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CHAPTER

Antibodies increase adherence of *Staphylococcus epidermidis* to biomaterial in experimental biomaterial-associated infection in mice
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Antibodies increase adherence of S. epidermidis to biomaterial

ABSTRACT

Biomaterial-associated infection (BAI) is a major problem in modern medicine, and is often caused by Staphylococcus epidermidis. We aimed to raise monoclonal antibodies (mAbs) against major surface protein antigens of S. epidermidis, and to assess their possible protective activity in experimental BAI. Mice were vaccinated with a cell wall protein preparation of S. epidermidis. A highly immunodominant antigen was identified as Accumulation-associated protein (Aap). Mabs against Aap and against surface-exposed lipoteichoic acid (LTA) were used for passive immunization of mice in experimental biomaterial-associated infection. Neither anti-Aap nor anti-LTA mAbs showed protection. Either with or without antibodies, tissue surrounding the implants was more often culture positive than the implants themselves, but bacterial adherence to the implants was significantly increased in mice injected with anti-LTA. In vitro, anti-Aap and anti-LTA did show binding to S. epidermidis, but no opsonic activity was observed. We conclude that antibodies against S. epidermidis LTA or Aap showed no opsonic activity and did not protect mice against BAI. Moreover, the increase in binding to implanted biomaterial suggests that passive immunization may increase the risk for BAI.

INTRODUCTION

Staphylococcus epidermidis, commensal bacteria of the skin, are the predominant cause of BAI. These infections increase morbidity and costs of care, and may even require removal of the implanted device. The pathogenesis of these infections includes the formation of biofilms consisting of bacteria, bacterial products and host proteins and cells on the biomaterial surface. Biofilm formation starts with direct adherence of S. epidermidis to the biomaterial through autolysin AtlE and to deposited host proteins such as fibronectin and fibrinogen, through fibronectin-binding and fibrinogen-binding protein, respectively. Subsequently a biofilm accumulates, involving polysaccharide intercellular adhesin (PIA) and the fibrillar accumulation-associated protein (Aap).

Previously we have shown that in addition to biofilm formation S. epidermidis persists in peri-implant tissue in mice, even inside macrophages. This is true
for different biomaterials, mouse strains and \textit{S. epidermidis} strains \textsuperscript{14,16}. The tissue-residing bacteria are more resistant to antibiotics than adherent bacteria \textsuperscript{17}. Moreover, tissue surrounding biomaterials also is a niche for bacteria in humans, as we recently assessed by culture and immunohistology in a study on catheters and surrounding tissue from deceased patients \textsuperscript{18}.

To prevent BAI, passive immunization may be applied. Antibodies developed until now mostly are directed against polysaccharide antigens \textsuperscript{19-21}. The aim of the present investigation was to raise antibodies against \textit{S. epidermidis} major surface protein antigens and test their possible protective activity in a mouse BAI model.

**MATERIALS AND METHODS**

\textit{S. epidermidis} strain. The study was performed with \textit{S. epidermidis} strain AMC5, a clinical isolate causing persistent biomaterial-associated infection in our mouse BAI model \textsuperscript{16}. AMC5 produces slime, as determined by the method of Christensen et al. \textsuperscript{22}, δ-toxin as determined according to Hebert and Hancock \textsuperscript{23} and is positive for the biofilm-associated \textit{icaA}, \textit{atlE}, \textit{sarA} and \textit{Aap} genes by PCR. Primers for \textit{icaA}, \textit{atlE} and \textit{Aap} were according to Vandecasteele et al. \textsuperscript{24}. \textit{SarA} was detected using primers sarA\textsubscript{d} (AGCAAATGCTACATTGCTAATTC) and sarA\textsubscript{r} (ATTTGCTTCTGTGATACGGTTG). MIC values (µg/ml) according to standard E-test (AB Biodisk N.A.Inc, Solna, Sweden) were: rifampicin < 0.016, teicoplanin < 1.5, gentamicin 0.125, and vancomycin 4. Antibiotic tablets were used for antibiograms of challenge bacteria and cfu recovered from the mice \textsuperscript{16,17}, the challenge strain was susceptible to all antibiotics tested.

\textit{S. epidermidis} cell wall protein preparations for immunization. Bacteria were cultured overnight on tryptic soy agar (TSA; Oxoid, Badhoevedorp, The Netherlands) plates supplemented with 1 mM EDDA to provide iron-limiting conditions expected to induce expression of proteins also expressed in patients. Bacteria were harvested in 30\% (w/v) raffinose in 10 mM Tris buffer pH 8.0. PMSF (final conc. 5 mM), benzamidine (final conc. 10 mM) and lysostaphin (Sigma; final conc. 1 µg/ml) were added and the suspension was incubated at 37°C for 1 h. The supernatant containing the cell surface proteins was harvested by centrifugation at 8000 rpm for 15 min at 4°C, passed through a 0.2 µm filter and concentrated by ultrafiltration using a 5
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kDa cutoff membrane (Vivaflow 200, Sartorius, Epsom Surrey, United Kingdom). To obtain antigen preparations with differing composition, the concentrated surface protein preparation was applied to a monoQ anion exchange column in 20 mM Tris pH 8.6 (buffer A) and protein was eluted with a gradient of 10 mM Tris 1M NaCl pH 8.6 (buffer B). Pools of fractions A-E (Figure 1) of 16 separate chromatographic runs were prepared, dialyzed against PBS and stored at -20°C.

Polyclonal and monoclonal antibodies. Each of the cell surface protein fractions A-E (Figure 1) obtained after ion exchange chromatography was used to immunize BALB/c mice. Polyclonal sera were tested for reactivity with S. epidermidis total cell wall protein preparations in Western Blots. Based on the antibody responses 5 mice were chosen for fusion of their B-cells to produce mAbs. Monoclonal antibodies against S. epidermidis lipoteichoic acid (LTA) (IgG1) were from QED Biosciences Inc. (Autogen Bioclear, Calne, Wiltshire, UK), and isotype control mouse IgG1 and IgG2b from BD Biosciences (Breda, The Netherlands), and IgG2a from DAKO (Heverlee, Belgium).

SDS-PAGE and Western blot. SDS-PAGE gels (15%, BioRad, Hertfordshire, United Kingdom) were run for 4 h and blotted onto nitrocellulose overnight at 10 V and 50 mA. Blots were blocked with 1% bovine serum albumine (BSA), and incubated with mouse sera (diluted 1:10,000 in PBS) or mAbs (final concentration of 4 µg/ml). Bound antibodies were detected using alkaline phosphatase-conjugated goat-anti-mouse IgG (Sigma, Steinheim, Germany), and NBT/BCIP (nitroblue tetrazoliumchloride/5-Bromo-4-chloro-3-indolyl phosphate) (Promega, Leiden, The Netherlands) as the substrate.

Identification of immunodominant protein. The 175 kDa protein recognized by antibodies in Western blots was excised from Coomassie-stained SDS-PAGE gels run in parallel. The protein in the gel band was denatured, carboxymethylated and subjected to in-gel tryptic digestion. The resulting peptides were extracted from the gel and purified using a C18 cartridge. The peptide-containing fractions were analysed by Delayed Extraction-Matrix Assisted Laser Desorption Ionisation-Time of Flight (DE-MALDI-TOF) mass spectrometry. Some of the peptides were also subjected to sequencing using Nanospray-MS/MS.
Whole cell ELISA. *S. epidermidis* cultured on TSA plates were harvested in Dulbecco's PBS (Gibco Invitrogen, Breda, The Netherlands), centrifuged, and resuspended in Dulbecco's PBS to an optical density at 620 nm wavelength (OD$_{620}$) of 3.0. Microtiter plates (Nunc-Immuno Maxisorb; Nunc, Roskilde, Denmark) were incubated with 50 µl of this suspension per well for 4 h at 4°C, to allow adherence of the bacteria. Then, the plates were washed three times with PBS containing 0.05% Tween 20 (PBS-Tween) and the bacteria were killed and fixed overnight under UV light in a laminar flow cabinet. The plates were blocked with PBS containing 3% (w/v) BSA for 1 h at 37°C, and washed 3 times with PBS-Tween. Doubling dilutions of primary antibody (100 µl) were added, plates were incubated for 1 h at 37°C, washed 3 times with PBS-Tween, and incubated with 100 µl of alkaline phosphatase-conjugated goat-anti-mouse IgG for 1 h at 37°C. Then, plates were washed 4 times with PBS-Tween, developed with 100 µl Fast P-nitro Phosphatase substrate (Sigma) for 30 min at 37°C, and the OD at 405 nm was measured.

Mouse biomaterial-associated infection model. Mouse BAI studies were approved by the Animal Ethical Committee of the University of Amsterdam, and were performed as described earlier 15,16,25. In brief, specified pathogen-free C57Bl/6 female mice (Harlan, Horst, The Netherlands) were anesthetized with FFM-mix (1 ml fentanyl-citrate, 1 ml midazalam, and 2 ml distilled water) and a one cm long polyvinylpyrrolidone-grafted Silicon Elastomer (SEpvp, 2.5 mm diameter, wall thickness 0.6 mm; Medtronic PS Medical, Goleta, CA, USA) catheter segment was inserted subcutaneously at each side of the back. The incisions were closed with a stitch 14-16,26. A single dose (25 or 50 µl) of antibody or saline was injected alongside each catheter segment.

Fifty ml of a logarithmic culture of *S. epidermidis* AMC5 in TSB was centrifuged at 2200xg for 10 min. The pelleted bacteria were washed twice with 30ml pyrogen-free NaCl 0.9% (saline) (Fresenius Kabi, Sèvres, France) and resuspended. The optical density at 620 nm was measured and inoculum suspensions containing 1.2x10⁹ or 4x10⁸ cfu/ml saline were prepared based on this OD$_{620}$.

Thirty minutes after injection of the antibody, the bacterial inoculum of 3x10⁷ (anti-LTA experiment) or 1x10⁷ (anti-Aap experiment) cfu in 25 µl saline was injected along the implants. The anti-LTA experiment was set up as 3 groups of 6 mice; one group received a saline injection (control group). The second and 3rd group received
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an anti-LTA injection (40 µg / 25 µl), and in the 3rd group this anti-LTA injection was repeated after 3 days. For the anti-Aap experiment 9 mice received an anti-Aap injection (325 µg / 50 µl), and 9 mice a saline injection (50 µl, control group).

All mice were sacrificed 8 days after challenge. Mice were anesthetized with FFM-mix, and a standardized biopsy of 12 mm in diameter was taken from each implantation site comprising the implant with surrounding tissue \(^{15-17}\). The right side biopsy was used for quantitative culture; the left side biopsy was cut in two halves, 1 for quantitative culture, and 1 for histology. Cardiac puncture was performed to collect blood and terminate the mice.

The implants were rinsed and subsequently sonicated for 30 s in 500 µl saline. The sonicate was cultured quantitatively by plating 6 droplets of 10 µl of the undiluted sonicate (detection limit of 8 cfu) and of ten-fold serial dilutions. The implant itself was cultured in 80 ml of liquid Brewer Tween broth (BT), containing 3% (w/v) thioglycolate, 0.03% (w/v) polyanetholesulfonic acid, and 0.5% Tween 80, adjusted to pH 7.6 with 1M NaOH, for upto 14 days at 37°C. When plate cultures were negative, while the corresponding liquid culture was positive, we defined the implants to have been colonized with 5 cfu. The tissue samples were homogenized and cultured quantitatively. In addition, 1/10 volume of the homogenate was cultured in 80 ml BT for upto 14 days at 37°C. If the BT culture was positive and the plate cultures were negative, the total homogenate was considered to have contained 10 cfu, since one tenth of the homogenate had been cultured. Positive BT cultures of any of the implants or tissue samples were streaked on blood agar plates which were incubated overnight at 37°C.

Opsonophagocytosis. HL60 cells (a human granulocyte cell-line; ATCC CCL-240) were grown in complete RPMI medium without phenol red with 20% FBS (both from Gibco Invitrogen). Cells were centrifuged at 160xg for 7 min, resuspended in RPMI with N,N-dimethylformamide (100 mM) and incubated for 6 days to allow differentiation into polymorphonuclear leucocytes (PMN’s). S. epidermidis grown in TSB to midlog fase, were harvested by centrifugation at 4000xg for 5 min, washed in 0.1M NaHCO\(_3\) and resuspended in 5 ml of this solution. Twentyfive µl of 10 mg 5-(6-) carboxyfluorescein succinimidyl ester (FAM-SE; Molecular Probes, Leiden, The Netherlands) per ml dimethylsulfoxide was added, and the mixture was incubated
for 1 h at 37°C in a 5% CO₂ atmosphere. The labeled bacteria were washed 5 times with opsonization buffer (10 ml Hanks balanced salt solution with Ca²⁺ and Mg²⁺ containing 0.2% BSA). The bacteria were diluted to 4x10⁷ cfu/ml opsonization buffer and kept on ice.

Opsonophagocytosis assay. Aliquots of 20 µl of labeled bacterial suspension were incubated shaking with 10 µl sera or antibodies at 37°C for 30 min. Ten µl of rabbit complement (Harlan, Loughborough, UK) was added and incubation was continued for 15 min at 37°C. This was followed by addition of 40 µl of differentiated HL60 cells (10⁵ cells) and the mixture was incubated shaking for 25 min at 37°C. The reaction was stopped by placing the mixture on ice. After addition of a volume of 160 µl opsonization buffer, the opsonophagocytosis was assessed by FACS analysis and quantified as the percentage of FL1- positive HL60 cells.

Assessment of antibody binding. To verify antibody binding to the bacteria, *S. epidermidis* inocula were prepared as described for opsonophagocytosis. Anti-LTA was labeled with Zenon-alexa-488 using the Zenon Mouse IgG labeling kit, and anti-Aap with the alexa-488 fluor mAb labeling kit (Molecular Probes, Leiden, The Netherlands), according to the manufacturer's protocols. *S. epidermidis* samples were incubated with 0.6 µg of labeled antibody for 30 min at room temperature shielded from light, and analysed by FACS (FACS-calibur, BD, Breda, The Netherlands).

Statistics. Fisher's exact test was used to assess significances in differences in the frequencies of positive cultures of the biopsies and biomaterial implants from different groups of mice. Differences in numbers of cfu were analyzed with the Mann-Whitney test. For both tests, *P* < 0.05 was considered significant.

RESULTS

Production of antibodies against *S. epidermidis* AMC5 outer surface proteins. Cell wall protein fractions A-E obtained from strain AMC5 (Figure 1A) were used to immunize Balb/c mice and the resulting sera were tested against fractions A-E in Western blots. All sera recognized a protein with an apparent molecular weight (MW) of 175 kDa present in different amounts in each of the fractions A-E (Figure 1B).
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Figure 1: Antibodies against S. epidermidis AMC5 outer surface proteins. A; fractionation of concentrated outer surface protein preparation by MonoQ anion exchange chromatography. Fractions A-E of 16 separate chromatographic runs were pooled and used to immunize Balb/c mice. B; western blots of sera from mice immunized with fractions A-E (α-fraction A-E) tested against the cell wall protein fractions A-E. C; reaction of 15 monoclonal antibodies obtained from hybridoma’s of B-cells from immunized mice. Twenty-one of 27 hybridoma’s of mice immunized with either of the S. epidermidis protein fractions A-E produced antibodies recognizing a protein with an apparent molecular weight of 175 kDa.
Figure 1C shows the reaction of 15 mAbs obtained from hybridoma’s of B-cells from immunized mice. Twenty-one out of 27 hybridoma’s of mice immunized with the *S. epidermidis* protein fractions A-E, produced antibodies recognizing a protein with an apparent MW of 175 kDa which was also recognized by the polyclonal sera. Peptide mass fingerprinting identified this protein as the Accumulation-associated protein Aap. Aap was a highly immunodominant protein, since even cell wall protein preparations depleted of Aap predominantly induced anti-Aap antibodies in 13/15 mice (data not shown). We therefore continued our experiments using the anti-Aap antibodies MS1-34, MS1-47 and MS2-89 (Figure 1), designated as anti-Aap1, 2 and 3, respectively.

**Whole cell ELISA using mAbs against *S. epidermidis AMC5***. A whole cell ELISA was performed to test the antigen-binding activity of anti-Aap1 (IgG2a), 2 (IgG2a) and 3 (IgG2b), anti-LTA (IgG1), and of isotype control antibodies. Anti-Aap1, 2 and 3 showed similar dose-response curves, and showed significant binding at approximately 4-fold lower concentrations than anti-LTA (Figure 2).

![Figure 2: Antigen-binding activity of anti-Aap1, 2, 3 and anti-LTA in a bacterial whole-cell ELISA.](image)

The signal of anti-LTA continued to increase with increasing antibody concentration, whereas all 3 anti-Aap antibodies reached a maximum level of binding at approximately 0.4 mg/ml antibody, possibly indicating saturation. Control IgG1, IgG2a and IgG2b showed no binding, indicating that the binding of the anti-Aap and anti-LTA antibodies to *S. epidermidis* immobilized to the microtitre plates was specific.
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Effect of anti-LTA and anti-Aap-1 on colonization of biomaterial and surrounding tissue by *S. epidermidis* AMC5. The effect of anti-LTA and anti-Aap on tissue colonization as well as on *in vivo* biomaterial adherence in our mouse BAI model was investigated in 2 separate experiments. In the anti-LTA experiment mice received either a single injection of 40 µg of the antibody prior to injection of the bacterial inoculum, or one injection prior to challenge and a repeat injection after 3 days. Controls received a saline injection prior to bacterial challenge. After 8 days, all 12 tissue samples of each group were culture positive. In the control group 5/12 of the implants were culture-positive, versus 10/12 and 11/12 (p = 0.03 versus control group) of the implants of the anti-LTA single and repeat injection groups, respectively (Figure 3).

Figure 3: Effect of anti-LTA (A) and anti-Aap-1 (B) on biomaterial and tissue colonization by *S. epidermidis* AMC5. Frequencies of positive cultures and numbers of cfu recovered from tissue biopsies (T) and biomaterial implants (BM) of C57Bl/6 mice, 8 days after challenge with 3x10^7 and 1x10^7 cfu of *S. epidermidis* AMC5 are indicated. Frequencies of positive cultures are given above the lanes. * indicates p < 0.05.
Since no protection was seen in the anti-LTA experiments at the concentrations used, we gave the mice a higher concentration of antibody in the experiment with anti-Aap1, in a larger volume. In anti-Aap1-injected mice 16/18 tissue samples yielded growth compared to 10/16 in the control group. The control group only yielded one culture-positive implant, whereas in the anti-Aap1 group 6 implants with bacterial growth were recovered (P = 0.09, NS) (Figure 3).

Tissues contained higher numbers of cfu than the corresponding biomaterial implants in all groups at 8 days (P < 0.05), and higher frequencies of colonization were also observed for the anti-Aap experiment. Blood cultures were negative for all samples.

**Figure 4:** Effect of antibodies on complement-mediated opsonophagocytosis. A, fluorescently labeled bacteria were opsonized with increasing concentrations of sera. The opsonized bacteria were then incubated with the human granulocyte cell-line HL-60 and opsonophagocytosis was assessed by FACS analysis. The percentage of fluorescent phagocytic cells at each antibody concentration is plotted in the graph. B, alexa-488-labeled anti-LTA and anti-Aap-1 mAbs efficiently bound to S. epidermidis AMC5 cells (filled histograms). Open histograms, controls without antibody. Results are representative of three independent experiments. The fluorescence intensity of bacteria with bound anti-Aap-1 is relatively low due to indirect labeling of the antibody with Zenon.

**Effect of antibodies on complement-mediated opsonophagocytosis.** Since bacteria persisted in the mice despite antibody immunization, we assessed the opsonic capacity of anti-LTA and anti-Aap1 *in vitro*. FAM-SE-labeled *S. epidermidis* AMC5 bacteria were opsonized with different concentrations of anti-Aap1, anti-LTA or pooled human serum (expected to contain anti-*S. epidermidis* antibodies) and subsequently incubated with the HL-60 cell-line.
Opsonization by heat inactivated pooled human serum (HI-PHS) resulted in a serum concentration-dependent uptake of labeled bacteria by the HL-60 cells (Figure 4). Anti-Aap, anti-LTA and mouse preimmune (PI) serum did not show any opsonic activity. To assess whether the lack of opsonic activity might be due to a lack of binding of the antibodies to the bacteria, fluorescently labeled anti-LTA and anti-Aap1 were applied to *S. epidermidis* AMC5 and the bacteria were analysed by FACS. The clear shift in FL1 fluorescence (Figure 4B) showed that the antibodies did bind to the bacterial cells in suspension.

**DISCUSSION**

Aap appeared to be a highly immunodominant *S. epidermidis* protein, but antibodies against Aap were not opsonic and did not protect against mouse experimental BAI. Antibodies against LTA did not protect either, and even significantly increased bacterial colonization of SEpvp catheter segments, indicating a possible risk of passive immunization in presence of biomaterial.

To induce a maximum diversity of antibodies, mice were vaccinated with *S. epidermidis* cell wall proteins separated in different fractions. Even when Aap was depleted the antibodies induced were mostly Aap-specific. Aap is a surface-exposed protein of *S. epidermidis*, present in the form of fibrils covering the bacterial cell surface. Although after depletion Aap was not detected on SDS-Page gels, fragments of the protein carrying antigenic sequences not efficiently removed, may have accounted for the observed antibody response.

Earlier studies suggest that the Aap protein may also be immunodominant when whole bacteria are used as the antigen. These investigators obtained only 2 specific antibodies from mice vaccinated with *S. epidermidis* whole cells, one recognizing a 220 kDa protein, and one recognizing this same protein band, and also a band of 160-180 kDa apparent MW. Full length Aap is 220 kDa in size, and is processed to 180 and 140 kDa forms, the latter considered responsible for biofilm formation. It is therefore tempting to speculate that these antibodies recognize Aap.

The Aap gene is expressed *in vivo* in rat experimental BAI, and is highly prevalent in *S. epidermidis* isolated from patients with various BAI. Assuming that also in patients the bacteria express Aap and display Aap as a fibrillar protein
extending from the surface, one might expect that antibodies to Aap are present in sera of patients recovered from infection. Pourmand et al. probed a bacteriophage lambda expression library of *S. epidermidis* 138 in *Escherichia coli* with sera of patients recovered from staphylococcal infection. They did not identify Aap or other surface proteins as antigens, but this may have been due to low efficiency of cloning of genes encoding staphylococcal surface proteins and/or of their expression in *E. coli*.

Using an ELISA screen, Bowden et al. detected high levels of anti-Aap antibodies in sera of only 2/25 patients recovered from an *S. epidermidis* infection, but their approach may have failed to detect anti-Aap antibodies. The Aap protein contains an N-terminal A region followed by a variable number of B-repeats and smaller C-repeats, and a C-terminal peptidoglycan-binding region. The A-region needs to be removed by proteases in order for Aap to function as an intercellular adhesin. It is therefore possible that antibodies would predominantly be elicited against other parts of Aap than the A-region. As Bowden only used a recombinant A-region fragment from one specific *S. epidermidis* strain as antigen, anti-Aap antibodies present in the sera may not have been detected. In addition, surface-exposed proteins such as fibronectin-binding in *S. aureus* (FnB; ) and fibrinogen-binding protein in *S. epidermidis* (Fbe;) display antigenic variation. The C-repeat regions of the Aap gene have sufficient genetic variation to allow strain typing, and it may well be that such variation also exists in the B-region-encoding sequence of Aap, resulting in antigenic variation in B-repeat epitopes. Therefore, Aap of multiple strains should be used as antigen to screen for the presence of anti-Aap antibodies in patient sera.

In our study anti-Aap antibodies did not protect mice, nor did they enhance opsonophagocytosis. *In vitro*, the antibodies effectively bound whole *S. epidermidis* cells. We applied amounts of antibody to the mice similar to those of other antibodies conferring protection in mouse infection models not involving biomaterials. Possibly, the anti-Aap antibodies were not accessible for FC-receptors of phagocytic cells.

Sun et al. demonstrated that the Aap not only resides on the bacterial cell wall, but is also secreted into extracellular fluids. As we showed that Aap is immunodominant, and antibodies induced against Aap are not protective, expression and shedding of Aap may be a way of *S. epidermidis* to divert the
Antibodies increase adherence of S. epidermidis to biomaterial by inducing mass production of antibodies that do not contribute to opsonophagocytosis.

Anti-Aap and anti-LTA antibodies not only failed to provide protection against S. epidermidis experimental BAI, but they increased binding of the bacteria to the implanted SEpvp segments. The mechanism for this enhancement of binding is still elusive. A direct binding effect is unlikely, since the anti-Aap and anti-LTA antibodies did not influence adherence of S. epidermidis to SEpvp in vitro (not shown). In vivo, biomaterials are covered with many host components and cells, to which bacteria may directly or indirectly adhere. S. aureus, for instance, binds to blood platelets deposited onto biomaterial surfaces through soluble fibrin. Similarly, antibodies may function as bridging molecules attaching S. epidermidis indirectly to biomaterial surfaces, e.g. by binding to deposited complement components, or to Fc-receptors of attached host cells or cell residues. This adherence-enhancing effect of antibodies has until now gone unnoticed, since most passive immunization studies have been performed in animal models without biomaterials.

Our anti-LTA antibody did not protect against infection, and enhanced in vivo binding of S. epidermidis. LTA is also the target for a humanized mouse chimeric mAb in development for clinical use (Pagibaximab). Pagibaximab is reported to recognize a large selection of coagulase-negative staphylococcal clinical isolates, to be opsonic in vitro, to prevent infection in several animal models, and to be safe for humans in a phase II trial. The Pagibaximab studies have not been published in experimental detail, but to our knowledge, no experiments involving biomaterials have been reported. It will be highly relevant to assess the influence of Pagibaximab on in vivo colonization of biomaterials.

Thus, antibodies may increase rather than reduce binding of S. epidermidis to biomaterials in vivo. Although this may depend on the nature of the biomaterial, bacterial strain, and the antibody used, our results indicate a possible risk associated with the use of such antibodies in clinical applications.

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