Published in final edited form as:

J Surg Res. 2011 May 1; 167(1): 9–13. doi:10.1016/j.jss.2010.12.039.

Role of *Staphylococcus aureus* Protein A in Adherence to Silastic Catheters¹

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Abstract

Background—Communities of bacteria, termed biofilms, frequently develop on central venous catheters, and bacterial contamination of central venous catheters is the most common cause of nosocomial blood-stream infections. Little is known about the initial events in bacterial adherence to the catheter surface, and experiments were designed to clarify the role of staphylococcal protein A, serum, and immunoglobulin in adherence of *Staphylococcus aureus* to silastic catheters. We hypothesized that *S. aureus* protein A in the presence of serum and immunoglobulin would alter *S. aureus* adherence to silastic catheters.

Materials and Methods—Three strains of *S. aureus* with varying expression of staphylococcal protein A were incubated 15 min at room temperature with silastic catheters, and bacterial adherence to the catheter surface was quantified. In addition, the effects of serum, albumin, and purified IgG on bacterial adherence were assessed.

Results—Both serum and albumin had an inhibitory effect on *S. aureus* adherence to the catheter surface, and protein A expression did not appreciably modulate these effects. Purified serum IgG also inhibited *S. aureus* adherence, with IgG having a greater inhibitory effect on the adherence of an *S. aureus* strain deficient in protein A compared with an *S. aureus* strain expressing high levels of protein A.

Conclusion—*S. aureus* adherence to silastic catheters was inhibited by whole serum, albumin, and purified IgG. Expression of staphylococcal protein A interfered with IgG mediated inhibition of bacterial binding to the catheter surface. Protein A altered *S. aureus* adherence to silastic catheters in the presence of immunoglobulin, but not in the presence of serum or albumin.

Keywords

Staphylococcus aureus; biofilm; catheter; protein A; silastic; adherence

¹Presented at the 5th Annual Academic Surgical Congress (Association for Academic Surgery), San Antonio, TX, February 3–5, 2010.

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INTRODUCTION

Catheter-related infections are associated with severe morbidity and mortality, especially in postsurgical patients, and these infections are becoming increasingly prevalent and problematic due to ongoing emergence of antimicrobial resistance [1]. Central venous catheters (CVC) are indispensable in modern medicine and are the most common cause of bloodstream infections [2]. In the United States, it is estimated that 250,000 CVC-associated bloodstream infections occur annually with attributable mortality of 12% to 25%; the cost per catheter-related blood stream infection is high and is estimated to be \$34,508 to \$56,000, with annual costs ranging from \$296 million to \$2.3 billion (reviewed in [3]). Coagulase-negative staphylococci, enterococci, and *Staphylococcus aureus* remain the most common causes of bloodstream infections in the USA [4]. Much attention has been given to the management and prevention of CVC-associated infections [3], but the basic mechanisms underlying the initiation of CVC-associated infection remain unclear.

Once firm bacterial adherence to a catheter surface has been achieved, bacteria colonize catheters as communities of organisms within vegetations described as biofilms, with the term biofilm defined as a population of bacteria growing on a surface and enclosed in a complex extracellular polymeric substance composed of proteins, lipids, polysaccharides, and DNA [5, 6]. Biofilms are notoriously difficult to eradicate and often lead to chronic infections. It is generally recognized that biofilm communities of microbes are more antibiotic resistant than planktonic (freely floating) microbes [5, 7], and that resolution of biofilm infections of indwelling devices, including CVCs, often requires removal of the device. Although the mechanisms involved in biofilm formation, structure, and physiology are currently receiving much attention from the research community, relatively little attention is being given to mechanisms that promote initial bacterial adherence to a device such as a CVC.

Little is known about mechanisms by which bacteria adhere to the surface of silastic catheters. Bacteria, including *S. aureus*, have a variety of strategies to facilitate adherence. *S. aureus* in particular has surface proteins that bind various matrix and serum molecules, such as fibronectin, fibrinogen, vitronectin, collagen, immunoglobulin, and others (reviewed in [8]). Because indwelling vascular catheters are routinely and consistently exposed to serum components, investigations of the interplay between the catheter material, serum components, and bacterial factors may help to determine mechanisms by which bacteria adhere to catheters, and help to guide strategies to prevent bacterial adherence to catheters.

Using an *in vitro* model of silastic catheters incubated with *S. aureus*, we recently noted that pretreatment of the catheter with serum, but not heparin or albumin, inhibited bacterial adherence to the catheter surface [9]. Because *S. aureus* expresses protein A, which binds the Fc component of immunoglobulin [8], and because pretreatment of silastic catheters with serum (which contains immunoglobulin) appeared to decrease *S. aureus* adherence to the catheter surface, we designed experiments using a refined *in vitro* model, to clarify the role of immunoglobulin and protein A in *S. aureus* adherence to silastic catheter material. Specifically, we designed experiments to test the hypothesis that *S. aureus* protein A in the presence of serum and immunoglobulin would alter *S. aureus* adherence to silastic catheters.

MATERIALS AND METHODS

S. aureus Strains

S. aureus RN6390 is considered a virulent strain and has been used to study interactions of *S. aureus* with a variety of cultured human cells [10–12]. *S. aureus* Cowan 1 is a wild type strain known to overexpress staphylococcal protein A. (Protein A is considered a virulence

factor because it binds the Fc portion of mammalian IgG, making IgG unavailable for opsonophagocytosis; the Cowan strain expresses such high amounts of protein A that it is often used in immunological reagents designed to detect specific antigen-antibody reactions.) *S. aureus* DU5875 is a *Aspa* deletion mutant, deficient in expression of staphylococcal protein A [13, 14]. All *S. aureus* strains were kindly provided by Dr. Patrick Schlievert, University of Minnesota. Stock cultures were maintained at -80°C in brain heart infusion broth supplemented with 10% glycerol. For experiments, bacteria were incubated overnight in tryptic soy broth, washed, and diluted to the appropriate concentration (10^8 CFU/mL) in Hank's balanced salt solution (HBSS). *S. aureus* concentrations were assessed by densitometry and confirmed by serial dilution followed by quantitative culture on both tryptic soy agar and colistin-nalidixic acid agar supplemented with 5% sheep red blood cells.

***S. aureus* Adherence to Silastic Catheters**

Four cm segments of sterile, silastic tubing (1.47 mm i.d., Cole Parmer, Vernon Hills, IL) attached to 15 gauge tubing adapters (Becton Dickinson and Co., Franklin Lakes, NJ) were preincubated 15 min with HBSS (control), normal goat serum (NGS), or HBSS supplemented with 4 g/dL bovine serum albumin (BSA) or 20 mg/mL purified IgG, with all reagents purchased from Sigma-Aldrich, St. Louis, MO. The concentration of BSA (4 g/dL) was chosen to approximate the total protein content of NGS, and the concentration of IgG (20 mg/mL) was chosen to approximate the concentration of IgG in NGS. The tubing was then flushed with 1 mL/cm HBSS, followed by a similar flushing with 10^8 /mL *S. aureus* and bacteria were allowed to dwell in the catheter for 15 min. Preliminary experiments were performed to establish the optimal inoculum at which to determine differences in adherence at 15 min. Catheters were then washed three times with 3 mL/cm HBSS to remove nonadherent bacteria, cut into 1 cm segments, placed in 2 mL HBSS, sonicated with a Sonics Vibra-Cell ultrasonic processor (Sonics and Materials, Newtown, CT) at 20 kHz at 100% amplitude under a power output of approximately 13 W for 5 s, and quantitatively cultured on agar media. In some protocols, the bacterial inoculum was also preincubated 15 min with NGS or IgG, and washed, before use in experiments.

Statistical Analysis

Bacterial numbers were converted to $\log_{10}$ prior to statistical analysis, and significance was set at $P < 0.05$. Differences between two treatment groups were analyzed by unpaired Student's *t*-test, and more than two treatment groups were analyzed by one-way analysis of variance followed by Fisher's test for significant difference.

RESULTS

Effect of Protein A on IgG-Mediated Inhibition of *S. aureus* Adherence

Although serum contains numerous components, the most prominent are albumin and immunoglobulin. Because immunoglobulin interacts directly with the *S. aureus* protein A molecule, we tested the effect of pre-incubation with purified IgG on subsequent adherence of the Cowan strain that expresses high amounts of protein A and the DU5875 strain deficient in protein A (Fig. 1). IgG pre-incubation of the catheter alone, but not the bacterial cells alone, resulted in decreased *S. aureus* DU5875 adherence, and IgG pre-incubation of both the catheter and the bacterial cells seemed to have a synergistic effect, decreasing adherence further. In contrast, IgG pre-incubation of the catheter alone or the bacterial cells alone had no apparent effect on adherence of the Cowan strain, but a decrease in adherence was noted following IgG pre-incubation of both the catheter and *S. aureus* Cowan cells. There was no difference between Cowan and DU5875 in the adherence of the control groups.

Effect of Protein A on Serum-Mediated Inhibition of *S. aureus* Adherence

Catheters are not exclusively exposed to IgG in the clinical setting. Because of the potential for other serum components to influence interactions between protein A and immunoglobulin, we tested *S. aureus* adherence in the presence of serum. To help clarify the effects of protein A and immunoglobulin on serum-mediated inhibition of *S. aureus* adherence, we tested the effect of NGS pre-incubation of catheters on *S. aureus* adherence using three strains known to vary with respect to protein A expression: RN6390 (wild-type strain), Cowan, (a strain characterized by relatively high protein A expression), and DU5875 (protein A deficient strain). Here, either the catheter or the bacterial cells, or both, were pretreated with NGS prior to incubation of the catheter with *S. aureus* (Fig. 2). Catheter pretreatment with NGS was associated with decreased adherence of each the three *S. aureus* strains tested, while pretreatment of the bacterial cells alone had no noticeable effect, except in the case of the DU5875 strain where a significant 3.7-fold decrease ($P < 0.05$) was observed. Pre-incubation of both the catheter and the bacterial cells resulted in decreased adherence that was greater than that noted after pre-incubation of the catheter alone.

Effect of Protein A on Albumin-Mediated Inhibition of *S. aureus* Adherence

To determine whether changes in *S. aureus* adherence to silastic catheters were simply due to non-specific protein effects, we tested the effect of albumin pretreatment of catheters on subsequent adherence of *S. aureus* RN6390, Cowan, and DU5875. BSA pretreatment inhibited *S. aureus* adherence to silastic catheters regardless of protein A expression (Fig. 3).

DISCUSSION

S. aureus is known to bind to silastic catheters [15] and to catheters made of other materials [16–18]. Host factors, including human serum [15], fibrinogen [16], and fibronectin [18] are reported to influence *S. aureus* binding to various catheter materials. However, the role of *S. aureus* protein A and the interaction between protein A and immunoglobulin have not been evaluated with regard to adherence to silastic catheters. To directly measure interactions between staphylococcal protein A and catheter bound IgG, catheters, bacteria, or both were pretreated with IgG prior to measuring the number of adherent *S. aureus*. Pretreatment of catheters with IgG did not reduce the adherence of *S. aureus* expressing abundant protein A (Cowan), but did reduce the adherence of protein A deficient *S. aureus* (DU5875). Pretreatment of both bacteria and catheters reduced *S. aureus* adherence for both strains. These results indicate that IgG can play a role in inhibiting *S. aureus* adherence, and that protein A expression can ameliorate this IgG-mediated inhibition. The amelioration of IgG inhibition may be due to protein A binding of the Fc region of immunoglobulin. These results could be explained by catheter-bound IgG facilitating binding by protein A itself, and inhibiting binding nonspecifically in the absence of protein A. However, when bacterial protein A is already occupied by IgG, as in the bacterial pretreatment group, then catheter-bound IgG appeared to inhibit bacterial binding regardless of protein A expression. Thus, IgG can play a role in inhibiting *S. aureus* adherence to silastic catheters, and this inhibition may be modulated by the level of protein A expression. There was no difference in the adherence of the Cowan and DU5875 strains when catheters and bacteria were pretreated with HBSS, indicating that protein A expression in the absence of IgG does not affect *S. aureus* adherence to silastic catheters.

The clinical relevance of protein A binding to IgG is not clear from the present investigation. When measured using the bacterial inoculum in these studies (10^8 CFU/mL), changes in adherence related to IgG are on the level of one $\log_{10}$. In the clinical setting, the numbers of organisms which initiate a catheter infection are likely far lower than the numbers used in these experiments. The results presented here indicate that protein A

expression can facilitate *S. aureus* binding to catheters with bound IgG. Thus, in the clinical setting, on a single cell level, *S. aureus* binding to a catheter surface may be facilitated by IgG bound to the catheter surface.

S. aureus adherence was measured after pretreating catheters with NGS to assess whether non-immunoglobulin components of serum would differently affect *S. aureus* adherence to the catheter surface. We previously found that fetal bovine serum inhibited *S. aureus* adherence to silastic catheters, however, NGS was used in these experiments because goat serum has a higher IgG concentration. As expected, *S. aureus* adherence to silastic catheters was reduced when catheters were pretreated with serum. Serum pretreatment of the bacterial inoculum alone did not reduce adherence, but when both the bacterial inoculum and the catheter were pretreated, there was an additional decrease in bacterial adherence to the catheter. This apparent synergistic effect of pretreating both the catheter and the bacterial cells, coupled with the lack of noticeable effect of NGS pretreatment of the bacterial cells alone, is difficult to explain but is consistent with the following scenario. NGS may inhibit *S. aureus* adherence by depositing an unidentified substance on the catheter surface. It is also possible that NGS deposits a different substance on the bacterial cells that somewhat inhibits adherence. Finally, these two inhibitory substances either repel each other, or act (actively or passively) together, to block binding site for the bacteria on the catheter surface, resulting in the apparent synergistic inhibition following incubation of both the catheter and the bacterial cells with NGS.

In contrast to the experiments performed with IgG alone, expression of protein A by the Cowan strain did not ameliorate the inhibition of *S. aureus* adherence to NGS-pretreated catheters. This indicates that the presence of other serum proteins may interfere with or attenuate the effect of protein A-IgG interactions on *S. aureus* adherence to the catheter surface.

To assess whether changes in *S. aureus* adherence to IgG-pretreated catheters might be due to nonspecific protein effects, we measured *S. aureus* adherence to catheters after catheters, bacteria, or both were pretreated with albumin in a concentration comparable to the level of protein in NGS. We previously reported that albumin did not reduce bacterial adherence to silastic catheters [9]. In the present study, using a comparatively simplified experimental system, we found that albumin did reduce *S. aureus* adherence to silastic catheters, and that this effect was consistent regardless of the level of protein A expression. The former experiments used an *in vitro* system that included silastic tubing attached to adapters that were, in turn, attached to 4-way stopcock manifolds. Because the manifold itself might have bound albumin and influenced this result, the present experiments in this manuscript eliminated the manifold and merely used silastic tubing attached to tubing adapters. Our previous observation was not incorrect *per se* because it was based on clearly described methodology, but this situation highlights how a reasonably designed *in vitro* system might introduce artifactual results, an important point because much current research involving microbial biofilms uses a variety of relatively complex *in vitro* model systems to facilitate biofilm formation. Our current observation is consistent with previous reports that human serum albumin inhibited *S. aureus* adherence to polyurethane catheters [16]. Curiously, Edmiston *et al.* [19] reported that BSA increased binding of *S. aureus* and *Escherichia coli* to polygalactin 910 suture segments, but the mechanism for this serum-induced augmentation of bacterial adherence was not investigated. The observation that albumin pretreatment decreased *S. aureus* adherence to catheters seemed logical because BSA is often used as a diluent in immunological assays to block nonspecific microbial binding, and one might thus expect BSA to decrease bacterial adherence. The present experiments did not demonstrate a difference in adherence based on the expression of protein A with albumin pretreatment.

One limitation of this study was that bacterial adherence after 15 min of incubation was tested, and later time points were not investigated. The 15-min time point was chosen because it is less than a generation time for *S. aureus*; thus, observed effects would not be attributable to bacterial replication. We are interested in studying specific binding events rather than transient alighting on the catheter surface. Fifteen minutes of exposure would be long enough to allow for binding interactions to occur, and flushing of the catheters would be expected to eliminate bacteria that were not truly bound to the surface. Investigation of the time course of biofilm development after adherence were beyond the scope of this investigation and presents difficulties in determining what effects are related to bacterial proliferation rather than to the propensity to adhere to a particular surface.

Because protein A expression appeared to facilitate adherence of *S. aureus* to catheters treated with IgG alone, protein A may have a potential clinical role in *S. aureus* infection of catheters. The practical clinical relevance of this interaction is uncertain because the presence of other serum components eliminated any observable effect of protein A-IgG interactions on *S. aureus* adherence to the catheter surface. However, binding interactions that occur below the level of detection of our assay could potentially lead to catheter infection.

Little is known about the initial events in biofilm formation on silastic catheters. Data presented herein indicated that serum played a role in inhibiting initial *S. aureus* adherence to the catheter surface, although expression of staphylococcal protein A played little role in modulating this adherence. As expected, albumin also played a role in inhibiting *S. aureus* adherence to the catheter surface. Serum IgG did inhibit *S. aureus* adherence to silastic catheters, and increased expression of protein A (known to bind the Fc portion of immunoglobulin), modulated the inhibitory effect of IgG. Further investigation of factors related to the interactions of catheter material, host factors (e.g., serum proteins), and bacterial factors may lead to novel measures to prevent bacterial adherence to catheters and subsequent catheter-related infections.

Acknowledgments

The authors acknowledge support in part for this work by the Department of Surgery, University of Minnesota, Minneapolis, Minnesota.

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FIG. 1.

Effect of purified immunoglobulin (IgG) pretreatment of silastic catheters alone (IgG-HBSS), *S. aureus* cells alone (HBSS-IgG), or both the catheter and bacterial cells (IgG-IgG) on adherence of *S. aureus* to the catheter wall after 15 min of bacterial incubation within the catheter lumen. Each bar represents the average of six 1 cm catheter segments. *Decreased at $P < 0.01$ versus each of the three other treatments within that *S. aureus* strain; †decreased at $P = 0.01$ versus HBSS-HBSS for that strain; ‡decreased at $P < 0.01$ versus HBSS-HBSS and HBSS-IgG for that strain.

FIG. 2.

Effect of normal goat serum (NGS) pretreatment of silastic catheters alone (NGS-HBSS), *S. aureus* cells alone (HBSS-NGS), or both the catheter and bacterial cells (NGS-NGS) on adherence of *S. aureus* to the catheter wall after 15 min of bacterial incubation within the catheter lumen. Each bar represents the average of nine 1 cm catheter segments. *Decreased at $P < 0.01$ versus HBSS-HBSS and HBSS-NGS within that *S. aureus* strain; †decreased at $P < 0.05$ versus NGS-HBSS within that strain; ‡decreased at $P < 0.01$ versus each of the three other treatments within that strain, except HBSS-HBSS versus HBSS-NGS for DU5875 where the difference is $P < 0.05$.

FIG. 3.

Effect of bovine serum albumin (BSA) pretreatment of silastic catheters on adherence of *S. aureus* to the catheter wall after 15 min of bacterial incubation within the catheter lumen. Each bar represents the average of six 1 cm catheter segments. *Significantly decreased compared with corresponding HBSS control at differences at $P < 0.01$.