Complement Activation Influences \textit{Staphylococcus aureus} Adherence to Endothelial Cells

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The ability of \textit{Staphylococcus aureus} to adhere to endothelial cells (EC) is a critical step in the development of metastatic infection. The role of complement in \textit{S. aureus} binding to EC remains uninvestigated. Log-phase \textit{S. aureus}, expressing minimal capsule, was incubated with serum under various conditions, washed, and then incubated at 37°C for 30 min with cultured human umbilical vein EC (ATCC CRL-1730). Adherence was scored visually after staining with acridine orange. Incubation in 10% heat-inactivated human serum increased adherence to endothelial cells by 488% compared to organisms incubated in buffer. Incubating \textit{S. aureus} in complement-active normal human serum (NHS) decreased binding to EC by 58% compared to organisms incubated in heat-inactivated serum. The importance of active complement was confirmed by experiments using serum with added EDTA or cobra venom factor, a protein that depletes C3. The expression of capsule by \textit{S. aureus} strongly interfered with adherence. It has been shown that an important protein for \textit{S. aureus} adhesion to EC is fibronectin. \textit{S. aureus} adherence to purified fibronectin increased by 511% after incubation in complement-active serum, compared to that of organisms incubated in buffer. This decreased by 56% in complement-active serum, suggesting that inhibition of \textit{S. aureus} adherence to EC is due, in part, to complement-mediated diminished binding to fibronectin. Interestingly, when EC were exposed to \textit{S. aureus}-activated serum and then washed, binding by \textit{S. aureus} was 234% higher than that of EC exposed to NHS. Thus, complement-activated EC have increased \textit{S. aureus} binding, while complement on the bacterial surface markedly reduces adherence.

\textit{Staphylococcus aureus} remains a frequent cause of community-acquired and nosocomial infections, accounting for considerable morbidity, mortality, and health care expense (20). Poor outcomes are common despite conventional antibiotic therapy, and the prevalence of antibiotic resistance continues to rise (3). Understanding the contributions of the various elements of host defense against \textit{S. aureus} may ultimately provide new immune-based therapies to aid the fight against staphylococcal infections.

The ability of \textit{S. aureus} to adhere to tissue is thought to play a critical role in its pathogenesis. In many types of infection, staphylococcal adherence to endothelial cells is believed to be the first step in allowing the organism to transition from a bacteremia stage to causing end-organ infections like infectious endocarditis, osteomyelitis, and pyarthritis (8). \textit{S. aureus} is one of the most common causes of these end-organ infections that frequently result in morbidity and death.

Previous studies have demonstrated that \textit{S. aureus} is able to bind to and be internalized by bovine cardiac endothelial cells (7, 26). It has also been shown to bind human endothelial cells in a saturable manner (24, 25). These findings suggested that \textit{S. aureus} adheres to endothelial cells by receptor-ligand interactions.

Subsequent research has focused on identifying the receptors and ligands responsible for \textit{S. aureus} adherence to endothelial cells, with particular attention to extracellular-matrix binding proteins expressed by staphylococci. Most recent attention has focused on the \textit{S. aureus} fibronectin-binding protein, which has been shown to be capable of binding to fibronectin on the surface of endothelial cells. Experiments have shown a major effect on adherence by knockout of the fibronectin-binding protein gene and by competitive inhibition assays with the bacterial protein (13). These results have been disputed when adherence assays were performed under flow conditions (18). It is of great interest that recent studies have shown that immunizing rats with a subunit of the fibronectin-binding protein decreases the risk of catheter-induced infectious endocarditis (19). In addition, adding the fibronectin-binding protein gene to \textit{Lactococcus lactis} is reported to increase the risk in animal models of infectious endocarditis for this normally low-pathogenicity organism (17). Limited and conflicting data exist with respect to the role of clumping factor and coagulase in \textit{S. aureus} adherence to endothelial cells (4, 13, 17).

The complement system is believed to be important in opsonizing \textit{S. aureus}. Thus, complement facilitates host defense by promoting phagocytosis (14). When \textit{S. aureus} activates complement in serum, multiple complement proteins deposit on its surface (5). However, no one to our knowledge has examined whether complement proteins deposited on the microbes' surfaces alters their ability to adhere to surfaces. We speculate that the coating of \textit{S. aureus} in the bloodstream with complement proteins affects staphylococcal adherence to endothelial cells.

Greater than 70% of \textit{S. aureus} clinical isolates are encapsulated strains of serotypes 5 and 8 (10, 12, 22). These studies test an encapsulated serotype 5 strain (CP'), its isogenic capsuleneutral mutant (CP'), and a mucoid strain (CP'). Complement activation has been demonstrated to upregu-
late the expression of a variety of molecules on the surface of endothelial cells. Clq bound to immune complexes causes increased expression of E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cellular adhesion molecule 1 (VCAM-1) (11). C5a increases the expression of P-selectin (6), and cytotactically inactive terminal complement complexes increase the expression of E-selectin, ICAM-1, and VCAM-1 (23). We speculated that exposing endothelial cells to serum activated by \textit{S. aureus} could cause changes on the endothelial cell surface that would alter binding by \textit{S. aureus}.

In this study we demonstrate that \textit{S. aureus} activation of complement markedly affects adherence to human endothelial cells. We evaluate the effect of complement on adherence under conditions of varied capsule expression. The effect of antibody on \textit{S. aureus} adherence to endothelial cells is investigated, and the effect of complement on \textit{S. aureus} adherence to surface-bound fibronectin is evaluated. Endothelial cells exposed to serum treated with \textit{S. aureus} are also tested for \textit{S. aureus} adherence.

**MATERIALS AND METHODS**

**Bacterial strains and growth.** Strain Reynolds is an encapsulated serotype 5 strain (9). Strain JL022 is a capsule-negative mutant constructed by allelic replacement mutagenesis of strain Reynolds (16). JL022, kindly provided by Jean C. Lee (Channing Laboratory, Boston, Mass.), carries a 727-bp deletion in the cap50 gene. Strain M is a mucoid or heavily encapsulated strain (ATCC 49951; serotype 1).

Unless otherwise stated, \textit{S. aureus} cultures were grown in Columbia–2% NaCl broth at 37 °C with shaking to mid-logarithmic phase. Liquid-grown bacteria were washed with GVBS\(^{-}\) buffer (Veronal buffered saline [VBS] with 0.1% gelatin, 0.15 mM CaCl\(_2\) and 1.0 mM MgCl\(_2\)) twice and then brought to a standardized concentration using spectroscopy at 600 nm. Agar-grown organisms were incubated on Columbia–2% NaCl agar plates at 37 °C for 16 h. Agar-grown organisms were suspended in GVBS\(^{-}\) and diluted to a standardized concentration.

**Complement buffers, complement sources, and immunoglobulin sources.** All complement activation experiments were performed in isotonic VBS. In GVBS\(^{-}\) buffer, all complement pathways are active. EDTA-GVBS\(^{-}\) buffer (VBS with 0.1% gelatin, 0.01 M EDTA) inhibits all complement pathways.

Normal human serum (NHS) was obtained from healthy volunteers and tested for normal total hemolytic complement (CH50) and normal alternative hemolytic pathway complement activity (AH50). Prior studies have shown that \textit{S. aureus} strain Reynolds causes minimal activation of the mannose-binding lectin pathway (5). Blood samples for serum were collected in Vacutainer tubes without additives (Becton Dickinson, Franklin Lakes, N.J.), allowed to clot, and centrifuged to remove cellular blood components. Samples were stored at −80 °C for up to 3 months.

Complement activity in serum was inactivated by heating NHS to 56 °C for 60 min. Alternatively, complement activity in serum was inactivated by treatment with cobra venom factor (CVF) by incubating 1 ml of NHS with 20 μg of CVF at 37 °C for 60 min. Cobra venom protein is a potent activator of the alternative pathway and depletes alternative pathway complement factors by massive activation and utilization.

Encapsulated \textit{S. aureus} (CP\(^{+}\))-treated serum was generated by incubating 1-ml samples of NHS, heat-inactivated serum, and CVF-inactivated serum with 10\(^{10}\) CFU of CP\(^{+}\) Reynolds for 60 min at 37 °C. Samples were then pelleted by centrifugation at 2,000 \(\times\) g for 5 min and the serum was recovered. Control serum samples of each type were incubated for 60 min at 37 °C without added bacteria.

To remove antibodies against strain Reynolds, 1-ml samples of serum were adsorbed with 10\(^{10}\) CFU of agaro-grown Reynolds strain for 1 h on ice to minimize complement activation; this was repeated for a total of three absorptions.

Immunoglobulin G was prepared from Gammimmune N for intravenous injection (IVig; Cutter Miles, Elkhart, Ind.). The IVig was dialyzed with VBS, filtered, and ultrafiltered prior to use. IVig was added to serum where indicated at 2 mg/ml.

The antcapsular antibody preparation Altastaph (Nabi Inc., Rockville, Md.) is a human polyclonal product derived from the pooled sera of individuals immunized with StaphVax. StaphVax contains conjugated capsular polysaccharide 5 (CP5) and 8 (CP8) linked covalently to recombinant \textit{Pseudomonas aeruginosa} exotoxin A (21). Altastaph, kindly provided by Ali Fattom, was added to serum where indicated to a concentration of 1%.

**Complement deposition on bacteria.** Aliquots of 0.1 ml of bacteria (10\(^{8}\) CFU/ml) were incubated with 0.1 ml of serum and 0.5 ml of buffer at 37°C for 30 min. This has been shown to maximize complement deposition (5). The bacteria were then washed in cold GVBS\(^{-}\) twice and suspended to 1 ml of EGM-2 medium (Clonetics, Walkersville, Md.) without serum for endothelial cell adherence assays or suspended in 1 ml of M199 (Gibco, Grand Island, N.Y.) for fibronectin binding assays. The bacteria were then washed through a 5-μm Minisart filter (Sartorius AG, Goettingen, Germany) to remove bacterial aggregates. The bacterial suspension was then diluted to 5 \(\times\) 10\(^{5}\) CFU/ml in EGM-2 medium (without serum) prior to use in endothelial cell adherence assays or suspended in M199 with 1% bovine serum albumin prior to use in fibronectin binding assays.

**Endothelial cells.** The human umbilical vein endothelial cells (HUVEC) used for these experiments were HUV-EC-C (ATCC CRL-1730). These cells were grown at 37 °C in a humidified incubator with 5% CO\(_2\) in complete EGM-2 medium containing 5% fetal bovine serum and growth factors. Cells were grown to confluence in Lab-Tek II chamber slides (Nalge Nunc Intl., Naperville, Ill.).

**Endothelial cell adherence under various conditions of \textit{S. aureus} opsonization.** Each well of HUVEC was washed with 0.5 ml of EGM-2 medium (without serum) prior to overlay with the bacterial suspension. An aliquot of 0.2 ml of the prepared bacterial suspension in EGM-2 medium (without serum) was added to each well and incubated for 30 min at 37 °C with 5% CO\(_2\). The suspension overlays were decanted prior to removal of the wells from the slide. The HUVEC were then washed by immersion 10 times in M199 medium. They were then dehydrated by 10 immersions each in 80% ethanol, 90% ethanol, and 100% ethanol. After drying, the slides were then stained with 0.01% acridine orange (Sigma-Aldrich, St. Louis, Mo.) in Hanks’ balanced salt solution for 70 s. The slides were then washed by 10 immersions in distilled H\(_2\)O (dH\(_2\)O) and air dried again.

Endothelial cells and bacteria were visualized by fluorescence microscopy by using acridine orange-specific filters. Each well of the slide was scanned under low power to ensure confluence and integrity of the monolayer. High-power fields were visualized under oil immersion with a 100× magnification lens. The numbers of endothelial cells and bacteria per high-power field were counted. The following five high-power fields were selected for counting: (i) the center of the well, (ii) two points between the center and left edge, and (iii) two points between the center and right edge (13). Selected areas were chosen without prior inspection under high power.

**Endothelial cell incubation with \textit{S. aureus}-treated serum.** In order to test whether exposing endothelial cells to serum activated by CP\(^{+}\) would change the endothelial cells’ ability to bind \textit{S. aureus}, the following experiments were performed. HUVEC were grown to confluence on chambered slides as described above. Complete EBM-2 medium was replaced by EBM-2 (without additives) plus 0.5% of one of the prepared human serum samples either treated or not with CP\(^{+}\). For CP\(^{+}\)-treated serum, 1 ml of serum was incubated with 10\(^{10}\) CFU of CP\(^{+}\) for 60 min at 37°C and centrifuged. Untreated serum was incubated without organisms for 60 min at 37°C. After 1 h, the HUVEC were then washed and incubated with washed mid-logarithmic-phase CP\(^{+}\), previously opsonized with 10% NHS, in EBM-2 medium (without additives) for 30 min at 37°C in 5% CO\(_2\). The slides were then washed, dehydrated, stained, and visualized as described above.

**Fibronectin adherence assay.** Purified human fibronectin (Sigma-Aldrich) was dissolved in Tris-buffered saline to a concentration of 100 μg/ml at 37 °C and then stored at 4°C. An aliquot of 0.2 ml of fibronectin solution was applied to each well of a Lab-Tek chamber Permanox slide (Nalge Nunc Intl.) to completely cover the bottom of each well for 2 h at room temperature. Each well was then flooded with 0.5 ml of 1% bovine serum albumin in Tris-buffered saline overnight at room temperature. The wells were rinsed with M199 prior to use. A 0.2-ml aliquot of the prepared bacterial suspension in M199 with 1% bovine serum albumin was added to each well and incubated at room temperature for 30 min. The suspension overlays were decanted prior to removal of the wells from the slide. The HUVEC were then washed by immersion 10 times in M199 medium. They were then fixed in M199 with 2% glutaraldehyde for 2 h at room temperature. The slides were then washed by immersion 10 times in dH\(_2\)O and stained with 0.01% acridine orange (Sigma-Aldrich) in Hanks’ balanced salt solution for 70 s. The slides were then washed by 10 immersions in dH\(_2\)O and air dried. The number of bacteria per high-power field was determined by fluorescence microscopy.

**Statistical analysis.** The unpaired two-tailed Student’s t test was used to calculate \( P \) values to evaluate for significant differences (Excel 98; Microsoft Co.,

**TABLE 1**

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<th>Condition</th>
<th>endothelial cell adherence</th>
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RESULTS

Adherence of serum-incubated log-phase S. aureus strains to endothelial cells. In order to evaluate the effect of serum proteins and complement activation and binding on the adherence of S. aureus to endothelial cells, the following experiments were performed. Encapsulated, unencapsulated, and mucoid S. aureus strains were grown to mid-logarithmic phase in liquid medium; under these conditions minimal capsule expression is observed with strain Reynolds (5). The bacteria were incubated under various conditions, washed, and allowed to bind to HUVEC (Fig. 1). The mucoid strain (CP/H11001/H11001) demonstrated minimal binding to HUVEC under all conditions, suggesting that the mucoid capsule or other surface characteristic of this serotype 1 strain may impair adherence to endothelial cells. The CP+ and CP− strains showed very similar binding characteristics, as expected because they are isogenic except for the ability to produce capsule and little capsule is produced in mid-logarithmic phase. Incubation of CP+ in 10% heat-inactivated serum increased adherence to endothelial cells by 488% compared to organisms incubated in buffer alone (P < 0.001). Incubating CP+ in fresh NHS with complement activity decreased binding to endothelial cells by 28% compared to that in serum inactivated by ED TA (P = 0.04). Control experiments were performed without serum, comparing endothelial cell binding for CP+ incubated in CVF, CP− incubated in EDTA-containing buffer, and CP+ incubated in buffer without additives (data not shown). CP+ binding to endothelial cells was the same for the three conditions, suggesting that CVF and EDTA did not change the binding characteristics of CP+ under the conditions of these experiments.

Adherence of log-phase S. aureus to endothelial cells after incubation in serum under conditions that prevent complement activation. The mid-logarithmic-phase CP+ strain was incubated in serum in EDTA-GVBS− buffer or in CVF-treated serum, preventing complement activation, and was allowed to adhere to HUVEC (Fig. 2). CP+ incubated in fresh human serum with complement activity decreased binding to HUVEC by 36% compared to organisms incubated in serum inactivated by EDTA-GVBS− buffer (P = 0.03). CP+ incubated in fresh human serum decreased binding to HUVEC by 28% compared to that in serum inactivated by CVF (P = 0.04). Control experiments were performed without serum, comparing endothelial cell binding for CP+ incubated in CVF, CP− incubated in EDTA-containing buffer, and CP+ incubated in buffer without additives (data not shown). CP+ binding to endothelial cells was the same for the three conditions, suggesting that CVF and EDTA did not change the binding characteristics of CP+ under the conditions of these experiments.

Adherence of S. aureus to endothelial cells after incubation in staphylococcus-adsorbed serum. The effect of antibody on S. aureus adherence to endothelial cells was tested under a variety of conditions that did not show statistically significant differences (Fig. 3). Ice-cold serum was adsorbed with CP+ strain Reynolds to remove antistaphylococcal antibodies. Mid-logarithmic-phase CP+ incubated in adsorbed serum showed no significant difference (P = 0.2) in binding to HUVEC compared to bacteria incubated in normal serum. CP+ incubated
in adsorbed serum with added pooled IVIg bound in similar quantities to endothelial cells as after incubation in normal serum. Staphylococci were also tested after incubation in hypogammaglobulinemic serum and found to bind endothelial cells in similar quantities as after incubation in NHS (data not shown). Staphylococci incubated in serum containing human anticapsule (CP⁺) antibodies (Altastaph) adhered to HUVEC in similar quantities as after incubation in normal serum. Thus, under these conditions, anticapsule antibodies had no effect beyond the complement effect described.

Adherence of agar-grown S. aureus to endothelial cells. The CP⁺ and CP⁻ strains were grown on agar to encourage capsule production, incubated with serum, and allowed to adhere to HUVEC (Fig. 4). Under these conditions promoting capsule production, incubated with serum, and allowed to adhere to HUVEC. Each value represents five fields evaluated from each of four separate experiments. Error bars represent SEM.

Adherence of log-phase S. aureus to fibronectin after incubation in serum. Mid-logarithmic-phase of growth S. aureus strain Reynolds (CP⁺) was tested for adherence to purified human fibronectin on a solid support (Fig. 5). After incubation in heat-inactivated serum, 370% more CP⁺ bound to fibronectin than when organisms were incubated in buffer (P < 0.001). After incubation in fresh serum with complement activity, 55% fewer CP⁺ cells bound to fibronectin than after incubation in heat-inactivated serum (P < 0.001). After incubation in fresh human serum, 62% fewer CP⁺ cells bound to fibronectin than after incubation in CVF-inactivated serum (P < 0.001). This was a pattern of binding affinity for the various conditions found for CP⁺ adherence to HUVEC. However, in contrast to what was observed for HUVEC, incubation in fresh serum did not decrease CP⁺ binding compared to organisms incubated in serum inactivated in EDTA-GVBS⁻ buffer.

Adherence to endothelial cells after exposure to S. aureus-treated serum. Endothelial cells were then incubated with serum activated by S. aureus to evaluate whether this would change the endothelial cells’ binding characteristics for complement-coated S. aureus. Fresh human serum was incubated with CP⁺ to allow complement to activate and then the bacteria were removed. Control sera were inactivated by heat or CVF prior to incubation with CP⁺. Endothelial cells were then exposed to these sera or sera not incubated with organisms and washed before incubation with complement-coated CP⁺, as described above.

Preincubating HUVEC in serum treated with CP⁺ and allowing complement to activate increased adherence by opsonized S. aureus by 234% (P < 0.001) compared to HUVEC exposed to fresh human serum without complement activation.
Incubation of HUVEC with heat-inactivated serum treated with CP/H11001 showed a nonsignificant (P = 0.4) increase in adherence to HUVEC compared to heat-inactivated serum not treated with CP/H11001. A moderate, but not statistically significant (P = 0.1), increase in CP/H11001 binding to HUVEC was also noted for CVF-inactivated serum treated with CP/H11001, compared to CVF-inactivated serum not treated with CP/H11001.

**DISCUSSION**

Complement is believed to play an important role in innate immunity against *S. aureus*. To date, the role of complement in affecting *S. aureus* adherence to endothelial cells, an important mechanism for establishing metastatic infection, has not been studied. There are several reasons to believe that complement may play a role in *S. aureus* attachment to endothelial cells. First, pathogenic *S. aureus* strains activate complement in serum, and significant quantities of complement proteins are deposited on the surface of the bacteria. Second, complement proteins on the surface of *S. aureus* strongly influence attachment to and phagocytosis by neutrophils. It is important to note that *S. aureus* in the bloodstream will activate complement in the plasma, releasing reactive complement fragments that may affect endothelial cells’ susceptibility to adherence by *S. aureus* at potential sites for metastatic infection.

Our preliminary experiments demonstrated that incubation in heat-inactivated serum caused CP* S. aureus* to increase attachment affinity for endothelial cells by 488% compared to organisms incubated in buffer alone. It is unknown whether the increased binding to endothelial cells by organisms exposed to heat-inactivated serum is caused by *S. aureus* changing its surface upon exposure to serum or whether the change is caused by the deposition of noncomplement serum proteins on the *S. aureus* surface. The high quantities of CP* binding to endothelial cells after the bacteria were incubated in heat-inactivated serum was shown to decrease by 58% when the CP* cells were incubated in fresh human serum with intact complement activity. Significantly, incubating CP* in fresh serum and allowing complement activation also decreased endothelial cell adherence by *S. aureus* compared to organisms incubated in serum inactivated by EDTA-GVBS buffer or CVF, two additional methods to inhibit complement activity. These findings suggest that complement activation and binding to *S. aureus* inhibit its ability to adhere to endothelial cells and may serve as a mechanism to prevent *S. aureus* in the bloodstream from causing metastatic infection. It is possible that complement proteins directly bind to the *S. aureus* surface structures necessary for attachment to endothelial cells, sterically hinder receptor-ligand interactions, or even change the charge of the *S. aureus* surface. It is also possible that complement-inactivated serum contains components not present in normal serum that alter the *S. aureus* surface, causing increased adherence to endothelial cells.
We also examined the role of antibody in S. aureus adherence to endothelial cells. It has been shown by other investigators that endothelial cells poorly express Fc receptors, but after infection of endothelial cells with S. aureus Fc receptor expression increases markedly (1). In our experiments, CP− incubated with adsorbed serum, with the antistaphylococcal antibodies removed, showed no statistically significant difference in endothelial cell adherence compared to organisms incubated in NHS. The addition of pooled IVIg to the adsorbed serum again did not change the amount of endothelial cell adherence. The addition of antcapsular antibody to serum also did not change binding of S. aureus to endothelial cells. This suggests that antibody binding to S. aureus does not play a major role in the bacterium’s affinity for binding endothelial cells.

When grown on agar, encapsulated S. aureus expresses much greater quantities of capsule than when grown in liquid medium to mid-logarithmic phase. Agar-grown CP− attached to endothelial cells very poorly, whether or not incubated in serum. This is consistent with the findings of previous investigators showing that capsule expression by S. aureus impedes binding to endothelial cells (15). This suggests that when the capsule is expressed it interferes with S. aureus surface proteins binding to the endothelial cell surface. Agar-grown CP− adherence to endothelial cells increased markedly after incubation in heat-inactivated serum compared to that of bacteria incubated in fresh human serum with complement activity. This is consistent with the previous findings suggesting that complement activation decreases the ability of mid-logarithmic-phase S. aureus to adhere to endothelial cells.

Given the strong evidence suggesting that S. aureus fibronectin-binding protein plays an important role in the organism’s ability to bind endothelial cells, we tested whether complement activation would affect S. aureus binding to fibronectin. Mid-logarithmic-phase organisms showed greatly increased binding after incubation in heat-inactivated serum compared to organisms incubated in buffer alone, similar to what was found for attachment to endothelial cells. Incubating CP− cells in fresh human serum with intact complement activity markedly decreased their ability to bind fibronectin compared to bacteria incubated in serum inactivated by heat or CVF, but not for bacteria incubated in serum in EDTA-buffer. EDTA-buffer inhibits the enzymatic activities of C1, C2, and factor B, inhibiting activation of the classical and alternative pathways. It is unclear why this method of inhibiting complement activation would affect adherence to fibronectin differently than CVF inactivation or heat inactivation, which have more direct effects on C3. Overall, the findings for S. aureus adherence to fibronectin are quite similar to the findings for S. aureus adherence to endothelial cells. This suggests that the increased binding to endothelial cells after incubation in complement-inactivated serum and decreased binding to endothelial cells after complement deposition on S. aureus may be due to changes affecting the ability of S. aureus to adhere to fibronectin.

We also studied whether serum treated with S. aureus under conditions allowing complement activation would alter the staphylococcal binding properties of endothelial cells. Endothelial cells were exposed to fresh serum samples treated with CP−, allowing complement to activate, or untreated fresh human serum; washed; and evaluated for adherence by opsonized organisms. HUVEC exposed to CP−-treated serum and then washed showed 234% more adherence by the bacteria compared to HUVEC incubated in untreated NHS. These experiments were then repeated with serum that had been inactivated by heat or CVF and then incubated with CP+. The endothelial cells were then exposed to these CP−-treated inactivated sera or inactivated sera not treated with CP+, washed, and tested for adherence by opsonized bacteria. HUVEC exposed to complement-inactivated serum treated with CP+ compared to complement-inactivated serum not treated with CP+ did not show statistically significant differences in binding S. aureus. This is surprising, since CVF is a complement activator via the alternative pathway, and raises the possibility that the interaction of complement and staphylococci does more than simply release active complement fragments. Perhaps complexes of complement and staphylococcal surface molecules are released or perhaps classical pathway peptides are required for activation. Nevertheless, these data suggest that S. aureus activation of complement in serum increases endothelial cell susceptibility to adherence by S. aureus. It is possible that this effect is also due in part to staphylococcal factors released into serum. Interestingly, it has been reported that that the terminal complement complex in sub-lytic concentrations increases fibronectin synthesis by tubular epithelial cells (2).

In summary, in this report we show that complement-mediated opsonization of S. aureus inhibits binding to endothelial cells. Complement-mediated opsonization of S. aureus also inhibits binding to fibronectin, which may represent the mechanism by which adherence to endothelial cells is impeded. Complement activation of serum by S. aureus directly affects endothelial cells, increasing their susceptibility to adherence by S. aureus.


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