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(54) Title: ANTIBODIES OR FRAGMENTS THEREOF DIRECTED AGAINST A STAPHYLOCOCCUS AUREUS EPITOPE OF ISAA OR ISAB

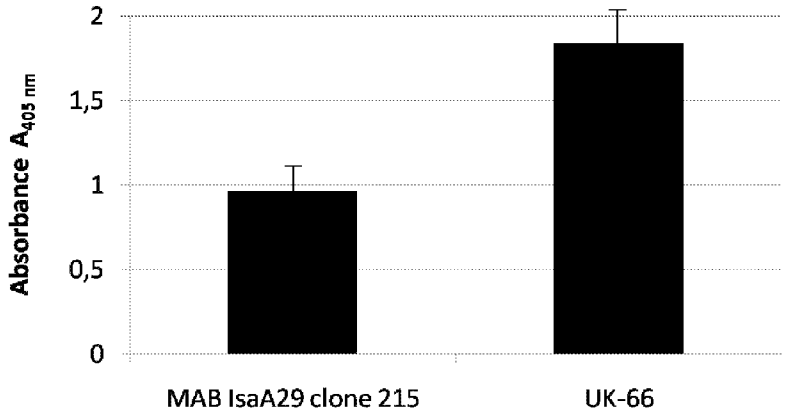

Fig. 2

(57) Abstract: The invention concerns antibodies or fragments thereof that are directed against a Staphylococcus aureus epitope.

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ANTIBODIES OR FRAGMENTS THEREOF DIRECTED AGAINST A STAPHYLOCOCCUS AUREUS EPITOPE OF ISAA OR ISAB

The invention concerns antibodies or fragments thereof that are directed against a Staphylococcus aureus (= S. aureus) epitope, a kit containing these antibodies or fragments, a use of these antibodies or fragments, a hybridoma cell line which produces these antibodies and a method of treatment.

From FEMS Imunol. Med. Microbial. 2000 Oct.; 29(2), pages 145 to 153 immunodominant structures which were expressed in vivo during sepsis caused by methicillin resistant Staphylococcus aureus (MRSA) are known. These structures are the 29 kDa protein IsaA and the 17 kDa protein IsaB. It is stated that these proteins may serve as potential targets for the development of antibody based therapy against MRSA.

From the abstract "Development of antibody-based therapy targeting immunodominant antigens of Staphylococcus aureus", Ohlsen, K. et al., page 128 of the abstract book published for the 59th annual conference of the Deutsche Gesellschaft für Hygiene und Mikrobiologie e.V. on September 2007 and from Lorenz, U. et al., "Therapeutische Effektivität von monoklonalen Antikörpern gegen Staphylococcus aureus in einem Sepsis- und Abszess-Mausmodell", Chirurgisches Forum 2008, Springer Berlin Heidelberg, 29th May 2008, issue 17, pp. 225-226 the application of a first murine monoclonal antibody targeting the immunodominant antigen IsaA in two animal infection models is known. The study revealed that application of anti-IsaA MAB lowers the infection burden in both infection models.

The object of the present invention is to provide novel antibodies or fragments thereof that are well suited for a treatment of infections caused by Staphylococcus aureus and for a

detection of S. aureus. Furthermore, a kit containing these antibodies or fragments, a use of these antibodies or fragments, a hybridoma cell line secreting these antibodies or fragments and a method of treatment shall be provided.

This object is solved by the subject-matter of claims 1, 8, 15, 16, 17 and 19. Embodiments of the invention are disclosed in claims 2 to 7, 9 to 14, 18 and 20 to 24.

According to the invention antibodies or fragments thereof are provided, wherein said antibodies or fragments are directed against a Staphylococcus aureus epitope that is recognized by monoclonal further antibodies which are secreted by the hybridoma cell line deposited at the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstr. 7B, D-38124 Braunschweig, Germany" (DSMZ) under accession number DSM ACC2987 or DSM ACC2988. The hybridoma cell line deposited at the DSMZ under accession number DSM ACC2987 is further designated as "cell line DSM ACC2987" and the hybridoma cell line deposited at the DSMZ under accession number DSM ACC2988 is further designated as "cell line DSM ACC2988". The monoclonal further antibodies which are secreted by cell lines DSM ACC2987 and DSM ACC2988 are monoclonal mouse antibodies.

The antibodies according to the invention comprise full immunoglobulin molecules, preferably IgMs, IgDs, IgEs, IgAs or IgGs, more preferably lgG1 , lgG2a, lgG2b, lgG3 or lgG4, whereas the fragments comprise parts of such immunoglobulin molecules, like Fab fragments or V-, VH- or CDR-regions. Furthermore, the antibodies comprise modified and/or altered antibodies, like chimeric and humanized antibodies. The antibodies also comprise modified or altered monoclonal or polyclonal antibodies as well as recombinantly or synthetically generated or synthesized antibodies. The fragments comprise antibody fragments as well as parts thereof, like, separated

light and heavy chains, Fab, Fab/c, Fv, Fab', $F(ab')_2$. The antibodies according to the invention also comprise antibody derivatives like bifunctional antibodies and antibody constructs, like single chain Fvs (scFv), bispecific scFvs or antibody-fusion proteins. All antibody derivatives exhibit the binding specificity of the antibodies they are derived from, i.e. they are directed against a Staphylococcus aureus epitope that is recognized by the monoclonal further antibodies secreted by the hybridoma cell lines DSM ACC2987 or DSM ACC2988.

The epitope that is recognized by the monoclonal further antibodies which are secreted by the cell line DSM ACC2987 is located on the immunodominant S. aureus antigen IsaA. The epitope that is recognized by the monoclonal further antibodies which are secreted by the cell line DSM ACC2988 is located on the immunodominant S. aureus antigen IsaB. The inventors of the present invention found that the epitopes recognized by the antibodies according to the invention are particularly exposed on S. aureus. Antibodies or fragments thereof that are directed against these epitopes are well suited for the detection of S. aureus and for the treatment of an infection with S. aureus. Owing to the high variability of S. aureus that causes different extends of expression and mutations of the antigens on different strains every antibody that recognizes an additional epitope not recognized by other antibodies is useful for the detection of S. aureus as well as for the treatment of a S. aureus infection.

The antibodies may be polyclonal or monoclonal antibodies. In particular the antibodies may be the monoclonal further antibodies, i.e. the antibodies which are secreted by the hybridoma cell line DSM ACC2987 or DSM ACC2988. These antibodies are very useful for the detection of S. aureus as well as for the treatment of an infection. They exhibit very high affini-

ties and specificities. The high affinity of the antibodies which are secreted by the hybridoma cell line DSM ACC2987 to the epitope that is recognized by these antibodies is indicated by a low K_D value for the binding of said antibody to said epitope. Depending on the method of determination K_D values of ≤ 18 pM and 1.7 nM have been determined for this binding (see Figs. 6 and 7 and corresponding text).

The monoclonal antibodies or the antibodies which are secreted by the hybridoma cell line DSM ACC2987 and/or DSM ACC2988 may be antibodies of the IgG type, in particular of the IgG1 type or the IgG2b type. The fragments may be Fab fragments, Fab/c fragments, Fv fragments, Fab' fragments or $F(ab')_2$ fragments. These fragments are particularly useful for the detection of S. aureus because the cell wall of S. aureus contains protein A which unspecifically binds immunglobulins via their Fc-parts.

The antibodies may be animal antibodies, i.e. antibodies produced in an animal, especially murine, bovine, or camel antibodies, human antibodies, antibodies produced in a plant, an egg or a fungus, in particular a Saccharomyces, recombinant antibodies produced in cells of a cell line, chimeric antibodies, or humanized antibodies. A humanized antibody may be a monoclonal antibody that contains the binding portion of a monoclonal mouse antibody, e.g. the monoclonal antibody secreted by the hybridoma cell line DSM ACC2987 or DSM ACC2988, and the none binding portion of a human antibody.

Each of the antibodies may have a heavy chain with a first variable region and a light chain with a second variable region,

wherein the hybridoma cell line is DSM ACC2987 and wherein the first variable region comprises an amino acid sequence

that is at least 90% identical, in particular at least 92.5% identical, in particular at least 95% identical, in particular at least 97.5% identical, in particular 100% identical, with sequence SEQ ID NO: 2 and wherein the second variable region comprises an amino acid sequence that is at least 90% identical, in particular at least 92.5% identical, in particular at least 95% identical, in particular at least 97.5% identical, in particular 100% identical, with sequence SEQ ID NO: 4

or

wherein the hybridoma cell line is DSM ACC2988 and wherein the first variable region comprises an amino acid sequence that is at least 90% identical, in particular at least 92.5% identical, in particular at least 95% identical, in particular at least 97.5% identical, in particular 100% identical, with sequence SEQ ID NO: 6 and wherein the second variable region comprises an amino acid sequence that is at least 90% identical, in particular at least 92.5% identical, in particular at least 95% identical, in particular at least 97.5% identical, in particular 100% identical, with sequence SEQ ID NO: 8.

The first variable region and the second variable region as characterized above together form a binding site having high affinity to and specifity for the epitope.

One possible DNA sequence encoding the first variable region according to SEQ ID NO: 2 is sequence SEQ ID NO: 1. SEQ ID NO: 1 is the sequence encoding the first variable region of the antibodies secreted by cell line DSM ACC2987. The second variable region according to SEQ ID NO: 4 may be encoded by sequence SEQ ID NO: 3. SEQ ID NO: 3 is the sequence encoding

the second variable region of the antibodies secreted by cell line DSM ACC2987.

The first variable region according to SEQ ID NO: 6 may be encoded by sequence SEQ ID NO: 5 which is the sequence encoding the first variable region of the antibodies secreted by cell line DSM ACC2988. The second variable region according to SEQ ID NO: 8 may be encoded by sequence SEQ ID NO: 7. SEQ ID NO: 7 is the sequence encoding the second variable region of the antibodies secreted by cell line DSM ACC2988.

If the hybridoma cell line is DSM ACC2987, i.e., if the epitope is located on immunodominant S. aureus antigen IsaA, the epitope may comprise at least one amino acid sequence which is identical in at least 10, in particular in at least 11, in particular in at least 12, in particular in at least 13, in particular in at least 14, in particular in 15, amino acids with one of the sequences SEQ ID NO: 15, 17 to 19, 21 to 26, 32 to 34 and 57 according to the sequence listing.

Epitope mapping revealed that sequences SEQ ID NO: 15, 17 to 19, 21 to 26, 32 to 34 and 57 are involved in the binding of the antibodies or fragments to the epitope. The epitope may comprise more than one amino acid sequence as specified above. The epitope may even be formed by two or more sequences located apart from each other in the amino acid sequence of IsaA.

The antibodies or fragments according to the invention may be used as a medicament. Especially they may be used as a medicament for the treatment of a human being or an animal which human being or animal has an infection with S. aureus, especially methicillin resistant or methicillin sensitive S. aureus, or is at risk of getting such an infection. The human being or the animal may have a mastitis or a sepsis caused by

the infection. The mastitis may be a bovine mastitis. If a cow has bovine mastitis no useable milk is produced by the cow and if the cow is treated with antibiotics as it is usual in this case the milk produced by this cow has to be discarded until no antibiotics are contained in the milk of this cow. This disadvantage of the usual treatment may be avoided by use of the antibodies or fragments according to the invention as a medicament for the treatment of the bovine mastitis.

The medicament may be a medicament that is prepared for systemic and/or local application. The inventors have recognized that the treatment of a severe S. aureus infection with the antibodies or fragments according to the invention results in a significant reduction of the mortality rates and number of S. aureus in the organs of the treated human being or animal. Furthermore, the inventors have recognized that phagocytotic killing of S. aureus bacteria by polymorphonuclear leukocytes is significantly enhanced if antibodies according to the invention are bound to S. aureus bacteria compared to S. aureus bacteria without these antibodies.

The antibodies or fragments may be present in a mixture with other antibodies or fragments of these other antibodies which other antibodies are directed against at least one further epitope of Staphylococcus aureus. This further epitope may be located on the antigen on which the epitope is located, i.e. IsaA or IsaB, or on a further antigen. The use of such a mixture as a medicament may be more efficient than the use of a medicament which solely contains the antibodies or fragments according to the invention. This may be owing to the high variability of S. aureus that causes different extents of expression of the antigens on different strains such that more bacteria are recognized by the mixture of antibodies or fragments than by the antibodies or fragments alone.

The antibodies or fragments may be present in a mixture with at least one antibiotic. In the human being or animal to be treated with the medicament mutated S. aureus may be present in addition to common S. aureus. The mutated S. aureus may have mutated IsaA and/or IsaB that cannot be recognized by the antibodies or fragments according to the invention. In this case the antibiotic may be effective against the mutated S. aureus.

The antibodies or fragments according to the invention may be present in a mixture with plasma of blood of a mammal, especially with plasma of blood of a human being. The inventors found, that antibodies or fragments according to the invention mixed with plasma may be much more efficient than antibodies or fragments according to the invention contained in a saline solution.

The invention also concerns a kit containing antibodies or fragments according to the invention for the detection of S. aureus. Such a kit may be used for diagnostic purposes.

The invention further concerns the use of antibodies or fragments according to the invention for the detection, especially a highly specific detection, of S. aureus.

Furthermore, the invention concerns a hybridoma cell line which produces antibodies according to the invention. The hybridoma cell line may be the cell line deposited at the DSMZ under accession number DSM ACC2987 or DSM ACC2988.

The invention further concerns a method of treatment of a human being or an animal which human being or animal has an infection with Staphylococcus aureus, especially methicillin resistant or methicillin sensitive Staphylococcus aureus, or

is at risk of getting such an infection, wherein antibodies or fragments according to the invention are administered to the human being or the animal. The antibodies or fragments are administered in a dosage that is sufficient to reduce the amount of S. aureus or to cause an elimination of S. aureus in the human being or the animal. The antibodies or fragments may be mixed with a suitable carrier.

The human being or the animal may have a mastitis or a sepsis caused by the infection. The antibodies or fragments may be present in a mixture with other antibodies or fragments of these other antibodies which other antibodies are directed against at least one further epitope of Staphylococcus aureus. Furthermore, the antibodies or fragments may be mixed with plasma or blood of a mammal, especially a human being, before they are administered. The antibodies or fragments may be administered systemically, in particular intravenously, nasally or sublingually. They may also be administered together with at least one antibiotic.

Embodiments of the invention

Fig. 1a, 1b, 1c show immunofluorescence stainings of S. aureus strain MA12 (Fig. 1a), S. aureus IsaA knock out strain MA12Δ*isaA* (Fig. 1b) and S. aureus protein A knock out strain Cowan I Δ*spa*::Tc^{r} (Fig. 1c) with antibodies according to the invention.

Fig. 2 shows ELISA data of IsaA binding of MAB-IsaA29 clone 215 compared to MAB-UK-66.

Fig. 3 shows survival of mice after i.v. challenge with *S. aureus* strain USA300 and i.v. treatment with monoclonal antibodies according to the invention or isotype control antibodies.

Fig. 4 shows the recovery of S. aureus, strain MA12 from a central venous catheter and organs of mice treated with S. aureus and monoclonal antibodies according to the invention.

Fig. 5 shows the phagocytosis of S. aureus by polymorphonuclear leukocytes in presence and absence of antibodies according to the invention.

Fig. 6 shows the kinetics of the binding of monoclonal antibodies MAB-UK-66 to immobilized IsaA.

Fig. 7 shows the kinetics of the binding of IsaA to immobilized monoclonal antibodies MAB-UK-66.

Figs. 1a-1c show the result of stainings with monoclonal antibodies directed against epitopes of IsaA as primary antibodies that were produced by the hybridoma cell line DSM ACC2987 and that are designated as MAB-UK-66. FITC conjugated antibodies directed against mouse IgG were used as secondary antibodies.

Figures 1a and 1c show positive immunofluorescence stainings of S. aureus, strains MA12 and Cowan I Δ*spa*::Tc^r whereas Fig. 1b shows no immunofluorescence staining of S. aureus, strain MA12Δ*isaA*. In contrast to native S. aureus bacteria the bacteria of S. aureus strain Cowan I Δ*spa*::Tc^r do not produce protein A. Protein A has a high affinity to the Fc-part of antibodies. The presence of protein A on the bacteria would result in a strong unspecific binding of the primary and secondary antibodies to the bacteria. Strain Cowan I Δ*spa*::Tc^r binds the primary antibodies indicating the presence of IsaA but no antibody cross reactivity with protein A.

Fig. 2 shows the result of an Enzyme Linked Immuno Sorbent Assay (ELISA) that was performed to compare IsaA binding of known monoclonal antibody MAB-IsaA29 clone 215 with that of MAB-UK-66. MAB-IsaA29 clone 215 is the monoclonal antibody described in the abstracts "Development of antibody-based therapy targeting immunodominant antigens of Staphylococcus aureus", Ohlsen, K. et al., page 128 of the abstract book published for the 59th annual conference of the Deutsche Gesellschaft für Hygiene und Mikrobiologie e.V. on September 2007 and Lorenz, U. et al., "Therapeutische Effektivität von monoklonalen Antikörpern gegen Staphylococcus aureus in einem Sepsis- und Abszess-Mausmodell", Chirurgisches Forum 2008, Springer Berlin Heidelberg, 29th May 2008, issue 17, pp. 225-226. The ELISA was performed as follows:

After overnight coating of each well of a microtitre plate with a 100 µl sample of recombinant IsaA-protein (rIsaA) at a concentration of 0.5 µg/ml in phosphate-buffered saline (PBS, pH 7.4) the wells were blocked with 1% bovine serum albumin for 2 h. Above mentioned anti-IsaA antibodies were diluted in a ratio of 1 to 4,000 and added to the wells. After incubation for 1 h horseradish peroxidase-conjugated rabbit anti-mouse IgG (DAKO, Glostrup, Denmark) was added and incubated for 1 h. Then ABTS [2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid)] substrate (Sigma Chemical Co., Deisenhofen, Germany) was added and incubated for 1 h. Absorbance was detected at 405 nm using a microplate autoreader. As can be seen from Fig. 1 the binding of MAB-UK-66 to rIsaA is much more intense than the binding of known antibody MAB-IsaA29 clone 215 to rIsaA.

Effective anti-*S. aureus* immunotherapy should protect mice against a lethal challenge of *S. aureus*. To investigate the efficiency of the antibodies according to the invention in

vivo a survival model of S. aureus sepsis was established in mice as follows:

Age and gender matched NMRI mice (Charles River, Sulzfeld, Germany) were challenged on day 0 by intravenous injection with 5×10^8 colony forming units (cfu) of *S. aureus* USA300 (ATCC No. BAA-1556). Treated mice received intravenously MAB-UK-66 or isotype matched antibody as control (double dose regimen: 15 mg/kg in a volume of 100 µl PBS, pH 7.4 immediately and 24 h after bacterial challenge). Animals were monitored for 8 days, and lethal disease was recorded.
The significance of protection was measured with the Log-Rank/Mantel-Cox Test: P=0.022. The result is shown in Fig. 3.

For further investigation of the efficiency of the antibodies according to the invention in vivo a catheter related S. aureus sepsis model was established in mice as follows:

Age, gender and weight matched NMRI mice (Charles River Wiga Deutschland GmbH, 97633 Sulzfeld, Germany) were used in the experiment. Mice were intraperitoneally anesthetized with xylazin (8 mg/kg body weight)/ketamine (100 mg/kg body weight) and a minimal horizontal skin incision was made at the left side of the shaved neck. Using an operating microscope (Carl Zeiss Jena GmbH, 07745 Jena, Germany) under 10 – 16 x magnification, the submaxillary gland was isolated to expose the bifurcation of anterior and posterior facial vein. A venotomy between loose ligatures on the isolated anterior facial vein was executed. A sterile single lumen polyethylene catheter (inner diameter 0.28 mm x outer diameter 0.6 mm) was inserted through the incision and advanced toward the superior vena cava. The ligatures were tied and the catheter was subcutaneously tunneled and exteriorized through midline scapular incision. The patency was tested, the catheter filled with heparin solution, sealed with a plug and left in place

throughout the experiment. Twenty-four hours after surgery the mice were inoculated via the catheter with 100 µl of a S. aureus suspension, containing $1x10^7$ cfu S. aureus bacteria, strain MA12. MA12 is a mucosal isolate from nursing staff described in Ohlsen, K., Ziebuhr, W., Koller, K. P., Hell, W., Wichelhaus, T. A., and Hacker, J. "Effects of subinhibitory concentrations of antibiotics on alpha-toxin (hla) gene expression of methicillin-sensitive and methicillin-resistant Staphylococcus aureus isolates", Antimicrob. Agents Chemother. (1998), 42, pages 2817 to 2823. The bacterial suspension was allowed to dwell within the catheter lumen for 15 minutes. The content of the catheter was then flushed in the mice with 0.2 ml 0.9% saline. Treated mice received the antibodies produced by the hybridoma cell line DSM ACC2987 i.v. (double dose regimen: 15 mg/kg in a volume of 100 µl immediately and 24 h after bacterial challenge) or saline i.v. (control group). Body weight and general appearance was assessed daily during the experiment. Five days post inoculation the mice were euthanized by CO_2 inhalation.

Organs were aseptically harvested from euthanized mice and homogenized in 2 ml saline. Furthermore, the location of the catheter in the superior vena cava was confirmed and the explanted catheter irrigated with 2 ml saline and the irrigation fluid collected. Serial dilutions of the organ homogenates and catheter fluid collections were cultured on mannitol salt phenol red agar plates for at least 48 h at 37°C. Colony forming units were calculated as cfu/organ or cfu/catheter. The results are shown in Fig. 4. The data show that the treatment with the antibodies according to the invention resulted in a significant reduction of the bacterial load of the organs.

To investigate the effect of the antibodies according to the invention on phagocytosis human neutrophils were isolated us-

ing Polymorphprep (Nycomed, Oslo, Norway) in accordance with manufacturer's instructions. $1x10^7$ cfu S. aureus MA12 in 1 ml Hanks' Balanced Salt Solution (HBSS) supplemented with 0,1% (wt/vol) gelatin (HBSS-gel) and 15% (vol/vol) purified MAB-UK-66 antibodies produced by the hybridoma cell line DSM ACC2987 or PBS (control) were incubated for 30 minutes at 37°C in a slow shaking water bath. Equal volumes of $5x10^6$ antibody- and PBS-treated S. aureus and $1x10^6$ PMN-cells/ml HBSS-gel in a final volume of 1.5 ml were incubated at 37°C under slow shaking. At intervals from zero to 60 minutes a sample of this suspension was removed, centrifuged for 4 minutes at 250 x g, and the number of bacteria in the supernatant was determined by cfu counting. Phagocytosis is expressed as means of triplicate determinations ± SD of the percentage number of extracellular bacteria. Statistical analysis was performed using the non-parametric Mann-Whitney *U* test. For all comparisons, a *P* value of < 0.05 was considered statistically significant. Values were expressed as means ± SD. The result shown in Fig. 5 demonstrates that S. aureus was phagocytized by polymorphonuclear leukocytes regardless of the presence of the antibodies according to the invention. However, with the antibodies according to the invention the phagocytosis process was significantly accelerated compared to the controls. The antibodies act as an opsonin for S. aureus phagocytosis by polymorphonuclear leukocytes.

To determine the affinity of the monoclonal antibodies MAB-UK-66 to IsaA the kinetics of the binding of these antibodies to immobilized IsaA was determined by means of measuring label-free surface plasmon resonance using the BIACORE®2000 system (GE Healthcare Europe GmbH, Munzinger Strasse 5, 79111 Freiburg, Germany). For the immobilization of the antigen IsaA was N-biotinylated by incubation with equimolar concentrations of sulfo-NHS-LC-biotin (Thermo Fisher Scientific,

p/a Perbio Science, Adenauerallee 113, 53113 Bonn, Germany). Under these conditions the majority of the molecules was biotinylated only at a single site leaving the majority of the epitopes recognized by monoclonal antibodies MAB-UK-66 unaffected. Immobilization of the antigen to streptavidin coated matrices of biosensor CM5 chips was carried out as described in Nickel, J., Kotzsch, A., Sebald, W., and Mueller, T. D. "A single residue of GDF-5 defines binding specificity to BMP receptor IB" J. Mol. Biol. (2005), 349, pages 933 to 947. The amount of the immobilized antigen corresponds to about 100 resonance units [RU] measured by means of the BIACORE®2000 system.

Interaction analyses were performed using HBS150 buffer (10 mM HEPES pH7.4, 150 mM NaCl, 3.4 mM EDTA). Sensorgrams were recorded at a flow rate of 10 µl/min at 25°C. The association and dissociation time was set to 10 min. The chips were regenerated after each cycle with different regeneration solutions (A: 1 mM CH_3COOH, 1 M NaCl, pH 3; B: 4 M $MgCl_2$; C: 1 mM CH_3COOH, 1 M NaCl, 6 M Urea, pH 3) for 2 min. The kinetics of the binding of these antibodies to immobilized IsaA is shown in Fig. 6.

All apparent binding affinities were calculated using the Biaevaluation software 2.2.4. Affinities of the interactions k_{on} (< 10^6 $M^{-1}s^{-1}$) and k_{off} (< 10^{-2} s^{-1}) were calculated by fitting the kinetics data k_{on} and k_{off} to a 1:1 Langmuir binding model. In this way a dissociation constant K_D was determined. The dissociation constant K_D indicates the affinity between two interacting molecules (such as an antibody and the respective antigen). A low K_D value indicates a high affinity whereas a high K_D value indicates a low affinity. The standard deviation of K_D values determined in this way is below 50 %. Differences in binding affinities of more than a factor of two are therefore considered to be significant.

Under the measure conditions described above, the MAB-UK-66 antibodies interact with the 29 kDa IsaA antigens irreversibly due to not evaluatable slow off-rates. Since the evaluation of the kinetic rate constant is limited to 10^{-5} sec^{-1} the observed off-rate has to be smaller than that value. By setting the off rate to 10^{-5} sec^{-1} the on rate could be determined to be 5.6 10^{5} $M^{-1}sec^{-1}$ resulting in a value for the dissociation constant of 1.8 10^{-11} M. Therefore, the K_D value for this interaction is $\leq$ 1.8 10^{-11} M. Importantly, the antibody could not be removed after interaction with the antigen from the chip surface using all regeneration solutions described above. This indicates a very strong and highly specific interaction.

To confirm the high affinity of the monoclonal antibody MAB-UK-66 to IsaA the kinetics of binding of IsaA to immobilized antibodies was determined by means of label-free surface plasmon resonance using the BIACORE®2000 system (GE Healthcare Europe GmbH, Munzinger Strasse 5, 79111 Freiburg, Germany). Reversible immobilization of the antibody MAB-UK-66 was performed using an anti mouse Fc antibody covalently coupled in high density (18700 resonance units RU) to a CM5 sensor surface according to manufacturer's instructions (Mouse Antibody Capture Kit, GE Healthcare). The average amount of captured antibody MAB-UK-66 onto the anti mouse Fc surface corresponds to about 640 RU. A blank anti mouse Fc surface was used as control surface for monitoring unspecific binding and performing reference subtraction. Interaction analyses were performed using HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20). Sensorgrams were recorded at a flow rate of 30 µl/min at 25°C. Association and dissociation times were set to 3 and 15 min, respectively. The anti-Fc capturing surfaces were regenerated after each cycle using short pulses of 10 mM glycine pH 1.7. The

kinetics of the binding of IsaA to immobilized monoclonal antibodies MAB-UK-66 is shown in Fig. 7.

Affinities and rate constants for association (k_{on}) and for dissociation (k_{off}) were calculated using the BIAevaluation software 4.0.1 fitting the obtained sensorgrams to a 1:1 Langmuir binding model. In this way a dissociation constant K_D of 1.7 nM was determined in two independent measurements. Rate constants for association and dissociation of the interaction between MAB-UK-66 and IsaA were determined to be 1.8 10^5 $M^{-1}s^{-1}$ (k_{on}) and 2.9 10^{-4} s^{-1} (k_{off}), respectively.

Under the measure conditions described above, the MAB-UK-66 antibody interacts with the 29 kDa IsaA antigen with a high affinity and slow off-rate confirming a strong and highly specific interaction as already determined by the binding of MAB-UK-66 antibodies to immobilized IsaA.

For characterizing the epitope of IsaA that is recognized by the antibodies or fragments according to the invention an epitope mapping was performed. For this oligopeptides of 15 amino acids in length have been synthesized. The sequence of each of the oligonucleotides is identical with a sequence of 15 amino acids of IsaA. Each oligonucleotide has an overlap of 11 amino acids with the oligonucleotide representing a subsequent part of the total sequence. The sequences of the oligonucleotides are sequences SEQ ID NO: 9 to 64 of the sequence listing.

Each oligonucleotide was immobilized on a small spot on a glass slide. Binding of the monoclonal antibodies secreted by the hybridoma cell line DSM ACC2987 and of control antibodies to these spots was examined upon incubation with these antibodies by binding of fluorescence labeled secondary antibo-

dies and detecting fluorescence intensities. The results are shown in the following table:

SEQ ID NO:	Binding of Antibodies Secreted by Cell Line DSM ACC2987	Binding of Control Antibodies
9	273,3	70
10	227,7	159,7
11	101,3	-11
12	679,7	-22
13	5748	366,7
14	3190	-35
15	11718,3	107
16	1951	117
17	17670,7	48
18	25327,7	-118,7
19	31946,3	83,7
20	1053	105,7
21	33295	182,3
22	21481,7	26,3
23	63890,7	366,7
24	9359,3	79,3
25	49296	-61,7
26	51825	261,3
27	441,7	81
28	3173,3	77,3
29	2486,3	85,7
30	1665,3	-7
31	2935	-20
32	59456	98,3
33	55515	-0,7
34	29452,3	98,7
35	505,7	110,3
36	2745	-14,3

37	139	-27,3
38	975,3	97,7
39	491,3	109,3
40	6010	-27
41	421	29,3
42	578,7	35,7
43	370,7	44,3
44	485,7	114,3
45	235	-4,3
46	587	-24,3
47	252	14,7
48	1168,3	397,7
49	399,3	40
50	192	-49,7
51	139,3	58
52	284,7	-60
53	577,3	70,3
54	569,7	-51,7
55	1033,3	36
56	959	-17,3
57	16756	312,7
58	1317,7	46,3
59	1404,3	132,3
60	3067,3	18,7
61	479	30,3
62	551,3	6,3
63	645,3	57,7
64	379	42

As can be seen from the above table sequences SEQ ID NO: 15, 17 to 19, 21 to 26, 32 to 34 and 57 are involved in the epitope binding of the antibodies secreted by cell line DSM ACC2987.

PCT

Print Out (Original in Electronic Form)
(This sheet is not part of and does not count as a sheet of the international application)

0-1	**Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.219 MT/FOP 20020701/0.20.5.9
0-2	**International Application No.**	
0-3	**Applicant's or agent's file reference**	506664EH

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	**page**	2
1-2	**line**	17
1-3	**Identification of deposit**	
1-3-1	Name of depositary institution	DSMZ DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
1-3-2	Address of depositary institution	Inhoffenstr. 7B, D-38124 Braunschweig, Germany
1-3-3	Date of deposit	17 February 2009 (17.02.2009)
1-3-4	Accession Number	DSMZ DSM ACC2987
1-5	**Designated States for Which Indications are Made**	All designations
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	**page**	2
2-2	**line**	17
2-3	**Identification of deposit**	
2-3-1	Name of depositary institution	DSMZ DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
2-3-2	Address of depositary institution	Inhoffenstr. 7B, D-38124 Braunschweig, Germany
2-3-3	Date of deposit	17 February 2009 (17.02.2009)
2-3-4	Accession Number	DSMZ DSM ACC2988
2-5	**Designated States for Which Indications are Made**	All designations

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0-4	**This form was received with the international application:** (yes or no)	YES
0-4-1	Authorized officer	Koestel, Gilbert

FOR INTERNATIONAL BUREAU USE ONLY

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Patent Claims

1. Antibodies or fragments thereof that are directed against a Staphylococcus aureus epitope that is recognized by monoclonal further antibodies which are secreted by the hybridoma cell line deposited at the DSMZ under accession number DSM ACC2987 or DSM ACC2988.

2. Antibodies or fragments as claimed in claim 1, wherein the antibodies are monoclonal antibodies, especially the monoclonal further antibodies.

3. Antibodies as claimed in claim 2, wherein the monoclonal antibodies or the monoclonal further antibodies are antibodies of the IgG type, in particular of the IgG1 type or the IgG2b type.

4. Fragments as claimed in any of the preceding claims, wherein the fragments are Fab fragments, Fab/c fragments, Fv fragments, Fab' fragments or $F(ab')_2$ fragments.

5. Antibodies or fragments as claimed in any of the preceding claims, wherein the antibodies are animal antibodies, especially murine, bovine, or camel antibodies, human antibodies, antibodies produced in a plant, an egg or a fungus, in particular a Saccharomyces, recombinant antibodies produced in cells of a cell line, chimeric antibodies, or humanized antibodies.

6. Antibodies or fragments as claimed in any of the preceding claims, wherein each of the antibodies has a heavy chain with a first variable region and a light chain with a second variable region,

wherein the hybridoma cell line is DSM ACC2987 and wherein the first variable region comprises an amino acid sequence that is at least 90% identical, in particular at least 92.5%

identical, in particular at least 95% identical, in particular at least 97.5% identical, in particular 100% identical, with sequence SEQ ID NO: 2 and wherein the second variable region comprises an amino acid sequence that is at least 90% identical, in particular at least 92.5% identical, in particular at least 95% identical, in particular at least 97.5% identical, in particular 100% identical, with sequence SEQ ID NO: 4

or

wherein the hybridoma cell line is DSM ACC2988 and wherein the first variable region comprises an amino acid sequence that is at least 90% identical, in particular at least 92.5% identical, in particular at least 95% identical, in particular at least 97.5% identical, in particular 100% identical, with sequence SEQ ID NO: 6 and wherein the second variable region comprises an amino acid sequence that is at least 90% identical, in particular at least 92.5% identical, in particular at least 95% identical, in particular at least 97.5% identical, in particular 100% identical, with sequence SEQ ID NO: 8.

7. Antibodies or fragments as claimed in any of the preceding claims, wherein the hybridoma cell line is DSM ACC2987 and wherein the epitope comprises at least one amino acid sequence which is identical in at least 10, in particular in at least 11, in particular in at least 12, in particular in at least 13, in particular in at least 14, in particular in 15, amino acids with one of the sequences SEQ ID NO: 15, 17 to 19, 21 to 26, 32 to 34 and 57.

8. Antibodies or fragments as claimed in any of the preceding claims for use as a medicament.

9. Antibodies or fragments as claimed in claim 8, wherein the medicament is a medicament for the treatment of a human

being or an animal which human being or animal has an infection with Staphylococcus aureus, especially methicillin resistant or methicillin sensitive Staphylococcus aureus, or is at risk of getting such an infection.

10. Antibodies or fragments as claimed in claim 9, wherein the human being or the animal has a mastitis or a sepsis caused by the infection.

11. Antibodies or fragments as claimed in any of claims 8 to 10, wherein the antibodies or fragments are present in a mixture with other antibodies or fragments of these other antibodies which other antibodies are directed against at least one further epitope of Staphylococcus aureus.

12. Antibodies or fragments as claimed in any of claims 8 to 11, wherein the antibodies or fragments are present in a mixture with at least one antibiotic.

13. Antibodies or fragments as claimed in any of claims 8 to 12, wherein the antibodies or fragments are present in a mixture with plasma of blood of a mammal, especially a human being.

14. Antibodies or fragments as claimed in any of claims 8 to 13, wherein the medicament is a medicament for systemic and/or local application.

15. Kit containing antibodies or fragments as claimed in any of claims 1 to 7 for the detection of Staphylococcus aureus.

16. Use of antibodies or fragments as claimed in any of claims 1 to 7 for the detection of Staphylococcus aureus.

17. Hybridoma cell line which produces antibodies according to any of claims 1 to 7.

18. Hybridoma cell line as claimed in claim 17, wherein the hybridoma cell line is the cell line deposited at the DSMZ under accession number DSM ACC2987 or DSM ACC2988.

19. Method of treatment of a human being or an animal which human being or animal has an infection with Staphylococcus aureus, especially methicillin resistant or methicillin sensitive Staphylococcus aureus, or is at risk of getting such an infection, wherein antibodies or fragments as claimed in any of claims 1 to 7 are administered to the human being or the animal.

20. Method according to claim 19, wherein the human being or the animal has a mastitis or a sepsis caused by the infection.

21. Method as claimed in claim 19 or 20, wherein the antibodies or fragments are present in a mixture with other antibodies or fragments of these other antibodies which other antibodies are directed against at least one further epitope of Staphylococcus aureus.

22. Method as claimed in any of claims 19 to 21, wherein the antibodies or fragments are mixed with plasma of blood of a mammal, especially a human being, before they are administered.

23. Method as claimed in any of claims 19 to 22, wherein the antibodies or fragments are administered systemically, in particular intravenously, nasally or sublingually.

24. Method as claimed in any of claims 19 to 23, wherein the antibodies or fragments are administered together with at least one antibiotic.

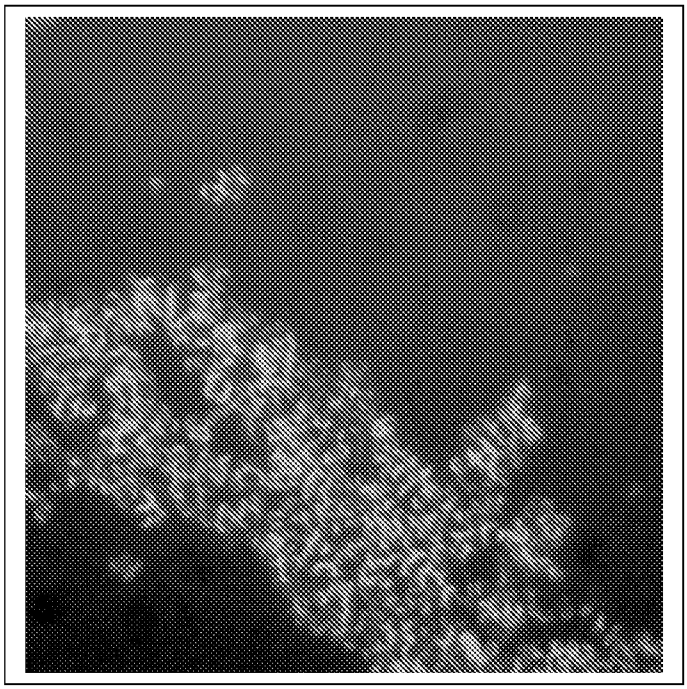

Fig. 1 a

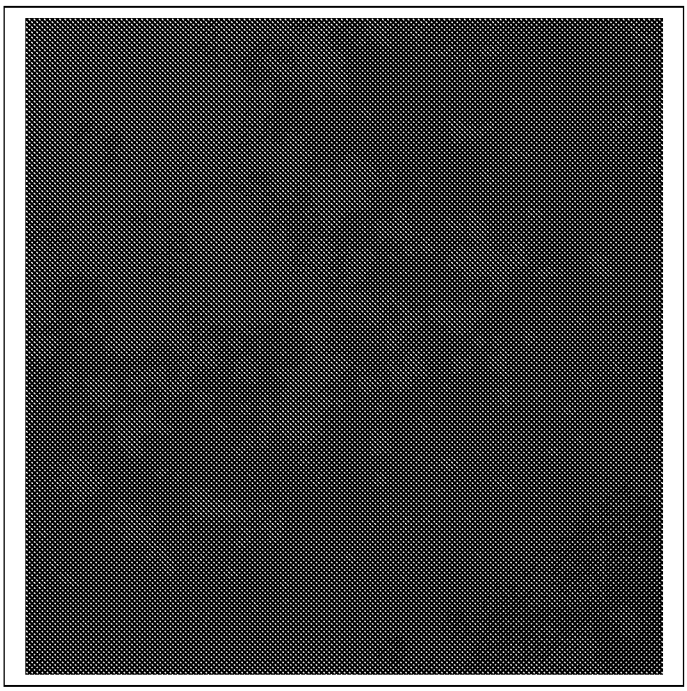

Fig. 1 b

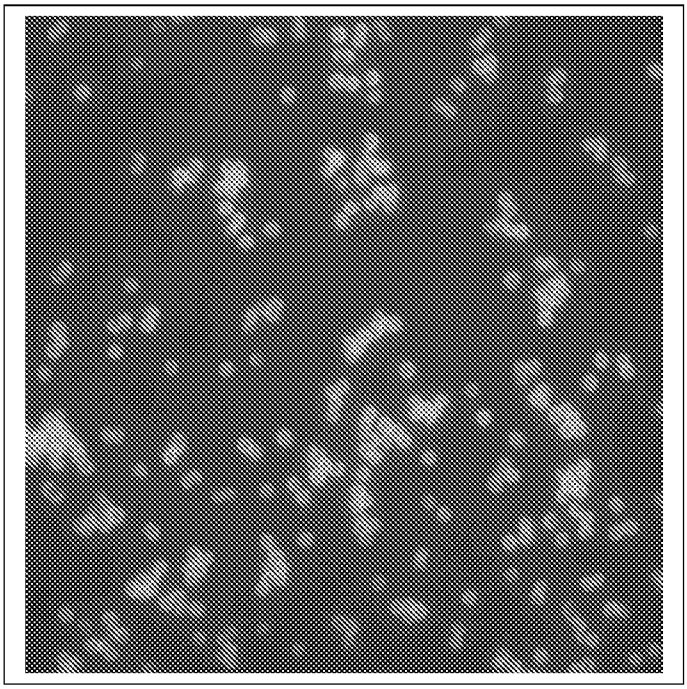

Fig. 1c

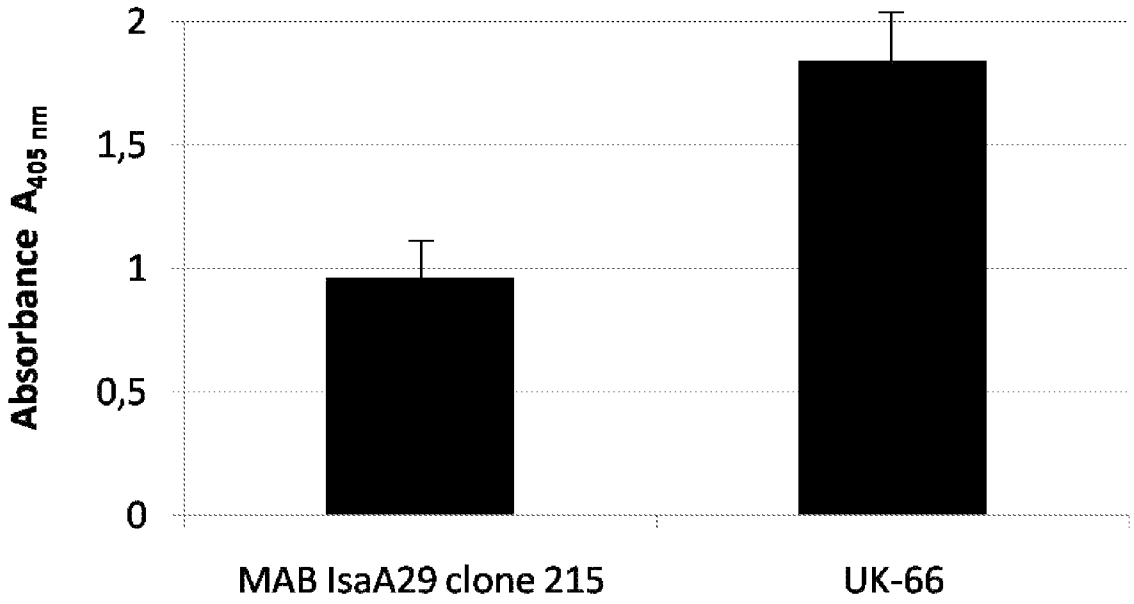

Fig. 2

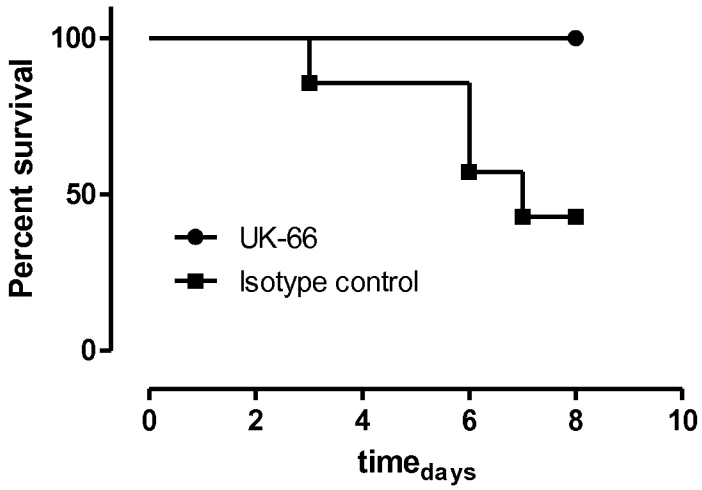
Percent survival
100
50
0
UK-66
Isotype control
0
2
4
6
8
10
time days

Fig. 3

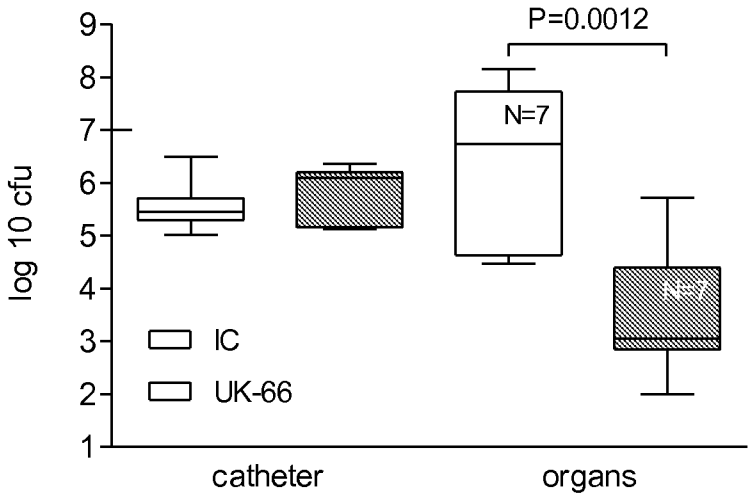
P=0.0012
N=7
N=7
log 10 cfu
9
8
7
6
5
4
3
2
1
IC
UK-66
catheter
organs

Fig. 4

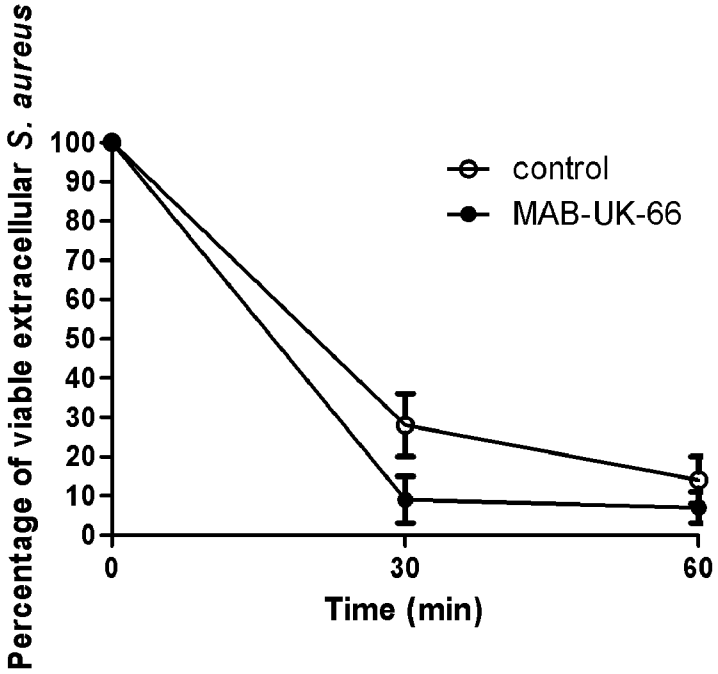
Percentage of viable extracellular S. aureus
100
90
80
70
60
50
40
30
20
10
0
0
30
60
Time (min)
control
MAB-UK-66

Fig. 5

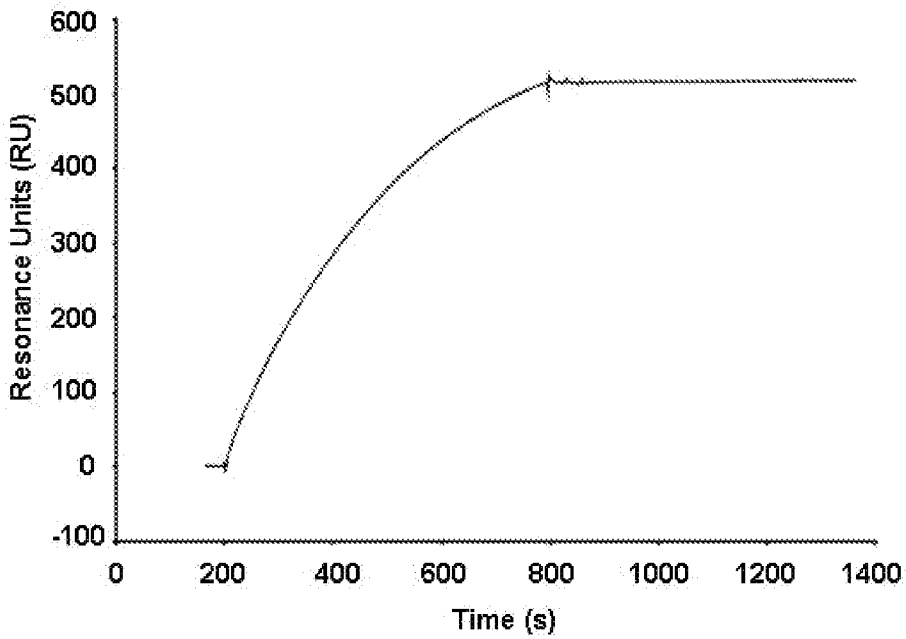
Resonance Units (RU)
600
500
400
300
200
100
0
-100
0
200
400
600
800
1000
1200
1400
Time (s)

Fig. 6

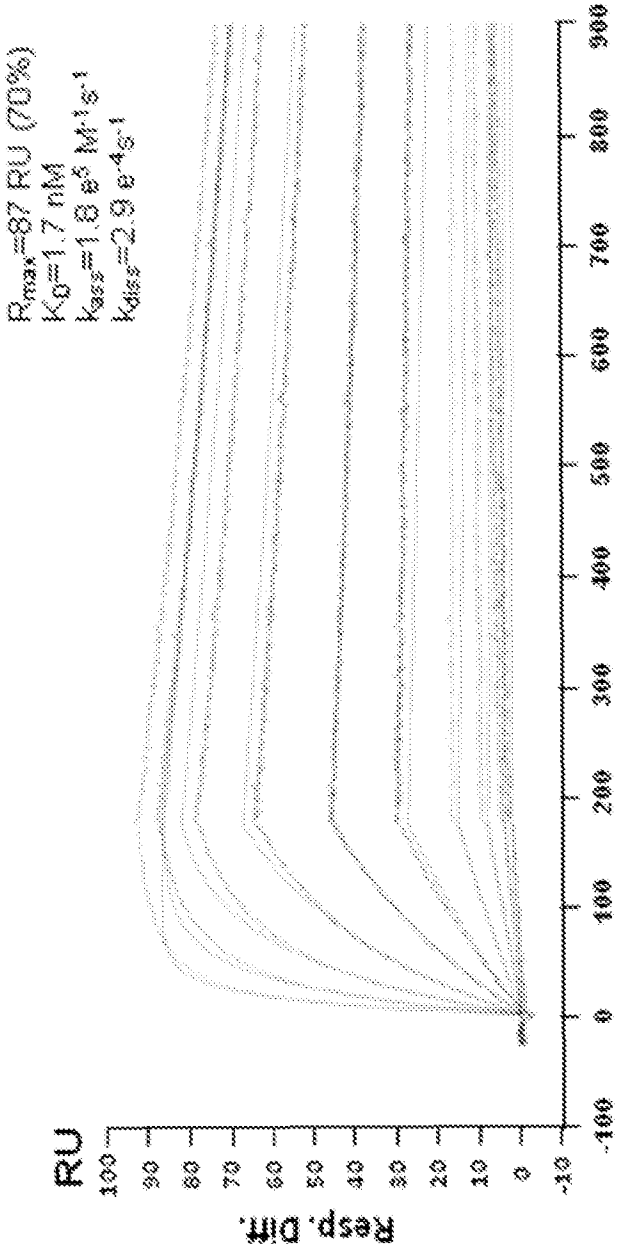
R_{max}=87 RU (70%)
K_D=1.7 nM
k_{ass}=1.8 e^5 M^{-1}s^{-1}
k_{diss}=2.9 e^{-4}s^{-1}
Resp. Diff.
RU
100
90
80
70
60
50
40
30
20
10
0
-10
-100
0
100
200
300
400
500
600
700
800
900
Time in s

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/056827

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/12
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LORENZ U ET AL: "Immunodominant proteins in human sepsis caused by methicillin resistant Staphylococcus aureus." ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY 2000 LNKD- PUBMED:11109116, vol. 485, 2000, pages 273-278, XP008124405 ISSN: 0065-2598 the whole document ----- -/--	1-5, 7-17, 19-24

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

[X] See patent family annex.

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 July 2010	11/08/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Le Flao, Katell

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LORENZ U ET AL: "Human antibody response during sepsis against targets expressed by methicillin resistant Staphylococcus aureus." FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY OCT 2000 LNKD- PUBMED:11024354, vol. 29, no. 2, October 2000 (2000-10), pages 145-153, XP002593023 ISSN: 0928-8244 * abstract	1,5,7
X	OHLSEN K ET AL: "Development of antibody-based therapy targeting immunodominant antigens of Staphylococcus aureus" IJMM INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, vol. 297, no. Suppl. 43, September 2007 (2007-09), page 128, XP008124403 & 59TH ANNUAL MEETING OF THE DEUTSCHEN-GESELLSCHAFT-FUR-HYGIENE-UND-MIKROBIOLOGIE; GOTTINGEN, GERMANY; SEPTEMBER 30 -OCTOBER 04, 2007 ISSN: 1438-4221 * abstract	1-5, 7-17, 19-24
A	WO 03/046007 A2 (NEUTEC PHARMA PLC [GB]; BURNIE JAMES P [GB]; MATTHEWS RUTH CHRISTINE [] 5 June 2003 (2003-06-05) examples 1-5,8	1-24
A	DE 42 38 806 A1 (DAINABOT CO [JP]) 27 May 1993 (1993-05-27) claims 1-14; examples 1-11	1-24
T	QUIEL ANNETT ET AL: "Electrical protein array chips for the detection of staphylococcal virulence factors." APPLIED MICROBIOLOGY AND BIOTECHNOLOGY FEB 2010 LNKD- PUBMED:19957082, vol. 85, no. 5, February 2010 (2010-02), pages 1619-1627, XP002593024 ISSN: 1432-0614 * abstract page 1620, right-hand column, last paragraph	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/056827

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