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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 March 2013 (28.03.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/041707 A1

(51) International Patent Classification:
C07K 16/12 (2006.01) *A61P 31/04* (2006.01)

(21) International Application Number:
PCT/EP2012/068703

(22) International Filing Date:
21 September 2012 (21.09.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11182598.0 23 September 2011 (23.09.2011) EP
61/545,763 11 October 2011 (11.10.2011) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: PEPTIDE OR ARRANGEMENT OF PEPTIDES FORMING A STAPHYLOCOCCUS AUREUS EPITOPE BINDING SITE

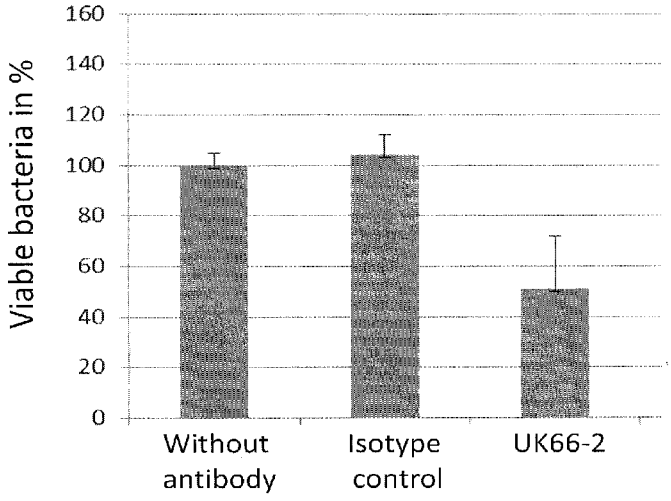

Fig. 3

(57) **Abstract**: The invention concerns a peptide or arrangement of peptides forming a Staphylococcus aureus epitope binding site comprising a first amino acid sequence and a second amino acid sequence, wherein the first amino acid sequence is at least 88% identical to sequence SEQ ID N0:1 and wherein the second amino acid sequence is at least 88% identical to sequence SEQ ID NO: 2.

WO 2013/041707 A1

PEPTIDE OR ARRANGEMENT OF PEPTIDES FORMING A STAPHYLOCOCCUS AUREUS EPITOPE BINDING SITE

The invention concerns a peptide or arrangement of peptides forming a Staphylococcus aureus (= S. aureus) epitope binding site, a kit containing this peptide or arrangement of peptides, a use of this peptide or arrangement of peptides, a cell line which produces antibodies comprising this peptide or arrangement of peptides and a method of treatment.

From WO 2010/133600 A1 antibodies or fragments thereof directed against an S. aureus epitope of IsaA are known. These antibodies have a binding site formed by a heavy chain with a first variable region and a light chain with a second variable region wherein the sequence of the first variable region may be SEQ ID NO:13 and the sequence of the second variable region may be SEQ ID NO:14. The effectiveness of antibodies vis-à-vis S. aureus in a mammal depends on killing of S. aureus by phagocytosis by phagocytizing blood cells. The antibodies known from WO 2010/133600 A1 accelerated the phagocytosis process. After 30 minutes of incubation the killing of S. aureus by human neutrophils in the presence of the antibodies specific for an epitope of IsaA has been enhanced by about 25% to 30% compared to an unspecific control antibody.

The object of the present invention is to provide a S. aureus epitope binding site that is very effective in an antibody or fragment of antibody with respect to the killing of S. aureus by phagocytizing blood cells and therefore is well suited for a treatment of infections caused by Staphylococcus aureus. Furthermore, the binding site should be well suited for a detection of S. aureus. A further object of the present inven-

tion is to provide a kit containing the binding site, a use of the binding site, a cell line secreting antibodies, antibody fragments, ScFvs or ScFvFcs comprising the binding site and a method of treatment.

This object is solved by the subject-matter of claims 1, 9, 16, 17, 18 and 19. Embodiments of the invention are disclosed in claims 2 to 8, 10 to 15 and 20 to 24.

According to the invention a peptide or arrangement of peptides forming a Staphylococcus aureus epitope binding site comprising a first amino acid sequence and a second amino acid sequence is provided. The first amino acid sequence is at least 88% identical to sequence SEQ ID NO:1 and the second amino acid sequence is at least 88% identical to SEQ ID NO:2.

In an embodiment the first amino acid sequence 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, to sequence SEQ ID NO:1. The second amino acid sequence 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, to sequence SEQ ID NO:2.

The first amino acid sequence may be part of the heavy chain and/or the second amino acid sequence may be part of the light chain of an antibody or antibody fragment. In this case the first amino acid sequence and the second amino acid sequence form the variable region of the antibody or antibody fragment. The binding site can also be formed by a single chain variable fragment. In this case the first amino acid

sequence and the second amino acid sequence are comprised by a single chain variable fragment (scFv) or by a single chain variable fragment comprising an Fc fragment of an antibody (scFvFc). The Fc fragment enhances phagocytosis of S. aureus to which the scFvFc has bound.

The inventors modified the binding region of one of the antibodies known from WO 2010/133600 A1 and thereby developed a binding site that is more effective in support of killing of S. aureus by phagocytosis by phagocytizing blood cells in heparinized human whole blood than the known antibody. As can be seen from the following alignment sequence SEQ ID NO:1 differs in 17 form 118 amino acids from the corresponding sequence SEQ ID NO:13 and SEQ ID NO:2 differs in 8 from 113 amino acids from SEQ ID NO:14 known from WO 2010/133600 A1:

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SEQ ID NO:1    EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYYMSWVRQAPGKGLEWVSDINGNGGSTYY  60
                V L ESGGGLV  GGSL LSC ASGFTFSNYYMSWVRQ P K LE V DINGNGGSTYY
SEQ ID NO:13 MADVKLVESGGGLVKLGGSLKLSCSASGFTFSNYYMSWVRQTPEKRLELVADINGNGGSTYY  62

SEQ ID NO:1  PDTVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVRRGGYYALDYWGQGTTVTVSS  118
             PDTVKGRFTISRDN KNTLYLQM SL  EDTA YYCVRRGGYYALDYWGQGTTVTVSS
SEQ ID NO:13 PDTVKGRFTISRDNAKNTLYLQMSSLKSEDTALYYCVRRGGYYALDYWGQGTTVTVSS  120


SEQ ID NO:2  DVVMTQTPLSLSVTPGQPASISCRSSQSLVHINGNTYLHWYLQKPGQSPQLLIYRVSNRF  60
             DVVMTQTPLSL V  G  ASISCRSSQSLVHINGNTYLHWYLQKPGQSP LLIYRVSNRF
SEQ ID NO:14 DVVMTQTPLSLPVSLGDQASISCRSSQSLVHINGNTYLHWYLQKPGQSPKLLIYRVSNRF  60

SEQ ID NO:2  SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHVPWTFGGGTKLELKR  113
             SGVPDRFSGSGSGTDFTLKISRVEAED GVY CSQSTHVPWTFGGGTKLELKR
SEQ ID NO:14 SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKLELKR  113
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Identical amino acids are displayed in the interspace between the sequences.

Antibodies containing the variable region comprising the first amino acid sequence and the second amino acid sequence exhibit high affinity to the immunodominant structure IsaA in methicillin resistant and methicillin sensitive S. aureus and

a high specificity with respect to the binding to this structure.

The antibody may be a monoclonal antibody, in particular an antibody of the IgG type, in particular of the IgG1 type, the IgG2 type, or the IgG4 type. The fragment may be an Fab fragment, Fab/c fragment, Fv fragment, Fab' fragment or $F(ab')_2$ fragment. 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.

In an embodiment of the invention the antibody is a recombinant antibody produced in cells of a cell line, in particular an insect cell line or a mammalian cell line, in particular a Chinese hamster ovary (CHO) cell line or a hybridoma cell line. The part of the antibody which is not formed by the first amino acid sequence and the second amino acid sequence is at least 85% identical, in particular 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, to the corresponding part of a human antibody. The light chain of the antibody can comprise sequence SEQ ID NO:6, in particular sequence SEQ ID NO:7 and the heavy chain can comprise the sequence SEQ ID NO:4, in particular SEQ ID NO:5, sequence SEQ ID NO:9, in particular sequence SEQ ID NO:10, or sequence SEQ ID NO:11, in particular SEQ ID NO:12. Sequences SEQ ID NO:7, SEQ ID NO:5, SEQ ID NO:10 and SEQ ID NO:12 comprise the leader sequence SEQ ID NO:8 which is from the MOPC 63, Ig kappa chain V-III of KV3A9_mouse. This leader sequence enables a good expression in mammalian cells. The sequence SEQ ID NO:4 comprises sequence SEQ ID NO:1 and an IgG1 heavy chain, human γ1 allotype

Gm 1,17. Sequence SEQ ID NO:6 comprises sequence SEQ ID NO:2 and the IgG light chain K. Sequence SEQ ID NO:9 comprises sequence SEQ ID NO:1 and the IgG2 heavy chain, allotype G2m(23). Sequence SEQ ID NO:11 comprises SEQ ID NO:1 and an IgG4 heavy chain.

The peptide or arrangement of peptides 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 treatment in the sense of this invention comprises prophylaxis. The animal may be a mammal. The human being or the animal may have a mastitis, an S. aureus bacteremia, in particular a primary or secondary bacteremia, a blood stream infection, in particular a primary or secondary blood stream infection, a prosthetic infection, a graft infection, a soft tissue infection, a surgery associated infection, an infant or newborn infection, a dialysis associated infection, a pneumonia, a bone infection, 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 peptide or arrangement of peptides according to the invention as a medicament for the treatment of the bovine mastitis.

The peptide or arrangement of peptides may be present in mixture with at least one other peptide or arrangement of pep-

tides directed against at least one further epitope of S. aureus. This further epitope may be located on the antigen on which the epitope is located, i. e. IsaA, 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 peptide or arrangement of peptides 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 peptide or arrangement of peptides can 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 that cannot be recognized by the peptide or arrangement of peptides according to the invention. In this case the antibiotic may be effective against the mutated S. aureus.

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

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 peptide or arrangement of peptides 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.

The invention also concerns a kit containing the peptide or arrangement of peptides according to the invention for the detection, especially a highly specific detection, of S. aureus.

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

Furthermore, the invention concerns a cell line, in particular an insect cell line or a mammalian cell line, in particular a Chinese hamster ovary (CHO) cell line or a hybridoma cell line, which produces an antibody, antibody fragment, ScFv or ScFvFc as specified above.

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 the peptide or arrangement of peptides according to the invention is administered to the human being or the animal. The peptide or arrangement of peptides 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 peptide or arrangement of peptides may be mixed with a suitable carrier.

The human being or the animal may have mastitis, an S. aureus bacteremia, in particular a primary or secondary bacteremia, a blood stream infection, in particular a primary or secondary blood stream infection, a prosthetic infection, a graft infection, a soft tissue infection, a surgery associated infection, an infant or newborn infection, a dialysis associated infection, a pneumonia, a bone infection, or a sepsis caused by the infection.

The peptide or arrangement of peptides may be present in a mixture with at least one other peptide or arrangement of peptides directed against at least one further epitope of S. aureus. The peptide or arrangement of peptides may be mixed with plasma or blood of a mammal, especially of a human being, before it is administered. The peptide or arrangement of peptides may be administered topically or systemically, in particular intravenously, intrapulmonary, intraperitoneally, nasally or sublingually. They may also be administered together with at least one antibiotic.

Embodiments of the invention

Fig. 1 shows the result of a competetive ELISA to determine binding of different anti-IsaA antibodies to the IsaA antigen.

Fig. 2 shows a bacterial cell ELISA to determine binding of different anti-IsaA antibodies to different S. aureus strains.

Fig. 3 shows the quantification of killing of S. aureus strain Newman by phagocytosis by phagocytizing blood cells in heparinized human whole blood.

Fig. 4 shows the quantification of killing of S. aureus strain Newman by phagocytosis by phagocytizing blood cells in heparinized human whole blood from healthy blood donators (n=15).

Fig. 5 shows the quantification of killing of S. aureus strain Newman by phagocytosis by phagocytizing blood cells in heparinized human whole blood from dialysis patients.

Fig. 6 shows the opsonophagocytic killing of bioluminescent S. aureus strain Newman (Newlux) in the presence of two concentrations of anti-IsaA antibody UK66-2 versus isotype control in HL-60 cells.

ScFv molecules containing sequences SEQ ID NOs:1 and 2, SEQ ID NOs:1 and 3 as well as other sequences have been expressed in E. coli and tested for binding and affinity in ELISA and competetive ELISA. The results showed that affinity of an ScFv molecule containing sequences SEQ ID NO:1 and SEQ ID NO:2 is about 10 times higher than affinity of an ScFv molecule containing sequences SEQ ID NO:1 and SEQ ID NO:3.

Vector constructs for the expression of complete antibodies has been transfected in CHO cells. IgG1 heavy chain, human γ1 allotype Gm1,17 according to sequence SEQ ID NO:4 (comprising sequence SEQ ID NO:1) with the Igk leader sequence SEQ ID NO:8 (resulting in sequence SEQ ID NO:5) and IgG light chain K according to SEQ ID NO:6 (comprising sequence SEQ ID NO:2) with the Igk leader sequence SEQ ID NO:8 (resulting in sequence SEQ ID NO:7) have been expressed to form antibody UK66-2. To investigate the influence of the isotype on functional activity IgG2 and IgG4 isotypes have been synthesized.

For this the IgG1 heavy chain has been replaced by IgG2 heavy chain, allotype G2m (23) according to sequence SEQ ID NO:9 with the Igk leader sequence SEQ ID NO:8 (resulting in sequence SEQ ID NO:10) or IgG4 heavy chain according to sequence SEQ ID NO:11 with the Igk leader sequence SEQ ID NO:8 (resulting in sequence SEQ ID NO:12).

After expression IgG1 antibodies have been purified from the supernatant of the CHO cells via a protein A column. The purified antibodies have been tested for the kinetics of binding, binding in ELISA, competitive ELISA, Western Blot and immunofluorescence und for function in phagocytosis assays with human phagocytizing blood cells. In funktional assays the antibody comprising sequences SEQ ID NOs:1 and 2 (UK66-2) enhanced oxidative burst und killing of S. aureus significantly more than known antibody UK66.

The kinetics of binding of IsaA to immobilized antibody UK66-2 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 UK66-2 was performed using an anti Fab antibody. Interaction analyses were performed using HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.005% Tween 20). Sensorgrams were recorded at a flow rate of 30 µl/min at 25°C.

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 4.8 nM was determined in two independent measurements. Rate constants for association and dissociation of the inter-

action between UK66-2 and IsaA were determined to be $3.7x10^{5}$ $M^{-1}s^{-1}$ (k_{on}) and $1.8x10^{-3}$ s^{-1} (k_{off}), respectively.

Figure 1 shows the result of a competetive ELISA to determine binding of different anti-IsaA antibodies to soluble recombinant IsaA antigen. The optical density at 450 nm indicates binding of the antibodies to IsaA. Soluble IsaA was added in different concentrations. The three lines represent the results received with the following anti IsaA antibodies:

- Upper line at 0.01 µM soluble IsaA: UK66 (reference antibody known from WO 2010/133600 A1)

- Middle line at 0.01 µM soluble IsaA: UK66-2 (antibody with a binding site comprising sequences SEQ ID NO:1 and SEQ ID NO:2)

- Lower line at 0.01 µM soluble IsaA: UK66-3 (antibody with a binding site comprising sequences SEQ ID NO:1 and SEQ ID NO:3)

Method description:
Nunc-Maxisorp 96-well plates were coated with 50 µl/well of IsaA (0.5 µg/well in 1xPBS) and incubated at 4°C overnight. The next day the plates were washed three times with PBS pH 7.4 containing 0.05% Tween 20 (PBST). After washing blocking was performed by addition of 200 µl 5% skimmed milk powder/PBS and incubated for 1 h at room temperature. The wells were washed twice with PBST (0.05%) and primary anti-IsaA antibody was added in serial concentrations ranging from 0.4 µM to 0.01 µM. The primary anti-IsaA-IgG1 antibodies were diluted in 2.5% skimmed milk powder/PBS and incubated for 1h at 37°C. The wells were then washed three times with PBST

(0.05%) and 50 µl of horseradish peroxidase linked secondary antibody 1:5000 diluted in 2.5% skimmed milk powder/PBS was added and incubated for 1h at 37°C. The wells were washed with PBST (0.05%) four times and 50 µl of TMB (Thermo Scientific Pierce ELISA substrate) was added and incubated for 15 min at 37°C. The reaction was stopped with 100 µl of 1N H_2SO_4 and optical density of the substrate reaction was analyzed with an ELISA plate reader at OD 450 nm.

Figure 2 shows a bacterial cell ELISA to determine binding of different anti-IsaA antibodies to the S. aureus strains USA300, SH1000, RN4220, E, MA12 and MA12isaA-. In MA12isaA- the immunodominant structure IsaA has been deleted. The optical density at 450 nm indicates binding of the antibodies to bacterial cells. The three columns represent the results received with the following anti IsaA antibodies:

- Left column: antibody UK66 (reference antibody)
- Middle column: antiboy UK66-2
- Right column: antibody UK66-3.

Method description:
The strains of S. aureus were cultured in B media at 37°C overnight. The bacteria were pelleted by centrifugation at 13000 rpm for 1 minute and washed with PBS (phosphate buffered saline). After the centrifugation step the pellet was resuspended in 1 ml PBS. A bacteria suspension containing $5x10^7$ bacteria/50 µl was prepared. Nunc-Maxisorp 96-well plates were coated with 50 µl/well of the bacteria suspension and incubated at 4°C overnight. The next day the plates were washed three times with PBS pH 7.4 containing 0.05% Tween 20 (PBST). After washing blocking was performed by addition of 200 µl 5% skimmed milk powder/PBS and incubated for 1 h at

room temperature. The wells were washed twice with PBST (0.05%) and primary anti-IsaA antibody was added. The primary anti-IsaA-IgG1 antibodies were diluted 1:2000 in 2.5% skimmed milk powder/PBS and 50 µl/well were added and incubated for 1h at 37°C. The wells were then washed three times with PBST (0.05%) and 50 µl of horseradish peroxidase linked secondary antibody 1:5000 diluted in 2.5% skimmed milk powder/PBS was added and incubated for 1h at 37°C. The wells were washed with PBST (0.05%) four times and 50 µl of TMB (Thermo Scientific Pierce ELISA substrate) was added and incubated for 15 min at 37°C. The reaction was stopped with 100 µl of 1N H_2SO_4 and optical density of the substrate reaction was analyzed with an ELISA plate reader at OD 450 nm.

Figure 3 shows the quantification of killing of S. aureus strain Newman by phagocytosis by phagocytizing blood cells in heparinized human whole blood. Bacteria were incubated 30 min with the heparinized human whole blood. The number of viable bacteria after incubation without antibody solution was set 100% (left column). Killing was significantly increased in the presence of UK66-2 (right column) compared to isotype control antibodies (middle column).

Method description:

S. aureus strain Newman was cultured in B medium at 37°C overnight. The bacteria were pelleted by centrifugation at 13000 rpm for 1 minute and washed with PBS. The centrifugation step was repeated and the bacteria were resuspended in 1 ml PBS. Bacteria solution of $5x10^7$ bacteria/20 µl was prepared. 100 µl of heparinized blood was added into 1.5 ml tubes and stored on ice. 20 µl of bacterial suspension and antibody solution were added, excluded the negative control sample which contained bacteria but no antibodies. The sam-

ples were incubated at 37°C for 30 min with constant movement overhead in a hybridisation oven. Phagocytosis was stopped by placing the samples on ice. Blood cells were lysed with 0.1% fresh prepared Saponin (20 min on ice). Two serial dilutions of the samples were prepared. 20 µl of 10^{-2}, 10^{-3} and 10^{-4} dilution, respectively were plated in duplicate on LB plates and incubated at 37°C for 24 h. The colonies were counted and killing was calculated setting the number of viable bacteria in blood without antibody solution as 100%.

Figure 4 shows the quantification of killing of S. aureus strain Newman by phagocytosis by phagocytizing blood cells in heparinized human whole blood from healthy blood donators (n=15). Figure 5 shows the quantification of killing of S. aureus strain Newman by phagocytosis by phagocytizing blood cells in heparinized human whole blood from dialysis patients (n=7). In both cases bacteria were incubated 60 min with the heparinized blood. The number of viable bacteria after incubation without antibody solution was set 100% (left scatter plot "Placebo"). Killing was significantly increased in the presence of UK66-2 (third and fourth scatter plot "UK66-2[75 µg/ml]" and "UK66-2[900 µg/ml]") compared to isotype control antibodies (second scatter plot "Isotype control [900 µg/ml]").

Method description:
S. aureus strain Newman was cultured in LB medium at 37°C overnight. The bacteria were pelleted by centrifugation at 13000 rpm for 1 minute and washed with PBS. The centrifugation step was repeated and the bacteria were resuspended in 1 ml PBS. Bacteria solution of $5x10^7$ bacteria/20 µl was prepared. 100 µl of heparinized blood was added into 1.5 ml tubes and stored on ice. 20 µl of bacterial suspension and

antibody solution were added, excluded the negative control sample which contained bacteria but no antibodies. The samples were incubated at 37°C for 60 min with constant movement overhead in a hybridisation oven. Phagocytosis was stopped by placing the samples on ice. Blood cells were lysed with 0.1% fresh prepared Saponin (20 min on ice). Two serial dilutions of the samples were prepared. 20 µl of 10^{-2}, 10^{-3} and 10^{-4} dilution, respectively were plated in duplicate on LB plates and incubated at 37°C for 24 h. The colonies were counted and killing was calculated. The number of viable bacteria in blood without antibody solution was set 100%.

Figure 6 shows killing of bioluminescent S. aureus (S. a.) strain Newman (Newlux) in the presence of two concentrations of anti-IsaA antibody UK66-2 (20 µg/ml and 200 µg/ml versus isotype control (200 µg/ml) in HL-60 cells. Determination of relative number of surviving bacteria was performed by measurement of bioluminescence. Surviving bacteria are given as light emission (RLU = relative light units). Bacterial killing is concentration dependent with UK66-2 and is not observed with an isotype-matched human IgG1 control antibody.

Method description:
A single colony of S. aureus strain Newman harbouring the luxABCED operon was used to inoculate 5 ml LB medium. Since the luxABCED operon causes a luminescence in living but not in dead bacteria the luminescence correlates with the number of living bacteria. The bacteria were cultivated overnight and 50 µl of this culture were used to inoculate 5 ml LB medium supplemented with 30 µg/ml kanamycin. The culture was cultivated on a rotary shaker at 200 rpm for 4-6 h at 37°C. Bioluminescence of the bacteria was determined using a Lumat LB 9501 luminometer (Berthold Technologies, Bad Wildbad, Ger-

many). The culture was ready for performing the assay when 100 µl of the culture generated bioluminescence signals ranged between 16000 – 24000 relative light units (RLU). Following cultivation, the bacteria were washed twice in phosphate buffered saline (PBS) and resuspended in Opti-MEM® medium (Life Technologies, Darmstadt, Germany) to a final concentration of $1x10^9$/ml. Phagocytic HL-60 cells were differentiated with 0.8% DMF for 5 days and resuspended to $1x10^8$ cells/ml in Opti-MEM®, and 50 µl per well were seeded in a 96-well tissue culture plate (Greiner Bio-One, Frickenhausen, Germany). Antibody solution (50 µl) was added followed by 100 µl of S. aureus ($1x10^9$/ml). HL-60 cells, antibody and bacteria were incubated at 37°C and bioluminescence was measured continuously at 15 min intervals for 240 min to determine the optimal signal-noise ratio. All assays were performed in triplicate and repeated at least three times. Bioluminescence was determined using the multi-mode reader Infinite 200 Pro (TECAN, Männedorf, Switzerland).

Patent Claims

1. A peptide or arrangement of peptides forming a Staphylococcus aureus epitope binding site comprising a first amino acid sequence and a second amino acid sequence, wherein the first amino acid sequence is at least 88% identical to sequence SEQ ID NO:1 and wherein the second amino acid sequence is at least 88% identical to sequence SEQ ID NO:2.

2. The peptide or arrangement of peptides as claimed in claim 1, wherein the first amino acid sequence is at least 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, to sequence SEQ ID NO:1 and/or wherein the second amino acid sequence 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, to sequence SEQ ID NO:2.

3. The peptide or arrangement of peptides as claimed in any of the preceding claims, wherein the first amino acid sequence is part of the heavy chain and/or the second amino acid sequence is part of the light chain of an antibody or antibody fragment or wherein the first amino acid sequence and the second amino acid sequence are comprised by a single chain variable fragment (scFv) or by a single chain variable fragment comprising an Fc fragment of an antibody (scFvFc).

4. The peptide or arrangement of peptides as claimed in claim 3, wherein the antibody is a monoclonal antibody, in

particular an antibody of the IgG type, in particular of the IgG1 type, the IgG2 type, or the IgG4 type.

5. The peptide or arrangement of peptides as claimed in claim 3 or 4, wherein the fragment is an Fab fragment, Fab/c fragment, Fv fragment, Fab' fragment or $F(ab')_2$ fragment.

6. The peptide or arrangement of peptides as claimed in any of claims 3 to 5, wherein the antibody is a recombinant antibody produced in cells of a cell line, in particular an insect cell line or a mammalian cell line, in particular a Chinese hamster ovary (CHO) cell line or a hybridoma cell line.

7. The peptide or arrangement of peptides as claimed in any of claims 3 to 6, wherein the part of the antibody which is not formed by the first amino acid sequence and the second amino acid sequence is at least 85% identical, in particular 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, to the corresponding part of a human antibody.

8. The peptide or arrangement of peptides as claimed in any of claims 3 to 7, wherein the light chain comprises sequence SEQ ID NO:6, in particular sequence SEQ ID NO:7, and the heavy chain comprises sequence SEQ ID NO:4, in particular sequence SEQ ID NO:5, sequence SEQ ID NO:9, in particular sequence SEQ ID NO:10, or sequence SEQ ID NO:11, in particular sequence SEQ ID NO:12.

9. Peptide or arrangement of peptides as claimed in any of the preceding claims for use as a medicament.

10. Peptide or arrangement of peptides as claimed in claim 9, 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.

11. Peptide or arrangement of peptides as claimed in claim 10, wherein the human being or the animal has a mastitis, an S. aureus bacteremia, a blood stream infection, a prosthetic infection, a graft infection, a soft tissue infection, a surgery associated infection, an infant or newborn infection, a dialysis associated infection, a pneumonia, a bone infection, or a sepsis caused by the infection.

12. Peptide or arrangement of peptides as claimed in any of claims 9 to 11, wherein the peptide or arrangement of peptides is present in a mixture with at least one other peptide or arrangement of peptides directed against at least one further epitope of Staphylococcus aureus.

13. Peptide or arrangement of peptides as claimed in any of claims 9 to 12, wherein the peptide or arrangement of peptides is present in a mixture with at least one antibiotic.

14. Peptide or arrangement of peptides as claimed in any of claims 9 to 13, wherein the peptide or arrangement of peptides is present in a mixture with plasma of blood of a mammal, especially a human being.

15. Peptide or arrangement of peptides as claimed in any of claims 9 to 14, wherein the medicament is a medicament for systemic and/or local application.

16. Kit containing the peptide or arrangement of peptides as claimed in any of claims 1 to 8 for the detection of Staphylococcus aureus.

17. Use of the peptide or arrangement of peptides as claimed in any of claims 1 to 8 for a detection of Staphylococcus aureus.

18. Cell line which produces an antibody, antibody fragment, ScFv or ScFvFc as specified in any of claims 3 to 8.

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 the peptide or arrangement of peptides as claimed in any of claims 1 to 8 is administered to the human being or the animal.

20. Method according to claim 19, wherein the human being or the animal has a mastitis, an S. aureus bacteremia, a blood stream infection, a prosthetic infection, a graft infection, a soft tissue infection, a surgery associated infection, an infant or newborn infection, a dialysis associated infection, a pneumonia, a bone infection, or a sepsis caused by the infection.

21. Method as claimed in claim 19 or 20, wherein the peptide or arrangement of peptides is present in a mixture with at least one other peptide or arrangement of peptides directed against at least one further epitope of Staphylococcus aureus.

22. Method as claimed in any of claims 19 to 21, wherein the peptide or arrangement of peptides is mixed with plasma of blood of a mammal, especially a human being, before it is administered.

23. Method as claimed in any of claims 19 to 22, wherein the peptide or arrangement of peptides is administered topically or systemically, in particular intravenously, intrapulmonary, intraperitoneally, nasally or sublingually.

24. Method as claimed in any of claims 19 to 23, wherein the peptide or arrangement of peptides is administered together with at least one antibiotic.

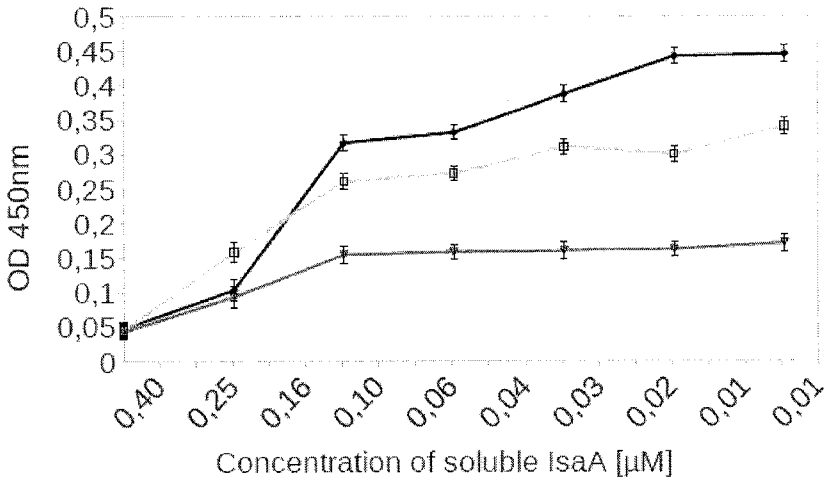
0,5
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
OD 450nm
0,40
0,25
0,16
0,10
0,06
0,04
0,03
0,02
0,01
0,01
Concentration of soluble IsaA [µM]

Fig. 1

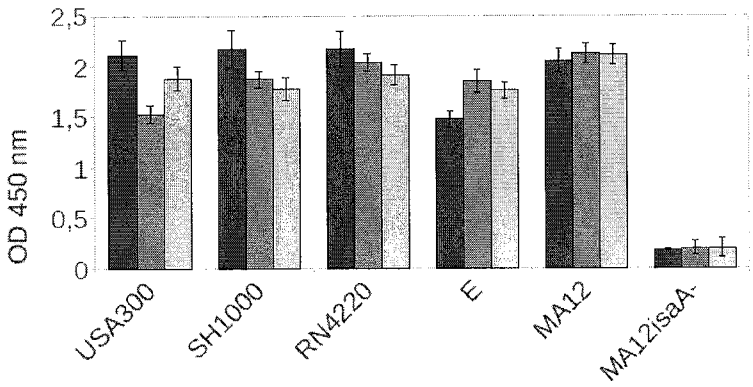
2,5
2
1,5
1
0,5
0
OD 450 nm
USA300
SH1000
RN4220
E
MA12
MA12isaA-

Fig. 2

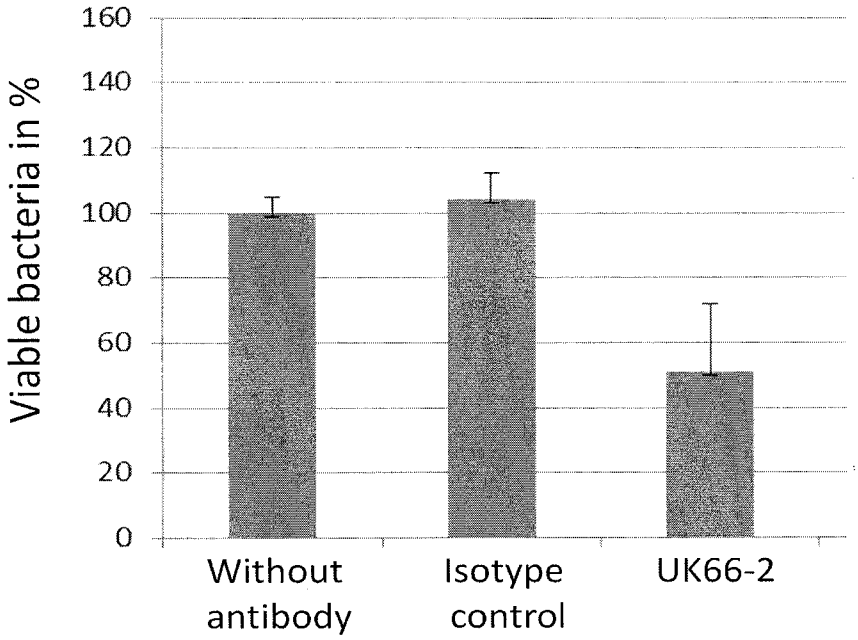
160
140
120
100
80
60
40
20
0
Viable bacteria in %
Without antibody
Isotype control
UK66-2

Fig. 3

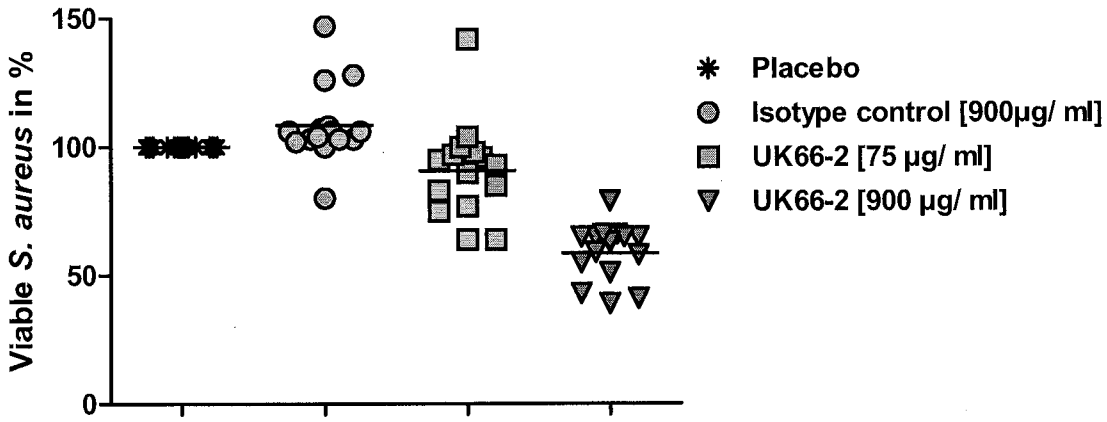
150
100
50
0
Viable S. aureus in %
Placebo
Isotype control [900µg/ ml]
UK66-2 [75 µg/ ml]
UK66-2 [900 µg/ ml]

Fig. 4

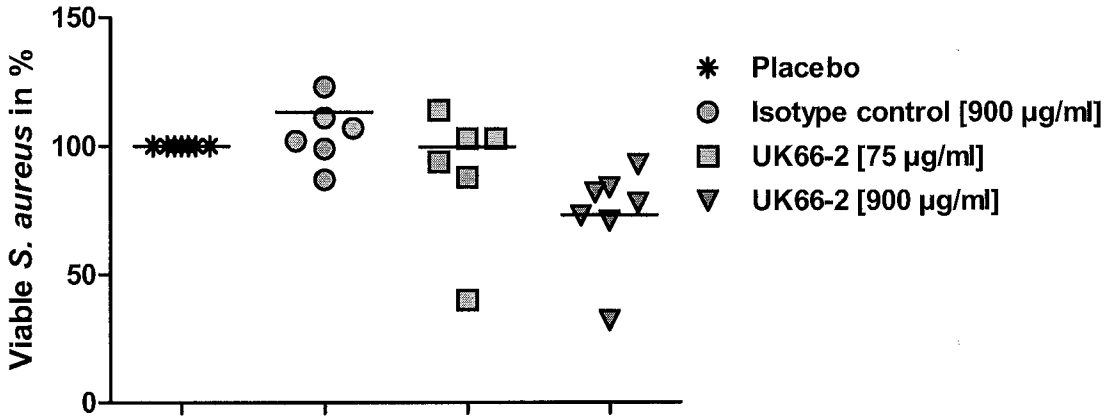
150
100
50
0
Viable S. aureus in %
Placebo
Isotype control [900 µg/ml]
UK66-2 [75 µg/ml]
UK66-2 [900 µg/ml]

Fig. 5

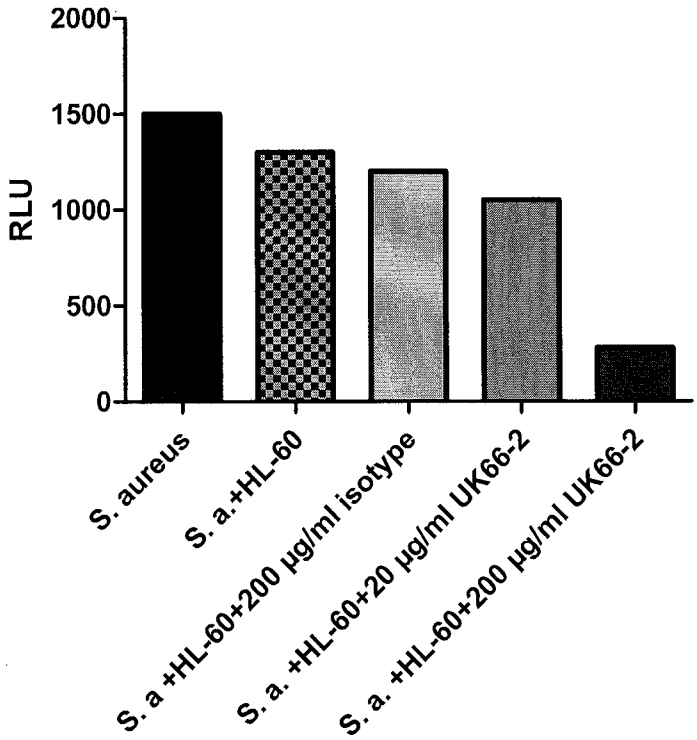
2000
1500
1000
500
0
RLU
S. aureus
S. a.+HL-60
S. a +HL-60+200 µg/ml isotype
S. a. +HL-60+20 µg/ml UK66-2
S. a. +HL-60+200 µg/ml UK66-2

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/068703

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/12 A61P31/04
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 practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/133600 A1 (UNIV WUERZBURG J MAXIMILIANS [DE]; OHLSEN KNUT [DE]; LORENZ UDO [DE]) 25 November 2010 (2010-11-25) pages 14-17	1-24
Y	LORENZ UDO ET AL: "Functional Antibodies Targeting IsaA of Staphylococcus aureus Augment Host Immune Response and Open New Perspectives for Antibacterial Therapy", ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 55, no. 1, January 2011 (2011-01), pages 165-173, XP002687630, ISSN: 0066-4804 pages 167-170	1-24

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

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier 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	Date of mailing of the international search report
21 November 2012	06/12/2012

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	Domingues, Helena

1

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.
Y	OHLSEN K ET AL: "Development of antibody-based therapy targeting immunodominant antigens of Staphylococcus aureus", INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY; 59TH ANNUAL MEETING OF THE DEUTSCHEN-GESELLSCHAFT-FUR-HYGIENE-UND-MIKROBIOLOGIE; GOTTINGEN, GERMANY; SEPTEMBER 30 -OCTOBER 04, 2007, URBAN UND FISCHER, DE, vol. 297, no. Suppl.1, 1 September 2007 (2007-09-01), page 128, XP008124403, ISSN: 1438-4221 the whole document -----	1-24
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Y	PATTI JOSEPH M: "A humanized monoclonal antibody targeting Staphylococcus aureus.", VACCINE 6 DEC 2004, vol. 22 Suppl 1, 6 December 2004 (2004-12-06), pages S39-S43, XP002687631, ISSN: 0264-410X the whole document -----	1-24
A	OHLSEN KNUT ET AL: "Immunotherapeutic strategies to combat staphylococcal infections.", INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY : IJMM AUG 2010, vol. 300, no. 6, August 2010 (2010-08), pages 402-410, XP002687632, ISSN: 1618-0607 the whole document -----	1-24

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/068703

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