binding to the antibody. An FcR corresponds to a subclass of an antibody, and IgG, IgE, IgA and IgM specifically

binds to FcγR, FcεR, FcαR and FcμR, respectively. Moreover, an FcγR has subtypes of FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) and these have isoforms of FcγRIA, FcγRIB, FcγRIC, FcγRIIA, FcγRIIB, FcγRIIC, FcγRIIIA and FcγRIIIB, respectively.

The above different FcγRs exist on different cells (*Annu. Rev. Immunol.*, 9:457-492

5 (1991)). In human, FcγRI specifically expresses on a macrophage and FcγRIIA specifically expresses on a neutrophil, macrophage and FcγRIIIB specifically expresses on a neutrophil and FcγRIIIA expresses on a monocyte, a Natural Killer cell (NK cell) and some part of T cell. Binding of an antibody through FcγRI, FcγRIIA, FcγRIIIB, leads to an immune cell-dependent phagocytotic activity.

10 In the present invention, the term “complement deposition activity” refers to an activity which leads to the complement C3b deposition on a target bacterial cell by such a manner that an antibody bound to an antigen on the target cell activates a series of cascades (complement activation pathways) containing complement-related protein groups in blood. In addition, protein fragments generated by the activation of a complement can induce the
15 migration, phagocytosis and activation of immune cells.

Further, among the antibodies of the present invention, examples of an antibody which recognizes *S. aureus* CP5 include a monoclonal antibody in which VH of the antibody comprises the amino acid sequences of CDRs 1 to 3 represented by SEQ ID
20 NOs:1 to 3, respectively, and VL of the antibody comprises the amino acid sequences of CDRs 1 to 3 represented by SEQ ID NOs:4 to 6, respectively, and the like. Examples of an antibody which recognizes *S. aureus* CP8 include a monoclonal antibody in which VH of the antibody comprises the amino acid sequences of CDRs 1 to 3 represented by SEQ ID NOs:7 to 9, respectively, and VL of the antibody comprises the amino acid sequences of CDRs 1 to 3 represented by SEQ ID NOs:10 to 12, respectively, and the like.

25 Moreover, specific examples of the anti-CP5 monoclonal antibody of the present invention include a monoclonal antibody wherein VH of the antibody comprises the amino acid sequence represented by SEQ ID NO:13 or NO:15 and VL of the antibody comprises the amino acid sequence represented by SEQ ID NO:14 and the like. Specific examples of the anti-CP8 monoclonal antibody of the present invention include a
30 monoclonal antibody wherein VH of the antibody comprises the amino acid sequence represented by SEQ ID NO:16 and VL of the antibody comprises the amino acid sequence represented by SEQ ID NO:17 and the like.

In addition, examples of the monoclonal antibody of the present invention include a monoclonal antibody which competes with the above monoclonal antibody in the
35 binding of the *S. aureus* CP, a monoclonal antibody which binds to the same epitope as an epitope in a *S. aureus* CP to which the above monoclonal antibody binds.

The hybridoma can be prepared, for example, by preparing the above *Staphylococcus aureus* cell or purified soluble CP as an antigen (immunogen), inducing an antibody-producing cell having antigen specificity from an animal immunized with the antigen, and fusing the antibody -producing cell with a myeloma cell. The anti-CP monoclonal antibody can be obtained by culturing the hybridoma or administering the hybridoma cell into an animal to cause ascites tumor in the animal and separating and purifying the culture or the ascites.

The animal immunized with an antigen may be any animal, so long as a hybridoma can be prepared, and mouse, rat, hamster, chicken, rabbit, or the like is suitably used. Also, the antibody of the present invention includes an antibody produced by a hybridoma obtained by fusion of the cell having antibody-producing activity can be obtained from such an animal, and immune *in vitro* with a myeloma cell.

In the present invention, the recombinant antibody includes an antibody produced by gene recombination, such as a human chimeric antibody, a humanized antibody (CDR-grafted antibody), a human antibody and an antigen-binding fragment thereof. Among the recombinant antibodies, one having characters of a monoclonal antibody, low immunogenicity and prolonged half-life in blood is preferable as a therapeutic agent. Examples of the recombinant antibody include an antibody in which the above monoclonal antibody of the present invention is modified by gene recombination technology.

CH of the recombinant antibody of the present invention is preferably of human origin and includes CH1 domain, hinge domain, CH2 domain and CH3 domain.

Fc region of the recombinant antibody of the present invention may include one or more amino acid modification, so long as it has binding activity to FcγRs.

The human chimeric antibody is an antibody comprising a heavy chain variable region VH and a light chain variable region VL of an antibody of a non-human animal and CH and CL of a human antibody. Specifically, the human chimeric antibody of the present invention can be produced by obtaining cDNAs encoding VH and VL from a hybridoma which produces a monoclonal antibody which specifically recognizes *S. aureus* CP, inserting each of them into an expression vector for animal cell comprising DNAs encoding CH and CL of human antibody to thereby construct a vector for expression of human chimeric antibody, and then introducing the vector into an animal cell to express the antibody.

As the CH of the human chimeric antibody, any CH can be used, so long as it belongs to human immunoglobulin (hereinafter referred to as "hIg"), and those belonging to the hIgG class are preferred, and any one of the subclasses belonging to the hIgG class, such as hIgG1, hIgG2, hIgG3 and hIgG4, can be used. Additionally an engineered

constant region of human IgG1/IgG3 chimeric isotypes can be also used [*Cancer Res.* 2008 May 15;68(10):3863-72.]. As the CL of the human chimeric antibody, any CL can be used, so long as it belongs to the hIg class, and those belonging to κ class or λ class can be used.

5 Specific examples of the mouse/human chimeric anti-CP5 antibody of the present invention include a human chimeric antibody wherein VH of the antibody comprises the amino acid sequence represented by SEQ ID NO:13 or 15 and VL of the antibody comprises the amino acid sequence represented by SEQ ID NO:14, and the like. Specific examples of the mouse/human chimeric anti-CP8 antibody of the present

10 invention include a human chimeric antibody wherein VH of the antibody comprises the amino acid sequence represented by SEQ ID NO:16 and VL of the antibody comprises the amino acid sequence represented by SEQ ID NO:17, and the like.

In addition, examples of the chimeric antibody of the present invention include a chimeric antibody which competes with the above chimeric antibody in the binding of *S. aureus* capsular polysaccharide type 5 or type 8 and a chimeric antibody which binds to the same epitope as an epitope in the *S. aureus* CP to which the above chimeric antibody binds.

A humanized antibody is an antibody in which amino acid sequences of CDRs of VH and VL of an antibody derived from a non-human animal are grafted into appropriate positions of VH and VL of a human antibody. The humanized antibody of the present invention can be produced by constructing cDNAs encoding a V region in which the amino acid sequences of CDRs of VH and VL of an antibody derived from a non-human animal produced by a hybridoma which produces a monoclonal antibody which specifically recognizes and binds to *S. aureus* CP are grafted into frame work region (hereinafter referred to as "FR") of VH and VL of any human antibody, inserting each of them into a vector for expression of animal cell comprising genes encoding CH and CL of a human antibody to thereby construct a vector for expression of humanized antibody, and introducing it into an animal cell to thereby express and produce the humanized antibody.

As the CH of the humanized antibody, any CH can be used, so long as it belongs to the hIg class, and those of the hIgG class are preferred and any one of the subclasses belonging to the hIgG class, such as hIgG1, hIgG2, hIgG3 and hIgG4 can be used. Additionally an engineered constant region of human IgG1/IgG3 chimeric isotypes can be also used [*Cancer Res.* 2008 May 15;68(10):3863-72.]. As the CL of the humanized antibody, any CL can be used, so long as it belongs to the hIg class, and those belonging to the κ class or λ class can be used.

Examples of the humanized anti-CP5 antibody of the present invention include a humanized antibody wherein CDRs 1 to 3 of VH of the antibody comprise the amino

acid sequences represented by SEQ ID NOs:1 to 3, respectively, and CDRs 1 to 3 of VL of the antibody comprise the amino acid sequences represented by SEQ ID NOs:4 to 6.

Examples of the humanized anti-CP8 antibody of the present invention include a humanized antibody wherein CDRs 1 to 3 of VH of the antibody comprise the amino acid sequences represented by SEQ ID NOs:7 to 9, respectively, and CDRs 1 to 3 of VL of the antibody comprise the amino acid sequences represented by SEQ ID NOs:10 to 12.

In addition, examples of the humanized antibody of the present invention include a humanized antibody which competes with the above humanized antibody in the binding of *S. aureus* CP and a humanized antibody which binds to the same epitope as an epitope in a *S. aureus* CP to which the above humanized antibody binds.

A human antibody is originally an antibody naturally existing in the human body, and it also includes an antibody obtained from a human antibody phage library or a human antibody-producing transgenic animal, which is prepared based on the recent advanced techniques in genetic engineering, cell engineering and developmental engineering.

The human antibody can be prepared from the human body, for example by isolating a human peripheral blood lymphocyte, immortalizing it by infecting with EB virus or the like and then cloning it to thereby obtain lymphocytes capable of producing the antibody, culturing the lymphocytes thus obtained, and purifying the antibody from the supernatant of the culture.

The human antibody phage library is a library in which antigen-binding fragments such as Fab and scFv are expressed on the phage surface by inserting a gene encoding an antibody prepared from a human B cell into a phage gene. A phage expressing an antigen-binding fragment having the desired antigen binding activity can be recovered from the library, using its activity to bind to an antigen-immobilized substrate as the index. The antigen-binding fragment can be converted further into a human antibody molecule comprising two full H chains and two full L chains by genetic engineering techniques.

A human antibody-producing transgenic animal is an animal in which a human antibody gene is integrated into cells. Specifically, a human antibody-producing transgenic animal can be prepared by introducing a gene encoding a human antibody into a mouse ES cell, grafting the ES cell into an early stage embryo of other mouse and then developing it. A human antibody is prepared from the human antibody-producing transgenic non-human animal by obtaining a human antibody-producing hybridoma by a hybridoma preparation method usually carried out in non-human mammals, culturing the obtained hybridoma to form and accumulate the human antibody in the supernatant of the culture.

In the amino acid sequence constituting the above antibody or antigen-binding fragment, a monoclonal antibody or antigen-binding fragment thereof in which one or more amino acids are deleted, substituted, inserted or added, having activity similar to the above antibody or antigen-binding fragment is also included in the monoclonal antibody or antigen-binding fragment of the present invention.

The number of amino acids which are deleted, substituted, inserted and/or added is one or more, and is not specifically limited, but it is within the range where deletion, substitution or addition is possible by known methods such as the site-directed mutagenesis [*Molecular Cloning* 2nd Edition, Cold Spring Harbor Laboratory Press (1989), *Current protocols in Molecular Biology*, John Wiley & Sons (1987-1997), *Nucleic Acids Research*, 10, 6487 (1982), *Proc. Natl. Acad. Sci., USA*, 79, 6409 (1982), *Gene*, 34, 315 (1985), *Nucleic Acids Research*, 13, 4431 (1985), *Proc. Natl. Acad. Sci USA*, 82, 488 (1985)] or the like. For example, the number is 1 to dozens, preferably 1 to 20, more preferably 1 to 10, and most preferably 1 to 5.

The expression "one or more amino acids are deleted, substituted, inserted or added" in the amino acid sequence of the above antibody means the followings. That is, it means there is deletion, substitution, insertion or addition of one or plural amino acids at optional positions in the same sequence and one or plural amino acid sequences. Also, the deletion, substitution, insertion or addition may occur at the same time and the amino acid which is substituted, inserted or added may be either a natural type or a non-natural type. The natural type amino acid includes L-alanine, L-asparagine, L-aspartic acid, L-glutamine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, L-cysteine, L-arginine and the like.

Preferable examples of mutually substitutable amino acids are shown below. The amino acids in the same group are mutually substitutable.

Group A: leucine, isoleucine, norleucine, valine, norvaline, alanine, 2-aminobutanoic acid, methionine, O-methylserine, t-butylglycine, t-butylalanine, cyclohexylalanine

Group B: aspartic acid, glutamic acid, isoaspartic acid, isoglutamic acid, 2-aminoadipic acid, 2-aminosuberic acid

Group C: asparagine, glutamine

Group D: lysine, arginine, ornithine, 2,4-diaminobutanoic acid, 2,3-diaminopropionic acid

Group E: proline, 3-hydroxyproline, 4-hydroxyproline

Group F: serine, threonine, homoserine

Group G: phenylalanine, tyrosine

The antigen-binding fragment of the present invention includes Fab, F(ab')₂, Fab', scFv, diabody, dsFv, a peptide comprising CDR and the like.

An Fab is an antibody-binding fragment having a molecular weight of about 50,000 and having antigen binding activity, in which about a half of the N-terminal side of H chain and the entire L chain, among fragments obtained by treating an IgG antibody molecule with a protease, papain (cleaved at an amino acid residue at position 224 of the H chain), are bound together through a disulfide bond.

An F(ab')₂ is an antigen-binding fragment having a molecular weight of about 100,000 and antigen binding activity and comprising two Fab regions which are bound in the hinge position obtained by digesting the lower part of two disulfide bonds in the hinge region of IgG, with an enzyme, pepsin. The F(ab')₂ of the present invention can be produced by treating a monoclonal antibody which specifically recognizes and binds to *S. aureus* CP with a protease, pepsin. Also, the F(ab')₂ can be also produced by binding Fab' described below via a thioether bond or a disulfide bond.

An Fab' is an antigen-binding fragment having a molecular weight of about 50,000 and antigen binding activity, which is obtained by cleaving a disulfide bond at the hinge region of the above F(ab')₂. The Fab' of the present invention can be produced by F(ab')₂ which specifically recognizes SACP and binds to the proline-rich region, with a reducing agent, such as dithiothreitol. Also, the Fab' can be produced by inserting DNA encoding Fab' fragment of the antibody into an expression vector for prokaryote or an expression vector for eukaryote, and introducing the vector into a prokaryote or eukaryote to express the Fab'.

An scFv is a VH-P-VL or VL-P-VH polypeptide in which one chain VH and one chain VL are linked using an appropriate peptide linker (hereinafter referred to as "P") and is an antigen-binding fragment having antigen binding activity. The scFv of the present invention can be produced by obtaining cDNAs encoding VH and VL of a monoclonal antibody which specifically recognizes and binds to *S. aureus* CP5 or CP8, constructing DNA encoding scFv, inserting the DNA into an expression vector for prokaryote or an expression vector for eukaryote, and then introducing the expression vector into a prokaryote or eukaryote to express the scFv.

A diabody is an antigen-binding fragment wherein scFv is dimerized, is an antigen-binding fragment having divalent antigen binding activity. In the divalent antigen binding activity, two antigens may be the same or different. The diabody of the present invention can be produced by obtaining cDNAs encoding VH and VL of a monoclonal antibody which specifically recognizes and binds to *S. aureus* CP5 or CP8, constructing DNA encoding scFv so that the length of the amino acid sequence of the peptide linker is 8 or less residues, inserting the DNA into an expression vector for prokaryote or an

expression vector for eukaryote, and then introducing the expression vector into a prokaryote or eukaryote to express the diabody.

A dsFv is obtained by binding polypeptides in which one amino acid residue of each of VH and VL is substituted with a cysteine residue via a disulfide bond between the cysteine residues. The amino acid residue to be substituted with a cysteine residue can be selected based on a three-dimensional structure estimation of the antibody in accordance with a known methods [*Protein Engineering*, 7, 697 (1994)]. The dsFv of the present invention can be produced by obtaining cDNAs encoding VH and VL of a monoclonal antibody which specifically recognizes SACP and binds to the proline-rich region, constructing DNA encoding dsFv, inserting the DNA into an expression vector for prokaryote or an expression vector for eukaryote, and then introducing the expression vector into a prokaryote or eukaryote to express the dsFv.

A peptide comprising CDR is constituted by including one or more regions of CDRs of VH or VL. Peptide comprising plural CDRs can be bound directly or via an appropriate peptide linker. The peptide comprising CDR of the present invention can be produced by constructing DNA encoding CDRs of VH and VL of a monoclonal antibody which specifically recognizes and binds to *S. aureus* CP5 or CP8, inserting the DNA into an expression vector for prokaryote or an expression vector for eukaryote, and then introducing the expression vector into a prokaryote or eukaryote to express the peptide. The peptide comprising CDR can also be produced by a chemical synthesis method such as Fmoc method or tBoc method.

The monoclonal antibody of the present invention includes an antibody conjugate in which a monoclonal antibody or an antigen-binding fragment thereof which specifically recognizes and binds to *S. aureus* capsular polysaccharide type 5 or type 8 is chemically or genetically bound to a radioisotope, an agent having a low molecular weight, an agent having a high molecular weight, a protein, a therapeutic antibody or the like.

The antibody conjugate of the present invention can be produced by chemically conjugating a radioisotope, an agent having a low molecular weight, an agent having a high molecular weight, a protein, a therapeutic antibody or the like to the N-terminal side or C-terminal side of an H chain or an L chain of the monoclonal antibody or the antigen-binding fragment thereof, an appropriate substituent or side chain of the antibody or the antigen-binding fragment, a sugar chain in the antibody or the antigen-binding fragment or the like, which specifically recognizes and binds to *S. aureus* capsular polysaccharide type 5 or type 8 in the present invention [*Antibody Engineering Handbook*, published by Chijin Shokan (1994)].

Also, the antibody conjugate can be genetically produced by linking a DNA encoding the monoclonal antibody or the antigen-binding fragment thereof which

specifically recognizes and binds to *S. aureus* capsular polysaccharide type 5 or type 8 in the present invention to other DNA encoding a protein or a therapeutic antibody to be conjugated, inserting the DNA into a vector for expression, and introducing the expression vector into an appropriate host cell.

5 The radioisotope includes ^{131}I , ^{125}I , ^{90}Y , ^{64}Cu , ^{99}Tc , ^{77}Lu , ^{111}In , ^{188}Re , ^{211}At , ^{213}Bi , and the like. The radioisotope can directly be conjugated with the antibody by Chloramine-T method. Also, a substance chelating the radioisotope can be conjugated with the antibody. The chelating agent includes 1-isothiocyanate benzyl-3-methyldiethylene-triaminepentaacetic acid (MX-DTPA) and the like.

10 The agent having a low molecular weight includes an anti-bacterial agent such as an antibiotic penicillin, cephalosporins, macrolides (such as erythromycin), tetracycline, clindamycin, quinolones, vancomycin, beta-lactam antibiotics (cephalosporins), fluoroquinolones such as levofloxacin and moxifloxacin, daptomycin, linezolid, and derivatives thereof.

15 The method for conjugating the agent having low molecular weight with the antibody includes a method in which the agent and an amino group of the antibody are conjugated through glutaraldehyde, a method in which an amino group of the agent and a carboxyl group of the antibody are conjugated through water-soluble carbodiimide, and the like.

20 The agent having a high molecular weight includes polyethylene glycol (hereinafter referred to as "PEG"), albumin, dextran, polyoxyethylene, styrene-maleic acid copolymer, polyvinylpyrrolidone, pyran copolymer, hydroxypropylmethacrylamide, and the like. By binding these compounds having a high molecular weight to an antibody or antigen-binding fragment, the following effects are expected: (1) improvement of stability against various chemical, physical or biological factors, (2) remarkable prolongation of half life in blood, (3) disappearance of immunogenicity, suppression of antibody production, and the like [*Bioconjugate Drug*, Hirokawa Shoten (1993)]. For example, the method for binding PEG to an antibody includes a method in which an antibody is allowed to react with a PEG-modifying reagent [*Bioconjugate Drug*, Hirokawa Shoten (1993)].

25 30 The PEG-modifying reagent includes a modifying agent of ϵ -amino group of lysine (Japanese Published Unexamined Patent Application No. 178926/86), a modifying agent of a carboxyl group of aspartic acid and glutamic acid (Japanese Published Unexamined Patent Application No. 23587/81), a modifying agent of a guanidino group of arginine (Japanese Published Unexamined Patent Application No. 117920/90) and the like.

35 The immunostimulator may be any natural products known as immunoadjuvants. Examples of an agent enhancing immunogen include $\beta(1\rightarrow3)$ glucan (lentinan, schizophyllan), α -galactosylceramide and the like.

The protein includes a cytokine or a growth factor which activates an immunocompetent cell, such as NK cell, macrophage or neutrophil, a toxic protein, and the like.

Examples of the cytokine or the growth factor include interferon (hereinafter referred to as "INF")- α , INF- β , INF- γ , interleukin (hereinafter referred to as "IL")-2, IL-12, IL-15, IL-18, IL-21, IL-23, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), macrophage-colony stimulating factor (M-CSF) and the like. The toxic protein includes ricin, diphtheria toxin, ONTAK and the like, and also includes a toxic protein wherein mutation is introduced into a protein in order to control the toxicity.

A fusion antibody with a protein or therapeutic antibody can be produced by linking a cDNA encoding a monoclonal antibody or an antigen-binding fragment to a cDNA encoding the protein, constructing a DNA encoding the fusion antibody, inserting the DNA into an expression vector for prokaryote or eukaryote, and then introducing the expression vector into a prokaryote or eukaryote to express the fusion antibody.

In the case where the above antibody conjugate is used for the detecting method, method for quantitative determination, detection reagent, reagent for quantitative determination or diagnostic agent in the present invention, examples of the agent to which a monoclonal antibody or an antigen-binding fragment thereof of the present invention which specifically recognizes and binds to *S. aureus* capsular polysaccharide type 5 or type 8 is bound includes a label used in routine immunological detecting or measuring method. The label includes enzymes such as alkaline phosphatase, peroxidase and luciferase, luminescent materials such as acridinium ester and lophine, fluorescent materials such as fluorescein isothiocyanate (FITC) and tetramethyl rhodamine isothiocyanate (RITC), and the like.

In addition, the present invention relates to a therapeutic agent for a disease relating to a *Staphylococcus aureus* bacterial cell which comprises a monoclonal antibody which specifically recognizes and binds to *S. aureus* capsular polysaccharide type 5 or type 8, or an antigen-binding fragment thereof as an active ingredient.

The *Staphylococcus aureus* bacterial cell-associated disease may be any disease so long as it is an infectious disease relating to *Staphylococcus aureus*, and examples include sepsis, septic shock, bacteremia, pneumonia, skin ulcer, atopic dermatitis, meningitis, enteritis, cholangiolitis, and endocarditis.

Examples of the *Staphylococcus aureus* strain are any kind of SA strains such as established SA strains, isolated SA strains from clinical patients with infectious diseases. The example of the establish strains are ATCC-49521 (Lowenstein), ATCC-49525 (Wright), ATCC-43300 (F-182), NRS-102 (Reynolds), NRS-103 (Becker), ATCC-

25904 (Newman), NRS-281 (COLVA), SSI E-5975, SSI E-6657, SSI E-2286, MCL8538, CYL1892, CYL770, and the like.

It is also included as SA strains in the present invention, that SA strain is resistant to at least one antibiotics selected from b-lactam antibiotics such as meticillin, amoxicillin, macrolide antibiotics such as vancomycin. SA strain being resistant to
5 meticillin is known as meticillin resistant SA (MRSA), SA strain being resistant to low dose vancomycin is known as vancomycin intermediate SA (VISA) and SA strain being resistant to vancomycin is known as vancomycin resistant SA (VRSA) are also included in the present invention.

10 The therapeutic agent in the present invention includes a therapeutic agent comprising the above monoclonal antibody or an antigen-binding fragment of the present invention as an active ingredient.

The therapeutic agent comprising the antibody or antigen-binding fragment thereof, or conjugate thereof of the present invention may comprise only the antibody or
15 antigen-binding fragment thereof, or conjugate thereof as an active ingredient. It is generally preferred that the therapeutic agent is prepared as a pharmaceutical preparation produced by an appropriate method well known in the technical field of pharmaceuticals, and by mixing it with one or more pharmaceutically acceptable carriers.

It is preferred to administer the therapeutic agent by the route that is most
20 effective for the treatment. Examples include oral administration and parenteral administration, such as buccal, tracheal, rectal, subcutaneous, intramuscular or intravenous administration is preferred.

The therapeutic agent may be in the form of spray, capsules, tablets, granules, powder, syrup, emulsion, suppository, injection, ointment, tape, and the like.

25 A method for treating an infectious diseases caused by *S. aureus* comprising administration of the anti-CP antibody of the present invention can be conducted into any infectious diseases caused by *S. aureus*. Preferably, sepsis, septic shock, bacteremia, pneumonia, skin ulcer, atopic dermatitis, meningitis, enteritis, cholangiolitis, and endocarditis are applicable for the method of the present invention.

30 Although the dose or the frequency of administration varies depending on the objective therapeutic effect, administration method, treating period, age, body weight and the like, it is usually 10 µg/kg to 100 mg/kg per day and per person (child or adult).

Further, the present invention relates to a method for immunologically detecting or measuring CP, an agent for immunologically detecting or measuring CP, a
35 method for immunologically detecting or measuring *Staphylococcus aureus* cell, an agent for immunologically detecting or measuring a *Staphylococcus aureus* cell, and an agent for diagnosing a disease relating to a *Staphylococcus aureus* cell, comprising a monoclonal

antibody or an antigen-binding fragment thereof which specifically recognizes and binds to *S. aureus* capsular polysaccharide type 5 or type 8, as an active ingredient.

As a method for detection or determination of the amount of CP in the present invention, any known method may be included. For example, an immunological
5 detecting or measuring method may be exemplified.

An immunological detecting or measuring method is a method in which an antibody amount or an antigen amount is detected or determined using a labeled antigen or antibody. Examples of the immunological detecting or measuring method are radioactive substance-labeled immunoantibody method (RIA), enzyme immunoassay (EIA or ELISA),
10 fluorescent immunoassay (FIA), luminescent immunoassay, Western blotting method, physico-chemical means and the like.

The above disease relating to *S. aureus* can be diagnosed by detecting or measuring a *S. aureus* cell by using the monoclonal antibody or antigen-binding fragment of the present invention.

For the detection of the *S. aureus* cell, known immunological detecting methods can be used, and an immunoprecipitation method, a fluorescent cell staining method, an immune tissue staining method and the like are preferably used. In addition, a fluorescent antibody staining method using FMAT 8100 HTS system (Applied Biosystem) and the like can be used.

20 In the present invention, the living body sample to be used for detecting or measuring CP is not particularly limited, so long as it has a possibility of containing the polypeptide, such as tissue cells, blood, blood plasma, serum, pancreatic fluid, urine, fecal matter, tissue fluid or culture fluid.

The diagnostic agent containing the monoclonal antibody or an antigen-binding
25 fragment thereof, or conjugate thereof may further contain a reagent for carrying out an antigen-antibody reaction or a reagent for detection of the reaction depending on the desired diagnostic method. The reagent for carrying out the antigen-antibody reaction includes a buffer, a salt, and the like. The reagent for detection includes a reagent generally used for the immunological detecting or measuring method, such as labeled
30 secondary antibody which recognizes the monoclonal antibody, antigen-binding fragment thereof or conjugates thereof and substrate corresponding to the labeling.

A process for producing the antibody of the present invention, a method for treating the disease and a method for diagnosing the disease are specifically described below.

1. Preparation method of monoclonal antibody

(1) Preparation of antigen

CP5 or CP8 as an antigen is expressed on the surface of various strains of *S. aureus*. In addition, CP can be purified from various cultures with *Staphylococcus aureus* cells. Furthermore, a synthetic carbohydrate having a partial structure of CP can be prepared by a chemical synthesis method.

CP5 is purified, for example, from the *S. aureus* strain Reynolds according to published protocols [*Infect. Immun* 1984; 45(1): 87-93; *Proc. Natl. Acad. Sci. USA*; 2001, 98(16): 9365-70]. Briefly, the staphylococci are grown overnight on several Columbia CNA agar plates supplemented with 2.5% NaCl (w/v) and 0.1% glucose (w/v) at 37°C in filtered air to promote CP5 production. Then, the bacteria are harvested from the plates by washing them off with 50 mM sodium acetate, pH 6.0, pooled, and autoclaved for 20 min. Then, bacteria are pelleted by centrifugation and the supernatant is collected (= supernatant #1). The bacterial pellet is resuspended in 50 mM sodium acetate, pH 6.0, and autoclaved as above. Subsequently, the bacterial suspension is centrifuged and the supernatant is collected (= supernatant #2). Supernatants #1 and #2, which contain CP5, are pooled and concentrated using a tangential flow filtration (TFF) unit with a molecular weight cut-off (MWCO) of 10 kDa. The retentate of the filtration containing CP5 is collected and its pH is adjusted to 5.0. Then, it is treated with 0.2 M sodium metaperiodate for 72 h to oxidize teichoic acids and subsequently dialyzed against phosphate buffered saline (PBS), pH 7.4, using dialysis bags with MWCO of 10 kDa. To remove DNA, RNA, and protein, the CP5 containing mixture is treated with DNase, RNase, and pronase and then dialyzed against 50 mM sodium acetate, pH 6.0, using dialysis bags as above. Subsequently, the absence of phosphorus, which would be indicative of residual nucleic acids in the sample, is confirmed by a standard colorimetric assay for phosphorus detection. Then, the material is subjected twice to anion exchange chromatography on Diethylaminoethyl cellulose (DEAE) with NaCl gradient elution. CP5 content in individual fractions is determined using ELISA. Moreover, their content of peptidoglycan, protein, teichoic acids, and phosphorus is also measured for each fraction. CP5-rich fractions, in which none of the above impurities are detectable, were pooled, concentrated, and dialyzed against H₂O, and then lyophilized and stored at -80°C. Finally, ¹H NMR is conducted with de-O-acetylated CP5. The obtained spectra are consistent with those obtained for reference material.

CP8 is purified, for example, from the *S. aureus* strain Wright according to the same protocols as CP5 purification.

(2) Immunization of animal and preparation of antibody-producing cell for fusion

A mouse, rat or hamster 3 to 20 weeks old is immunized with the antigen prepared in the above (1), and antibody-producing cells are collected from the spleen, lymph node or peripheral blood of the animal.

The immunization is carried out by administering the antigen to the animal through subcutaneous, intravenous or intraperitoneal injection together with an appropriate adjuvant (for example, complete Freund's adjuvant, combination of aluminum hydroxide gel with pertussis vaccine, or the like). When the antigen is a partial peptide, a conjugate is produced with a carrier protein such as BSA (bovine serum albumin), KLH (keyhole limpet hemocyanin) or the like, which is used as the antigen.

The administration of the antigen is carried out 5 to 10 times every week or every two weeks after the first administration. On the 3rd to 7th day after each administration, a blood sample is collected from the fundus of the eye, the reactivity of the serum with the antigen is tested, for example, by enzyme immunoassay [*Antibodies-A Laboratory Manual*, Cold Spring Harbor Laboratory (1988)] or the like. An animal showing a sufficient antibody titer in their sera against the antigen used for the immunization is used as the supply source of antibody-producing cells for fusion.

Three to seven days after final administration of the antigen, tissue containing the antibody-producing cells such as the spleen from the immunized animal is excised to collect the antibody-producing cells. When the spleen cells are used, the spleen is cut out and loosened, followed by centrifugation. Then, antibody-producing cells for fusion are obtained by removing erythrocytes.

(3) Preparation of myeloma cell

An established cell line obtained from mouse is used as myeloma cells. Examples include 8-azaguanine-resistant mouse (derived from BALB/c) myeloma cell line P3-X63Ag8-U1 (P3-U1) [*Current Topics in Microbiology and Immunology*, 18, 1 (1978)], P3-NS1/1-Ag41 (NS-1) [*European J. Immunology*, 6, 511 (1976)], SP2/0-Ag14 (SP-2) [*Nature*, 276, 269 (1978)], P3-X63-Ag8653 (653) [*J. Immunology*, 123, 1548 (1979)], P3-X63-Ag8 (X63) [*Nature*, 256, 495 (1975)] and the like.

The myeloma cells are subcultured in a normal medium [a medium in which glutamine, 2-mercaptoethanol, gentamicin, FBS and 8-azaguanine are added to RPMI1640 medium] and they are subcultured in the normal medium 3 or 4 days before cell fusion to ensure the cell number of 2×10^7 or more on the day for fusion.

(4) Cell fusion and preparation of hybridoma for producing monoclonal antibody

The antibody-producing cells for fusion obtained by the above (2) and myeloma cells obtained by the above (3) were sufficiently washed with a minimum essential medium (MEM) or PBS (1.83 g of disodium hydrogen phosphate, 0.21 g of potassium dihydrogen phosphate, 7.65 g of sodium chloride, 1 liter of distilled water, pH 7.2) and mixed to give a ratio of the antibody-producing cells: the myeloma cells = 5 to 10:1, followed by centrifugation. Then, the supernatant is discarded. The precipitated cell group is sufficiently loosened. After loosening the precipitated cell, the mixture of polyethylene glycol-1000 (PEG-1000), MEM and dimethylsulfoxide is added to the cell under stirring at 37°C. In addition, 1 to 2 mL of MEM medium is added several times every one or two minutes, and MEM is added to give a total amount of 50 mL. After centrifugation, the supernatant is discarded. After the cells are gently loosen, the cells are gently suspended in HAT medium [a medium in which hypoxanthine, thymidine and aminopterin is added to the normal medium]. The suspension is cultured in a 5% CO₂ incubator for 7 to 14 days at 37°C.

After the culturing, a portion of the culture supernatant is sampled. A hybridoma which is reactive to an antigen preparation containing CP and is not reactive to an antigen preparation not containing CP is selected by binding assay as described below. Then, cloning is carried out twice by a limiting dilution method [Firstly, HT medium (HAT medium from which aminopterin is removed) is used, and secondly, the normal medium is used], and a hybridoma which shows a stably high antibody titer is selected as the monoclonal antibody-producing hybridoma.

(5) Preparation of purified monoclonal antibody

The hybridoma cells producing a monoclonal antibody obtained by the above (4) are administered by intraperitoneal injection into 8- to 10-week-old mice or nude mice treated with 0.5 mL of pristane (2,6,10,14-tetramethylpentadecane (pristane) is intraperitoneally administered, followed by feeding for 2 weeks). The hybridoma develops ascites tumor in 10 to 21 days. The ascitic fluid is collected from the mice, centrifuged to remove solids, subjected to salting out with 40 to 50% ammonium sulfate and then precipitated by caprylic acid, passed through a DEAE-Sepharose column, a protein A column or a gel filtration column to collect an IgG or IgM fraction as a purified monoclonal antibody.

Furthermore, a monoclonal antibody-producing hybridoma obtained by the above (4) is cultured in RPMI1640 medium containing FBS or the like and the supernatant is removed by centrifugation. The precipitated cells are suspended in Hybridoma SFM medium containing 5% DIGO GF21 and cultured for 3 to 7 days. The purified

monoclonal antibody can be obtained by centrifuging the obtained cell suspension, followed by purifying the resulting supernatant with Protein A column or Protein G column to collect the IgG fractions.

The subclass of the antibody can be determined using a subclass typing kit by enzyme immunoassay. The amount of the protein can be determined by the Lowry method or from the absorbance at 280 nm.

(6) Selection of monoclonal antibody

Selection of monoclonal antibody is carried out by the following binding assay using an enzyme immunoassay method and kinetic analysis with Biacore.

(6-a) Binding assay

As the antigen, a CP-positive *Staphylococcus aureus* cell or the like, or a purified CP obtained from the culture of the CP-positive *S. aureus* strain is used. When the antigen is a partial carbohydrate, a conjugate is prepared with a carrier protein such as BSA or KLH and is used.

After making these antigens into a solid layer by dispensing in a 96-well plate, a substance to be tested such as serum, a culture supernatant of a hybridoma or a purified monoclonal antibody is dispensed therein as the primary antibody and allowed to react.

After thoroughly washing with PBS or PBS-Tween, an anti-immunoglobulin antibody labeled with biotin, an enzyme, a chemiluminescent material, a radiation compound or the like is dispensed therein as the secondary antibody and allowed to react. After thoroughly washing with PBS-Tween, the reaction is carried out in response to the label of the secondary antibody to select a monoclonal antibody which specifically reacts with the antigen.

The antibody which competes with the anti-CP monoclonal antibody of the present invention can be prepared by adding an antibody to be tested to the above-mentioned binding assay system and carrying out reaction. That is, a monoclonal antibody which competes with the thus obtained monoclonal antibody for its binding to *S. aureus* CP can be prepared by carrying out a screening of an antibody by which the binding of the monoclonal antibody is inhibited when the antibody to be tested is added.

Furthermore, an antibody which binds to an epitope which is the same as the epitope recognized by the monoclonal antibody which binds to *S. aureus* CP of the present invention can be obtained by identifying the epitope of the antibody obtained in the above binding assay, and preparing a partial synthetic peptide, a synthetic peptide mimicking the conformational structure of the epitope or the like, followed by immunization.

(6-b) Kinetic analysis with Biacore

The kinetics between an antigen and a test substance is measured using Biacore T100 and then the obtained results are analyzed using analysis software accompanied with the apparatus. After anti-IgG mouse antibody is immobilized onto to a CM5 sensor chip by an amine coupling method, a test substance such as culture supernatant of a hybridoma, a purified antibody is allowed to flow, bind at an appropriate amount, and further flow an antigen at plural known concentrations, followed by measuring the binding and dissociation. Using the obtained data and the software accompanied with the apparatus, the kinetics analysis is carried out using the 1:1 binding model to obtain necessary parameters. Otherwise, after CP is immobilized onto the sensor chip by an amino coupling method, a purified monoclonal antibody is allowed to flow at plural known concentrations followed by measuring the binding and dissociation. Using the obtained data and the software accompanied with the apparatus, the kinetics analysis is carried out using bivalent analyte model to obtain necessary parameters.

2. Preparation of recombinant antibody

As production examples of recombinant antibodies, processes for producing a human chimeric antibody and a humanized antibody are shown below.

(1) Construction of vector for expression of recombinant antibody

A vector for expression of recombinant antibody is an expression vector for animal cell into which DNAs encoding CH and CL of a human antibody have been inserted, and is constructed by cloning each of DNAs encoding CH and CL of a human antibody into an expression vector for animal cell.

The C region of a human antibody may be CH and CL of any human antibody. Examples include CH belonging to $\gamma 1$ subclass, CL belonging to κ class, and the like. As the DNAs encoding CH and CL of a human antibody, the cDNA may be generally used and a chromosomal DNA comprising an exon and an intron can be also used. As the expression vector for animal cell, any expression vector can be used, so long as a gene encoding the C region of a human antibody can be inserted therein and expressed therein. Examples include pAGE107 [*Cytotechnol.*, 3, 133 (1990)], pAGE103 [*J. Biochem.*, 101, 1307 (1987)], pHSG274 [*Gene*, 27, 223 (1984)], pKCR [*Proc. Natl. Acad. Sci. USA*, 78, 1527 (1981)], pSG1bd2-4 [*Cytotechnol.*, 4, 173 (1990)], pSE1UK1Sed1-3 [*Cytotechnol.*, 13, 79 (1993)] and the like. Examples of a promoter and enhancer used for an expression vector for animal cell include an SV40 early promoter [*J. Biochem.*, 101, 1307 (1987)], a Moloney mouse leukemia virus LTR [*Biochem. Biophys. Res. Commun.*, 149, 960 (1987)],

an immunoglobulin H chain promoter [*Cell*, 41, 479 (1985)] and enhancer [*Cell*, 33, 717 (1983)] and the like.

The vector for expression of recombinant antibody may be either of a type in which a gene encoding an antibody H chain and a gene encoding an antibody L chain exist on separate vectors or of a type in which both genes exist on the same vector (tandem type). In respect of ease of construction of a vector for expression of recombinant antibody, ease of introduction into animal cells, and balance between the expression amounts of antibody H and L chains in animal cells, a tandem type of the vector for expression of recombinant antibody is more preferred [*J. Immunol. Methods*, 167, 271 (1994)]. Examples of the tandem type vector for expression of recombinant antibody include pKANTEX93 (WO 97/10354), pEE18 [*Hybridoma*, 17, 559 (1998)], and the like.

(2) Obtaining of cDNA encoding V region of antibody derived from non-human animal and analysis of amino acid sequence

Obtaining of cDNAs encoding VH and VL of a non-human animal antibody and analysis of amino acid sequence are carried out as follows.

mRNA is extracted from hybridoma cells producing an antibody derived from a non-human animal to synthesize cDNA. The synthesized cDNA is cloned into a vector such as a phage or a plasmid, to prepare a cDNA library. Each of a recombinant phage or recombinant plasmid containing cDNA encoding VH or VL is isolated from the library using DNA encoding a part of the C region or V region of a mouse antibody as the probe. The full length of the nucleotide sequences of VH and VL of a mouse antibody derived from a non-human animal of interest on the recombinant phage or recombinant plasmid are determined. Also, the full length of the amino acid sequences of VH and VL of a mouse antibody are deduced from the determined nucleotide sequences.

Examples of the non-human animal for preparing a hybridoma cell which produces a non-human antibody include mouse, rat, hamster, rabbit or the like. Any animals can be used so long as a hybridoma cell can be produced therefrom.

Examples of the method for preparing total RNA from a hybridoma cell include a guanidine thiocyanate-caesium trifluoroacetate method [*Methods in Enzymol.*, 154, 3 (1987)], the use of a kit such as RNA easy kit (manufactured by Qiagen) and the like.

Examples of the method for preparing mRNA from total RNA include an oligo (dT) immobilized cellulose column method [*Molecular Cloning, A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press (1989)], a method using a kit such as Oligo-dT30 <Super> mRNA Purification Kit (manufactured by Takara Bio) and the like. Also, examples of a kit for preparing mRNA from a hybridoma cell include Fast

Track mRNA Isolation Kit (manufactured by Invitrogen), Quick Prep mRNA Purification Kit (manufactured by Pharmacia) and the like.

Examples of the method for synthesizing cDNA and preparing a cDNA library include known methods [*Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Lab. Press (1989); *Current Protocols in Molecular Biology*, Supplement 1, John Wiley & Sons (1987-1997)]; a method using a kit such as Super Script Plasmid System for cDNA Synthesis and Plasmid Cloning (manufactured by GIBCO BRL), ZAP-cDNA Kit (manufactured by Stratagene), *etc.*; and the like.

The vector into which the synthesized cDNA obtainable by using mRNA extracted from a hybridoma cell as the template is inserted for preparing a cDNA library may be any vector, so long as the cDNA can be inserted. Examples include ZAP Express [*Strategies*, 5, 58 (1992)], pBluescript II SK(+) [*Nucleic Acids Research*, 17, 9494 (1989)], λzapII (manufactured by Stratagene), λgt10 and λgt11 [*DNA Cloning: A Practical Approach*, I, 49 (1985)], Lambda BlueMid (manufactured by Clontech), λExCell and pT7T3 18U (manufactured by Pharmacia), pcD2 [*Mol. Cell. Biol.*, 3, 280 (1983)], pUC18 [*Gene*, 33, 103 (1985)], and the like.

Any *Escherichia coli* for introducing the cDNA library constructed by a phage or plasmid vector may be used, so long as the cDNA library can be introduced, expressed and maintained. Examples include XL1-Blue MRF' [*Strategies*, 5, 81 (1992)], C600 [*Genetics*, 39, 440 (1954)], Y1088 and Y1090 [*Science*, 222: 778 (1983)], NM522 [*J. Mol. Biol.*, 166, 1 (1983)], K802 [*J. Mol. Biol.*, 16, 118 (1966)], JM105 [*Gene*, 38, 275 (1985)], and the like.

A colony hybridization or plaque hybridization method using an isotope- or fluorescence-labeled probe may be used for selecting cDNA clones encoding VH and VL of a non-human antibody or the like from the cDNA library [*Molecular Cloning, A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press (1989)].

Also, the cDNAs encoding VH and VL can be prepared through polymerase chain reaction (hereinafter referred to as "PCR"; *Molecular Cloning, A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press (1989); *Current Protocols in Molecular Biology*, Supplement 1, John Wiley & Sons (1987-1997)) by preparing primers and using cDNA prepared from mRNA or a cDNA library as the template.

The nucleotide sequence of the cDNA can be determined by digesting the cDNA selected with appropriate restriction enzymes and the like, cloning the fragments into a plasmid such as pBluescript SK(-) (manufactured by Stratagene), carrying out the reaction by a usually used nucleotide analyzing method. For example, a nucleotide analyze is carried out by using an automatic nucleotide sequence analyzer such as ABI PRISM3700 (manufactured by PE Biosystems) and A.L.F. DNA sequencer (manufactured

by Pharmacia) after a reaction such as the dideoxy method [*Proc. Natl. Acad. Sci. USA*, 74, 5463 (1977)].

Whether the obtained cDNAs encode the full amino acid sequences of VL and VL of the antibody containing a secretory signal sequence can be confirmed by estimating the full length of the amino acid sequences of VH and VL from the determined nucleotide sequence and comparing them with the full length of the amino acid sequences of VH and VL of known antibodies [*Sequences of Proteins of Immunological Interest*, US Dept. Health and Human Services (1991)]. The length of the secretory signal sequence and N-terminal amino acid sequence can be deduced by comparing the full length of the amino acid sequences of VH and VL of the antibody comprising a secretory signal sequence with full length of the amino acid sequences of VH and VL of known antibodies [*Sequences of Proteins of Immunological Interest*, US Dept. Health and Human Services (1991)], and the subgroup to which they belong can also be known. Furthermore, the amino acid sequence of each of CDRs of VH and VL can be found by comparing the obtained amino acid sequences with amino acid sequences of VH and VL of known antibodies [*Sequences of Proteins of Immunological Interest*, US Dept. Health and Human Services (1991)].

Moreover, the novelty of the full length of the amino acid sequence of VH and VL can be examined by carrying out a homology search with sequences in any database, for example, SWISS-PROT, PIR-Protein or the like using the obtained full length of the amino acid sequences of VH and VL, for example, according to the BLAST method [*J. Mol. Biol.*, 215, 403 (1990)] or the like.

(3) Construction of vector for expression of human chimeric antibody

cDNA encoding each of VH and VL of antibody of non-human animal is cloned in the upstream of genes encoding CH or CL of human antibody of vector for expression of recombinant antibody mentioned in the above (1) to thereby construct a vector for expression of human chimeric antibody.

For example, in order to ligate cDNA comprising a nucleotide sequence of 3'-terminal of VH or VL of antibody of non-human animal and a nucleotide sequence of 5'-terminal of CH or CL of human antibody, each cDNA encoding VH and VL of antibody of non-human animal is prepared so as to encode an appropriate amino acid sequence encoded by a nucleotide sequence of a linkage region and designed to have an appropriate recognition sequence of a restriction enzyme. The obtained cDNAs encoding VH and VL of antibody are respectively cloned so that each of them is expressed in an appropriate form in the upstream of gene encoding CH or CL of human antibody of the vector for expression of humanized antibody mentioned in the above (1) to construct a vector for expression of human chimeric antibody.

In addition, cDNA encoding VH or VL of a non-human animal antibody is amplified by PCR using a synthetic DNA having a recognition sequence of an appropriate restriction enzyme at both ends and each of them is cloned to the vector for expression of recombinant antibody obtained in the above (1).

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(4) Construction of cDNA encoding V region of humanized antibody

cDNAs encoding VH or VL of a humanized antibody can be obtained as follows.

Amino acid sequences of framework region (hereinafter referred to as "FR") in VH or VL of a human antibody to which amino acid sequences of CDRs in VH or VL of an antibody derived from a non-human animal antibody are transplanted are respectively selected. Any amino acid sequences of FR of a human antibody can be used, so long as they are derived from human. Examples include amino acid sequences of FRs of human antibodies registered in database such as Protein Data Bank or the like, and amino acid sequences common to subgroups of FRs of human antibodies [*Sequences of Proteins of Immunological Interest*, US Dept. Health and Human Services (1991)], and the like. In order to inhibit the decrease in the binding activity of the antibody, amino acid sequences having high homology (at least 60% or more) with the amino acid sequence of FR in VH or VL of the original antibody is selected.

Then, amino acid sequences of CDRs of the original antibody are grafted to the selected amino acid sequence of FR in VH or VL of the human antibody, respectively, to design each amino acid sequence of VH or VL of a humanized antibody. The designed amino acid sequences are converted to DNA sequences by considering the frequency of codon usage found in nucleotide sequences of genes of antibodies [*Sequence of Proteins of Immunological Interest*, US Dept. Health and Human Services (1991)], and the DNA sequence encoding the amino acid sequence of VH or VL of a humanized antibody is designed.

Based on the designed nucleotide sequences, several synthetic DNAs having a length of about 100 nucleotides are synthesized, and PCR is carried out using them. In this case, it is preferred that 6 synthetic DNAs per each of the H chain and the L chain are designed in view of the reaction efficiency of PCR and the lengths of DNAs which can be synthesized.

Furthermore, the cDNA encoding VH or VL of a humanized antibody can be easily cloned into the vector for expression of humanized antibody constructed in (1) by introducing the recognition sequence of an appropriate restriction enzyme to the 5' terminal of the synthetic DNAs existing on the both ends.

Otherwise, it can be carried out using a synthetic DNA as one DNA encoding each of the full-length H chain and the full-length L chain based on the designed DNA sequence.

After the PCR, an amplified product is cloned into a plasmid such as pBluescript SK (-) (manufactured by Stratagene) or the like, and the nucleotide sequence is determined according to a method similar to the method described in (2) to obtain a plasmid having a DNA sequence encoding the amino acid sequence of VH or VL of a desired humanized antibody.

10 (5) Modification of amino acid sequence of V region of humanized antibody

It is known that when a humanized antibody is produced by simply grafting only CDRs in VH and VL of an antibody derived from a non-human animal into FRs of VH and VL of a human antibody, its antigen binding activity is lower than that of the original antibody derived from a non-human animal [*BIO/TECHNOLOGY*, 9, 266 (1991)].

15 In humanized antibodies, among the amino acid sequences of FRs in VH and VL of a human antibody, an amino acid residue which directly relates to binding to an antigen, an amino acid residue which interacts with an amino acid residue in CDR, and an amino acid residue which maintains the three-dimensional structure of an antibody and indirectly relates to binding to an antigen are identified and modified to an amino acid residue which
20 is found in the original non-humanized antibody to thereby increase the antigen binding activity which has been decreased.

In order to identify the amino acid residues relating to the antigen binding activity in FR, the three-dimensional structure of an antibody is constructed and analyzed by X-ray crystallography [*J. Mol. Biol.*, 112, 535 (1977)], computer-modeling [*Protein Engineering*, 7, 1501 (1994)] or the like. In addition, various attempts must be currently
25 necessary, for example, several modified antibodies of each antibody are produced and the correlation between each of the modified antibodies and its antibody binding activity is examined.

The modification of the amino acid sequence of FR in VH and VL of a human
30 antibody can be accomplished using various synthetic DNA for modification according to PCR as described in (4). With regard to the amplified product obtained by the PCR, the nucleotide sequence is determined according to the method as described in (2) so that whether the objective modification has been carried out is confirmed.

35 (6) Construction of vector for expression of humanized antibody

A vector for expression of humanized antibody can be constructed by cloning each cDNA encoding VH or VL of a constructed recombinant antibody into upstream of

each gene encoding CH or CL of the human antibody in the vector for expression of recombinant antibody as described in (1).

For example, when recognizing sequences of an appropriate restriction enzymes are introduced to the 5'-terminal of synthetic DNAs positioned at both ends among synthetic DNAs used in the construction of VH or VL of the humanized antibody in (4) and (5), cloning can be carried out so that they are expressed in an appropriate form in the upstream of each gene encoding CH or CL of the human antibody in the vector for expression of a humanized antibody as described in (1).

(7) Transient expression of recombinant antibody

In order to efficiently evaluate the antigen binding activity of various humanized antibodies produced, the recombinant antibodies can be expressed transiently using the vector for expression of humanized antibody as described in (3) and (6) or the modified expression vector thereof.

Any cell can be used as a host cell, so long as the host cell can express a recombinant antibody. Generally, COS-7 cell (ATCC CRL1651) is used in view of its high expression amount [*Methods in Nucleic Acids Res.*, CRC Press, 283 (1991)].

Examples of the method for introducing the expression vector into COS-7 cell include a DEAE-dextran method [*Methods in Nucleic Acids Res.*, CRC Press, 283 (1991)], a lipofection method [*Proc. Natl. Acad. Sci. USA*, 84, 7413 (1987)], and the like.

After introduction of the expression vector, the expression amount and antigen binding activity of the recombinant antibody in the culture supernatant can be determined by the enzyme immunoassay [*Monoclonal Antibodies-Principles and practice*, Third edition, Academic Press (1996), *Antibodies-A Laboratory Manual*, Cold Spring Harbor Laboratory (1988), *Monoclonal Antibody Experiment Manual*, Kodansha Scientific (1987)] and the like.

(8) Obtaining transformant which stably expresses recombinant antibody and preparation of recombinant antibody

A transformant which stably expresses a recombinant antibody can be obtained by introducing the vector for expression of recombinant antibody described in (3) and (6) into an appropriate host cell.

Examples of the method for introducing the expression vector into a host cell include electroporation [Japanese Published Unexamined Patent Application No. 257891/90, *Cytotechnology*, 3, 133 (1990)] and the like.

As the host cell into which a vector for expression of a recombinant antibody is introduced, any cell can be used, so long as it is a host cell which can produce the

recombinant antibody. Examples include CHO-K1 (ATCC CCL-61), DUKXB11 (ATCC CCL-9096), Pro-5 (ATCC CCL-1781), CHO-S (Life Technologies, Cat # 11619), rat myeloma cell YB2/3HL.P2.G11.16Ag.20 (also referred to as YB2/0), mouse myeloma cell NSO, mouse myeloma cell SP2/0-Ag14 (ATCC No. CRL1581), mouse P3X63-Ag8.653 cell (ATCC No. CRL1580), CHO cell in which a dihydrofolate reductase gene (hereinafter referred to as "dhfr") is defective [*Proc. Natl. Acad. Sci. U.S.A.*, 77, 4216 (1980)], lection resistance-acquired Lec13 [*Somatic Cell and Molecular genetics*, 12, 55 (1986)], CHO cell in which α 1,6-fucosyltransferase gene is defected (WO 2005/35586, WO 02/31140), rat YB2/3HL.P2.G11.16Ag.20 cell (ATCC No. CRL1662), and the like.

In addition, host cells in which activity of a protein such as an enzyme relating to synthesis of an intracellular sugar nucleotide, GDP-fucose, a protein such as an enzyme relating to the modification of a sugar chain in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through α -bond in a complex type N-glycoside-linked sugar chain, or a protein relating to transport of an intracellular sugar nucleotide, GDP-fucose, to the Golgi body are introduced is decreased or deleted, preferably CHO cell in which α 1,6-fucosyltransferase gene is defected as described in WO05/35586, WO02/31140 or the like, can also be used.

After introduction of the expression vector, transformants which express a recombinant antibody stably are selected by culturing in a medium for animal cell culture containing an agent such as G418 sulfate (hereinafter referred to as "G418") or the like (Japanese Published Unexamined Patent Application No. 257891/90).

Examples of the medium for animal cell culture include RPMI1640 medium (manufactured by Invitrogen), GIT medium (manufactured by Nihon Pharmaceutical), EX-CELL301 medium (manufactured by JRH), IMDM medium (manufactured by Invitrogen), Hybridoma-SFM medium (manufactured by Invitrogen), media obtained by adding various additives such as fetal calf serum (hereinafter referred to as "FCS") to these media, and the like. The recombinant antibody can be produced and accumulated in a culture supernatant by culturing the selected transformants in a medium. The expression amount and antigen binding activity of the recombinant antibody in the culture supernatant can be measured by ELISA or the like. Also, in the transformant, the expression amount of the recombinant antibody can be increased by using DHFR amplification system or the like according to the method disclosed in Japanese Published Unexamined Patent Application No. 257891/90.

The recombinant antibody can be purified from the culture supernatant of the transformant by using a Staphylococcus Protein A column [*Monoclonal Antibodies-Principles and practice*, Third edition, Academic Press (1996), *Antibodies-A Laboratory Manual*, Cold Spring Harbor Laboratory (1988)]. For example, the recombinant antibody

can be purified by a combination of gel filtration, ion-exchange chromatography, ultrafiltration and the like.

The molecular weight of the H chain or the L chain of the purified recombinant antibody or the antibody molecule as a whole is determined by polyacrylamide gel electrophoresis (hereinafter referred to as "SDS-PAGE") [*Nature*, 227, 680 (1970)], Western blotting [*Monoclonal Antibodies-Principles and practice*, Third edition, Academic Press (1996), *Antibodies-A Laboratory Manual*, Cold Spring Harbor Laboratory (1988)], and the like.

3. Activity evaluation of the monoclonal antibody or antigen-binding fragment

The activity of the purified monoclonal antibody or antigen-binding fragment of the present invention can be evaluated in the following manner.

The binding activity to *Staphylococcus aureus* cell is evaluated by the binding assay described in the above 1-(6-a) and a surface plasmon resonance method using such as the Biacore system described in the above (6-b). Furthermore, it can be measured by fluorescent antibody technique [*Cancer Immunol. Immunother.*, 36, 373 (1993)], a surface plasmon resonance method using such as BIAcore system or the like. Furthermore, it can be measured by fluorescent antibody technique [*Cancer Immunol. Immunother.*, 36, 373 (1993)].

In addition, complement-deposition activity or antibody dependent phagocytotic activity against an antigen positive bacterial cell is evaluated by a known method [*Vaccine*. 2011 Feb 24;29(10):1929-34., *PLoS One*. 2011;6(10):e24581].

4. Method of controlling effector activity of antibody

As a method for controlling an effector activity of the anti-SACP monoclonal antibody of the present invention, a method for controlling an amount of fucose which is bound in α -1,6 linkage to N-acetylglucosamine (GlcNAc) present in a reducing end of a complex type N-linked sugar chain which is bound to asparagine (Asn) at position 297 of an Fc region of an antibody (hereinafter, referred to also as "core fucose") (WO2005/035586, WO2002/31140, and WO00/61739), a method for controlling an effector activity of a monoclonal antibody by modifying amino acid group(s) of an Fc region of the antibody, and the like are known. The effector activity of the anti-SACP monoclonal antibody of the present invention can be controlled by using any of the methods.

The "effector activity" means an antibody-dependent activity which is induced via an Fc region of an antibody. As the effector activity, an antibody-dependent phagocytotic activity, a complement-deposition activity, and the like are known.

By controlling an amount of core fucose of a complex type N-linked sugar chain of Fc of an antibody, an effector activity of the antibody can be increased or decreased. According to a method for lowering an amount of fucose which is bound to a complex type N-linked sugar chain bound to Fc of the antibody, an antibody to which
5 fucose is not bound can be obtained by the expression of an antibody using a CHO cell which is deficient in a gene encoding α 1,6-fucosyltransferase. The antibody to which fucose is not bound has a high antibody-dependent phagocytotic activity. On the other hand, according to a method for increasing an amount of fucose which is bound to a complex type N-linked sugar chain bound to Fc of an antibody, an antibody to which
10 fucose is bound can be obtained by the expression of an antibody using a host cell into which a gene encoding α 1,6-fucosyltransferase is introduced. The antibody to which fucose is bound has a lower antibody-dependent phagocytotic activity than the antibody to which fucose is not bound.

In the present invention, an antibody which has no core-fucose is defined as
15 non-fucosylated antibody, afucosylated antibody or Potelligent antibody in case.

Further, by modifying amino acid residue(s) in an Fc region of an antibody, the antibody-dependent phagocytotic activity or complement deposition activity can be increased or decreased. The antibody-dependent phagocytotic activity can be controlled by increasing or decreasing the binding activity to Fc γ R due to the modification(s) of
20 amino acid residue(s) in an Fc region. In addition, the complement deposition activity can be controlled by increasing or decreasing the binding activity of complement due to the modification(s) of amino acid residue(s) in an Fc region. For example, the binding activity to an antibody can be increased by using the amino acid sequence of the Fc region described in US published patent application No. 2007/0148165. Further, the antibody-
25 dependent phagocytotic activity or complement deposition activity can be increased or decreased by modifying the amino acid as described in US Patent Nos. 6,737,056, or 7,297,775 or WO2005/070963, and some scientific papers [*Mol Cancer Ther.* 2008 Aug;7(8):2517-27., *MAbs.* 2010 Mar-Apr;2(2):181-9].

The antibodies of the present invention also includes any antibodies improved
30 with the above identified technologies, such as an antibody comprising lowered or no fucose content in the sugar chain bound to the Fc region, an antibody comprising at least one amino acid substitution in the Fc region and an antibody comprising both of them.

In the present invention, it is particularly defined as AccretaMab or afucosylated Fc-engineered version (IgG1/IgG3) that the antibody which has a domain
35 exchanged Fc between human IgG1 and IgG3, and is afucoslated. The antibody has an enhanced ADCC activity and an enhanced CDC activity by both of glycoengineering and Fc engineering.

Furthermore, an antibody in which the effector activity is controlled can be obtained by combining the above methods; the method for controlling a sugar chain and the method for modifying amino acid(s) in an Fc region.

5 5. Method for treating disease using the anti-SACP monoclonal antibody or antigen-binding fragment thereof of the present invention

A monoclonal antibody which specifically recognizes and binds to *S. aureus* CP, or an antigen-binding fragment thereof of the present invention can be used for treating a disease relating to *Staphylococcus aureus*.

10 The method for treating an infectious disease caused by *S. aureus* of the present invention includes an administration of the antibody for SA and at least one of other pharmaceutical drugs as antibody, antibiotic, and so on, simultaneously or continuously.

Examples of a route of administration include oral administration and
15 parenteral administration, such as buccal, tracheal, rectal, subcutaneous, intramuscular or intravenous administration. In the case of an antibody or peptide formulation, intravenous administration is preferred. Examples of the dosage form includes sprays, capsules, tablets, powder, granules, syrups, emulsions, suppositories, injections, ointments, tapes and the like.

20 The pharmaceutical preparation suitable for oral administration includes emulsions, syrups, capsules, tablets, powders, granules and the like.

Liquid preparations such as emulsions and syrups can be produced using, as additives, water; sugars such as sucrose, sorbitol and fructose; glycols such as polyethylene glycol and propylene glycol; oils such as sesame oil, olive oil and soybean oil; antiseptics
25 such as p-hydroxybenzoic acid esters; flavors such as strawberry flavor and peppermint; and the like.

Capsules, tablets, powders, granules and the like can be produced using, as additives, excipients such as lactose, glucose, sucrose and mannitol; disintegrating agents such as starch and sodium alginate; lubricants such as magnesium stearate and talc; binders
30 such as polyvinyl alcohol, hydroxypropylcellulose and gelatin; surfactants such as fatty acid ester; plasticizers such as glycerin; and the like.

The pharmaceutical preparation suitable for parenteral administration includes injections, suppositories, sprays and the like.

35 Injections can be prepared using a carrier such as a salt solution, a glucose solution or a mixture of both thereof.

Suppositories can be prepared using a carrier such as cacao butter, hydrogenated fat or carboxylic acid.

Sprays can be prepared using the antibody or antigen-binding fragment as such or using it together with a carrier which does not stimulate the buccal or airway mucous membrane of the patient and can facilitate absorption of the compound by dispersing it as fine particles. The carrier includes lactose, glycerol and the like. It is possible to produce pharmaceutical preparations such as aerosols and dry powders.

In addition, the components exemplified as additives for oral preparations can also be added to the parenteral preparations.

6. Method for diagnosing disease using the anti-CP monoclonal antibody or antigen-binding fragment of the present invention

A disease relating to *Staphylococcus aureus* can be diagnosed by detecting or determining CP or a bacterial cell expressing CP using the monoclonal antibody or antigen-binding fragment of the present invention. The antibodies against CP of the present invention can bind to the purified CP and CP expressed on *S. aureus* and further bind to the immobilized CP on plastic plate or biotinylated CP on avidin plate through non-covalent bond. Therefore, the antibodies of the present invention can detect a solubilized CP in body fluid from infectious patients and can quantify the amount of CP.

A diagnosis of infectious disease, one of the diseases relating to *Staphylococcus aureus*, can be carried out by, for example, the detection or measurement of *Staphylococcus aureus* as follows.

The diagnosis of infectious disease can be carried out by detecting CP expressing on the bacterial cell in a patient's body by an immunological method such as a flow cytometer or immunoblotting.

An immunological method is a method in which an antibody amount or an antigen amount is detected or determined using a labeled antigen or antibody. Examples of the immunological method include radioactive substance-labeled immunoantibody method, enzyme immunoassay, fluorescent immunoassay, luminescent immunoassay, Western blotting method, physico-chemical means and the like.

Examples of the radioactive substance-labeled immunoantibody method include a method, in which the antibody or antigen-binding fragment of the present invention is allowed to react with an antigen, a cell expressing an antigen or the like, then anti-immunoglobulin antibody subjected to a radioactive labeling or a binding fragment thereof is allowed to react therewith, followed by determination using a scintillation counter or the like.

Examples of the enzyme immunoassay include a method, in which the antibody or antigen-binding fragment of the present invention is allowed to react with an antigen, a cell expressing an antigen or the like, then an anti-immunoglobulin antibody or

an binding fragment thereof subjected to antibody labeling is allowed to react therewith and the colored pigment is measured by a spectrophotometer, and, for example, sandwich ELISA may be used. As a label used in the enzyme immunoassay, any known enzyme label (*Enzyme Immunoassay*, published by Igaku Shoin, 1987) can be used as described already. Examples include alkaline phosphatase labeling, peroxidase labeling, luciferase labeling, biotin labeling and the like.

Sandwich ELISA is a method in which an antibody is bound to a solid phase, antigen to be detected or measured is trapped and another antibody is allowed to react with the trapped antigen. In the ELISA, two kinds of antibody which recognizes the antigen to be detected or measured or the antigen-binding fragment thereof in which antigen recognizing site is different are prepared and the first antibody or antigen-binding fragments is previously adsorbed on a plate (such as a 96-well plate) and the second antibody or antigen-binding fragment is labeled with a fluorescent substance such as FITC, an enzyme such as peroxidase, or biotin. The plate to which the above antibody is adsorbed is allowed to react with the cell separated from living body or disrupted cell suspension thereof, tissue or disintegrated solution thereof, cultured cells, serum, pleural effusion, ascites, eye solution or the like, then allowed to react with a labeled monoclonal antibody or an antigen-binding fragment and a detection reaction corresponding to the labeled substance is carried out. The antigen concentration in the sample to be tested can be calculated from a calibration curve prepared by a stepwise dilution of antigen of known concentration. As antibody used for sandwich ELISA, any of polyclonal antibody and monoclonal antibody may be used or antigen-binding fragments such as Fab, Fab' and F(ab)₂ may be used. As a combination of two kinds of antibodies used in sandwich ELISA, a combination of monoclonal antibodies or antigen-binding fragments recognizing different epitopes may be used or a combination of polyclonal antibody with monoclonal antibody or antigen-binding fragments may be used. Further two kinds of antibodies are same or not, can be used in the assay.

A fluorescent immunoassay includes a method described in the literatures [*Monoclonal Antibodies - Principles and practice*, Third Edition, Academic Press (1996); *Manual for Monoclonal Antibody Experiments*, Kodansha Scientific (1987)] and the like. As a label for the fluorescent immunoassay, any of known fluorescent labels [*Fluorescent Immunoassay*, by Akira Kawao, Soft Science, (1983)] may be used as described already. Examples of the label include FITC, RITC and the like.

The luminescent immunoassay can be carried out using the methods described in the literature [*Bioluminescence and Chemical Luminescence*, Rinsho Kensa, 42, Hirokawa Shoten (1998)] and the like. As a label used for luminescent immunoassay,

any of known luminescent labels can be included. Examples include acridinium ester, lophine or the like may be used.

Western blotting is a method in which an antigen or a cell expressing an antigen is fractionated by SDS-polyacrylamide gel electrophoresis [*Antibodies-A Laboratory Manual* (Cold Spring Harbor Laboratory, 1988)], the gel is blotted onto PVDF membrane or nitrocellulose membrane, the membrane is allowed to react with antigen-recognizing antibody or antigen-binding fragment, further allowed to react with an anti-mouse IgG antibody or antigen-binding fragment which is labeled with a fluorescent substance such as FITC, an enzyme label such as peroxidase, a biotin labeling, or the like, and the label is visualized to confirm the reaction.

The physicochemical method is specifically carried out by reacting CP as the antigen with the antibody or antigen-binding fragment of the present invention to form an aggregate, and detecting this aggregate. Other examples of the physicochemical methods include a capillary method, an one-dimensional immunodiffusion method, an immunoturbidimetry, a latex immunoturbidimetry [*Handbook of Clinical Test Methods*, Kanehara Shuppan, (1988)] and the like. For example, in a latex immunodiffusion method, a carrier such as polystyrene latex having a particle size of about of 0.1 to 1 μm sensitized with antibody or antigen may be used and when an antigen-antibody reaction is carried out using the corresponding antigen or antibody, scattered light in the reaction solution increases while transmitted light decreases. When such a change is detected as absorbance or integral sphere turbidity, it is now possible to measure antigen concentration, etc. in the sample to be tested.

For the detection of the bacterial cell expressing CP, known immunological detection methods can be used, and an immunoprecipitation method, an immuno cell staining method, an immune tissue staining method, a fluorescent antibody staining method and the like are preferably used.

An immunoprecipitation method is a method in which a cell expressing CP is allowed to react with the monoclonal antibody or antigen-binding fragment of the present invention and then a carrier having specific binding ability to immunoglobulin such as protein G-Sepharose is added so that an antigen-antibody complex is precipitated. Also, the following method can be carried out.

The above-described antibody or antigen-binding fragment of the present invention is solid-phased on a 96-well plate for ELISA and then blocked with BSA-PBS. When the antibody is in a non-purified state such as a culture supernatant of hybridoma cell, anti-mouse immunoglobulin or rat immunoglobulin or protein A or Protein G or the like is previously adsorbed on a 96-well plate for ELISA and blocked with BSA-PBS and a culture supernatant of hybridoma cell is dispensed thereto for binding. After BSA-PBS is

discarded and the residue is sufficiently washed with PBS, reaction is carried out with a dissolved solution of cells or tissues expressing CP. An immune precipitate is extracted from the well-washed plate with a sample buffer for SDS-PAGE and detected by the above-described Western blotting.

5 An immune cell staining method or an immune tissue staining method are a method where cells or tissues in which antigen is expressed are treated, if necessary, with a surfactant, methanol or the like to make an antibody easily permeate to the cells or tissues, then the monoclonal antibody of the present invention is allowed to react therewith, then
10 subjected to fluorescent labeling such as FITC, enzyme label such as peroxidase or biotin labeling and the label is visualized and observed under a microscope. In addition, cells of tissues can be detected by an immunofluorescent staining method where cells are allowed to react with a fluorescence-labeled antibody and analyzed by a flow cytometer
[*Monoclonal Antibodies - Principles and practice*, Third Edition, Academic Press (1996),
15 *Manual for Experiments of Monoclonal Antibodies*, Kodansha Scientific (1987)] in which cells are allowed to react with a fluorescence-labeled antibody and analyzed by a flow cytometer. Particularly, the monoclonal antibody or antigen-binding fragment of the present invention which binds to *S. aureus* CP can detect a cell expressing the polypeptide maintaining a natural structure.

20 In addition, in the case of using FMAT8100HTS system (manufactured by Applied Biosystems) and the like among fluorescent antibody staining methods, the antigen quantity or antibody quantity can be measured without separating the formed antibody-antigen complex and the free antibody or antigen which is not concerned in the formation of the antibody-antigen complex.

25 The present invention is described below by Examples; however, the present invention is not limited to the following Examples.

Example 1: Preparation of purified capsular polysaccharide 5 (CP5).

CP5 was purified from the *S. aureus* strain Reynolds according to published
30 protocols [*Infect. Immun* 1984; 45(1): 87-93; *Proc. Natl. Acad. Sci. USA*; 2001, 98(16): 9365-70]. Briefly, the staphylococci were grown overnight on several Columbia CNA agar (Beckton Dickinson) plates supplemented with 2.5% NaCl (w/v) and 0.1% glucose (w/v) at 37°C in filtered air to promote CP5 production. Then, the bacteria were
35 harvested from the plates by washing them off with 50 mM sodium acetate, pH 6.0, pooled, and autoclaved for 20 min. Then, bacteria were pelleted by centrifugation and the supernatant collected (= supernatant #1). The bacterial pellet was resuspended in 50 mM sodium acetate, pH 6.0, and autoclaved as above. Subsequently, the bacterial suspension

was centrifuged and supernatant #2 collected. Supernatants #1 and #2, which contained CP5, were pooled and concentrated using a tangential flow filtration (TFF) unit with a molecular weight cut-off (MWCO) of 10 kDa (Sartorius). The retentate of the filtration containing CP5 was collected and its pH was adjusted to 5.0. Then, it was treated with 0.2 M sodium meta-periodate for 72 h to oxidize teichoic acids and subsequently dialyzed against phosphate buffered saline (PBS), pH 7.4, using dialysis bags with MWCO of 10 kDa (Thermo Scientific). To remove DNA, RNA, and protein, the CP5 containing mixture was treated with DNase, RNase, and pronase (Sigma-Aldrich) and then dialyzed against 50 mM sodium acetate, pH 6.0, using dialysis bags as above. Subsequently, the absence of phosphorus, which would be indicative of residual nucleic acids in the sample, was confirmed by a standard colorimetric assay for phosphorus detection (detection limit: 4.7 ng/mL). Then, the material was subjected twice to anion exchange chromatography on Diethylaminoethyl cellulose (DEAE) with NaCl gradient elution. CP5 content in individual fractions was determined using ELISA. Moreover, their content of peptidoglycan (detection limit: 14 ng/mL), protein, teichoic acids (detection limit: 1.4 ng/mL), and phosphorus (detection limit: 4.7 ng/mL) was also measured for each fraction. CP5-rich fractions, in which none of the above impurities were detectable, were pooled, concentrated, and dialyzed against H₂O, and then lyophilized and stored at -80°C. Finally, ¹H NMR was conducted with de-O-acetylated CP5. The obtained spectra were consistent with those obtained for reference material. Final CP5 purity was found to be >95%.

Example 2: Preparation of immunogens for mouse immunizations aiming for anti-CP5 mAb generation (Partially purified CP5; CP5-BSA conjugates; formalin fixed *S. aureus* cells).

Three types of immunogens were prepared for the immunization of mice aiming for the generation of anti-CP5 mAbs. The immunogens and how they were prepared is described below.

Partially purified CP5 was isolated from the *S. aureus* strain Reynolds. Briefly, the CP5 expressing *S. aureus* strain Reynolds was grown, harvested and autoclaved twice to obtain supernatants #1 and #2. After supernatants #1 and #2 were pooled and concentrated, and the teichoic acids oxidized, the material was dialyzed against PBS, pH 7.4. Then, the material was characterized for protein, peptidoglycan, phosphorous and CP5 content by colorimetric assays or ELISAs. The material contained substantial amounts of all these components (Data not shown) and was designated as "CP5 autoclave prep". Subsequently, aliquots of the material were lyophilized and stored at -80°C until used for immunization of mice by reconstitution with H₂O.

CP5-rich semi-purified material was obtained by a precipitation method using trichloroacetic acid (TCA) and ethanol (EtOH). Briefly, CP5 expressing *S. aureus* Reynolds was grown overnight on several Columbia CNA agar plates supplemented with 2.5% NaCl (w/v) and 0.1% glucose (w/v) at 37°C in filtered air. Then, the bacteria were harvested by washing them off the plates with PBS. The cells were washed once in PBS, then pelleted and resuspended in PBS containing 2% TCA. The bacterial suspension was incubated overnight at 4°C with gentle stirring. Then, the suspension was centrifuged at 3,000 x g for 10 min. The supernatant was collected and mixed 1/5 with EtOH (Absolute-200 proof, Aaper Alcohol and Chemical Co.). The mixture was incubated overnight at 4°C with gentle stirring. To obtain the TCA/EtOH precipitate, the mixture was centrifuged at 750 x g for 10 min and the supernatant discarded. The precipitate was resuspended in PBS, pH 7.4. Aliquots of the material were lyophilized and stored at -80°C until used for immunization of mice by reconstitution with ddH₂O. The material was characterized for protein, peptidoglycan, phosphorous and CP5 content by colorimetric assays or ELISAs. The material contained substantial amounts of all these components (Data not shown) and was designated as "CP5 TCA/EtOH prep".

Conjugation of CP5 to bovine serum albumin (BSA) was performed using chemical crosslinkers through a thioether bond after introduction of a thiol group containing a linker to the polysaccharide and a haloacetyl group to the protein carrier. Briefly, 3-(2-Pyridyldithio)-propionyl hydrazide (PDPH) was used to modify CP5, whereby the carbodiimide-activated carboxylate groups of N-acetylmannosaminouronic acid in CP5 were coupled to the hydrazide group of PDPH. The carrier protein, namely BSA, was activated by bromoacetylation of the lysine residues with the N-hydroxysuccinimide ester of bromoacetic acid. The PDPH-thiolated CP5, generated by reduction with dithiothreitol (DTT) and purified by NAP-5 columns, was then conjugated to the activated BSA protein by displacement of bromine in the bromoacetylated protein with thiol, resulting in a stable thioether bond. After the reaction was completed, the CP5-BSA conjugate was separated from unconjugated BSA using size-exclusion chromatography (SEC). Briefly, SEC fractions were collected and analyzed by dot blot. The fractions double positive for CP5 and BSA were dialyzed against ddH₂O water and then lyophilized. Finally, the lyophilized material was re-suspended in PBS for quantitation; the CP5 concentration was calculated by ELISA, while the amount of conjugated BSA was determined using Lowry assay.

S. aureus strain Reynolds and Lowenstein were grown as described in Example 1, then harvested from the agar plates with PBS and washed twice with PBS. Then, the cells were mixed 1:1 (v/v) with 6% formaldehyde (Sigma Aldrich) and incubated overnight at 4°C to fix the cells. Then, the bacteria were washed twice with PBS and stored at 4°C

until used for immunization of mice. That the FF Reynolds and Lowenstein cells had CP5 on their surface was verified by immunofluorescence and flow cytometry (Data not shown).

5 Example 3: Immunization of mice, screening process, selection of candidate anti-CP5 mAbs, and isotyping.

To obtain mouse anti-CP5 mAbs, BALB/c mice were immunized with antigens that were prepared as described in Example 2. For each immunization except the final boost, the antigens were mixed 1:1 with Sigma Adjuvant System® (Sigma-Aldrich) and 10 200 µL of the emulsions were administered intraperitoneally to the respective mice. Three days after the final booster injection, which were given intravenously without adjuvant, mice were euthanized and the spleens collected and grinded. The splenocytes from the hyper-immunized mice and Sp2/0-Ag14 mouse myeloma cells (ATCC#: CRL-1581) were used as fusion partners for the production of hybridomas. Fusions were 15 conducted according to standard methods. After fusion, hybridoma supernatants were tested for the presence of anti-CP5 IgG-type antibodies with ELISA with immobilized CP5 as described elsewhere in this document. To isolate individual hybridoma cells secreting anti-CP5 mAbs, limiting dilution technique was applied according to standard techniques. To identify the isotype of isolated mouse mAbs, IsoQuick Strips® (Sigma-Aldrich) were 20 used. To test whether candidate mouse anti-CP5 mAbs can react with CP5 expressed on *S. aureus* whole cells, their binding to agar-grown CP5 expressing *S. aureus* Reynolds wild-type cells was assessed by flow cytometry as described elsewhere in this document. To determine specificity of the candidate antibodies for CP5, a genetically engineered isogenic *S. aureus* Reynolds CP5-negative capO mutant was included in the flow 25 cytometry analyses. Through these efforts, four IgG-type mouse CP5-specific antibodies were obtained (they bound to immobilized CP5 and *S. aureus* Reynolds wild-type, but not to the CP5-negative capO mutant strain); the antibody names, IgG subclass, light chain type, and the antigens used to immunize the mice are listed in Table 1.

30 Table 1:

Clone name	Isotype	Antigens used for immunization*
137F8	IgG2b/kappa	50 µg "CP5 TCA/EtOH prep" (4x) and 20 µg CP5-BSA conjugate (1x)
137G10	IgG1/kappa	50 µg "CP5 autoclave prep" (4x) and 20 µg CP5-BSA conjugate (2x)
137G18A	IgG1/kappa	50 µg "CP5 TCA/EtOH prep" (4x) and 20 µg CP5-BSA conjugate (1x)
35 137L2AA	IgG2a/kappa	20 µg CP5-BSA conjugate (3x) and 10 ⁷ <i>S. aureus</i> cells (1x)

*, The amounts of "CP5 TCA/EtOH prep", "CP5 autoclave prep", and CP5-BSA antigens indicated in Table 1 are protein contents of the samples.

Antibodies were purified by affinity chromatography on AKTA FPLC system controlled by Unicorn software. Depending on the antibody subclass, either protein A or protein G resin was used. MabSelect SuRe Protein A resin (GE Healthcare) was used for purification of mouse IgG2a, IgG2b, and IgG3 subclasses from culture supernatants of the selected hybridomas. After loading of the serum-free hybridoma supernatant, the resin was washed with 10 column volumes of PBS to remove the unbound proteins, and an antibody was eluted with 0.1 M Glycine-HCl, pH 3.4. The eluted fractions were immediately neutralized with 1 M Tris-HCl, pH 8.0. The antibody-containing fractions were pooled and buffer exchanged into PBS via dialysis with 10K MWCO Slide-A-Lyzers (Thermo Scientific). Protein G Sepharose 4 Fast Flow (GE Healthcare) was used for purification of mouse IgG1 subclass. Column was equilibrated in 50 mM Sodium Phosphate pH 6.0, 0.5 M NaCl. After loading of the serum-free hybridoma supernatant (adjusted to pH 6.0 with Citric acid – Sodium Citrate buffer pH 5.0), the resin was washed with 10 column volumes of equilibration buffer to remove the unbound proteins. An antibody was eluted with 0.1 M Glycine-HCl, pH 2.5. The eluted fractions were immediately neutralized with 1 M Tris-HCl, pH 8.0. The antibody-containing fractions were pooled and buffer exchanged into PBS via dialysis with 10K MWCO Slide-A-Lyzers (Thermo Scientific). The purified antibody samples were concentrated with a centrifugal concentrator (Vivaspin, 50K MWCO, Sartorius-Stedim Biotech SA) and sterilized with 0.22 μ m syringe filter. The protein concentration was determined by the Lowry assay using DC Lowry kit (Bio-Rad). SDS PAGE and analytical SEC analyses were used for quality control analysis of the purified antibodies. All antibody samples had the purity of $\geq 99\%$ and the percentage of aggregates of $< 1\%$. Endotoxin level was analyzed by Charles River Endosafe® - PTS spectrophotometer, which utilizes cartridges that contain FDA-licensed Limulus Amebocyte Lysate (LAL) formulations. For all of the samples the endotoxin level was shown to be very low (< 0.05 EU/mg).

Example 4: Comparison of mouse anti-CP5 mAb binding to immobilized CP5 by ELISA.

The binding of four mouse anti-CP5 mAbs to immobilized CP5 was compared by ELISA. Briefly, 96-well plates were coated overnight with purified CP5, washed, and blocked. Mouse anti-CP mAbs were added at the indicated concentrations to the CP5 coated wells for 1 h. Isotype mAbs served as negative controls. Antibodies bound to immobilized CP5 were detected with horseradish peroxidase (HRP)-conjugated secondary antibodies and TMB substrate. Reaction was stopped with sulfuric acid and the optical density (OD) read at 450 nm (OD_{450nm}).

As can be seen in FIG. 1, 137G18A was found to be the strongest binder to CP5; 137F8, 137G10, and 137L2AA exhibited similar reactivity profiles. Indicating

specificity of anti-CP5 mAb binding, negative control mouse isotype antibodies did not show any significant binding to immobilized CP5 in the ELISAs (Data not shown); furthermore, the anti-CP5 mAbs did not bind to uncoated wells, that were blocked, but did not contain any CP5 (Data not shown). 137G18A showed the best binding of the anti-CP5 mAbs to immobilized CP5.

Example 5: Preparation of biotinylated CP5 and affinity measurement for four mouse anti-CP5 mAbs using BIAcore system.

To enable binding of purified CP5 to BIAcore chips, it was biotinylated using EZ-Link® Hydrazide-Biotin reagent (Thermo Scientific). Briefly, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) was used to activate carboxyl groups of N-acetylmannosaminouronic acid residues in CP5 to make them reactive to bind to the amino groups of Hydrazide-Biotin, forming an amide linkage. The reaction was performed in acidic buffer (pH 4.7-5.5); the pH was maintained constant by the addition of HCl. Subsequently, the reaction mixture was dialyzed against ddH₂O to separate biotinylated CP5 from non-conjugated biotin. Finally, the concentration of biotinylated CP5 was calculated by ELISA.

In order to kinetically analyze the binding activity of anti-CP5 mouse antibodies 137G10, 137G18A, 137L2AA, and 137F8 to immobilized CP5, the binding activity was measured by surface plasmon resonance method (SPR). All of the following manipulations were carried out using a Biacore 3000 (manufactured by GE Healthcare Bio-Sciences). Biotinylated CP5 was captured with a SA chip (manufactured by GE Healthcare Bio-Sciences). Thereafter, the anti-CP5 antibodies diluted from a high concentration in seven steps were allowed to run at a flow rate of 20 μ L/min onto the chip, and the sensorgram corresponding to each concentration was obtained (FIGs. 2A to 2D). The analysis was carried out using a bivalent binding model, using the analysis software attached to the apparatus, Biacore 3000 Evaluation software (provided by Biacore), thereby calculating an association rate constant k_{a1} and a dissociation rate constant k_{d1} , as well as k_{a2} and k_{d2} of respective antibodies for immobilized CP5.

As a result of using a bivalent binding model, an association rate constant k_{a1} , a dissociation rate constant k_{d1} , and a dissociation constant K_{D1} (k_d/k_a), as well as an association rate constant k_{a2} , a dissociation rate constant k_{d2} , and a dissociation constant K_{D2} (k_d/k_a), of individual antibodies thus obtained are given in Table 2.

Table 2:

Ligand: CP5 (~172 RUs); Analyte: 137G10 (Concentration Range = 25 nM-0.1953 nM)							
ka1 (1/Ms)	kd1 (1/s)	KD1	ka2 (1/RUs)	kd2 (1/s)	KD2	Rmax (RU)	Chi2
6.1E+05	0.0345	5.66E-08	7.06E-06	1.96E-03	2.77E+02	359	1.77
Ligand: CP5 (~75 RUs); Analyte: 137G18A (Concentration Range = 25 nM-0.1953 nM)							
ka1 (1/Ms)	kd1 (1/s)	KD1	ka2 (1/RUs)	kd2 (1/s)	KD2	Rmax (RU)	Chi2
2.77E+05	1.46E-03	5.27E-09	4.56	381	8.36E+01	306	1.02
Ligand: CP5 (~340 RUs); Analyte: 137L2AA (Concentration Range = 50 nM-0.3906 nM)							
ka1 (1/Ms)	kd1 (1/s)	KD1	ka2 (1/RUs)	kd2 (1/s)	KD2	Rmax (RU)	Chi2
2.17E+05	0.0333	1.53E-07	1.01E-05	1.32E-03	1.31E+02	346	1.47
Ligand: CP5 (~340 RUs); Analyte: 137F8 (Concentration Range = 50 nM-0.3906 nM)							
ka1 (1/Ms)	kd1 (1/s)	KD1	ka2 (1/RUs)	kd2 (1/s)	KD2	Rmax (RU)	Chi2
3.38E+05	0.0917	2.71E-07	2.68E-06	5.33E-4	1.99E+02	564	0.81

Example 6: Competition ELISA with purified CP5 and two mouse anti-CP5 mAbs.

To determine whether the four mouse anti-CP5 mAbs compete for binding to immobilized CP5, ELISAs were conducted. Briefly, ELISA plates were coated with CP5. Then, primary anti-CP5 mAbs or isotype antibodies were added for 30 min. After that, unbound primary antibody was washed off the plates. Then, secondary anti-CP5 mAbs or isotype antibodies were added to determine whether the binding of the primary anti-CP5 mAb was affected by addition of another anti-CP5 mAb and vice versa. After 30 min incubation with secondary antibody, bound primary antibodies were detected with HRP-labeled anti-mouse isotype specific tertiary antibodies and TMB substrate.

As can be seen in FIG. 3A, the OD450nm values for 137G18A (mouse IgG1) binding to CP5 were reduced when 137L2AA (mouse IgG2a) was added as secondary antibody (Average OD450nm value: 1.47+0.06) as compared to addition of an isotype mouse IgG2a antibody (Average OD450nm value: 1.79+0.06) or vehicle alone (Average OD450nm value: 1.81+0.04). A ~60% lower average OD450nm value for 137L2AA binding to CP5 was observed when 137G18A was added as secondary antibody (Average OD450nm value: 0.73+0.05) as compared to addition of an isotype mouse IgG1 mAb (Average OD450nm value: 1.82+0.22) or vehicle alone (Average OD450nm value: 1.78+0.06) (FIG. 3B). Of note, primary mouse IgG1 and IgG2a isotype antibodies did not show any significant binding to CP5 (Data not shown) indicating CP5 specific binding of 137G18A and 137L2AA in the ELISAs. Regarding the other two anti-CP5 mAbs 137G10 and 137F8, they did not show any clear competitive effect on the binding of 137G18A and 137L2AA to immobilized CP5. Both anti-CP5 137G18A and 137L2AA

negatively influenced the binding of each other to immobilized CP5 indicating that they are either specific for the same CP5 epitope or that they sterically hinder each others binding to CP5.

5 Example 7: Capturing of soluble CP5 antigens by mouse anti-CP5 mAbs.

After having shown that the mouse anti-CP5 mAbs 137G18A and 137L2AA can bind to immobilized CP5, it was tested by sandwich ELISA whether they can capture soluble CP5.

10 The methods of the applied sandwich ELISA are described in detail in the legend of FIGs. 4A and 4B. Briefly, 96-well plates were coated with vehicle, isotype negative control mAbs or the mouse anti-CP5 mAbs 137G18A or 137L2AA. After blocking unspecific binding sites, CP5 in solution was added to each well. CP5, that was captured by the anti-CP5 mAbs or that unspecifically bound to the plates or the negative control antibodies was then detected with biotinylated anti-CP5 mAb 137G18A,
15 streptavidin-conjugated horse radish peroxidase and TMB substrate.

As can be seen in FIGs. 4A and 4B, both 137G18A and 137L2AA were able to capture solubilized CP5 as indicated by the highly elevated OD450nm values measured in wells that contained the anti-CP5 mAbs as capture antibodies as compared to wells coated with negative control antibodies or vehicle alone. The sandwich ELISA data proved that
20 the mouse anti-CP5 mAbs 137G18A and 137L2AA can capture immobilized/solubilized CP5.

Example 8: Detection of soluble CP5 antigens in plasma taken from *S. aureus* infected animals.

25 After showing that the mouse anti-CP5 mAb 137G18A can bind to immobilized and soluble CP5, it was determined whether it can be utilized to detect CP5 in rodents infected with an *S. aureus* strain expressing CP5.

C57BL/6 mice were infected intraperitoneally with CP5 expressing *S. aureus* Reynolds. 48 hours after infection, mice were euthanized and left kidneys and
30 heparinized cardiac blood collected. Bacterial burden in blood and kidneys was determined and CP5 in the plasma samples detected by biotinylated 137G18A.

As can be seen in FIG. 5 and Table 3, CP5 was detectable in the sterile-filtered plasma samples of all three *S. aureus* Reynolds infected animals 48 hours after infection. All three animals had bacteria in their kidneys and two of the three mice also had >100
35 CFU of bacteria in their peripheral blood. Of note, using the same sandwich ELISA system, it was possible to detect CP5 in sterile-filtered plasma of Sprague Dawley rats experimentally infected with *S. aureus* Reynolds (Data not shown).

The results with mice and rats show that CP5 can be detected in the plasma of rodents infected with CP5 expressing staphylococci. In the shown mouse example, bacteria were found in the kidneys and blood of the mice indicating that the host animals did not clear the infection during the 48-hour period. This suggests that CP5 might be shedded by the bacteria in the hosts and that thus CP5 might be used as biomarker to detect active infection caused by CP5 expressing *S. aureus* strains in mammals possibly including humans.

Table 3:

Bacteria in kidneys and blood of infected mice

Infected mouse #	Total CFU in left kidney	CFU/mL cardiac blood
1	1.8×10^7	<100
2	6.2×10^4	4.4×10^5
3	1.65×10^6	6.0×10^2

Example 9: Comparison of mouse anti-CP5 mAb binding to live antibiotic-resistant *S. aureus* cells (VRSA COLVA and MRSA BAA-41) by flow cytometry.

To test whether the mouse anti-CP5 mAbs 137F8, 137G10, 137G18A, and 137L2AA can react with CP5 expressed on antibiotic-resistant *S. aureus* whole cells, the binding of these mAbs to live cells of the CP5 expressing *S. aureus* strains VRSA COLVA and MRSA BAA-41 was assessed.

The binding of the mouse anti-CP5 mAbs to the above-mentioned *S. aureus* strains was tested by immunofluorescence. Briefly, live agar-grown staphylococci were incubated with various concentrations of mouse anti-CP5 or isotype control mAbs (= primary antibodies). Bound primary antibodies on the bacterial particles were detected with fluorescently labeled secondary antibodies. The fluorescence intensity of the bacterial particles was measured by flow cytometry using a BD FACSCalibur™ and data analyzed using FlowJo software.

As can be seen in FIGs. 6A and 6B, all four mouse anti-CP5 antibodies were able to bind to the VRSA and MRSA strains in a dose dependent fashion. Of note, isotype antibodies did not show any significant binding to the bacteria (Data not shown.). 137G18A and 137L2AA showed much stronger binding than the comparator mAbs 137G10 and 137F8 to the staphylococcal strains.

The flow cytometry experiments with strains COLVA and BAA-41 showed that 137L2AA and 137G18A could strongly bind to the clinically most relevant multidrug-resistant *S. aureus* types VRSA and MRSA.

Example 10: Activity of mouse anti-CP5 mAbs in opsonophagocytosis assays with mouse macrophages and CP5 expressing MSSA strain Reynolds.

Professional phagocytes such as macrophages and polymorphonuclear neutrophils (PMN) compose a critical first line host defense against invading microbial pathogens. It is thought that anti-bacterial antibodies can mediate protection via conferring uptake and destruction of bacteria by professional phagocytes. To assess whether the murine anti-CP5 mAbs 137F8, 137G10, and 137G18A can mediate uptake of *S. aureus* cells by phagocytes, their activity was assessed in opsonophagocytosis assays (OPAs) with the murine macrophage cell line J774 and the CP5 expressing strain *S. aureus* Reynolds. Briefly, live *S. aureus* cells were preopsonized with control or anti-CP5 mAbs in the absence of any complement source and then co-incubated with J774 cells to induce phagocytosis of the staphylococci. After 1 h, remaining extracellular bacteria were killed and then intracellular CFU numbers quantified. Importantly, macrophages are thought to kill staphylococci relatively slowly. Thus, it was expected that a high percentage of bacteria taken up by the phagocytes would survive intracellularly until quantification.

As can be seen in FIGs. 7 and 8, all three anti-CP5 mAbs were able to confer significant uptake of *S. aureus* Reynolds by murine macrophages as compared to negative control isotype antibodies. Of note, 137G18A showed higher activity than 137G10 and 137F8 in spite of its subclass (mouse IgG1).

The experiments demonstrated that anti-CP5 antibodies can confer uptake of CP5 expressing *S. aureus* cells by mouse macrophages in the absence of complement. Since phagocytosis is thought to be a crucial host defense mechanism against *S. aureus*, the OPA results described in this example indicate that anti-CP5 antibodies might also be protective *in vivo* in the host even at infection sites with low/no complement source.

Example 11: Mouse whole blood killing assays with mouse anti-CP5 antibodies.

After having established that the mouse anti-CP5 mAbs 137F8, 137G10, 137G18A, and 137L2AA can induce uptake of *S. aureus* cells by murine macrophages, it was tested if they can also mediate killing of staphylococci in an *ex vivo* whole blood killing experiments to further verify their anti-bacterial activities.

Briefly, live *S. aureus* cells were incubated with 50% pooled fresh heparinized mouse cardiac blood with overhead rotation. Then, the samples were diluted in distilled water to lyse blood cells and finally plated on agar plates to enumerate surviving CFU numbers.

As can be seen in FIGs 9A and 9B, all four mouse anti-CP5 mAbs led to reduced bacterial numbers as compared to the respective negative control isotype mAbs after 2 hours co-incubation with bacteria and mouse whole blood.

Further suggesting anti-bacterial therapeutic potential of the anti-CP5 mAbs 137F8, 137G10, 137G18A, and 137L2AA, the results outlined in this example indicate that the anti-CP5 mAbs facilitate killing of CP5 expressing *S. aureus* cells by mouse whole blood *ex vivo*.

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Examples 12: *In vivo* efficacy evaluation of mouse anti-CP5 mAbs in mouse systemic infection models of passive immunization and activity of mouse anti-CP5 mAbs in mouse kidney infection model of passive immunization.

The activities of 137F8, 137G10, 137G18A, and 137L2AA were tested in
10 mouse sepsis and kidney infection models to determine whether passive immunization with anti-CP5 mAbs is conferring protection from *S. aureus* infection.

Anti-CP5 antibody activity was tested in two sepsis models and a kidney infection model in mice. Briefly, antibodies or vehicle alone were administered to mice 24 hours before infection with the MSSA strains Reynolds or Lowenstein. As read-out
15 for antibody efficacy survival and bacterial burden in blood or kidneys were selected.

At 0.1 mg doses, passively administered 137G18A conferred significant protection in a sepsis model with the highly encapsulated CP5 *S. aureus* strain Reynolds (FIG. 10A). Correlating with their survival, 137G18A treated mice had lower CFU concentrations in their peripheral blood than isotype treated animals 24 hours after
20 infection (FIG. 10B). In contrast, 137G10 and 137F8 failed to confer a clear survival advantage and did not have any impact on the blood CFU numbers at 0.1 mg doses as compared to the respective isotype antibodies. 137L2AA exhibited similar activity as 137G18A at 0.1 mg (FIG. 10C). In antibody titration experiments, 25 and 50 microgram doses of 137G18A conferred protection and reduced bacterial burden in the blood (FIGS.
25 10D-10E). In a mouse sepsis model with the *S. aureus* strain Lowenstein, 137G10 and 137G18A conferred high protection at 0.2 mg doses (FIG. 10F) and reduced blood CFU numbers (FIG. 10G). 137L2AA treated mice also had a survival advantage in this model (FIG. 10H), whereas 137F8 did not show any efficacy (Data not shown).

In a *S. aureus* Reynolds kidney infection model, treatment with 0.1 mg
30 137G18A via the intraperitoneal (i.p.) route led to reduced bacterial numbers in the left kidneys 48 hours after infection compared to isotype control treated mice, whereas 137G10 and 137F8 did not have a significant effect on the bacterial burden in the kidneys of the mice (FIG. 11A). Of note, 137G18A also reduced the *S. aureus* CFU numbers in the kidneys when given subcutaneously instead of i.p. (FIG. 11B).

35 The mouse passive immunization experiments established that anti-CP5 mAbs can confer protection from *S. aureus* infection in a mammalian host. 137G18A showed efficacy in all of the three infection models. In the two sepsis models, the survival

advantage of 137G18A treated mice correlated well with a statistically reduced bacterial burden in the blood of the animals 24 hours after infection. This results suggests that 137G18A leads to enhanced clearance of the bacteria in the mouse host and thus to a survival advantage. The fact that 137G18A treatment led to strongly reduced bacterial numbers in the kidneys of mice in a kidney infection model further substantiated that this antibody has therapeutic potential by leading to bacterial clearance in the mammalian host.

Example 13: cDNA cloning and sequencing of VH and VL regions of candidate mouse anti-CP5 mAbs.

From 1×10^6 cells of hybridoma 137G18A, total RNA was purified from these cells using RNeasy kit (manufactured by QIAGEN Inc.) following the manufacturer's instructions.

Using the RNA obtained as a template, a SMART RACE cDNA Amplification Kit (manufactured by Clontech) with the reverse transcriptase SuperScriptTMII (manufactured by Invitrogen) was used, as per the manufacturer's written instructions, for 5' SMART-RACE-PCR to amplify the cDNA that encodes the VH and VL of the immunoglobulin genes. Briefly, first strand cDNA was prepared by reverse transcriptase from 1 μ g of total RNA following the manufacturer's instructions. This cDNA was used as a template for PCR amplification of the VH using a 5' universal primer mix supplied in the kit and a 3' gene-specific primer specific to mouse IgG. Also, the cDNA fragment of the VL was amplified using the same method using a mouse kappa chain specific 3' primer instead of the IgG-specific primer. PCR was carried out using KOD Hot Start DNA polymerase (manufactured by Novagen). The thermal cycling program was 1 cycle {94°C for 4min}; 5 cycles {94°C for 30 sec; 68°C for 2min}; 5 cycles {94°C for 30 sec, 66°C for 30 sec, 68°C for 1min}; 25 cycles {94°C for 30 sec, 64°C for 30 sec, 68°C for 1min}; 1 cycle {68°C for 5min}.

Amplified DNA fragments were isolated by agarose gel electrophoresis, and purified by QIAquick Gel Extraction Kit (manufactured by Qiagen). Purified DNA fragments of VH and VL were separately integrated into pCR®-Blunt II-TOPO® vector using the Zero® Blunt® TOPO PCR Cloning Kit (manufactured by Invitrogen) as per the manufacturer's instructions. Each plasmid was transformed into competent DH5 α -T1R *E. coli*, and plated on kanamycin-containing LB-Miller agar plates. Plasmids were isolated from bacterial colonies grown in LB-Miller medium with kanamycin grown overnight, using QIAprep Spin Miniprep kit (manufactured by QIAGEN). Plasmids were screened by *Eco*RI restriction digest, and those containing inserts were sequenced by Sanger-sequencing (performed by GENEWIZ Inc.). Nucleotide sequences of each insert in the construct plasmids were analyzed using the nucleotide-alignment software program

Sequencher (manufactured by Gene Codes Corp.). Based on consensus alignment of multiple plasmid clones obtained, cDNA sequences were identified for the VH and VL regions of the heavy and light chains, including the leader sequences, from a putative 5' start ATG codon.

5 Antibody VH and VL domains were analyzed for germline V, D and J genes using the IMGT/V-quest program (Brochet, Lefranc et al. 2008), and leader sequences were deduced. Antibody CDRs were defined using the Kabat method.

Nucleotide sequence of 137G18A VH without the leader sequence is represented in (SEQ ID NO:18), amino acid sequence of 137G18A VH without the leader
10 sequence is represented in (SEQ ID NO:13). Nucleotide sequence of 137G18A VL without the leader sequence is represented in (SEQ ID NO:19), amino acid sequence of 137G18A VL without the leader sequence is represented in (SEQ ID NO:14).

CDRs of the VH and VL of 137G18A were identified using the Kabat method. Amino acid sequences of CDR1, CDR2 and CDR3 of VH of 137G18A were represented
15 by SEQ ID NOs:1, 2 and 3, respectively, and amino acid sequences of CDR1, CDR2 and CDR3 of VL thereof are represented by SEQ ID NOs:4, 5 and 6, respectively.

Example 14: Preparation of mouse/human chimeric IgG1 antibodies for 137G18A and 137G18A (N107D).

20 The mouse/human chimeric 137G18A antibody prepared is comprised of a human IgG1 heavy chain constant region and human kappa constant region respectively ligated to variable regions of heavy and light chains of the mouse anti-CP5 antibody. Transient production of fucosylated antibody was performed by transfection of suspension cultures of FreeStyle™CHO-S cells (Invitrogen) maintained in FreeStyle™CHO
25 Expression Medium (Gibco) while shaking in an 8% CO₂ humidified incubator at 37°C. Cells were transfected with the desired expression vector DNA based on pKANTEX93 (*Mol. Immunol.*(2000), 37:1035–1046) using FreeStyle™MAX transfection reagent (manufactured by Invitrogen) following manufacturer's written instructions. Transfectants were incubated for six days, then supernatant was clarified by centrifugation
30 followed by 0.22 μm filtration (Millipore). This material was further processed for purified antibody.

Framework region three (FR3) of the VH of 137G18A was found to contain a possible Asn-linked glycosylation site asparagine-aspartic acid-threonine (Asn-Asp-Thr), the asparagine (N) residue located at amino acid #107 as numbered from the start
35 methionine of the complete sequence of 137G18A VH with the leader peptide. This amino acid corresponds to position 85 in Kabat method numbering. To eliminate the putative glycosylation site, the asparagine (N) 107 was mutated to aspartic acid (D)

(N107D) by PCR. Briefly, primers containing a mutation that would change the codon for asparagine (AAT) to a codon for aspartic acid (GAT) were designed overlapping the nucleotide in the codon for asparagine 107. The 137G18A(N107D) VH nucleotide sequence without leader sequence is represented by SEQ ID NO:20, and the VH amino acid sequence without leader sequence is represented by SEQ ID NO:15.

Culture supernatants from transfected CHO cells were first concentrated using a small- or medium-scale benchtop tangential flow filtration (TFF) system (Sartorius-Stedim Biotech SA) with 30K MWCO Sartocan® Slice Hydrosart cassette. The concentrated supernatants were filtered with a 0.22 µm vacuum filter unit (Millipore) and processed on AKTA FPLC chromatography system controlled by Unicorn software. MabSelect SuRe Protein A resin (GE Healthcare) was used for the first chromatography step to capture the antibody, and CHT ceramic hydroxyapatite resin (Bio-RAD laboratories) was used to remove residual amounts of leached protein A, host cell proteins, DNA, and the antibody aggregates. After loading of the concentrated supernatant, the resin was washed with 10 column volumes of PBS to remove the unbound proteins, and an antibody was eluted with 0.1 M Glycine-HCl, pH 3.6, 3.5, or 3.4. The eluted fractions were immediately neutralized with 1 M Tris-HCl, pH 8.0. Following analytical SEC analysis, the antibody-containing fractions with low percentage of the aggregates were pooled and buffer exchanged into 10 mM Sodium Phosphate, pH 6.5 via dialysis with 10K MWCO Slide-A-Lyzers (Thermo Scientific). CHT polishing chromatography step was conducted according to the manufacturer's instructions. After loading of the post-protein A material, the resin was washed with 10 mM Sodium Phosphate, pH 6.5 and an antibody was eluted with 20 CV gradient of 0 – 1 M NaCl. Following analytical SEC analysis, the antibody-containing fractions with low percentage of the aggregates were pooled and buffer exchanged into PBS via dialysis with 10K MWCO Slide-A-Lyzers (Thermo Scientific). The purified antibody was concentrated with a centrifugal concentrator (Vivaspin, 50K MWCO, Sartorius-Stedim Biotech SA), sterilized with 0.22 µm syringe filter, and the sample concentration was determined by the Lowry assay using DC Lowry kit (Bio-Rad). SDS PAGE and analytical SEC analyses were used for quality control analysis of the purified samples. All antibody samples had the purity of ≥ 99% and the percentage of aggregates of < 1%. Endotoxin level was analyzed by Charles River Endosafe® - PTS spectrophotometer that utilizes cartridges that contain FDA-licensed Limulus Amebocyte Lysate (LAL) formulations. For all of the samples the endotoxin level was shown to be very low (< 0.05 EU/mg).

Example 15: Binding of chimeric anti-CP5 antibody 137G18A to immobilized CP5 and capturing of soluble CP5 by the antibody.

To test the functionality of the mouse/human chimeric version of the anti-CP5 antibody 137G18A, it was tested if it could bind to immobilized CP5 and if it could capture soluble CP5 by ELISA. As can be seen in FIG. 12A, in contrast to the isotype antibody, chimeric 137G18A showed dose-dependent binding to the CP5 coated plates. Furthermore, it was demonstrated that chimeric 137G18A could capture soluble CP5 (FIG. 12B). The ELISA data suggest that the mouse/human chimeric version of the anti-CP5 antibody 137G18A retained its ability to strongly bind to immobilized CP5 and to capture soluble CP5.

Example 16: Antigen-binding detection for chimeric anti-CP5 antibodies 137G18A and 137G18A (N107D) by using Biacore system.

In order to compare the binding activity of anti-CP5 chimeric antibodies 137G18A and 138G18A (N107) to CP5, the binding activity was measured by surface plasmon resonance method (SPR). All of the following manipulations were carried out using a Biacore 3000 (manufactured by GE Healthcare Bio-Sciences). Biotinylated CP5 was captured with a SA chip (manufactured by GE Healthcare Bio-Sciences). Thereafter, the anti-CP5 antibodies and negative control antibodies were diluted to a concentration of 25 nM, allowed to run at a flow rate of 20 μ L/min onto the chip, and sensorgrams were obtained (FIG. 13). Mouse/human chimeric 138G18A (N107) showed a higher response than that of mouse/human chimeric 137G18A.

Example 17: Preparation of Pagibaximab and F598 antibodies

F598 is a human IgG1/lambda antibody against poly-N-acetyl glucosamine (PNAG) described in WO 2005/103084 A2 and a previous paper [*Infect Immun.* 2006, 74(5):2742-50]. A fully human antibody based on the VH and VL antibody sequences provided in WO 2005/103084 A2 was constructed. Pagibaximab (A110) is a chimeric mouse/human IgG1/kappa antibody against lipoteichoic acid (LTA) described in patent US 7250494 B2. We constructed a chimeric mouse/human antibody based on the VH and VL antibody sequences from clone A110 in the patent. Stable CHO-K1 cells for the expression of F598 and Pagibaximab were generated using the same method described in a previous paper [*Biotechnol Bioeng.* 2006, 94(4):680-8.]. These cells were used for the production of antibody using standard culture methods.

Example 18: Antigen-binding comparison between chimeric 137G18A and anti-LTA antibody Pagibaximab in human serum using *Staphylococcus haemolyticus* and MRSA BAA-41.

LTA is a conserved cell envelope component of Gram-positive bacteria. It is described in the literature that the binding of anti-LTA antibodies in serum can be impaired by serum ficolins by competing with binding sites on the LTA surface polymer. To assess whether the binding of anti-CP5 antibodies to staphylococci is affected by serum, the binding of the chimeric anti-CP5 antibody 137G18A to a *Staphylococcus haemolyticus* strain (ATCC-43252) was tested, because previously we found 137G18A bound to the strain with flow cytometry. Similar experiments were conducted with the MRSA strain BAA-41. For both assay systems, the chimeric anti-LTA antibody Pagibaximab was used as a control.

As can be seen in FIGs. 14A, 14B and 15, individually tested sera from various human donors affected the binding of the chimeric anti-CP5 antibody 137G18A to *S. haemolyticus* and MRSA significantly less than the binding of the chimeric anti-LTA antibody Pagibaximab. The serum inhibition data suggest that the anti-CP5 antibody 137G18A has superior antigen binding activity to staphylococci it reacts with than Pagibaximab in the presence of human serum.

Example 19: Activity comparison of chimeric 137G18A, anti-LTA antibody Pagibaximab, and anti-PNAG antibody F598 in opsonophagocytosis assays with mouse macrophages and MSSA Reynolds, and in those with human PMN and VRSA COLVA.

After having established that chimeric 137G18A retained its ability to bind to its antigen as shown by assays with immobilized CP5 and with *S. aureus* whole cells, the functionality of chimeric 137G18A was tested in several *in vitro* and *in vivo* assay systems. Firstly, the activity of chimeric 137G18A was evaluated in opsonophagocytosis assays with mouse macrophages or human PMN as effector phagocytic cells and the MSSA strain Reynolds or VRSA COLVA as target cells. In these assay systems, the activity of chimeric 137G18A was also compared with that of the anti-LTA mAb Pagibaximab and the anti-PNAG mAb F598, two anti-staphylococcal antibodies in clinical development. In OPAs with mouse macrophages and agar-grown (High CP5 expression) and exponential phase (Low CP5 expression) *S. aureus* Reynolds, chimeric 137G18A conferred uptake of the staphylococci in a dose-dependent fashion and showed superior activity over Pagibaximab and F598 (FIGs. 16 and 17). Similarly, chimeric 137G18A mediated a higher percentage of uptake than Pagibaximab and F598 in OPAs with human phagocytes and the VRSA strain COLVA (FIG. 18). The results of the phagocytosis assays demonstrated that chimeric 137G18A is able to mediate uptake of both CP5 expressing

MSSA and multi-drug resistant *S. aureus* strains. Moreover, in the tested assays systems it showed higher activity over Pagibaximab and F598. Since all experiments were conducted in the absence of any complement source, 137G18A has a high Fc receptor-mediated and antibody-mediated phagocytotic activity against opsonized staphylococci.

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Example 20: Activity of chimeric anti-CP5 antibody 137G18A in opsonophagocytic killing assays with human PMN.

Opsonophagocytic killing (OPK) experiments were conducted to assess whether the chimeric anti-CP5 antibody 137G18A cannot only confer uptake of *S. aureus* by human PMN, but also killing of staphylococci by these professional phagocytes. Of note, OPK experiments are often thought to be surrogate of protection assays that can reliably predict the *in vivo* activity of anti-bacterial antibodies. Briefly, agar-grown *S. aureus* Lowenstein cells (MSSA strain; high CP5 expression) were incubated with chimeric 137G18A or an isotype human IgG1 negative control antibody and freshly purified peripheral human PMN in the absence of complement. As a control, samples were included that did contain buffer instead of PMN. After 90 min, the numbers of surviving staphylococci were enumerated for each sample. As can be seen in FIG. 19, statistically significantly less *S. aureus* CFU were recovered from wells containing chimeric 137G18A and human PMN as compared to comparator wells with PMN and isotype mAb or wells without any PMN.

The results indicate that chimeric 137G18A conferred killing of MSSA Lowenstein in a PMN-dependent fashion. As illustrated in Example 19, chimeric 137G18A could also mediate uptake of CP5 expressing *S. aureus* cells by human PMN. Taken this into consideration, one of the modes of action of the anti-CP5 antibody is the uptake of antibody-opsonized staphylococci by the human phagocytes and subsequent destruction of the bacteria.

Example 21: *In vivo* efficacy comparison of chimeric 137G18A, anti-LTA antibody Pagibaximab, and anti-PNAG antibody F598 in mouse passive immunization experiments with MSSA strain Reynolds. And *in vivo* efficacy of chimeric 137G18A in two rat sepsis models.

To further demonstrate functionality of chimeric 137G18A, it was tested whether the antibody can confer protection in mouse and rat sepsis models of passive immunization. In the mouse experiments, the activity of the anti-CP5 antibody was also compared with those of the anti-LTA mAb Pagibaximab and anti-PNAG mAb F598. Briefly, mice were treated with 0.1 mg or 1.8 mg doses of negative control isotype mAb, or either chimeric 137G18A, Pagibaximab or F598; rats were treated with 2-5 mg of isotype

control mAb or chimeric 137G18A. Then, the animals were infected systemically with a LD50-100 dose of the *S. aureus* strain Reynolds (MSSA, high CP5 expression) and the survival of the animals was followed for 7-21 days after infection. 100- μ g and 1.8-mg doses of chimeric 137G18A were reproducibly found significantly more protective than
5 Pagibaximab and F598 in the *S. aureus* Reynolds sepsis model (FIGs. 20A and 20B). Chimeric 137G18A also showed strong activity in sepsis models with Sprague Dawley and Fischer F344 rats (FIGs. 21A and 21B). After having established that the chimeric anti-CP5 antibody 137G18A has favorable activity in *in vitro* functionality assays (antigen binding, OPAs and OPKs), the passive immunization experiments established its *in vivo*
10 activity. In accordance with the results obtained in OPAs, chimeric 137G18A exhibited superior activity compared to Pagibaximab and F598 in the mouse passive immunization experiments with a CP5 expressing *S. aureus* strain.

Example 22: Preparation of Fc-engineered versions of mouse/human chimeric 137G18A.

15 A version of the chimeric anti-CP5 antibody was prepared in which a heavy chain constant region (hereinafter, referred to as "113F type" or IgG1/IgG3 type") comprising an amino acid sequence in which a part of an Fc region of a human IgG1 antibody is substituted with Fc of a human IgG3 antibody disclosed in US2007/0148165 and in a previous paper [Cancer Res. 2008, 68(10):3863-72], was ligated to the heavy
20 chain variable region of the anti-CP5 antibody, and a human kappa constant region was ligated to variable regions of the light chain of the anti-CP5 antibody.

Transient production of non-fucosylated antibody was performed by transfection of an alpha-(1,6)-fucosyltransferase-double-knockout CHO cell line (herein called "CHO_{fut8}-"). It is known that fucose is not added to a core structure of a complex
25 type N-glycoside-linked sugar chain of the antibody expressed in this host cell [Biotechnol Bioeng. 2004, 87(5):614-22]. Cells were cultured in FreeStyle™CHO Expression Medium (Gibco) while shaking in an 8% CO₂ humidified incubator at 37°C. Expression vector DNA containing genes coding for the desired antibody (either standard IgG1 Fc or IgG1/IgG3-type Fc) was transfected using FreeStyle™MAX transfection reagent
30 (Invitrogen) following manufacturer's written instructions. Transfectants were incubated for six days. Supernatant was clarified by centrifugation followed by 0.22 μ m filtration (Millipore). This material was further processed for purified antibody. Supernatants were first concentrated using a small- or medium-scale benchtop tangential flow filtration (TFF) system (Sartorius-Stedim Biotech SA) with 30K MWCO Sartocoon® Slice Hydrosart
35 cassette. The concentrated supernatants were filtered with a 0.22 μ m vacuum filter unit (Millipore) and processed on AKTA FPLC chromatography system controlled by Unicorn software. MabSelect SuRe Protein A resin (GE Healthcare) was used for the first

chromatography step to capture the antibody, and CHT ceramic hydroxyapatite resin (Bio-RAD laboratories) was used to remove residual amounts of leached protein A, host cell proteins, DNA, and the antibody aggregates. Protein A chromatography was conducted according to the manufacturer's instructions. After loading of the concentrated
5 supernatant, the resin was washed with 10 column volumes of PBS to remove the unbound proteins, and an antibody was eluted with 0.1 M Glycine-HCl, pH 3.6, 3.5, or 3.4. The eluted fractions were immediately neutralized with 1 M Tris-HCl, pH 8.0. Following analytical SEC analysis, the antibody-containing fractions with low percentage of the aggregates were pooled and buffer exchanged into 10 mM Sodium Phosphate, pH 6.5 via
10 dialysis with 10K MWCO Slide-A-Lyzers (Thermo Scientific).

CHT polishing chromatography step was conducted according to the manufacturer's instructions. After loading of the post-protein A material, the resin was washed with 10 mM Sodium Phosphate, pH 6.5 and an antibody was eluted with 20 CV gradient of 0 – 1 M NaCl. Following analytical SEC analysis, the antibody-containing
15 fractions with low percentage of the aggregates were pooled and buffer exchanged into PBS via dialysis with 10K MWCO Slide-A-Lyzers (Thermo Scientific). The purified antibody was concentrated with a centrifugal concentrator (Vivaspin, 50K MWCO, Sartorius-Stedim Biotech SA), sterilized with 0.22 μ m syringe filter, and the sample concentration was determined by the Lowry assay using DC Lowry kit (Bio-Rad). SDS
20 PAGE and analytical SEC analyses were used for quality control analysis of the purified samples. All antibody samples had the purity of $\geq 99\%$ and the percentage of aggregates of $< 1\%$. Endotoxin level was analyzed by Charles River Endosafe® - PTS spectrophotometer that utilizes cartridges that contain FDA-licensed Limulus Amebocyte Lysate (LAL) formulations. For all of the samples the endotoxin level was shown to be
25 very low (< 0.05 EU/mg). Antigen binding of 4 versions including fucosylated IgG1/IgG3 (Complegent), afucosylated IgG1/IgG3 (AccretaMab), fucosylated IgG1 (Conventional), and afucosylated IgG1 (Potelligent) versions of 137G18A was analyzed by using Biacore system with immobilized CP5 as the ligand. All of four versions showed comparable binding activity to CP5 antigen (data not shown).

30 Example 23: Biacore-based evaluation of binding activity of Fc-engineered versions of mouse/human chimeric 137G18A to recombinant human Fc gamma receptor IIIa including V158 and F158 allotypes.

In order to kinetically analyze the binding activity of AccretaMab and Native
35 Chimeric 137G18A, the binding activity was measured by surface plasmon resonance method (SPR). All of the following manipulations were carried out using a Biacore 3000 (manufactured by GE Healthcare Bio-Sciences). Recombinant hFc γ RIIIa V158 and

hFcγRIII F158 were immobilized on a CM5 chip (manufactured by GE Healthcare Bio-Sciences) using amine coupling method in accordance with the protocols attached thereto. Thereafter, the afucosylated IgG1/IgG3 (AccretaMab), fucosylated IgG1 (Conventional) antibodies diluted to 0.5 μM, were allowed to run at a flow rate of 20 μL/min onto the chip, and each corresponding sensorgram was obtained (FIGs. 22A and 22B). The afucosylated IgG1/IgG3 (AccretaMab) showed an enhanced binding to both allotypes of Fc gamma receptor IIIa comparing to the fucosylated IgG1 (Conventional).

Example 24: Activity comparison of Fc-engineered versions of mouse/human chimeric 137G18A in complement deposition assays with human or rat serum and MSSA strain Lowenstein; C1q dependence.

It was tested *in vitro* and *in vivo*, if effector function of anti-CP5 mAbs can be enhanced through engineering of the antibody Fc part. Firstly, complement deposition assays were performed. Antibodies mediate complement deposition on bacteria via the classical complement activation pathway, which is initiated by binding of C1q molecules to antibody-opsonized bacteria. It is known that human IgG3 is stronger in complement activation than human IgG1. It was shown previously that engineered human IgG1/IgG3-type mAbs exhibit increased complement-dependent cytotoxicity towards cancer cells compared to human IgG1 by enhancing the activation of the classical complement pathway. Based on this, we hypothesized that IgG1/IgG3 anti-CP5 mAbs may confer enhanced activation of the classical complement pathway and deposition of C3b on the surface of staphylococci than IgG1 anti-CP5 antibodies.

The activity of several chimeric 137G18A versions was compared in C3b deposition assays with human and rat serum and the CP5 expressing MSSA strain Lowenstein. To show that anti-CP5 antibody mediated C3b deposition in human serum experiments was due to the classical pathway of complement activation, C1q-deficient serum was included to the study. To supplement the C1q deficient serum, commercially available purified C1q was added to the C1q-deficient serum at physiological concentration.

As can be seen in FIG. 23, a fucosylated (Fuc+) hIgG1/IgG3 version of 137G18A conferred strongly increased C3 deposition on live *S. aureus* cells compared to the regular Fuc+ hIgG1 and non-fucosylated (Fuc-) hIgG1 versions of the anti-CP5 antibody. Similar results were obtained in experiments with rat serum (FIG. 24). Results of a time-course experiment with human serum are shown in FIG. 25. A non-fucosylated hIgG1/IgG3 137G18A version also had higher activity than the Fuc+ hIgG1 comparator antibody showing that hIgG1/IgG3 anti-CP5 mAbs mediate enhanced C3b deposition on *S. aureus* irrespective of their fucosylation status (FIG. 26). No enhanced

activity of Fuc+ hIgG1/IgG3 137G18A in mediating C3b deposition was observed in C1q-depleted human serum (FIG. 27A), demonstrating that the higher activity of the hIgG1/IgG3 antibody was C1q- and thus classical complement pathway-dependent. The difference between IgG1 and IgG1/IgG3-type anti-CP5 antibodies was restored in reconstituted C1q-depleted serum (supplemented with purified C1q) (FIG. 27B).

Here, it was demonstrated that human C3b deposition on CP5 encapsulated *S. aureus* cells can be significantly enhanced through the classical pathway of complement activation by using hIgG1/IgG3-type anti-CP5 antibodies. Since, C3b and its degradation products deposited on bacteria is recognized by complement receptors on human phagocytes, the results obtained in the C3b deposition assays suggest that hIgG1/IgG3-type anti-CP5 antibodies have the potential to confer increased uptake and destruction of *S. aureus* cells by human phagocytes as compared to hIgG1 anti-CP5 antibodies.

Example 25: Activity comparison of Fc-engineered versions of mouse/human chimeric 137G18A in both opsonophagocytosis and opsonophagocytic killing assays with human PMN and MSSA Lowenstein or VRSA COLVA.

The Fc part of human IgG antibodies is usually fucosylated. It has been shown that non-fucosylated anti-B cell lymphoma human IgG1 had higher affinity to Fc gamma receptor IIIb than fucosylated comparator antibodies thereby potentiating phagocytosis of cancer cells by human PMN [*Exp Hematol.* 2009, 37(3):309-21]. Based on this, we hypothesized that non-fucosylated (Fuc-) anti-CP5 mAbs could confer increased uptake and destruction of CP5 encapsulated *S. aureus* cells by human phagocytes.

The activity of several chimeric 137G18A versions was compared in opsonophagocytosis assays (OPAs) with fluorescein-labeled MSSA Lowenstein and VRSA COLVA cells as target and purified human blood PMN as effector cells in the absence of a complement source. Moreover, the efficacy of Fuc+ hIgG1 and Fuc-hIgG1/IgG3 137G18A versions were compared in opsonophagocytic killing (OPK) experiments with MSSA Lowenstein and human PMN.

In the OPAs with MSSA Lowenstein (FIG. 28), both Fuc- hIgG1 and Fuc-hIgG1/IgG3 137G18A versions conferred increased phagocytosis of Lowenstein cells by human blood PMN compared to the respective fucosylated (Fuc+) hIgG1 and hIgG1/IgG3 comparator molecules showing that the degree of anti-CP5 antibody activity in complement-independent OPAs was dependent on the fucosylation status. Similar results were obtained in OPAs with VRSA COLVA (FIG. 29). In OPK experiments, Fuc-

hIgG1/IgG3 137G18A conferred a statistically significant higher CFU concentration reduction in the presence of PMN than Fuc+ hIgG1 137G18A (FIG. 30).

The OPA and OPK data described here suggest that non-fucosylated anti-CP5 antibodies have the potential to enhance the phagocytosis and killing of CP5 encapsulated *S. aureus* cells by human PMN, which are considered to be part of the first-line host defense against invading bacteria. These data demonstrated that non-fucosylated hIgG1 and hIgG1/IgG3 137G18A molecules had significantly increased activity in OPAs with human PMN compared to Fuc+ comparator molecules. Furthermore, it was shown that a Fuc- hIgG1/IgG3 version of the chimeric anti-CP5 antibody conferred more killing of MSSA Lowenstein cells by human PMN than Fuc+ hIgG1-type 137G18A. Since the OPAs and the OPK experiments were conducted in the absence of complement, it is assumed that the higher activity of the non-fucosylated immunoglobulins can be explained by Fc receptor-dependent uptake of the staphylococci and subsequent destruction.

Example 26: Activity comparison of chimeric 137G18A and AccretaMab 137G18A in RNU rat sepsis model with MSSA Reynolds.

To determine whether Fc part engineering can enhance the *in vivo* activity of anti-CP5 antibodies, the activities of regular fucosylated (Fuc+) hIgG1 137G18A and non-fucosylated (Fuc-) hIgG1/IgG3 137G18A were compared in a rat systemic infection model. The activity of the above mentioned anti-CP5 antibodies was compared in a Rowett Nude (RNU) rat model in which the bacteria were pre-opsonized with 1 microgram of the antibodies *in vitro* and then administered to the animals.

As can be seen in FIG. 31, rats infected with MSSA Reynolds cells that were preopsonized with Fuc- hIgG1/IgG3 137G18A survived at a higher percentage than rats that were infected with bacteria preopsonized with either isotype control hIgG1 or a Fuc+ hIgG1 version of 137G18A. The *in vivo* data suggests that Fuc- hIgG1/IgG3-type anti-CP5 antibodies have the potential to be more protective *in vivo* than regular Fuc+ hIgG1-type anti-CP5 antibodies.

Example 27: Immunization of mice, screening process, selection of candidate anti-CP8 mAbs, and isotyping.

To obtain mouse anti-CP8 mAbs, BALB/c mice were immunized with antigens that were prepared as described in Example 2. For each immunization except the final boost, antigens were mixed 1:1 with Sigma Adjuvant System® (Sigma-Aldrich) and 0.2 ml of the emulsions administered intraperitoneally to the mice. Three days after the final booster injection, which were given intravenously without adjuvant, mice were euthanized, their spleens collected and grinded. The splenocytes from these mice and Sp2/0-Ag14

mouse myeloma cells (ATCC#: CRL-1581™) were used as fusion partners for hybridoma production. Fusions were conducted according to standard methods. After fusion, hybridoma supernatants were tested for the presence of anti-CP8 IgG antibodies by ELISA with immobilized CP8 as described elsewhere in this document. To isolate individual hybridoma cells secreting anti-CP5 mAbs, limiting dilution technique was applied. To identify the isotype of isolated mouse mAbs, IsoQuick Strips (Sigma-Aldrich) were used. To test whether candidate mouse anti-CP8 mAbs can react with CP8 expressed on *S. aureus* cells, their binding to CP8 expressing *S. aureus* Wright wild-type cells was tested by flow cytometry as described elsewhere in this document. To determine specificity of the candidate antibodies for CP8, a genetically engineered isogenic *S. aureus* Wright CP8-negative capO mutant was included in the flow cytometry analyses. Through these efforts, eight IgG-type mouse CP8-specific antibodies were obtained (they bound to immobilized CP8 and *S. aureus* Wright wild-type, but not to the CP8-negative capO mutant strain); the antibody names, IgG subclass, light chain type, and the antigens used to immunize the mice are listed in Table 4.

Table 4:

Clone name	Isotype	Antigens used for immunization*
138O2	IgG3/kappa	10 ⁷ <i>S. aureus</i> cells (3x) and 20 µg CP8-BSA conjugate (2x)
138O5	IgG3/kappa	10 ⁷ <i>S. aureus</i> cells (3x) and 20 µg CP8-BSA conjugate (2x)
138P2	IgG1/kappa	10 ⁷ <i>S. aureus</i> cells (3x) and 20 µg CP8-BSA conjugate (3x)
138P10	IgG2b/kappa	10 ⁷ <i>S. aureus</i> cells (3x) and 20 µg CP8-BSA conjugate (3x)
138P11	IgG3/kappa	10 ⁷ <i>S. aureus</i> cells (3x) and 20 µg CP8-BSA conjugate (3x)
138P14	IgG2a/kappa	10 ⁷ <i>S. aureus</i> cells (3x) and 20 µg CP8-BSA conjugate (3x)
138T8E	IgG2b/kappa	10 ⁷ <i>S. aureus</i> cells (3x) and 20 µg CP8-BSA conjugate (3x)
138T13A	IgG2b/kappa	10 ⁷ <i>S. aureus</i> cells (3x) and 20 µg CP8-BSA conjugate (3x)

*, The amounts of CP8-BSA antigens indicated in Table 3 are protein contents of the samples.

Example 28

Comparison of mouse anti-CP8 mAb binding to immobilized CP8 by ELISA.

The binding of eight mouse anti-CP8 mAbs to immobilized CP8 was compared by ELISA. Briefly, 96-well plates were coated overnight with purified CP8, then washed, and blocked. Mouse anti-CP8 mAbs were added at the indicated concentrations to the CP8 coated wells for 1 h. Isotype antibodies served as negative controls. Bound antibodies were detected with anti-mouse IgG gamma chain specific horseradish peroxidase (HRP)-conjugated secondary antibody and TMB substrate. Reaction was stopped with sulfuric acid and the optical density (OD) read at 450 nm (OD_{450nm}). As

can be seen in FIG. 32, 138P2 was found to be the strongest binder to immobilized CP8, followed by 138P10. Indicating specificity of anti-CP8 mAb binding, negative control mouse isotype antibodies did not show any significant binding to immobilized CP8 in the ELISAs (Data not shown); furthermore, the anti-CP8 mAbs did not bind to uncoated wells, that were blocked, but did not contain any CP8 (Data not shown).

Example 29: Comparison of mouse anti-CP8 mAb binding to live antibiotic-resistant *S. aureus* cells (MRSA F-182 strain) by flow cytometry.

To test whether the eight mouse anti-CP8 mAbs in Table 3 can react with CP8 expressed on antibiotic-resistant *S. aureus* whole cells, the binding of these mAbs to live cells of the CP8 expressing *S. aureus* strains MRSA F-182 was assessed.

The binding of the mouse anti-CP8 mAbs to the above-mentioned *S. aureus* strains was tested by immunofluorescence. Briefly, live agar-grown staphylococci were incubated with various concentrations of mouse anti-CP8 or isotype control mAbs (= primary antibodies). Bound primary antibodies on the bacterial particles were detected with fluorescently labeled secondary antibodies. The fluorescence intensity of the bacterial particles was measured by flow cytometry using a BD FACSCalibur™ and data analyzed using FlowJo software.

As can be seen in FIG. 33, all eight mouse anti-CP8 antibodies were able to bind to the MRSA strain in a dose dependent fashion. Of note, isotype antibodies did not show any significant binding to the bacteria. 138P2 showed much stronger binding than the comparator mAbs to the staphylococcal strains.

Example 30: Affinity measurement for four mouse anti-CP8 mAbs using BIAcore system.

To enable binding of purified CP8 to BIAcore chips, it was biotinylated using EZ-Link® Hydrazide-Biotin reagent (Thermo Scientific). Briefly, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) was used to activate carboxyl groups of N-acetylmannosaminouronic acid residues in CP8 to make them reactive to bind to the amino groups of Hydrazide-Biotin, forming an amide linkage. The reaction was performed in acidic buffer (pH 4.7-5.5); the pH was maintained constant by the addition of HCl. Subsequently, the reaction mixture was dialyzed against ddH₂O to separate biotinylated CP8 from non-conjugated biotin. Finally, the concentration of biotinylated CP8 was calculated by ELISA.

In order to kinetically analyze the binding activity of anti-CP8 mouse antibodies 138P2 and 138P10 to immobilized CP8, the binding activity was measured by surface plasmon resonance method (SPR). All of the following manipulations were carried out using a Biacore 3000 (manufactured by GE Healthcare Bio-Sciences).

Biotinylated CP8 was captured with a SA chip (manufactured by GE Healthcare Bio-Sciences). Thereafter, the anti-CP8 antibodies diluted from a high concentration in seven steps were allowed to run at a flow rate of 20 μ L/min onto the chip, and the sensorgram corresponding to each concentration was obtained (FIGs. 34A and 34B). The analysis was carried out using a bivalent binding model, using the analysis software attached to the apparatus, Biacore 3000 Evaluation software (provided by Biacore), thereby calculating an association rate constant k_{a1} and a dissociation rate constant k_{d1} , as well as k_{a2} and k_{d2} of respective antibodies for immobilized CP8. As a result of using a bivalent binding model, an association rate constant k_{a1} , a dissociation rate constant k_{d1} , and a dissociation constant K_{D1} (k_{d1}/k_{a1}), as well as an association rate constant k_{a2} , a dissociation rate constant k_{d2} , and a dissociation constant K_{D2} (k_{d2}/k_{a2}), of individual antibodies thus obtained are given in Table 5. The K_{D1} value of 138P2 and 138P10 was 15 nM and 582 nM, respectively. The data demonstrated that 138P2 had a superior binding affinity for CP8 antigen.

Table 5:

Ligand: CP8 (RUs); Analyte: 138P2 (Concentration Range = 400 nM-3.125 nM)							
k_{a1} (1/Ms)	k_{d1} (1/s)	K_{D1}	k_{a2} (1/RUs)	k_{d2} (1/s)	K_{D2}	Rmax (RU)	Chi2
1.2E+05	1.8E-03	1.5E-08	0.049	0.49	1.0E+01	70	0.75
Ligand: CP8 (RUs); Analyte: 138P10 (Concentration Range = 400 nM-3.125 nM)							
k_{a1} (1/Ms)	k_{d1} (1/s)	K_{D1}	k_{a2} (1/RUs)	k_{d2} (1/s)	K_{D2}	Rmax (RU)	Chi2
5.5E+04	0.032	5.81E-07	5.3E-03	0.11	20.75	88	0.21

Example 31: Capturing of soluble CP8 antigens by mouse anti-CP8 mAbs.

Briefly, 96-well plates were coated with vehicle, isotype negative control mAbs or the mouse anti-CP8 mAbs 138P2 or 138P10. After blocking unspecific binding sites, CP8 in solution was added to each well. CP8, that was captured by the anti-CP8 mAbs or that unspecifically bound to the plates or the negative control antibodies was then detected with biotinylated anti-CP8 mAb 138P2, streptavidin-conjugated horse radish peroxidase and TMB substrate.

As can be seen in FIGs. 35A and 35B, both 138P2 and 138P10 were able to capture soluble CP8 as indicated by the highly elevated OD450nm values measured in wells that contained the anti-CP8 mAbs as capture antibodies as compared to wells coated with negative control antibodies or vehicle alone. The sandwich ELISA data proved that the mouse anti-CP8 mAbs 138P2 and 138P10 can capture/immobilize solubilized CP8.

Example 32: Detection of soluble CP8 antigens in plasma taken from *S. aureus* infected animals.

After showing that anti-CP8 mAb 138P2 can bind to immobilized and soluble CP8, it was determined whether it can be utilized to detect CP8 in rodents infected with an *S. aureus* strain expressing CP8. C57BL/6 mice were infected intraperitoneally with CP8 expressing *S. aureus* Wright. 48 hours after infection, mice were euthanized and left kidneys and heparinized cardiac blood collected. Bacterial burden in blood and kidneys was determined and CP8 in the plasma samples detected by biotinylated 138P2.

As can be seen in FIG. 36 and Table 6, CP8 was detectable in sterile-filtered plasma samples of all five *S. aureus* Wright infected animals 48 hours after infection. Four out of five animals had detectable bacteria in their kidneys and/or peripheral blood. Of note, using the same sandwich ELISA system, it was possible to detect CP8 in sterile filtered plasma of Sprague Dawley rats experimentally infected with *S. aureus* Wright (Data not shown).

The results with mice and rats show that CP8 can be detected in the plasma of rodents infected with CP8 expressing *S. aureus*. In the shown mouse example, bacteria were found in either kidneys or blood of most of the mice indicating that the host animals did not clear the infection during the 48-hour period. This suggests that CP8 might be shedded by the bacteria in the hosts and that thus CP8 might be used as biomarker to detect active infection caused by CP8 expressing *S. aureus* strains in mammals possibly including humans.

Table 6:
Bacteria in kidneys and blood of infected mice

Infected mouse #	Total CFU in left kidney	CFU/mL cardiac blood
1	8.6×10^6	<100
2	4.0×10^3	2×10^2
3	<100	<100
4	7.2×10^6	<100
5	1.3×10^3	<100

Example 33: Activity of mouse anti-CP8 mAbs in opsonophagocytosis assays with mouse macrophages and CP8 expressing MSSA strain Wright.

Professional phagocytes such as macrophages and polymorphonuclear neutrophils (PMN) compose a critical first line defense against invading microbial pathogens. It is thought that anti-*S. aureus* antibodies can mediate protection via

conferring uptake and destruction of bacteria by professional phagocytes. To assess whether the murine anti-CP8 mAbs 138P2 and 138P10 can mediate uptake of *S. aureus* cells by phagocytes, their activity was assessed in opsonophagocytosis assays (OPAs) with the murine macrophage cell line J774 and the CP8 expressing strain *S. aureus* Wright.

5 Briefly, live highly encapsulated *S. aureus* Wright cells were preopsonized with control or anti-CP8 mAbs in the absence of any complement source and then co-incubated with J774 cells to induce phagocytosis. After 1 h, remaining extracellular bacteria were killed and then intracellular CFU numbers quantified. Importantly, macrophages are thought to kill staphylococci relatively slowly so that a high percentage
10 of bacteria taken up by the phagocytes were expected to survive intracellularly during the duration of the procedure.

As can be seen in FIGs. 37A and 37B, 138P2 and 138P10 were able to confer significant dose dependent uptake of encapsulated *S. aureus* Wright by murine macrophages as compared to the respective negative control isotype antibodies.

15 The experiments demonstrated that anti-CP5 antibodies can confer uptake of highly encapsulated CP8 expressing *S. aureus* cells by mouse macrophages in the absence of complement. Since phagocytosis is thought to be a crucial host defense mechanism against *S. aureus*, the OPA results described in this example indicate that anti-CP8 antibodies might also be protective *in vivo* in the host even at infection sites with low/no
20 complement source.

Example 34: *In vivo* efficacy evaluation of mouse anti-CP8 mAbs in three mouse passive immunization models of systemic *S. aureus* infection. And activity of mouse anti-CP8 mAb 138P10 in mouse kidney infection model of passive immunization.

25 The activities of the mouse anti-CP8 mAbs 138P2 and 138P10 were tested in three mouse sepsis infection models to determine whether passive immunization with anti-CP8 mAbs is conferring protection from infection with CP8 encapsulated *S. aureus*. Moreover, 138P10 activity was determined in a mouse kidney infection model. Briefly, antibodies or vehicle alone were administered to CD-1 or C57BL/6 mice 24 hours before
30 infection with the MSSA strains Wright or Becker (Both show high CP8 expression) or the MRSA strain F-182. As read-out for antibody efficacy survival or bacterial burden in the kidneys were selected.

138P2 and 138P10 showed efficacy in all of the three sepsis models with survival as read-out for antibody efficacy. Specifically, in antibody dose titration
35 experiments, 25 microgram and 50 microgram of passively administered 138P2 and 138P10, respectively, were found to confer a statistically significant degree of protection from mortality in sepsis experiments with MSSA Wright (FIGs. 38A and 38B).

Moreover, both anti-CP8 mAbs were found to be protective in systemic infection models with MSSA Becker (FIG. 39A) and MRSA F-182 (FIG. 39B). As can be seen in FIG. 40, 138P10 also had significant activity in a kidney infection model with MSSA Wright when administered subcutaneously.

5 The mouse passive immunization experiments established that anti-CP8 mAbs can confer protection from *S. aureus* infection in a mammalian host. 138P2 and 138P10 showed efficacy in all of the three sepsis models with survival as read-out for antibody efficacy. The fact that 138P10 treatment led to strongly reduced bacterial numbers in the kidneys of mice in a kidney infection model suggests that anti-CP8 mAbs have therapeutic
10 potential by leading to bacterial clearance in the mammalian host.

Example 35: cDNA cloning and sequencing of VH and VL of candidate mouse anti-CP8 mAbs.

From 1×10^6 cells of hybridomas 138P2 and 138P10, total RNA was purified
15 from these cells using RNeasy kit (manufactured by QIAGEN Inc.) following the manufacturer's instructions. Using the RNA obtained as a template, a SMART RACE cDNA Amplification Kit (manufactured by Clontech) with the reverse transcriptase SuperScript™II (manufactured by Invitrogen) was used, as per the manufacturer's written instructions, for 5' SMART-RACE-PCR to amplify the cDNA that encodes the VH and VL
20 of the immunoglobulin genes. Briefly, first strand cDNA was prepared by reverse transcriptase from 1 µg of total RNA following the manufacturer's instructions. This cDNA was used as a template for PCR amplification of the VH using a 5' universal primer mix supplied in the kit and a 3' gene-specific primer specific to mouse IgG. Also, cDNA fragment of the VL of each of the antibodies was amplified using the same method using a
25 mouse kappa chain specific 3' primer. PCR was carried out using KOD Hot Start DNA polymerase (manufactured by Novagen). The thermal cycling program was 1 cycle {94°C for 4min}; 5 cycles {94°C for 30 sec; 68°C for 2min}; 5 cycles {94°C for 30 sec, 66°C for 30 sec, 68°C for 1min}; 25 cycles {94°C for 30 sec, 64°C for 30 sec, 68°C for 1min}; 1 cycle {68°C for 5min}. Amplified DNA fragments were isolated by agarose gel
30 electrophoresis, and purified by QIAquick Gel Extraction Kit (manufactured by Qiagen). Purified DNA fragments of VH and VL were separately integrated into pCR®-Blunt II-TOPO® vector using the Zero® Blunt® TOPO PCR Cloning Kit (manufactured by Invitrogen) as per the manufacturer's instructions. Each plasmid was transformed into competent DH5α-T1R *E. coli*, and plated on kanamycin-containing LB-Miller agar plates.
35 Plasmids were isolated from bacterial colonies grown in LB-Miller medium with kanamycin grown overnight, using QIAprep Spin Miniprep kit (manufactured by QIAGEN). Plasmids were screened by *Eco*RI restriction digest, and those containing inserts were

sequenced by Sanger-sequencing (performed by GENEWIZ Inc.). Nucleotide sequences of each insert in the construct plasmids were analyzed using the nucleotide-alignment software program Sequencher (manufactured by Gene Codes Corp.). Based on consensus alignment of multiple plasmid clones obtained from each antibody, cDNA sequences were identified for the VH and VL regions of the heavy and light chains, including the leader sequences, from a putative 5' start ATG codon.

Antibody VH and VL domains were analyzed for germline V, D and J genes using the IMGT/V-quest program (Brochet, Lefranc et al. 2008), and leader sequences were deduced. Antibody CDRs were defined using the Kabat method.

Nucleotide sequence of 138P2 VH without the leader sequence are represented in SEQ ID NO:21, and amino acid sequences of 138P2 VH without the leader sequence are represented in SEQ ID NO:16. Also nucleotide sequences of 138P2 VL without the leader sequence are represented in SEQ ID NO:22, and amino acid sequences of 138P2VL without the leader sequence are represented in SEQ ID NO:17.

CDRs of the VH and VL of the respective anti-CP8 antibodies were identified using the Kabat method. Amino acid sequences of CDR1, CDR2 and CDR3 of VH of 138P2 are represented by SEQ ID NOs:7, 8 and 9, respectively, and amino acid sequences of CDR1, CDR2 and CDR3 of VL thereof are represented by SEQ ID NOs:10, 11 and 12, respectively.

Example 36: Preparation of mouse/human chimeric IgG1 antibodies for 138P2.

The mouse/human chimeric anti-CP8 antibodies prepared are comprised of a human IgG1 heavy chain constant region and human kappa constant region respectively ligated to variable regions of heavy and light chains of the anti-CP8 antibodies. Transient production of anti-CP8 antibodies was performed by transfection of suspension cultures of FreeStyle™CHO-S cells (herein called "CHO-S") (Invitrogen) maintained in FreeStyle™CHO Expression Medium (Gibco) while shaking in an 8% CO₂ humidified incubator at 37°C. Cells were transfected with expression vector DNA containing genes coding for the desired antibody, using FreeStyle™MAX transfection reagent (manufactured by Invitrogen) following manufacturer's written instructions. Transfectants were incubated for six days. Supernatant was clarified by centrifugation followed by 0.22 µm filtration (Millipore). This material was further processed for purified antibody. Antibody purification from the culture supernatants was conducted according to the same method as described in Example 14.

Example 37: Binding of chimeric anti-CP8 antibody 138P2 and 138P10 to immobilized CP8 and capturing of soluble CP8 by the antibodies.

To test the functionality of the mouse/human chimeric version of the anti-CP8 antibody 138P2, it was tested if it could bind to immobilized CP8 and if it could capture soluble CP8 by ELISA. As can be seen in FIG. 41A, in contrast to the isotype antibody, chimeric 138P2 showed dose-dependent binding to immobilized CP8. Similarly, the chimeric 138P10 was able to bind to immobilized CP8 (Data not shown). Further the chimeric 138P2 was able to capture soluble CP8 antigen (FIG. 41B).

Example 38: Comparison of chimeric anti-CP8 antibody binding to live *S. aureus* cells by flow cytometry (MSSA ATCC-25923 and MRSA F-182).

To test whether the chimeric anti-CP8 mAbs 138P2 and 138P10 can react with CP8 expressed on *S. aureus* whole cells, their binding to live cells of the CP8 expressing MSSA ATCC-25923 and MRSA F-182 was assessed by flow cytometry. Briefly, live agar-grown staphylococci were incubated with various concentrations of chimeric anti-CP8 or isotype control antibodies (= primary antibodies). Bound primary antibodies on the bacterial particles were detected with fluorescently labeled secondary antibodies. The fluorescence intensity of the bacterial particles was measured by flow cytometry using a BD FACSCalibur™ and data analyzed using FlowJo software.

As can be seen in FIG. 42A and FIG. 42B, the two chimeric anti-CP8 antibodies bound to MSSA ATCC-25923 and MRSA F-182 in a dose dependent manner, whereas the negative control antibodies did not show any significant binding to the staphylococci. Of note, in both assay systems, the mean fluorescence intensity values for 138P2 were higher than that for 138P10. The flow cytometry experiments demonstrated that the chimeric anti-CP8 antibodies 138P2 and 138P10 bind to *S. aureus* whole cells including a representative strain of the clinically most relevant multidrug-resistant MRSA type.

Example 39: Activity comparison of chimeric anti-CP8 antibodies 138P2 and 138P10, and anti-LTA antibody Pagibaximab in opsonophagocytosis assays with mouse macrophages and MSSA Becker. And activity comparison of chimeric anti-CP8 antibodies 138P2 and 138P10, anti-LTA antibody Pagibaximab, and anti-PNAG antibody F598 in opsonophagocytosis assays with human PMN and MSSA Becker and MRSA F-182.

After having established that chimeric 138P2 and 138P10 retained their ability to bind to immobilized CP8 as shown by ELISAs and/or Biacore and to *S. aureus* whole cells, the functionality of the chimeric anti-CP8 antibodies was tested *in vitro*. Specifically, the activity of chimeric 138P2 and 138P10 was evaluated in

opsonophagocytosis assays with mouse macrophages and human PMN as effector phagocytic cells and MSSA Becker or MRSA F-182 as target cells. In these assay systems, the activity of chimeric 138P2 and 138P10 was also compared with that of the anti-LTA mAb Pagibaximab and anti-PNAG mAb F598, two anti-staphylococcal antibodies in clinical development. In OPAs with mouse macrophages and agar-grown *S. aureus* Becker cells (High CP8 expression), chimeric 138P2 and 138P10 conferred uptake of the staphylococci in a dose-dependent fashion and showed superior activity over Pagibaximab (FIG. 43). Similarly, chimeric 138P2 and 138P10 mediated more uptake than Pagibaximab and F598 in OPAs with human PMN and both MSSA Becker (FIG. 44) and MRSA F-182 (FIG. 45).

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skill in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

All references cited herein are incorporated in their entirety.

This application is based on U.S. provisional patent application No. 61/682,413 filed on August 13, 2012, the entire contents of which are incorporated hereinto by reference.

Industrial Applicability

According to the present invention, an antibody and antigen-binding fragment thereof which specifically recognizes an epitope comprising mannosaminuronic acid residue of capsular polysaccharide and binds to CP expressed on *Staphylococcus aureus*, a nucleotide encoding amino acid sequence of the antibody, a production method of the antibody and a method for treating a infectious diseases caused by *S. aureus* are provided.

Sequence Listing Free Text

SEQ ID NO:1 amino acid sequence of HCDR1 of 137G18A

SEQ ID NO:2: amino acid sequence of HCDR2 of 137G18A

SEQ ID NO:3: amino acid sequence of HCDR3 of 137G18A

SEQ ID NO:4: amino acid sequence of LCDR1 of 137G18A

SEQ ID NO:5: amino acid sequence of LCDR2 of 137G18A

SEQ ID NO:6: amino acid sequence of LCDR3 of 137G18A

SEQ ID NO:7: amino acid sequence of HCDR1 of 138P2

SEQ ID NO:8 amino acid sequence of HCDR2 of 138P2

SEQ ID NO:9: amino acid sequence of HCDR3 of 138P2

SEQ ID NO:10: amino acid sequence of LCDR1 of 138P2

SEQ ID NO:11: amino acid sequence of LCDR1 of 138P2

SEQ ID NO:12: amino acid sequence of LCDR3 of 138P2

SEQ ID NO:15: amino acid sequence of VH of 137G18A (N107D)

SEQ ID NO:20: nucleotide sequence of VH of 137G18A (N107D)

CLAIMS

1. A monoclonal antibody and antigen-binding fragment thereof, which recognizes an epitope comprising mannosaminuronic acid residue of capsular polysaccharide (hereinafter described as CP) and binds to CP expressed on *Staphylococcus aureus* (hereinafter, described as *S. aureus*).

2. The monoclonal antibody and antigen-binding fragment thereof according to claim 1, wherein the antibody binds to at least one CP selected from CP type 5 (CP5) and CP type 8 (CP8).

3. The monoclonal antibody and antigen-binding fragment thereof according to claim 1, wherein the antibody has a binding affinity for CP5 less than 10 nM.

4. The monoclonal antibody and antigen-binding fragment thereof according to claim 1, wherein the antibody has a binding affinity for CP8 less than 20 nM.

5. The monoclonal antibody and antigen-binding fragment thereof according to claim 1, wherein the antibody is any one of the antibody selected from (i) to (vi):

(i) a monoclonal antibody comprising complementarity determining regions (hereinafter, described as CDR) 1 to 3 of a heavy chain variable region (hereinafter, described as VH) comprising amino acid sequences of SEQ ID NOs:1 to 3, respectively, and CDRs 1 to 3 of a light chain variable region (hereinafter, described as VL) comprising amino acid sequences of SEQ ID NOs:4 to 6, respectively;

(ii) a monoclonal antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:13 and a VL comprising the amino acid sequence of SEQ ID NO:14;

(iii) a monoclonal antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:15 and a VL comprising the amino acid sequence of SEQ ID NO:14;

(iv) an antibody which competes with the monoclonal antibody according to (i);

(v) an antibody which comprises an epitope bound by the monoclonal antibody according to (i);

(vi) an antibody which binds to the same epitope bound by the monoclonal antibody according to (i).

6. The monoclonal antibody and antigen-binding fragment thereof according to claim 1, which is any one of the antibody selected from (i) to (v):

(i) a monoclonal antibody comprising CDRs 1 to 3 of VH comprising amino acid sequences of SEQ ID NOs:7 to 9, respectively, and CDRs 1 to 3 of VL comprising amino acid sequences of SEQ ID NOs:10 to 12, respectively;

(ii) a monoclonal antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:16 and a VL comprising the amino acid sequence of SEQ ID NO:17;

(iii) an antibody which competes with the monoclonal antibody according to (i);

(iv) an antibody which comprises an epitope bound by the monoclonal antibody according to (i);

(v) an antibody which binds to the same epitope bound by the monoclonal antibody according to (i).

7. The monoclonal antibody and antigen-binding fragment thereof according to claim 1, which is a recombinant antibody and antigen-binding fragment thereof.

8. The recombinant antibody and antigen-binding fragment thereof according to claim 7, which is at least one antibody selected from a chimeric antibody, a humanized antibody and human antibody.

9. A DNA encoding amino acid sequence of the monoclonal antibody and antigen-binding fragment thereof according to claim 1.

10. A method for producing the monoclonal antibody and antigen-binding fragment thereof according to claim 1.

11. A method for treating an infectious disease caused by *S. aureus* which comprises administration of the monoclonal antibody or antigen-binding fragment thereof according to claim 1.

12. The method for treating an infectious disease caused by *S. aureus* according to claim 11, wherein the infectious disease is at least one disease selected from sepsis, septic shock, bacteremia, pneumonia, skin ulcer, atopic dermatitis, meningitis, enteritis, cholangiolitis and endocarditis.

13. The method for treating an infectious disease caused by *S. aureus* according to claim 11, wherein *S. aureus* is resistant to at least one antibiotic selected from methicillin and vancomycin.

5 14. The method for treating an infectious disease caused by *S. aureus* according to claim 11, wherein the method is combined with administration of at least one antibiotic.

15. A recombinant vector which comprises the DNA according to claim 9.

10 16. A transformant obtainable by introducing the recombinant vector according to claim 15 into a host cell.

15 17. A process for producing the monoclonal antibody or the antigen-binding fragment thereof according to claim 1, which comprises culturing the transformant according to claim 16 to form and accumulate the monoclonal antibody or the antigen-binding fragment thereof according to claim 1 in the culture, and collecting the antibody or the antigen-binding fragment thereof from the culture.

FIG. 1

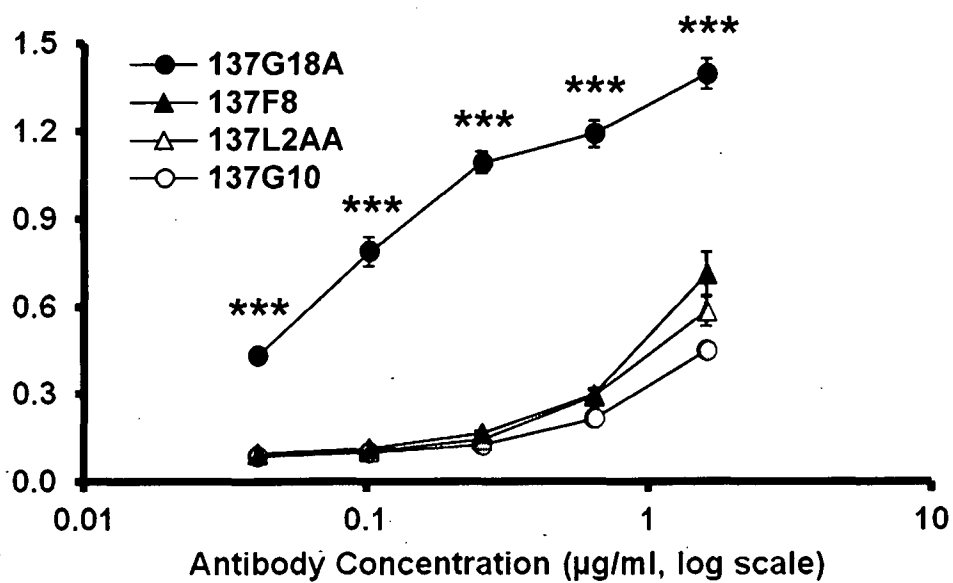
OD_{450nm} = Degree of anti-CP5 mAb binding to CP5

FIG. 2A

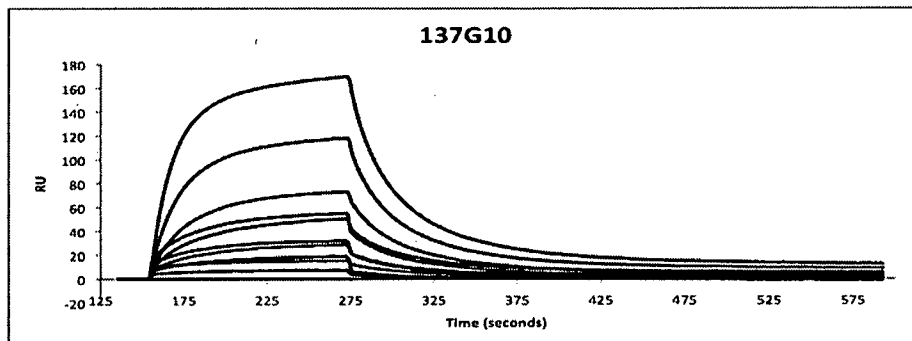


FIG. 2B

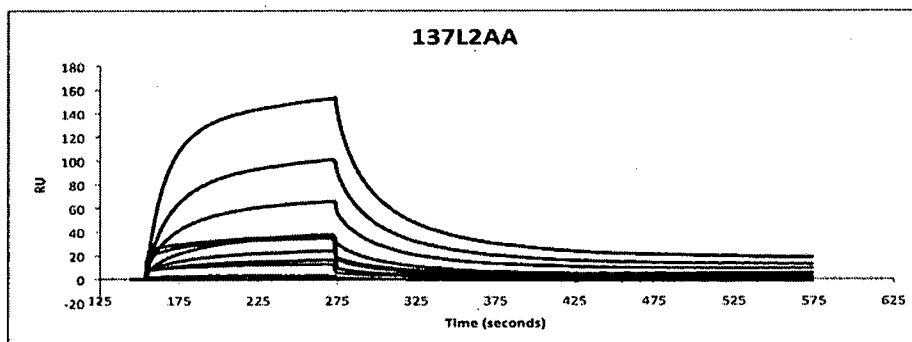


FIG. 2C

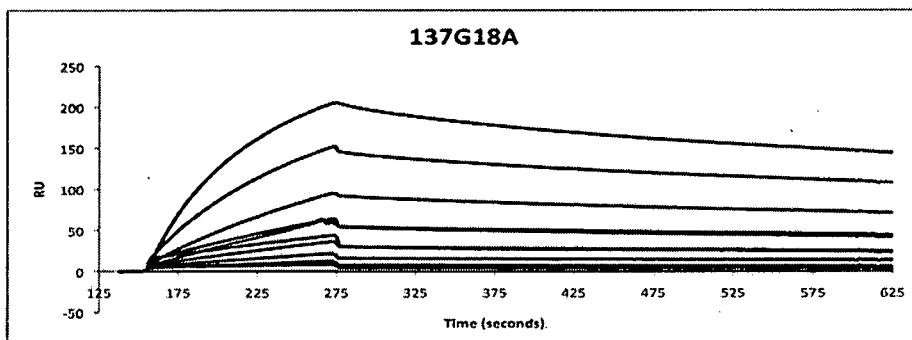


FIG. 2D

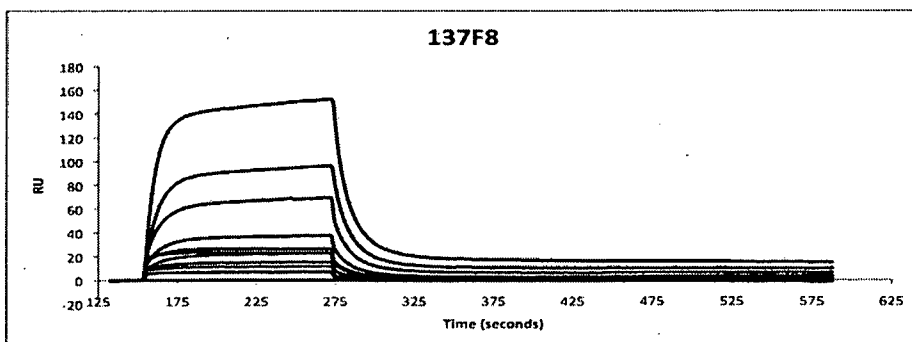


FIG. 3A

Anti-CP5 mAb 137G18A (Mouse IgG₁)

OD_{450nm} = residual binding of 137G18A to CP5 after addition of vehicle, isotype mAb, or 137L2AA

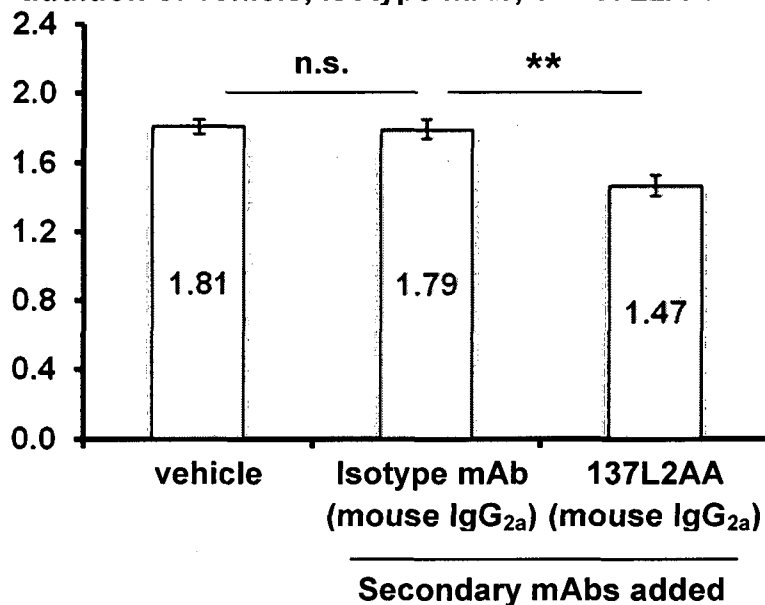


FIG. 3B

Anti-CP5 mAb 137L2AA (Mouse IgG_{2a})

OD_{450nm} = residual binding of 137L2AA to CP5 after addition of vehicle, isotype mAb, or 137G18A

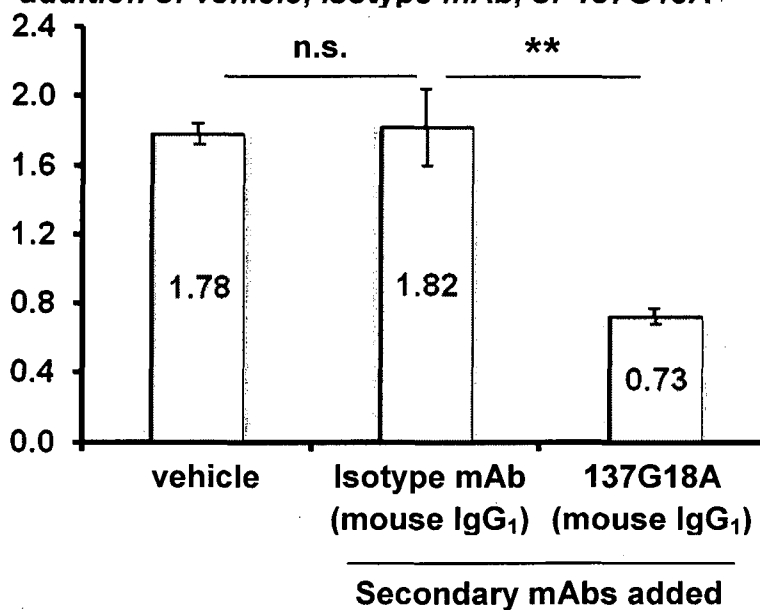


FIG. 4A

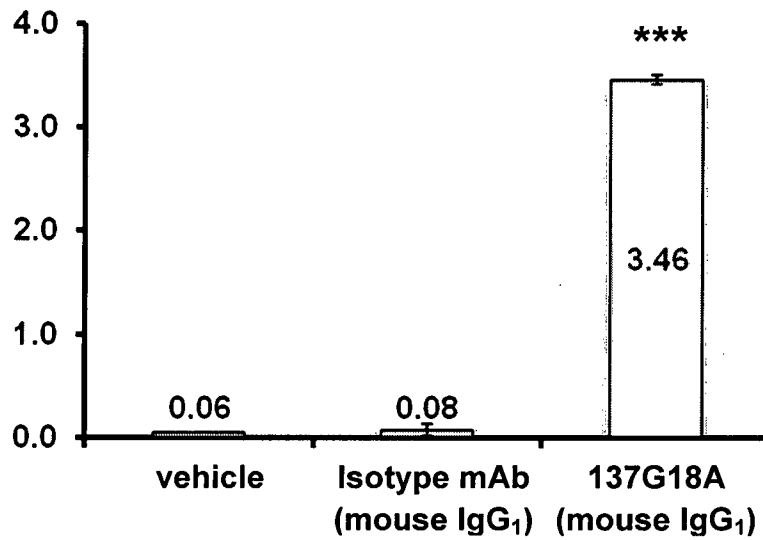
OD_{450nm} = Capturing of soluble CP5 by isotype and 137G18A

FIG. 4B

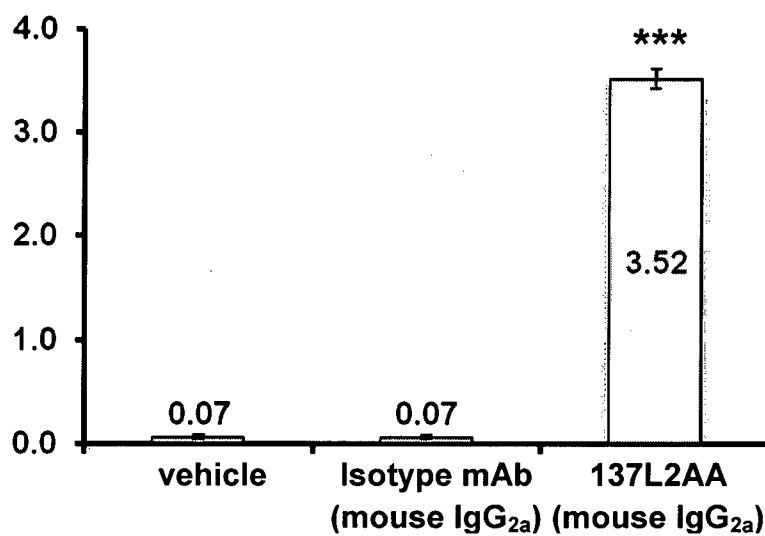
OD_{450nm} = Capturing of soluble CP5 by isotype and 137L2AA

FIG. 5

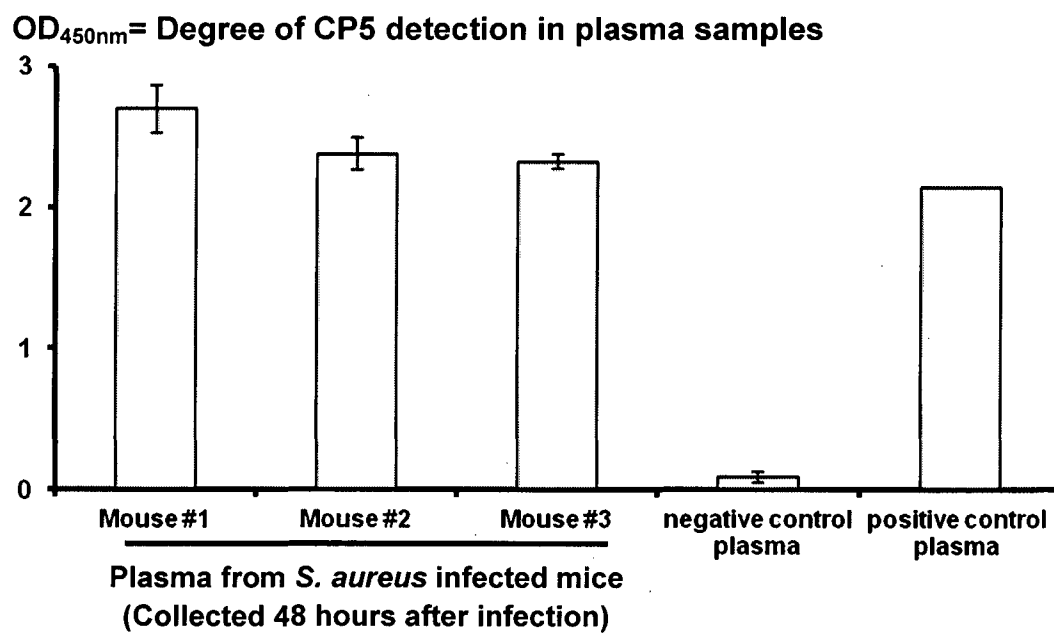


FIG. 6A

Binding to VRSA strain COLVA

MFI (FL-1)= Degree of anti-CP5 mAb binding to VRSA

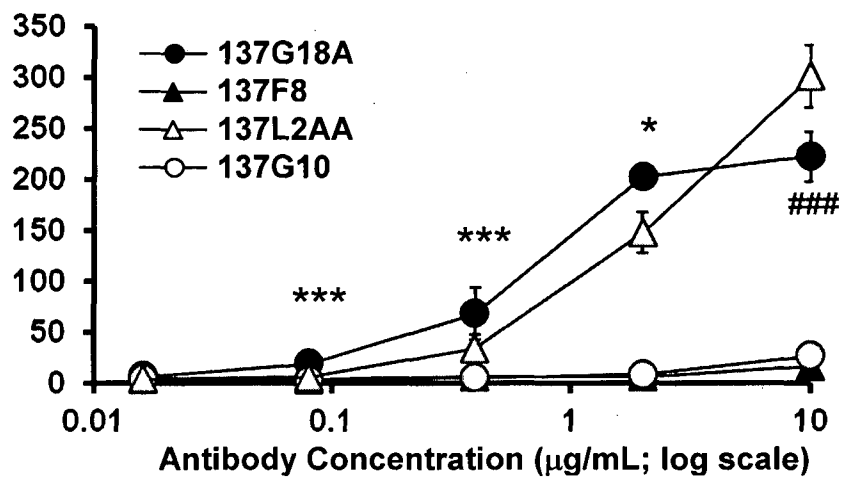


FIG. 6B

Binding to MRSA strain BAA-41

MFI (FL-1)= Degree of anti-CP5 mAb binding to MRSA

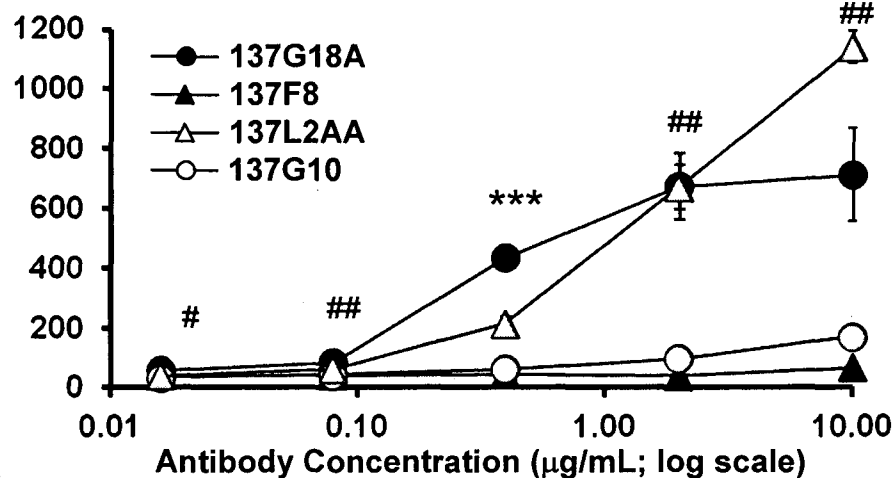


FIG. 7

% uptake of bacteria relative to initial

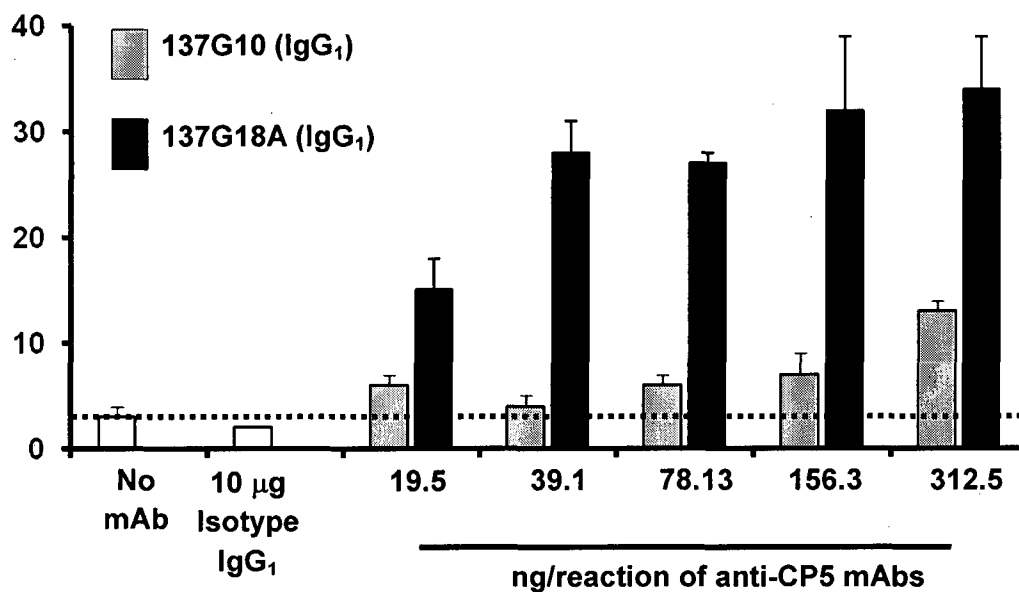


FIG. 8

% uptake relative to initial

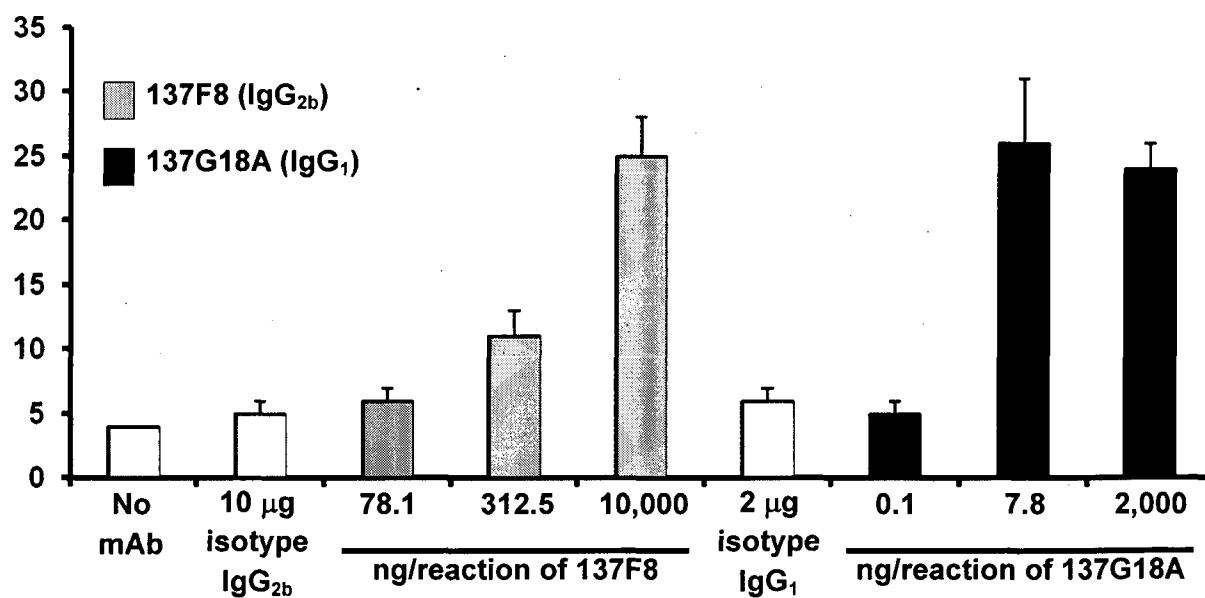


FIG. 9A

Activity comparison of anti-CP5 mAbs 137G10, 137G18A, and 137F8

% CFU found after 120 min compared to initial CFU

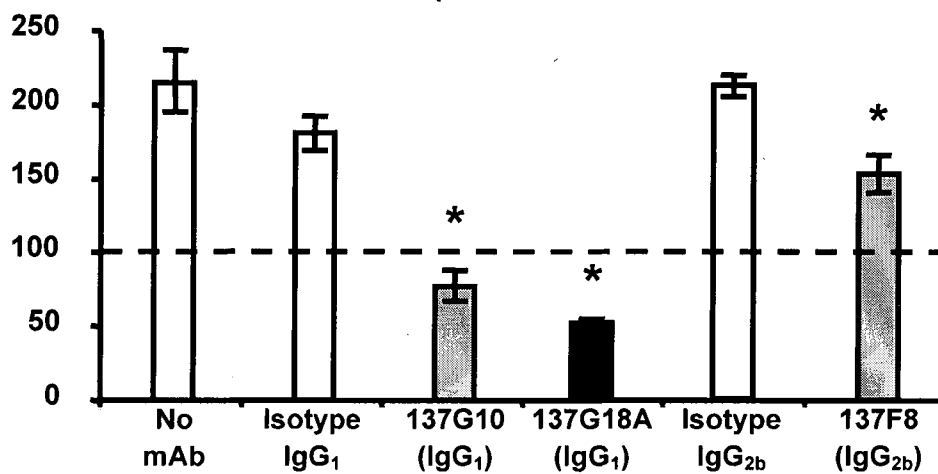


FIG. 9B

Activity comparison of anti-CP5 mAbs 137G18A and 137L2AA

% CFU found after 120 min compared to initial CFU

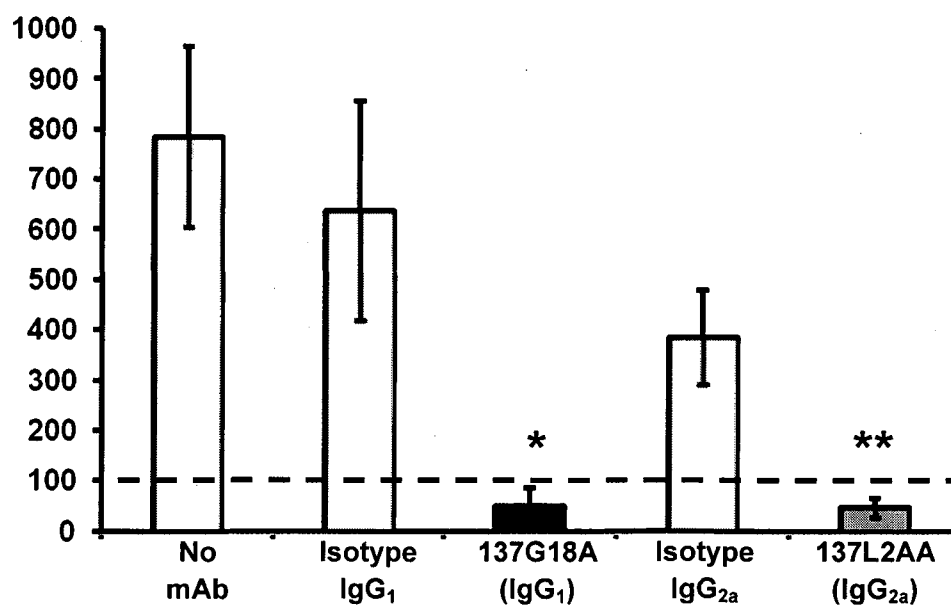


FIG. 10A

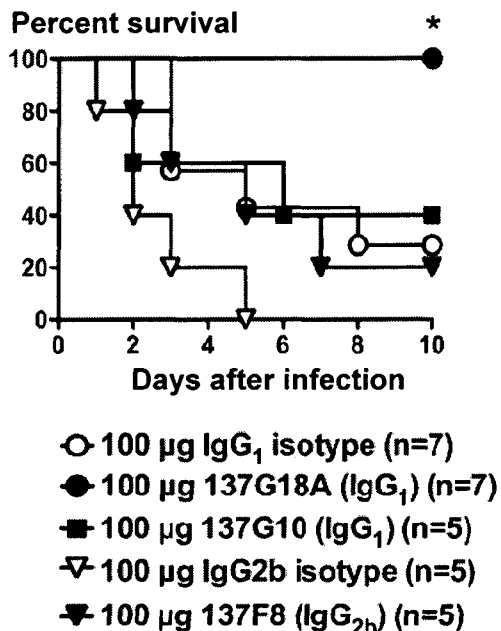


FIG. 10B

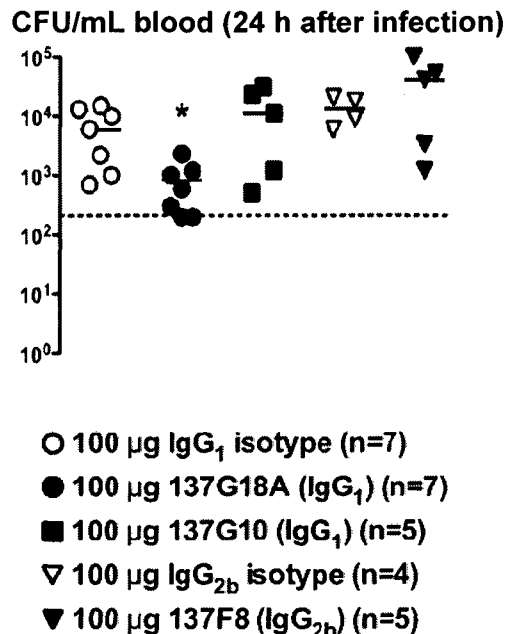


FIG. 10C

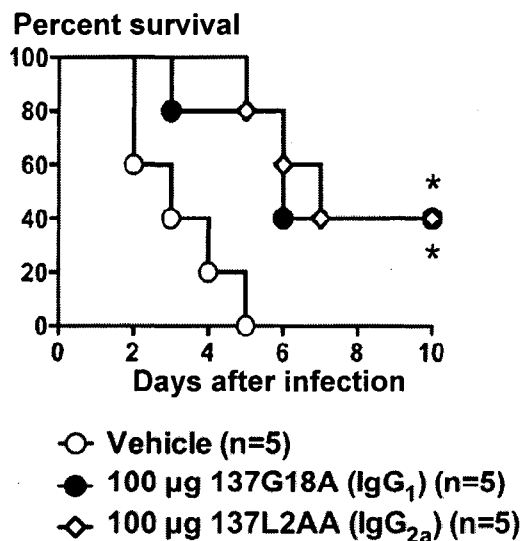


FIG. 10D

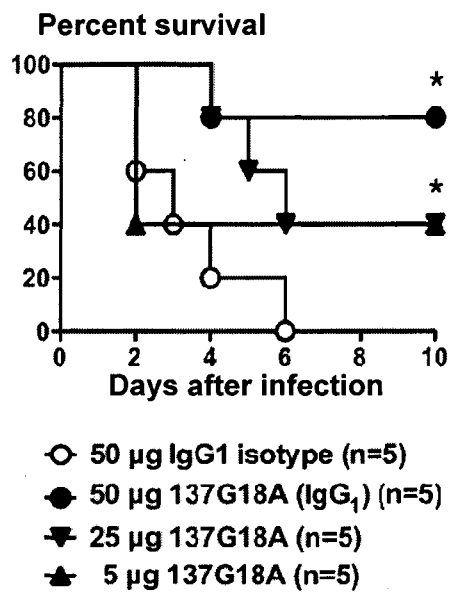


FIG. 10E

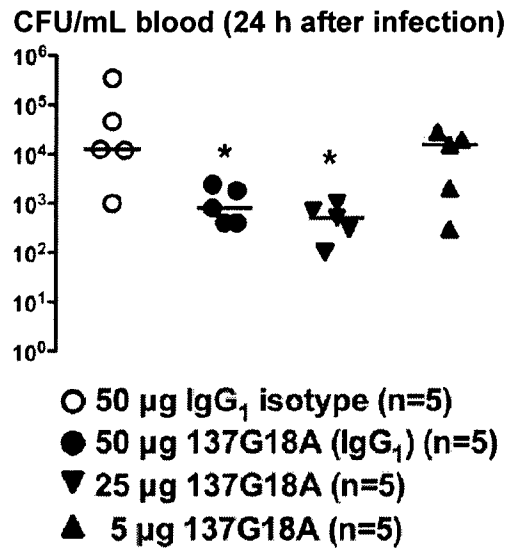


FIG. 10F

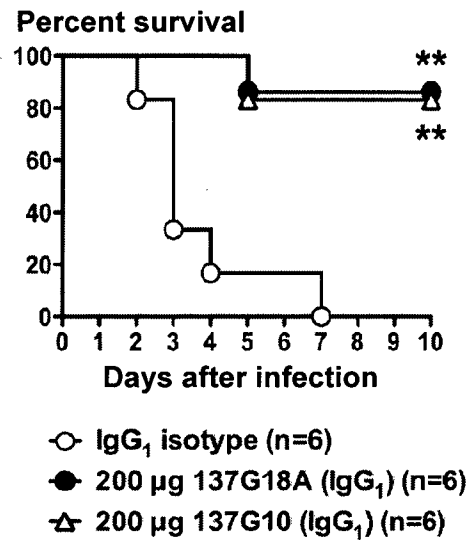


FIG. 10G

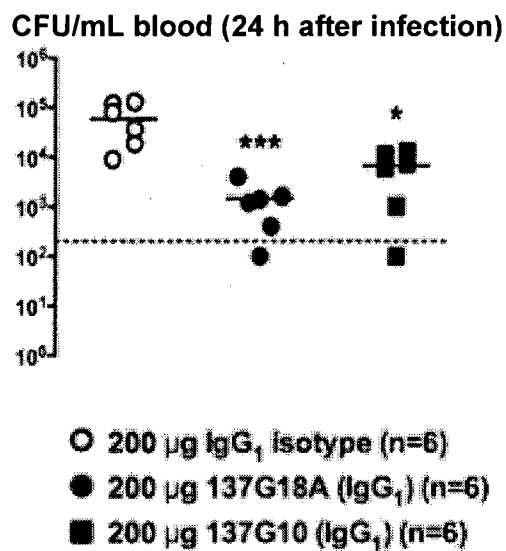


FIG. 10H

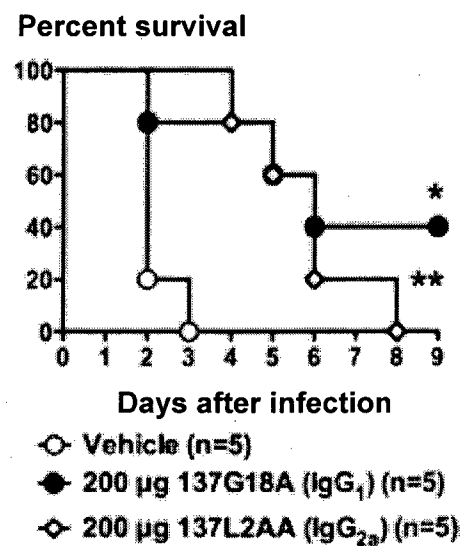


FIG. 11A

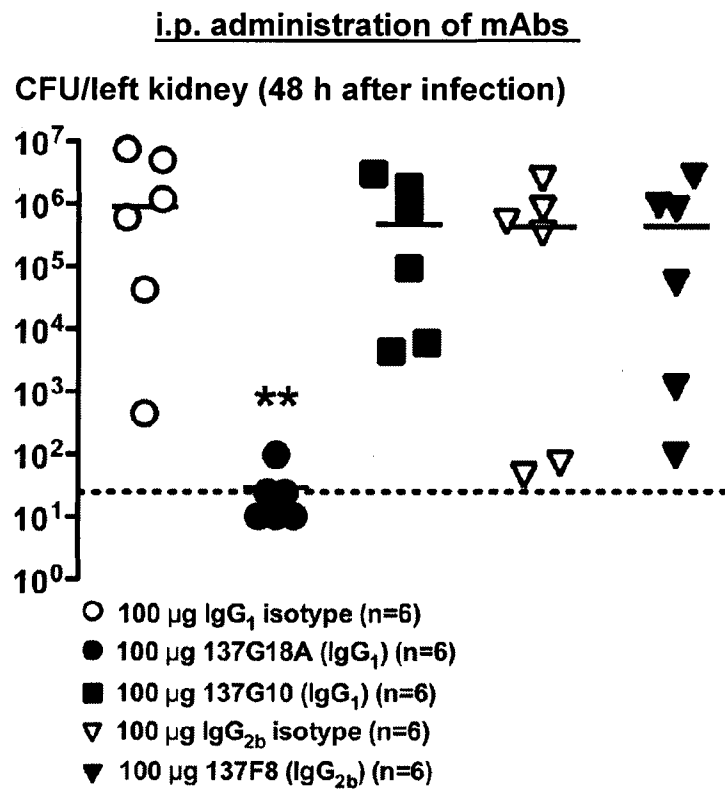


FIG. 11B

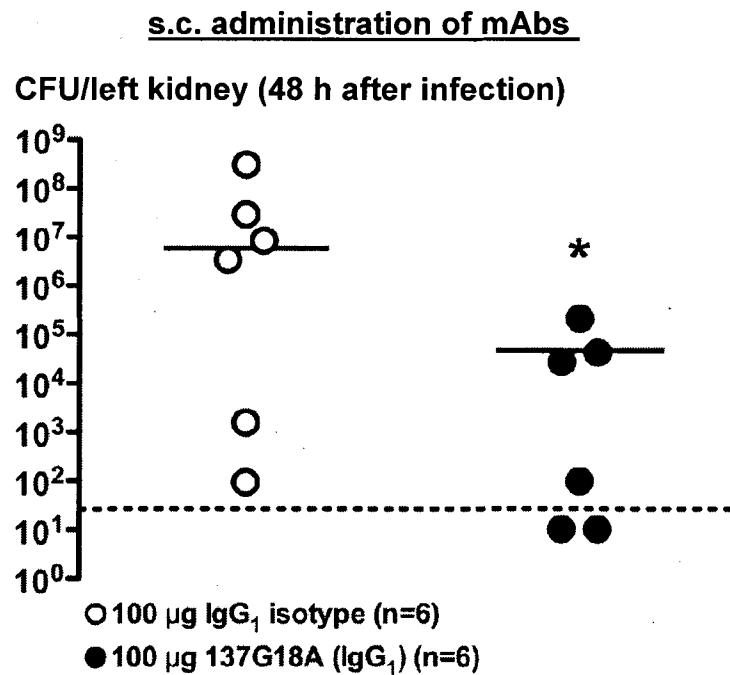


FIG. 12A

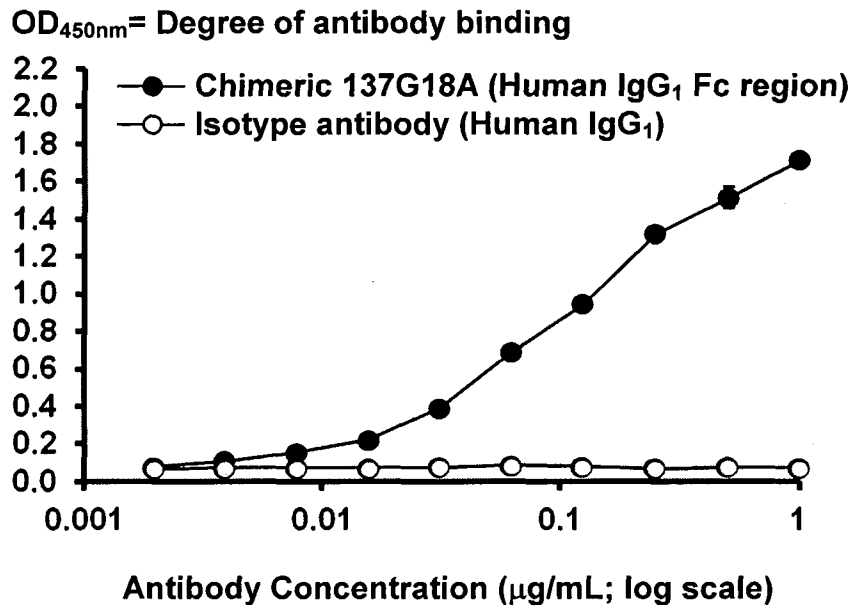


FIG. 12B

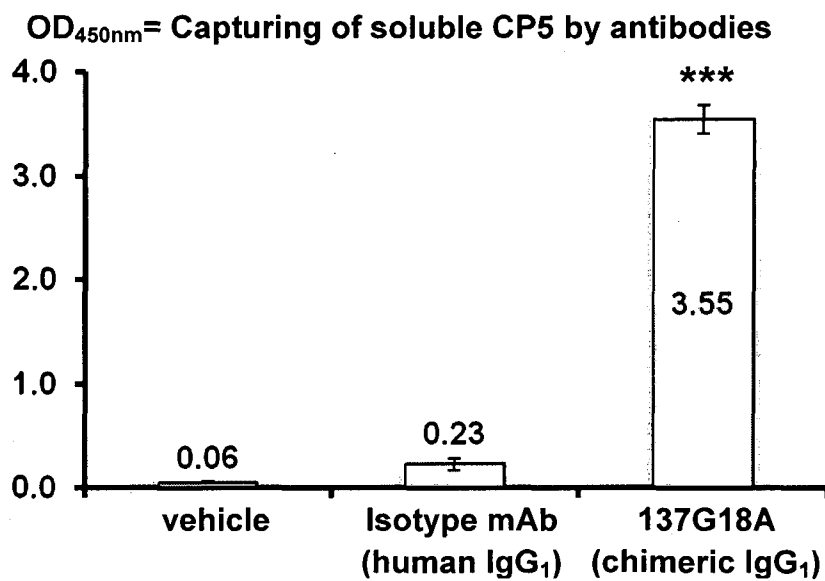


FIG. 13

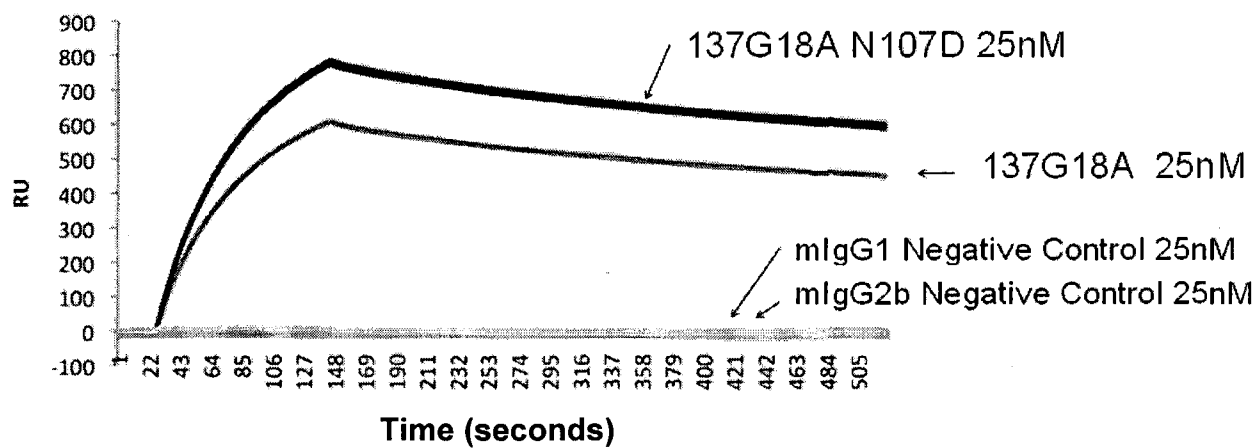


FIG. 14A

Antibody binding in presence or absence of 50% serum
(Example for one donor serum is shown)

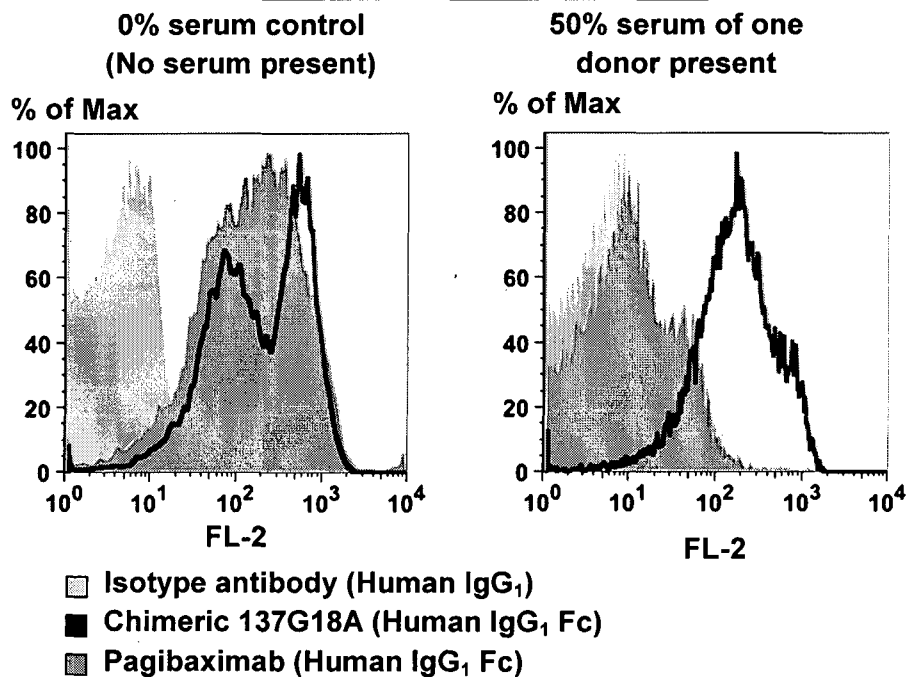


FIG. 14B

Relative antibody binding data for 5 donor sera

% binding of antibodies to live *S. haemolyticus* in presence serum of 5 human donors vs. 0% serum control

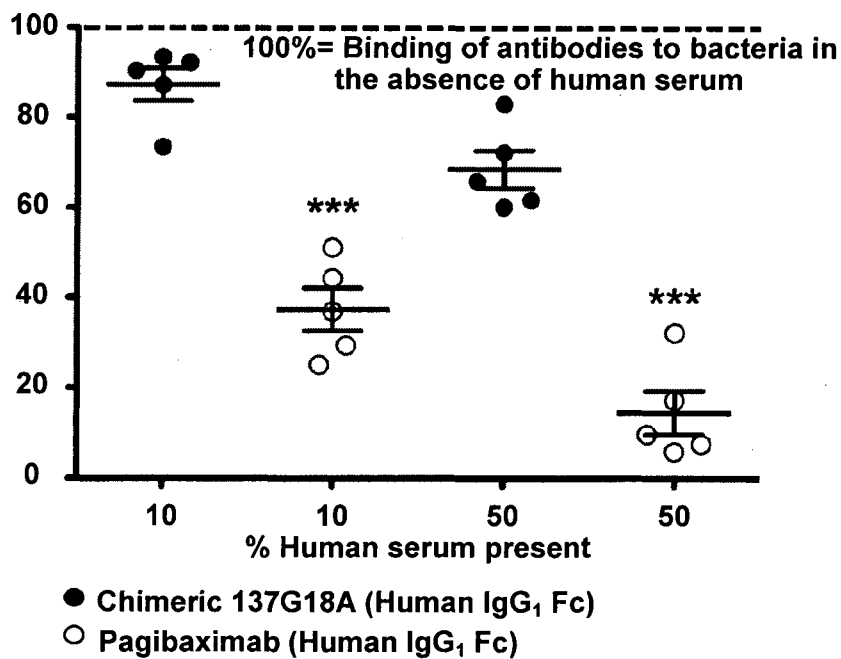


FIG. 15

% binding of antibodies to *S. aureus* BAA-41 in presence of 10% serum of 5 human donors vs. 0% serum control

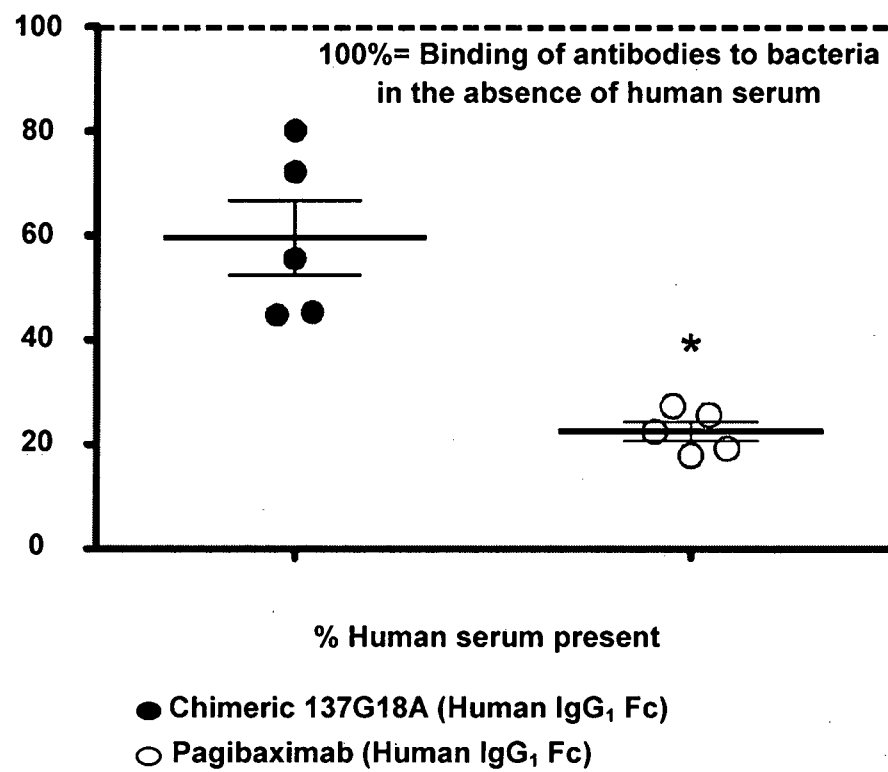


FIG. 16

OPA with agar-grown *S. aureus* Reynolds (MSSA) cells (High CP5 expression)

% uptake of bacteria by macrophages compared to initial inoculum

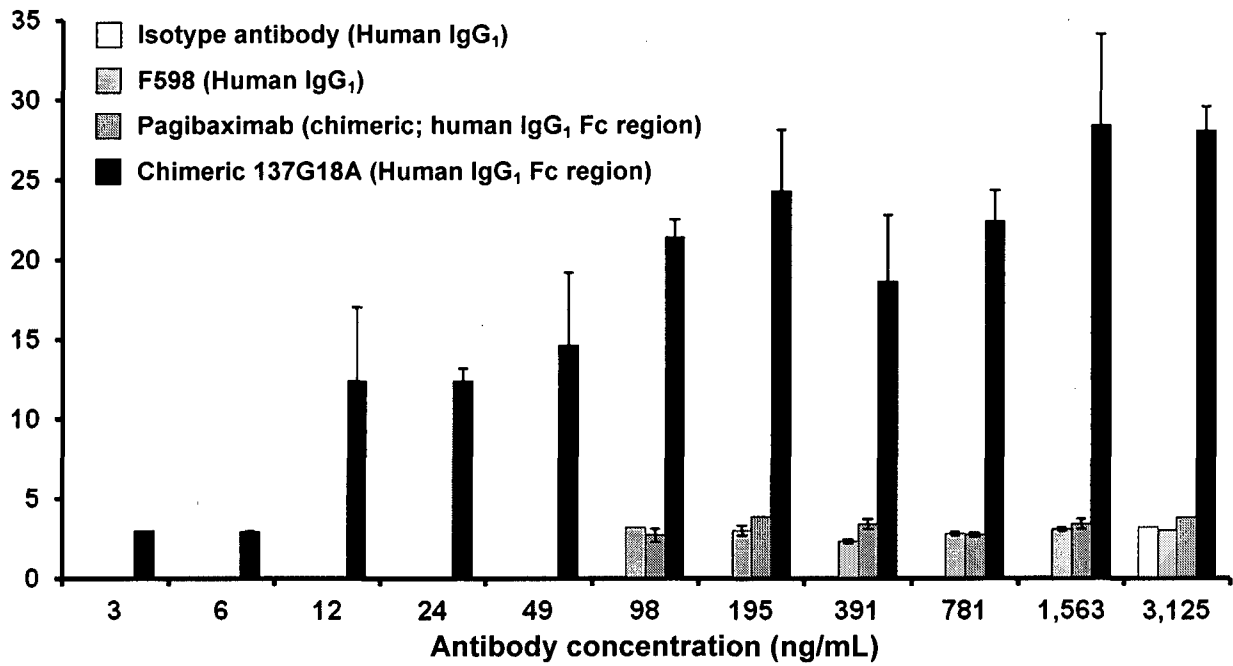


FIG. 17

OPA with exponential phase *S. aureus* Reynolds cells (Low CP5 expression)

% uptake of bacteria by macrophages compared to initial inoculum

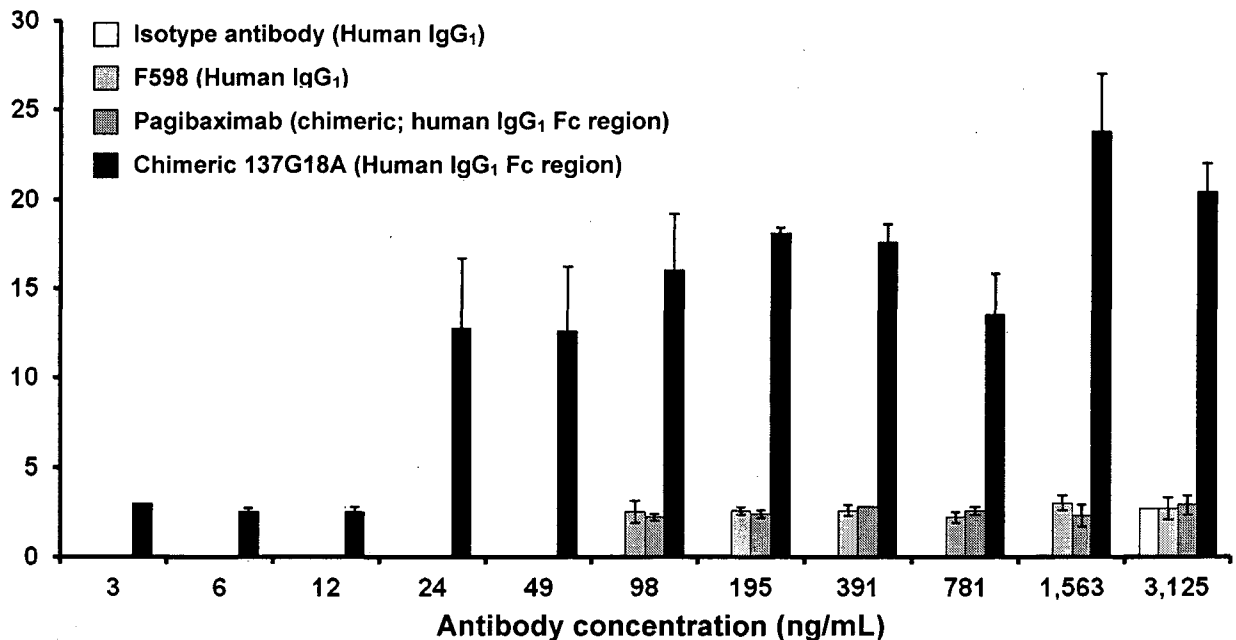


FIG. 18

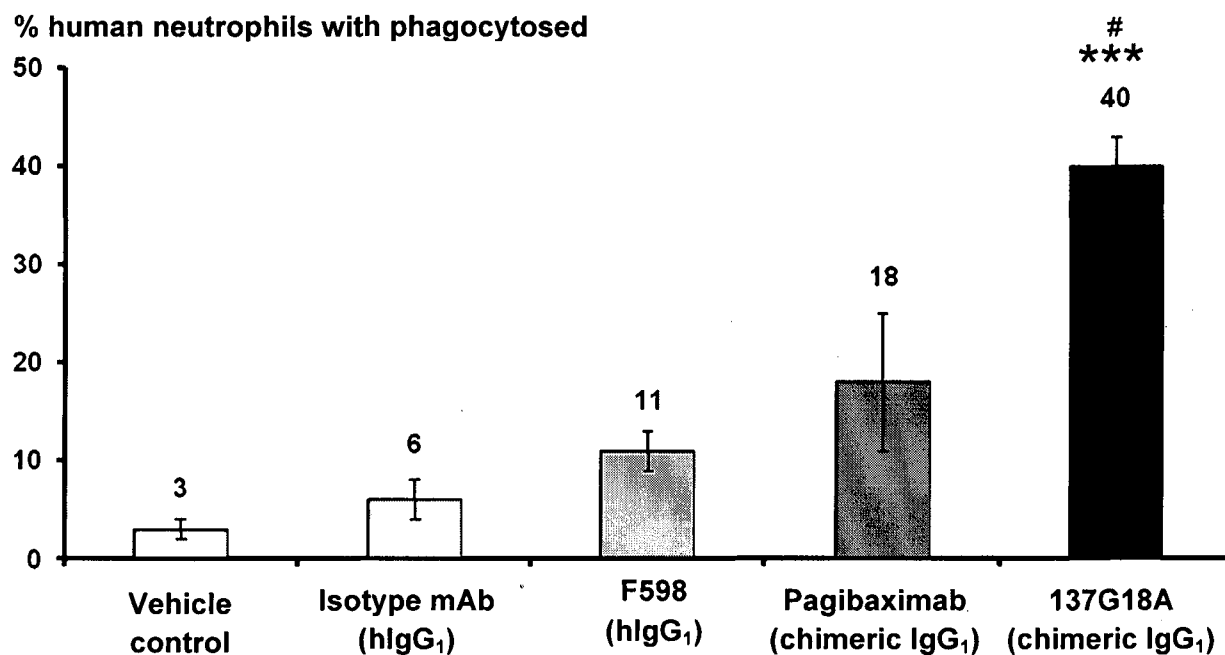


FIG. 19

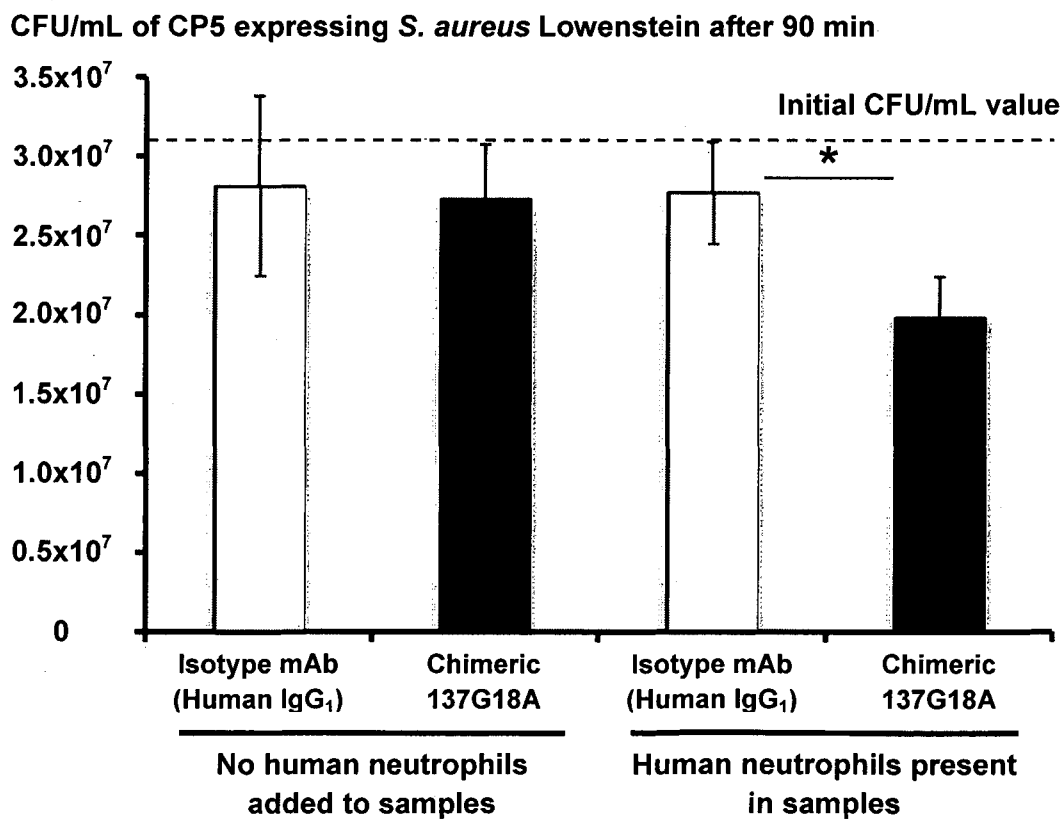


FIG. 20A

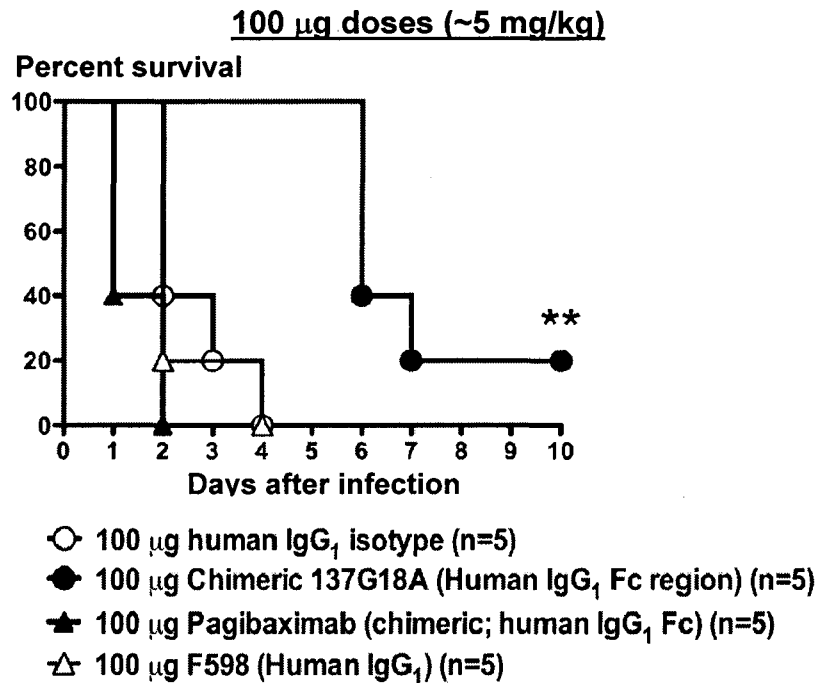


FIG. 20B

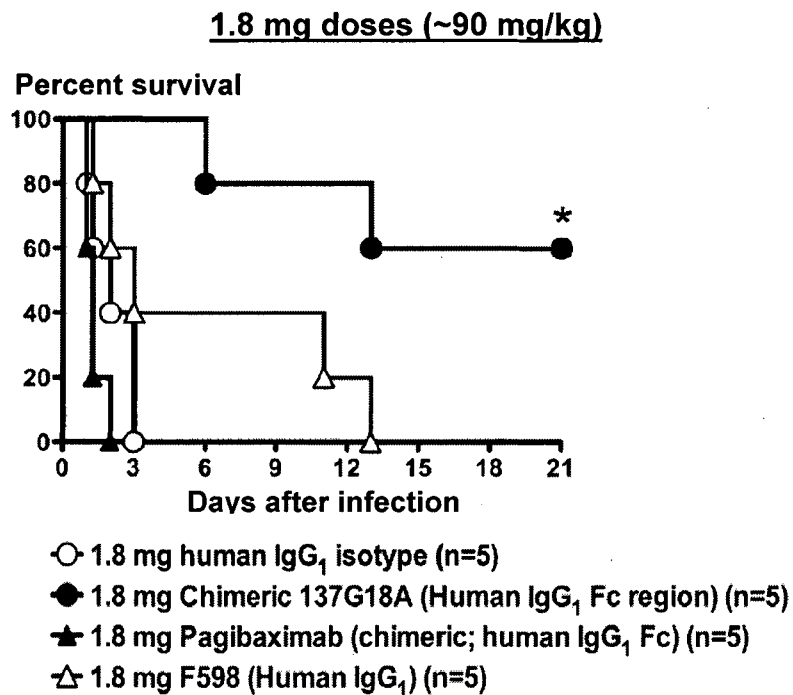
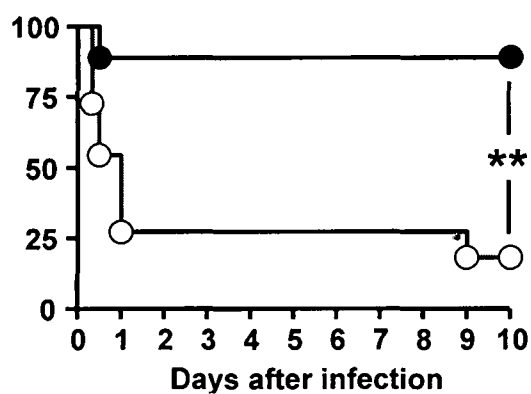


FIG. 21A

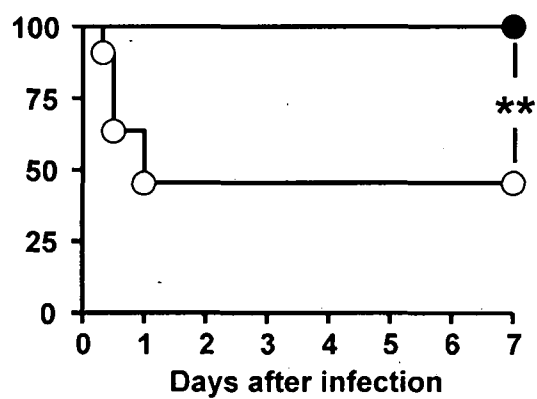
Percent survival of Sprague Dawley rats

○ 5 mg IgG₁ control antibody (n=11)

● 5 mg chimeric anti-CP5 antibody 137G18A (n=11)

FIG. 21B

Percent survival of Fischer F344 rats

○ 2 mg IgG₁ control antibody (n=11)

● 2 mg chimeric anti-CP5 antibody 137G18A (n=11)

FIG. 22A

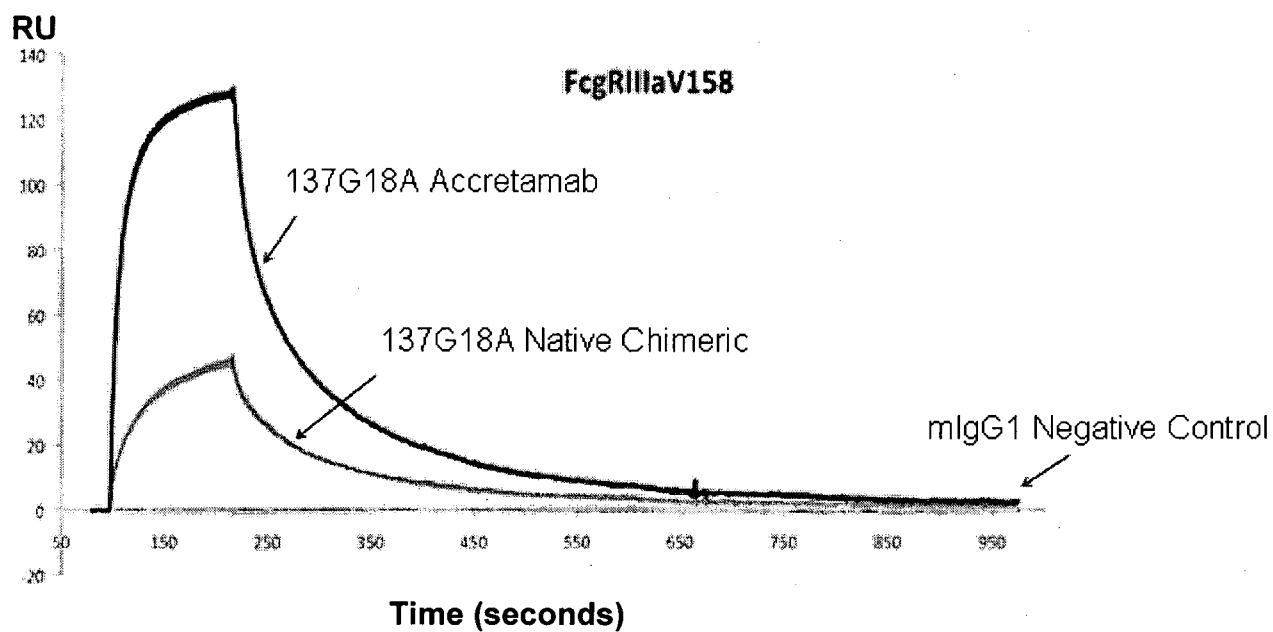


FIG. 22B

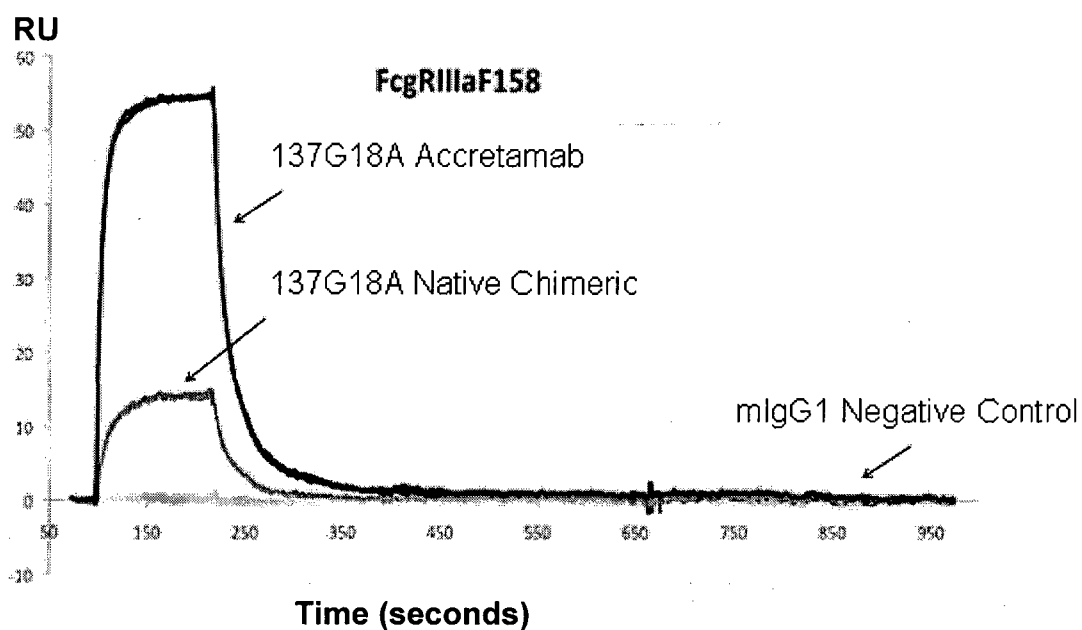


FIG. 23

Mean Fluorescence Intensity (MFI) = Human C3b deposition on *S. aureus* after 15 min

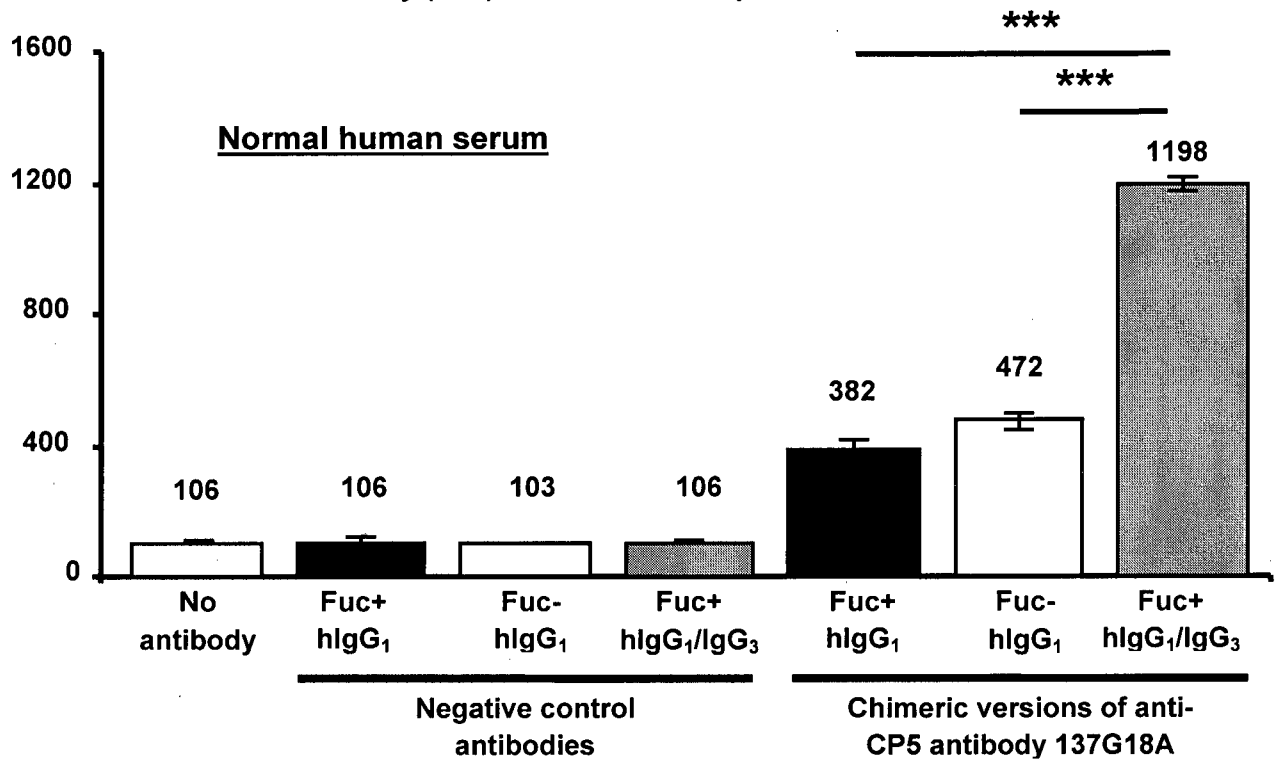


FIG. 24

MFI = Rat C3b deposition on *S. aureus* after 15 min

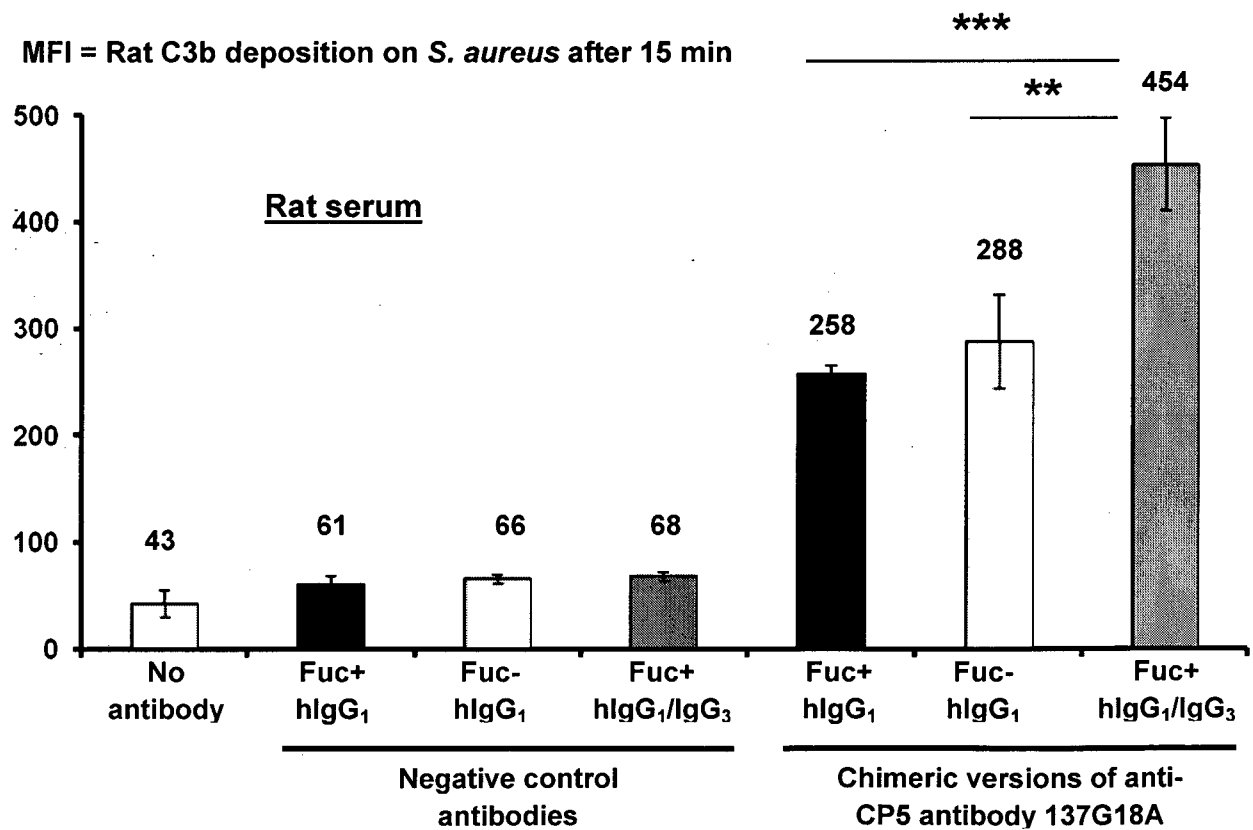


FIG. 25

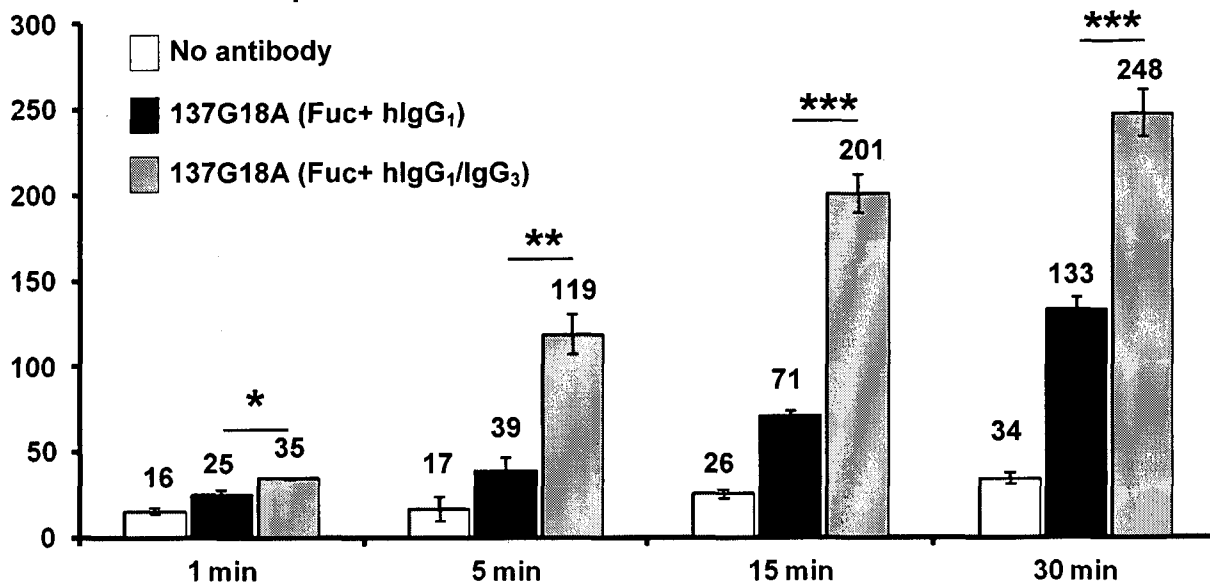
Time course with normal human serumMFI= Human C3b deposition on *S. aureus*

FIG. 26

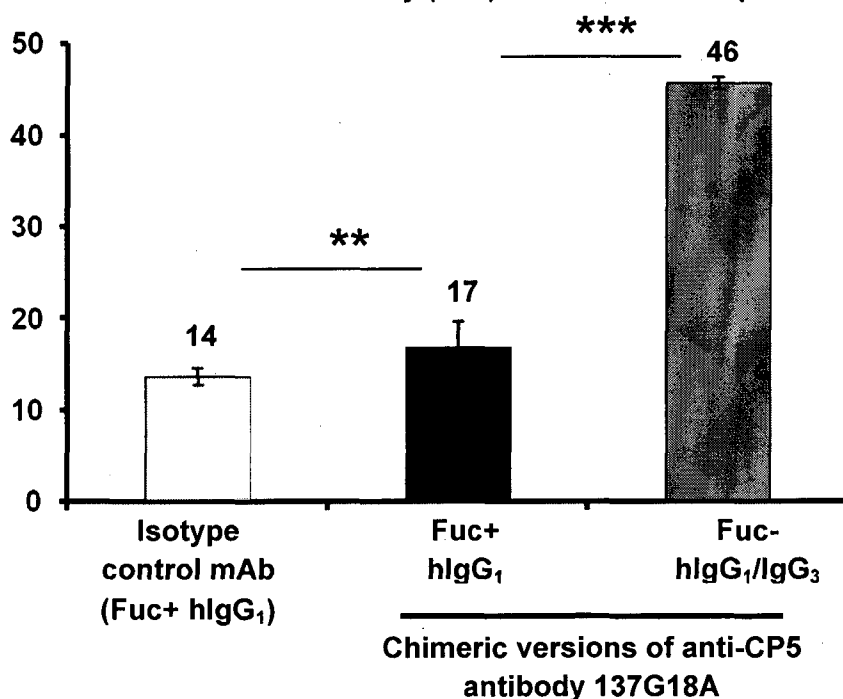
Normal human serumMean Fluorescence Intensity (MFI) = Human C3b deposition on *S. aureus* after 15 min

FIG. 27A

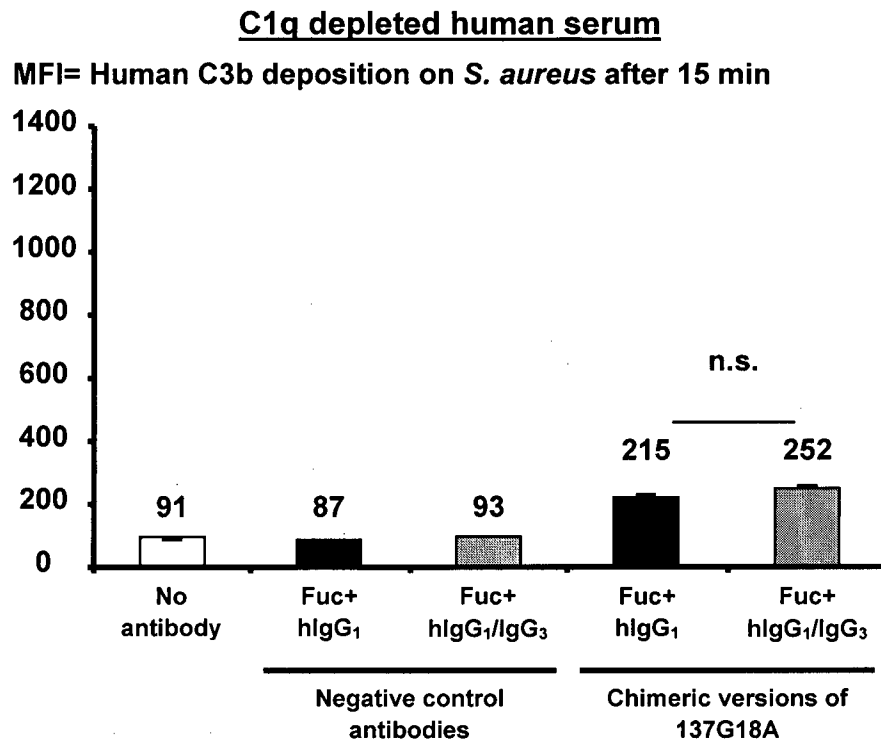


FIG. 27B

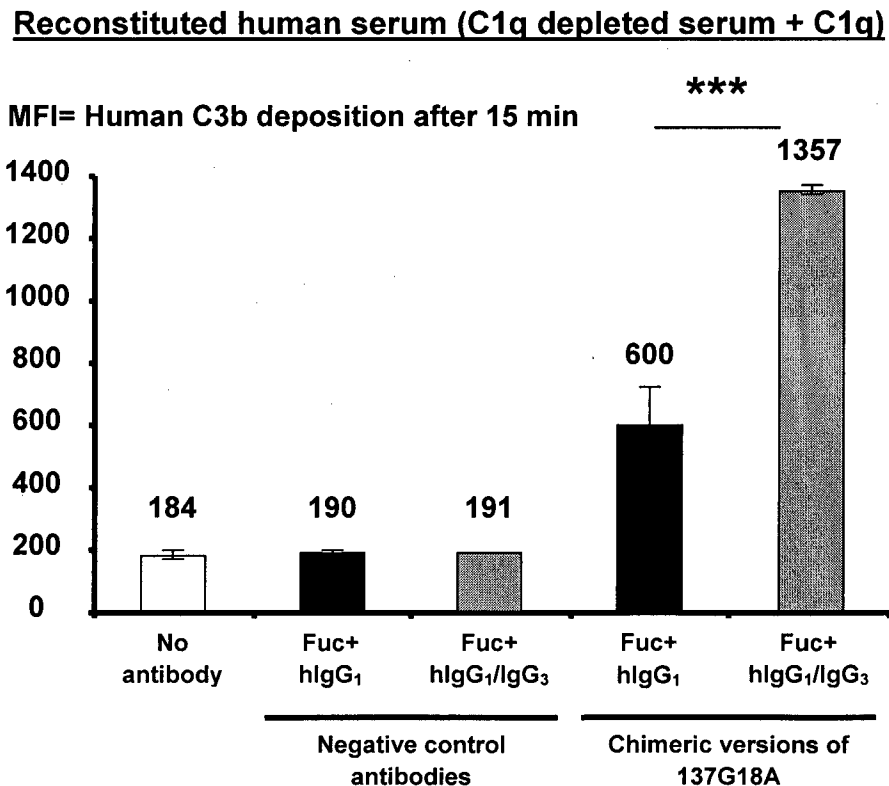


FIG. 28

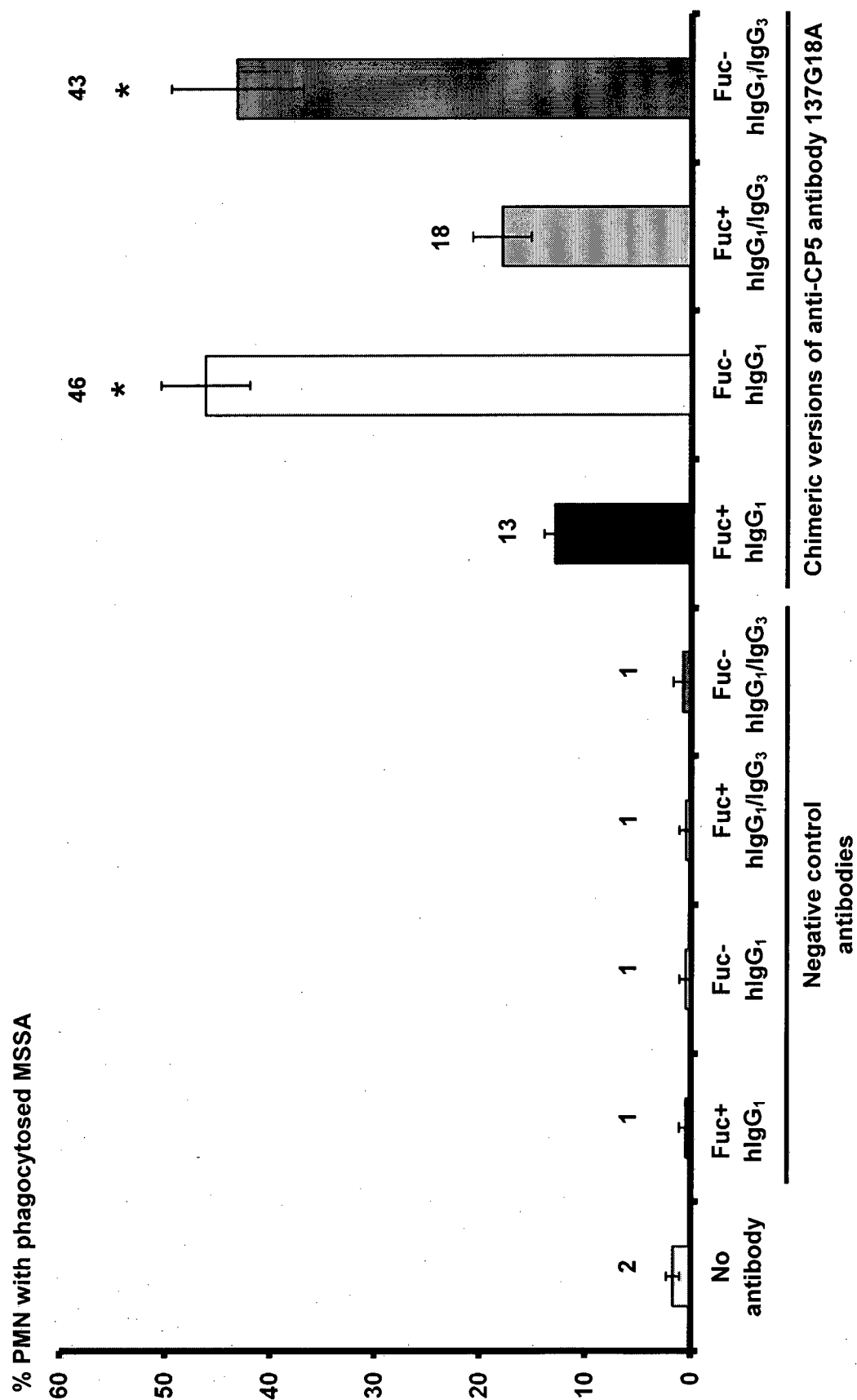


FIG. 29

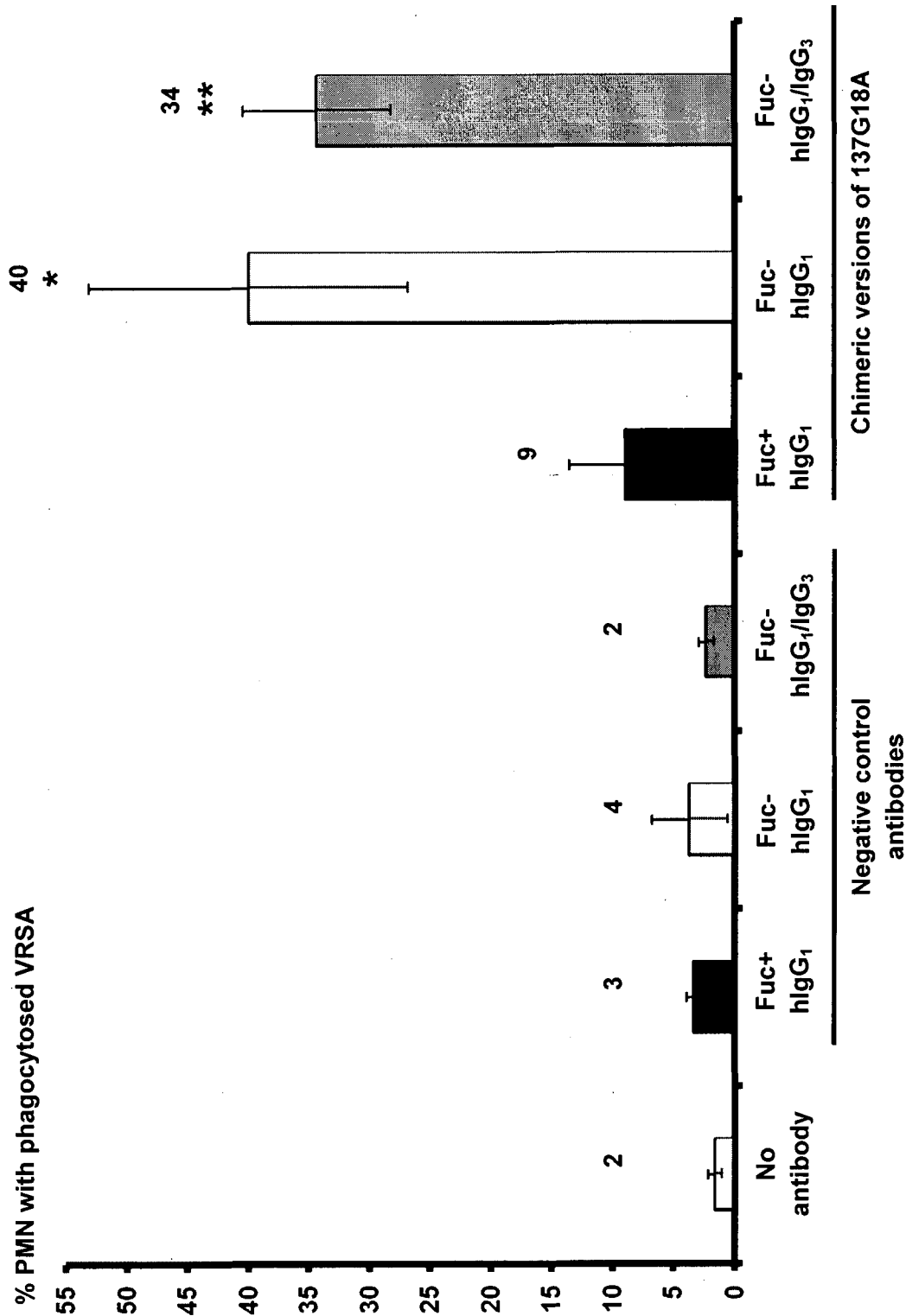


FIG. 30

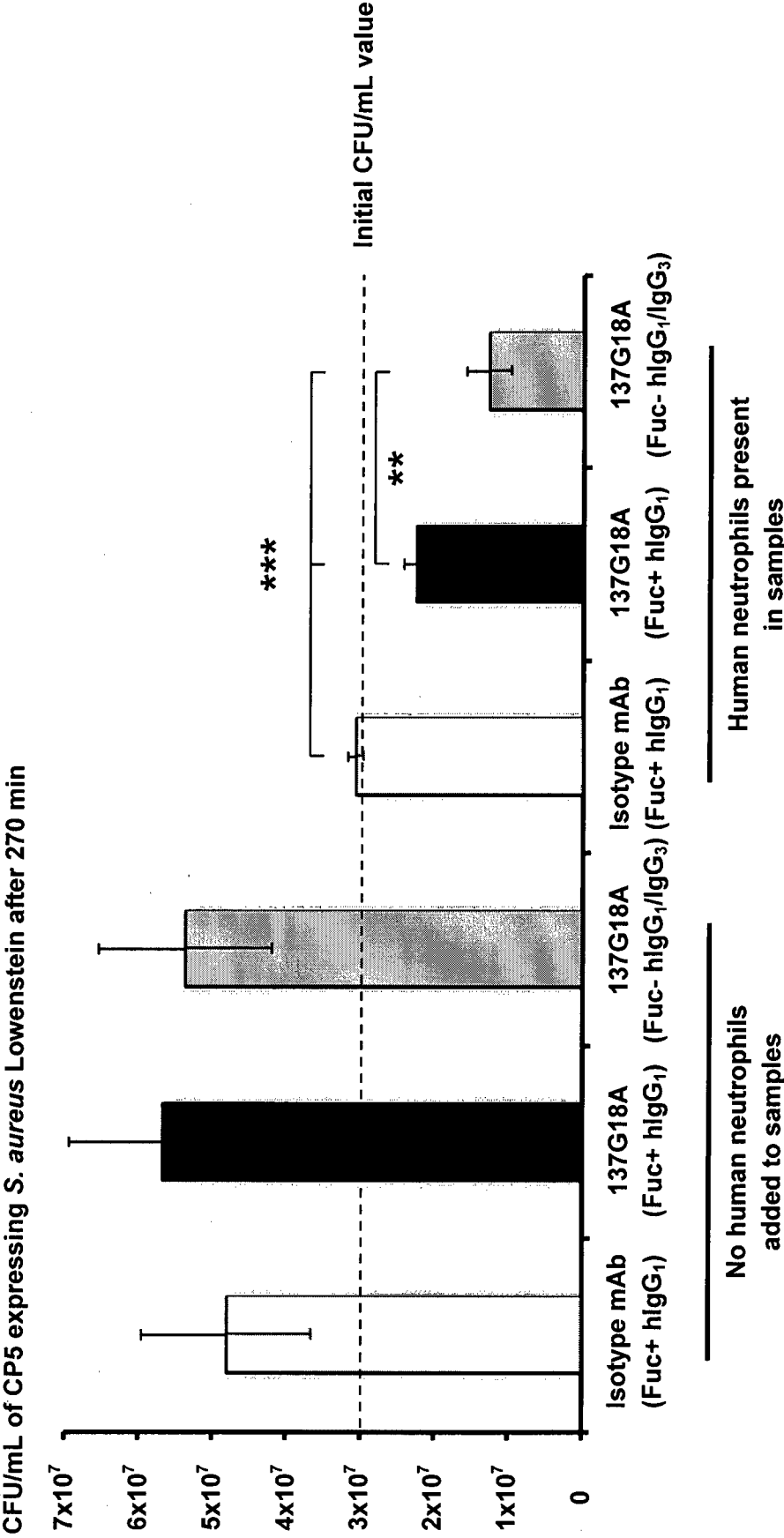
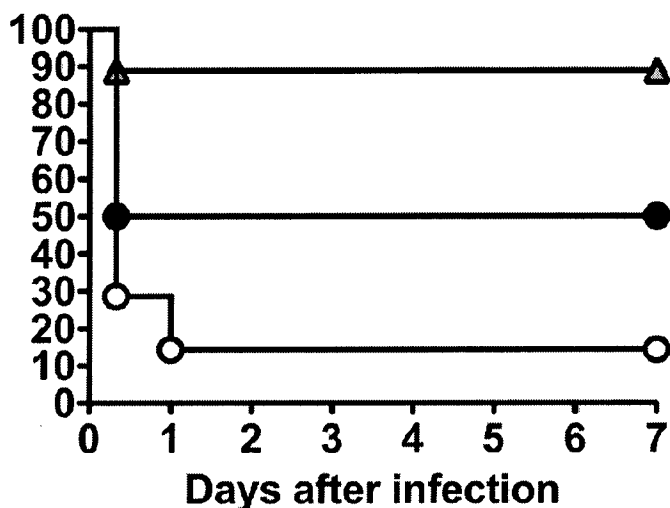


FIG. 31

Percent survival or RNU rats



- 1 µg isotype control mAb (Fuc+ hlgG₁) (n=7)
 ● 1 µg chimeric 137G18A (Fuc+ hlgG₁) (n=8)
 ▲ 1 µg chimeric 137G18A (Fuc- hlgG₁/lgG₃) (n=9)

FIG. 32

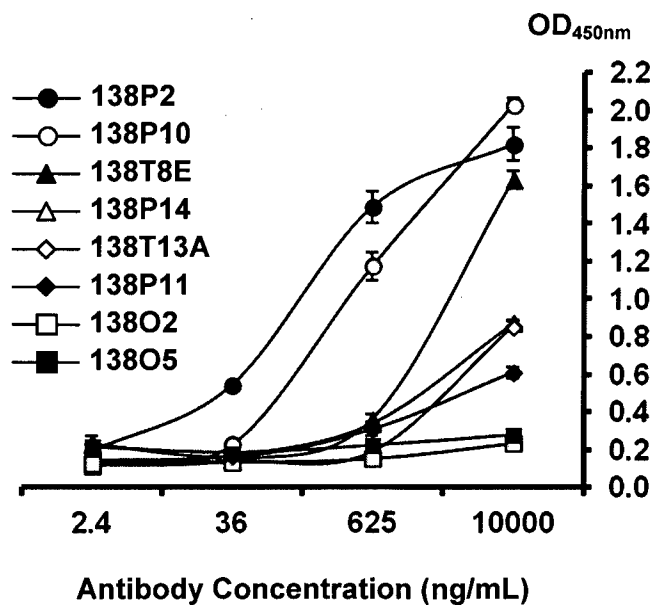


FIG. 33

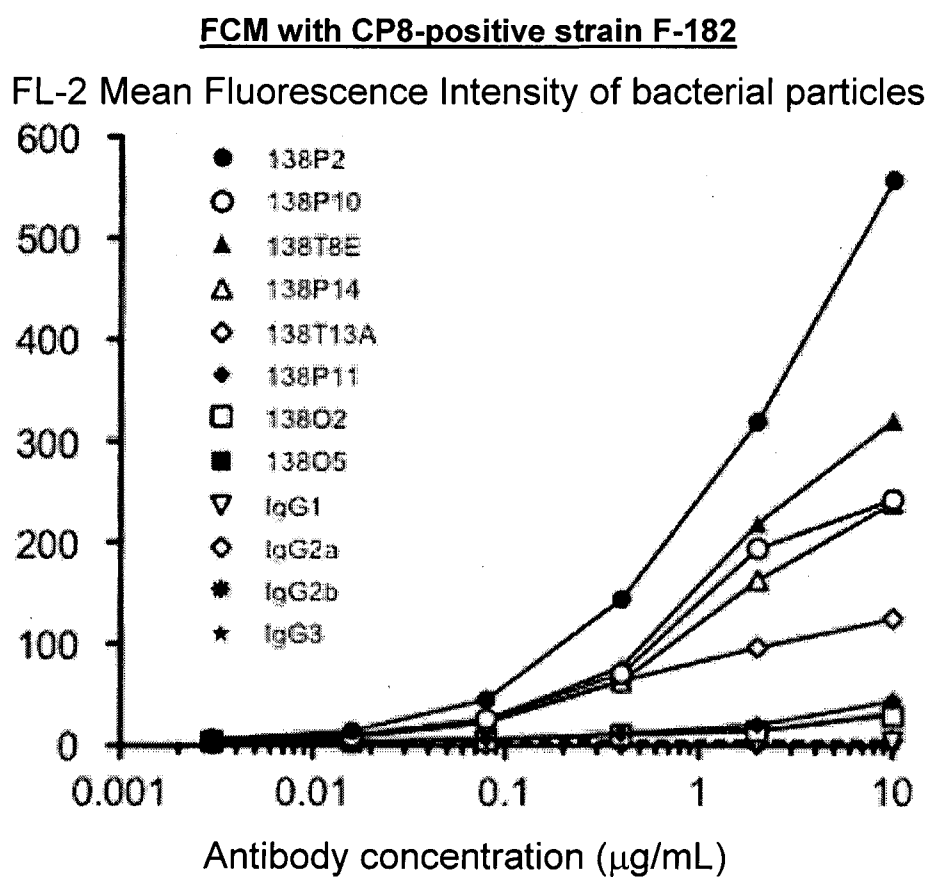


FIG. 34A

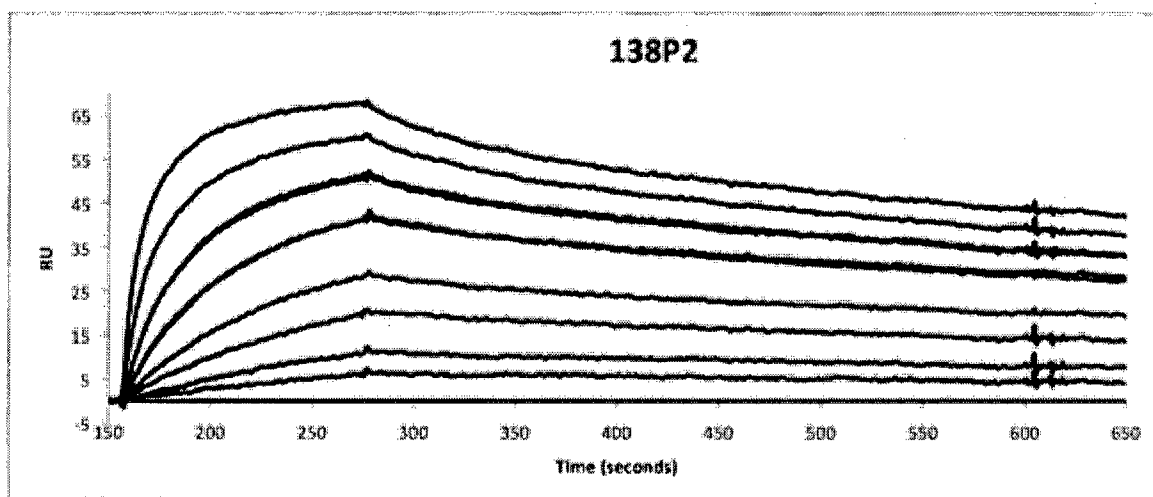


FIG. 34B

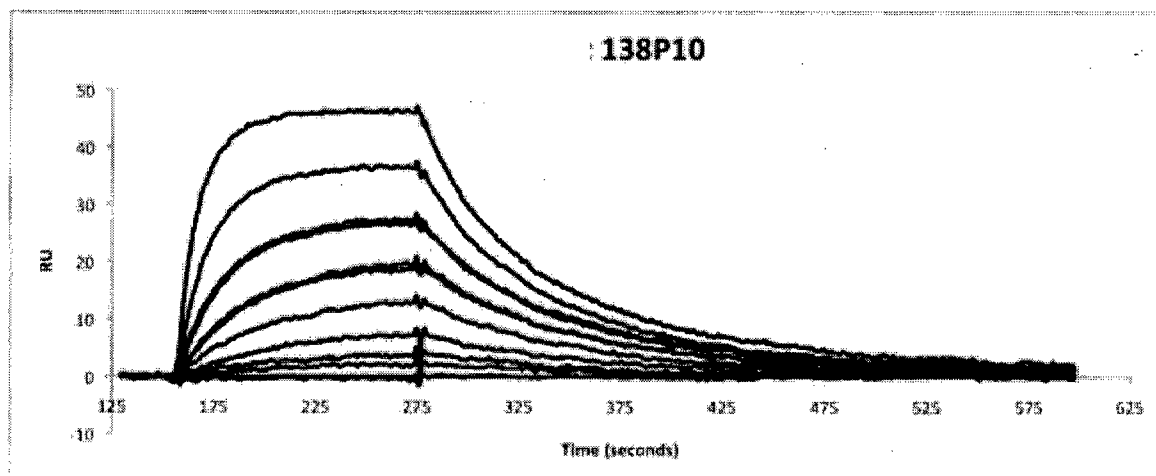


FIG. 35A

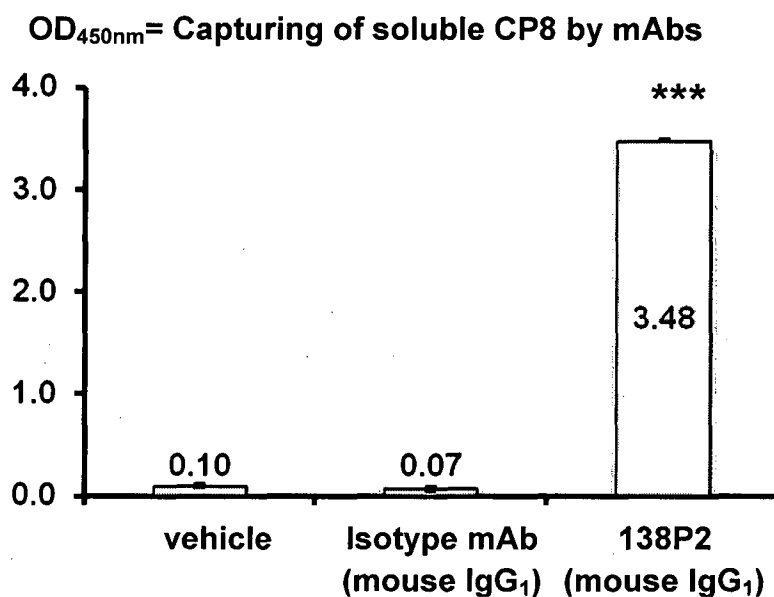


FIG. 35B

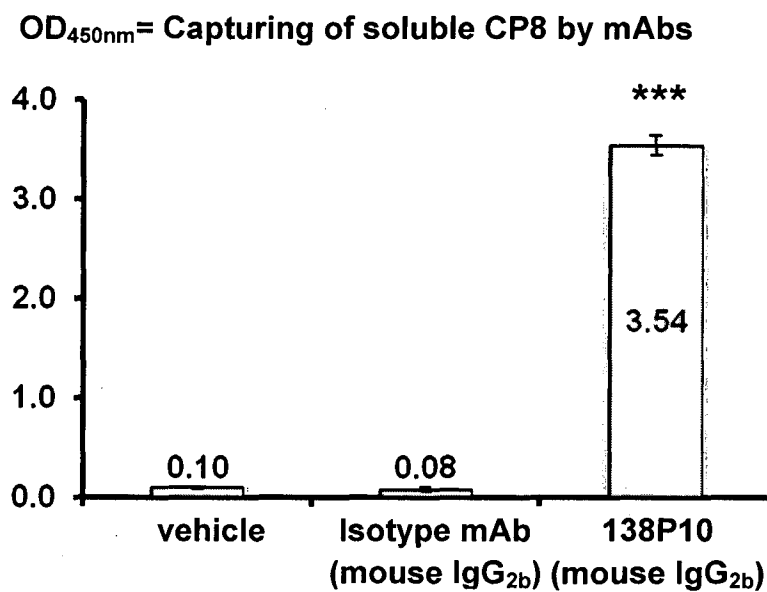


FIG. 36

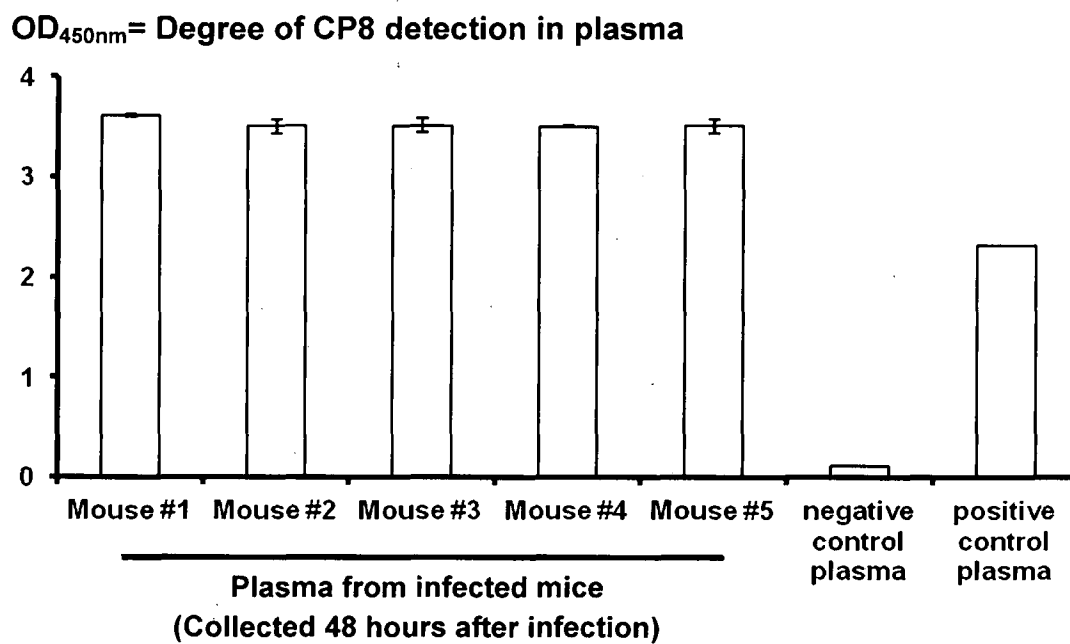


FIG. 37A

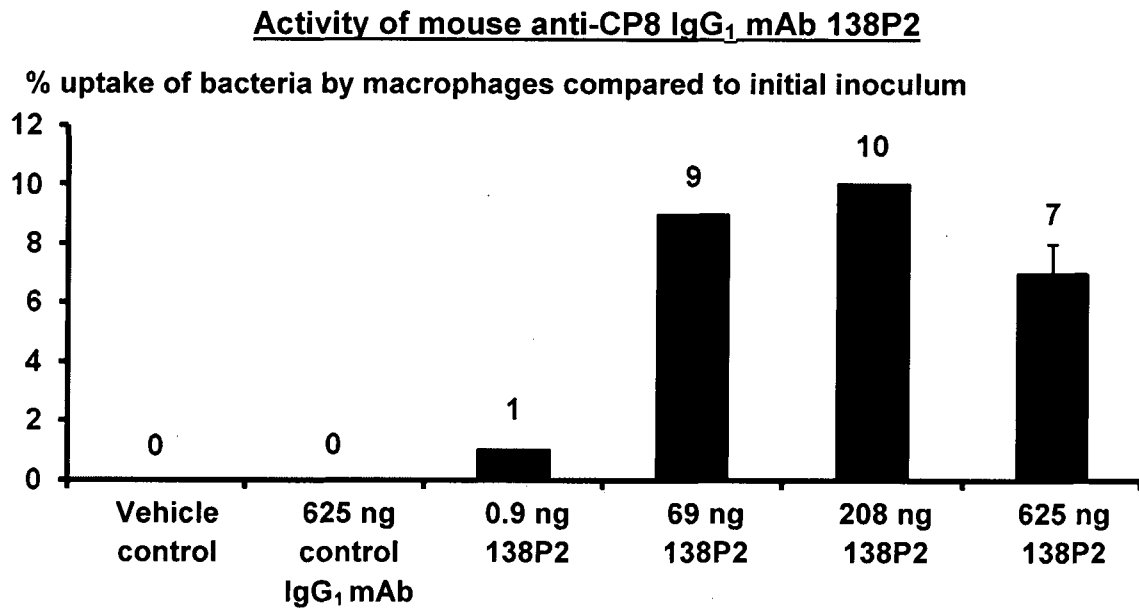


FIG. 37 B

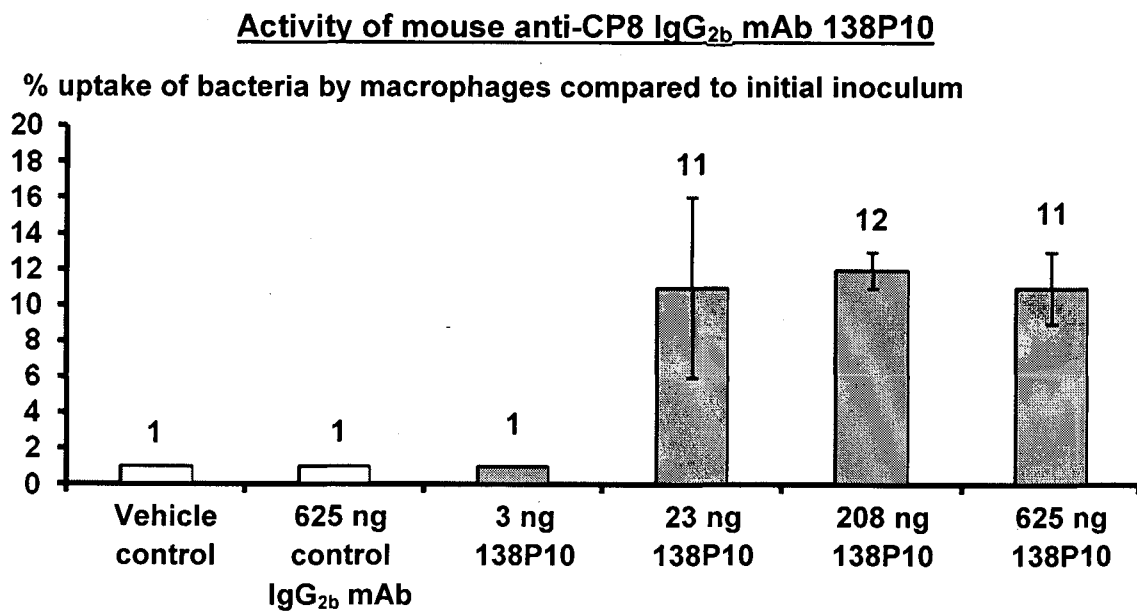


FIG. 38A

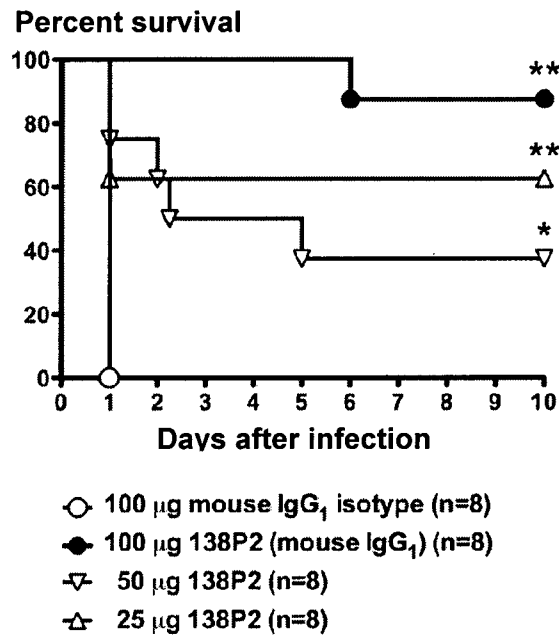


FIG. 38B

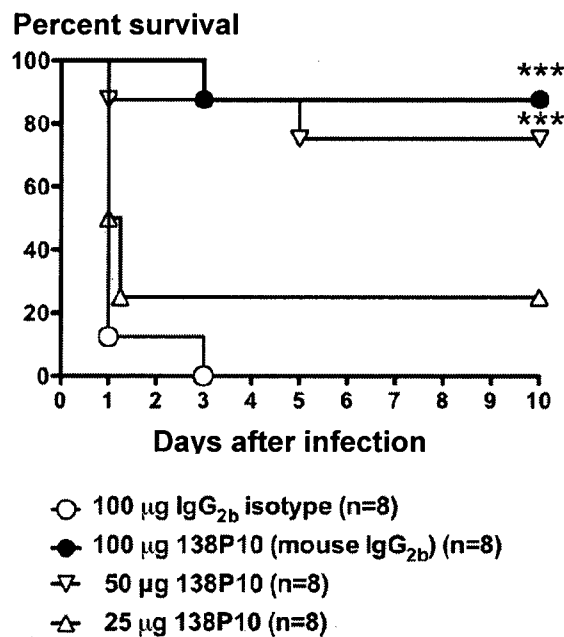


FIG. 39A

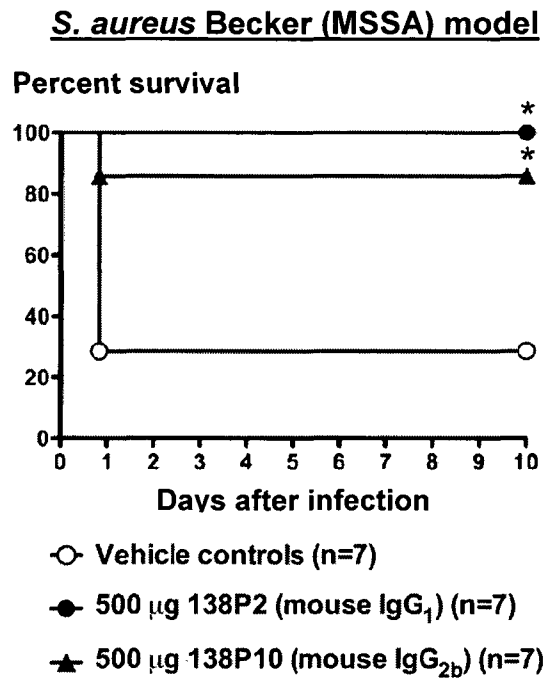


FIG. 39B

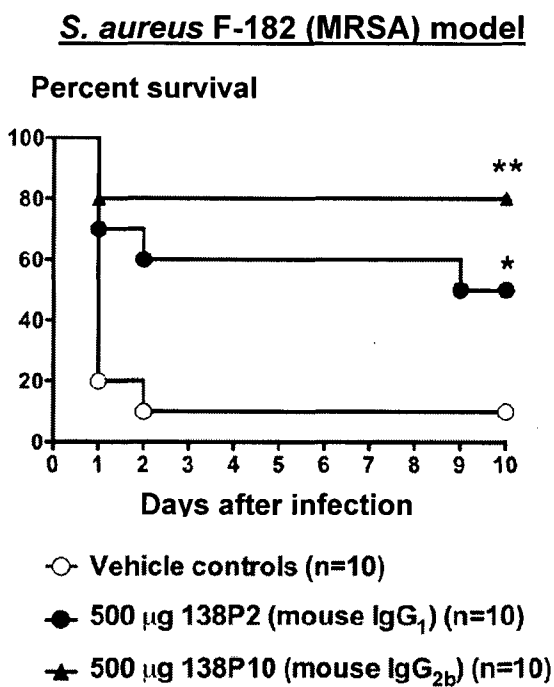


FIG. 40

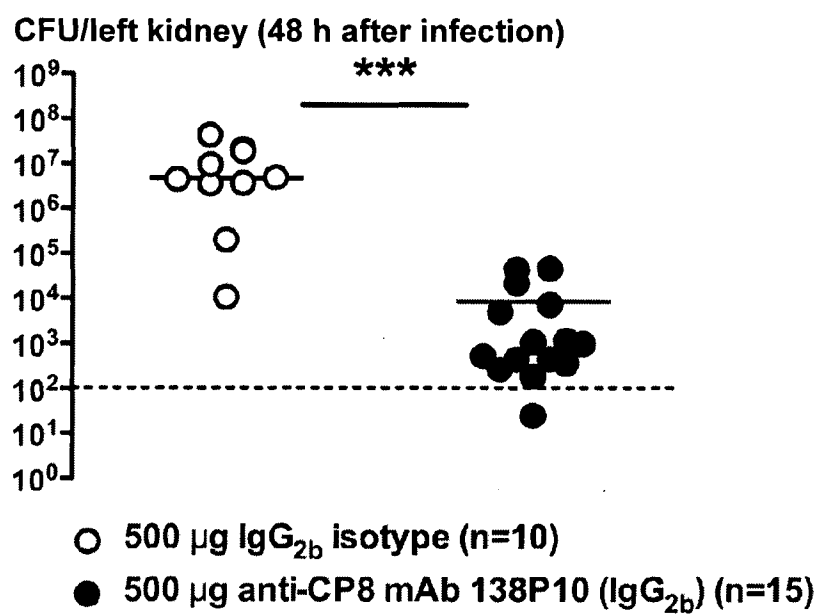


FIG. 41A

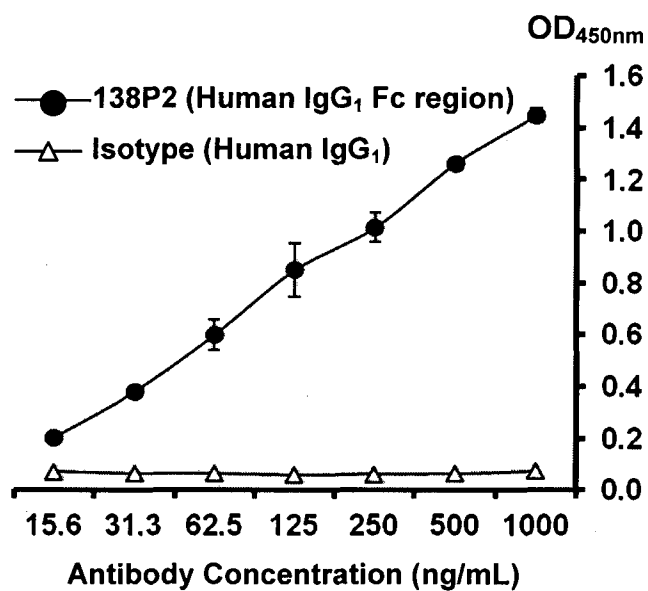


FIG. 41B

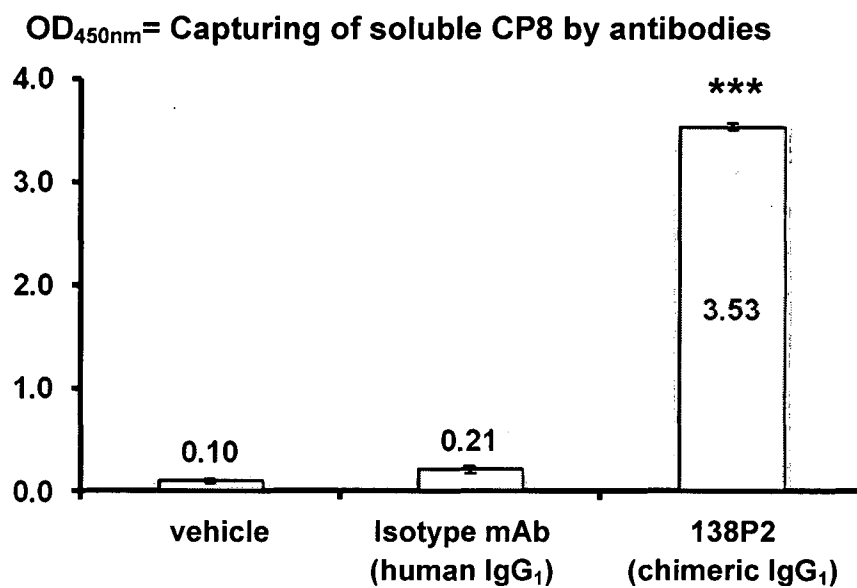


FIG. 42A

Binding to MSSA strain ATCC-25923

MFI (FL-1)= Degree of antibody binding to MSSA

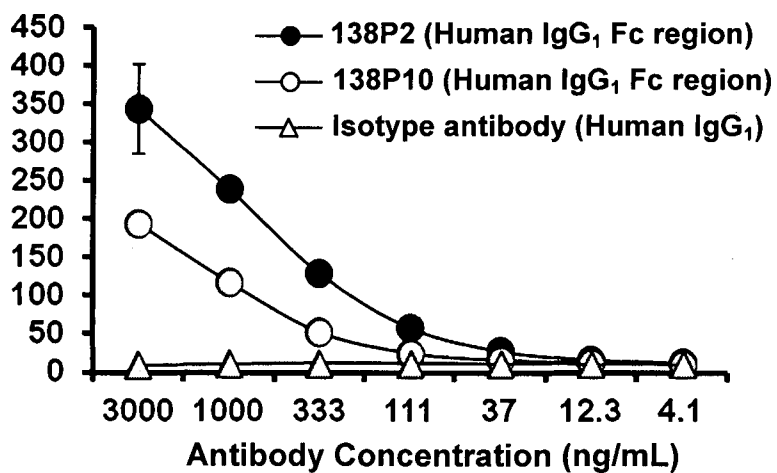


FIG. 42B

Binding to MRSA strain F-182

MFI (FL-1)= Degree of antibody binding to MRSA

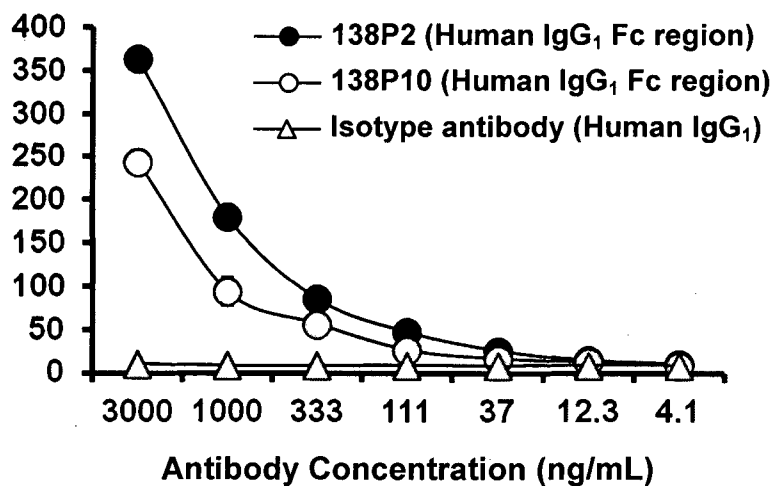


FIG. 43

% uptake of bacteria by macrophages compared to initial

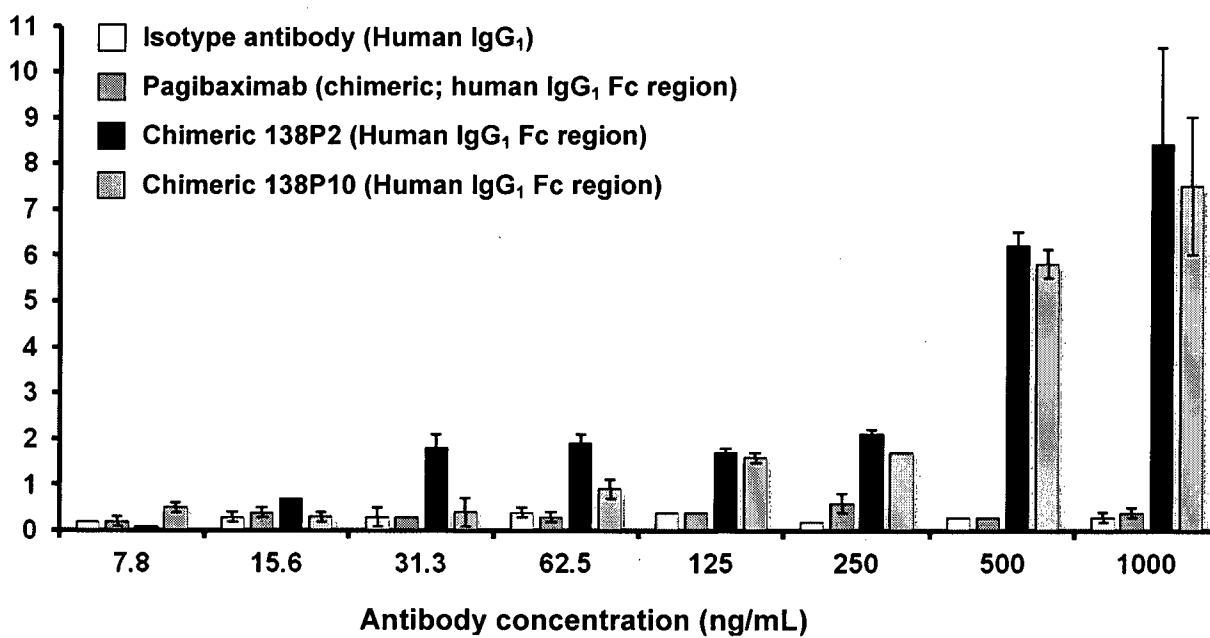


FIG. 44

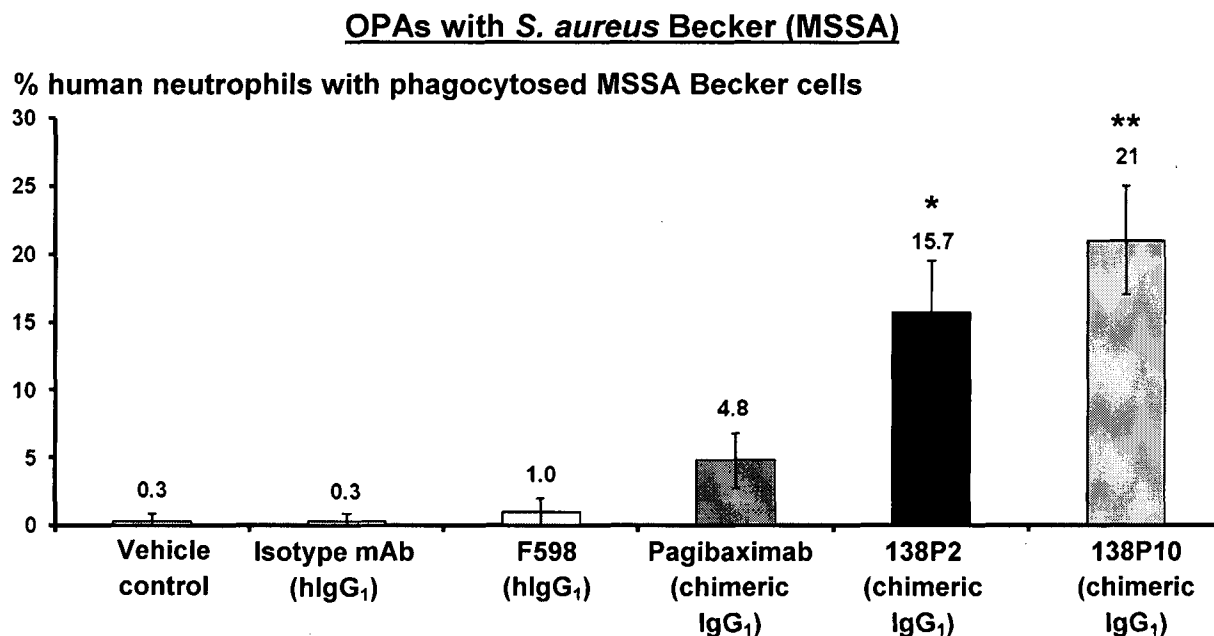
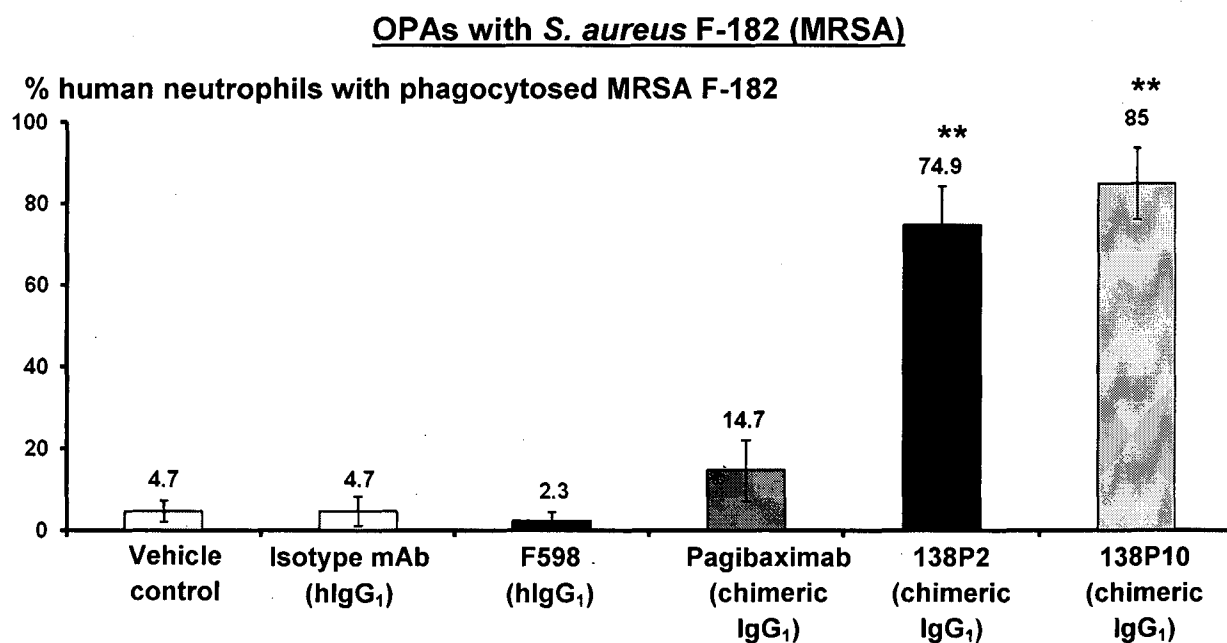


FIG. 45



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2013/072042

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. C07K16/12 (2006.01) i, C12N15/02 (2006.01) i, C12P21/08 (2006.01) i,
C12Q1/14 (2006.01) i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C07K16/12, C12N15/02, C12P21/08, C12Q1/14

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

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2013
Registered utility model specifications of Japan 1996-2013
Published registered utility model applications of Japan 1994-2013

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPplus/MEDLINE / BIOSIS (STN), JSTPlus/JMEDPlus/JST/580 (JDreamIII),
GenBank/EMBL/DBJ/GeneSeq, UniProt/GeneSeq, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M J Nelles et al., Reactivity of type-specific monoclonal antibodies with Staphylococcus aureus clinical isolates and purified capsular polysaccharide., Infection and Immunity, 1985, 49(1), pp.14-18	1-10, 15-17
X	Bernard Poutrel et al., Heterogeneity of Cell-Associated CP5 Expression on Staphylococcus aureus Strains Demonstrated by Flow Cytometry., Clinical and Diagnostic Laboratory Immunology, 1997, 4(3), pp.275-278	1-10, 15-17



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

12.11.2013

Date of mailing of the international search report

19.11.2013

Name and mailing address of the ISA/JP

Japan Patent Office

3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan

Authorized officer

Ito, Yuichi

Telephone No. +81-3-3581-1101 Ext. 3488

4N 3846

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2013/072042

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	James Cook et al., Staphylococcus aureus capsule type 8 antibodies provide inconsistent efficacy in murine Models of staphylococcal infection., Human Vaccines and Immunotherapeutics, 2009, 5(4), pp.254-263	1-10, 15-17
A	Ali I. Fattom et al., Antigenic Determinants of Staphylococcus aureus Type 5 and Type 8 Capsular Polysaccharide Vaccines., Infection and Immunity, 66(10), 1998, pp.4588-4592	1-10, 15-17
A	Ali I. Fattom et al., A Staphylococcus aureus Capsular Polysaccharide (CP)Vaccine and CP-Specific Antibodies Protect Miceagainst Bacterial Challenge., Infection and Immunity, 1996, 64(5), pp.1659-1665	1-10, 15-17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2013/072042

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

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

1. ☒ Claims Nos.: 11-14
because they relate to subject matter not required to be searched by this Authority, namely:
The subject matter of claim 11-14 relates to a method for treatment of the human or animal body by surgery or therapy, which does not require an international search by the International Searching Authority in accordance with PCT Article 17(2)(a)(i) and [Rule 39.1(iv)].
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.