Capsular Polysaccharide Masks Clumping Factor A–Mediated Adherence of *Staphylococcus aureus* to Fibrinogen and Platelets

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Background. Clumping factor A (ClfA) is a *Staphylococcus aureus* cell wall–associated adhesin that mediates staphylococcal binding to fibrinogen and platelets. Our goals were to determine whether expression of capsular polysaccharide (CP) affected ClfA-mediated adherence of *S. aureus* and to assess whether the length of the ClfA repeat region influenced this interaction.

Methods. ClfA constructs with repeat regions of different lengths were introduced into isogenic *S. aureus* strains that expressed CP5, CP8, or no CP. *S. aureus* binding to fibrinogen was assessed in rabbit plasma and on fibrinogen-coated microtiter plates. Adherence of *S. aureus* strains to platelets was evaluated by flow cytometry and confocal microscopy.

Results. As the length of the ClfA repeat region increased, binding of acapsular *S. aureus* to fibrinogen-coated microtiter plates was enhanced. By contrast, encapsulated *S. aureus* expressing the full-length ClfA were poorly adherent. The acapsular *S. aureus* mutant strain showed a 2-fold increase in platelet binding, compared with the isogenic encapsulated strains. By contrast, platelet aggregation was unaffected by CP production.

Conclusion. CP expression inhibits *S. aureus* ClfA-mediated binding to fibrinogen and platelets, and a full-length repeat region cannot overcome this inhibition. These findings have important implications for vaccine development, given that CP may mask surface adhesins.

Staphylococcus aureus causes a broad spectrum of infections, including abscesses, wound infections, and osteomyelitis, as well as infections associated with indwelling devices, such as catheters and prostheses. When implanted into the body, these medical devices become coated with host proteins such as fibronectin and fibrinogen (Fg), which promote bacterial adherence [1]. The capacity of *S. aureus* to adhere to plasma proteins is thought to be an important step in the establishment of foreign-body infections. Localized *S. aureus* infections may lead to bacteremia with life-threatening complications, such as pneumonia, endocarditis, and sepsis.

Staphylococcus aureus adheres to host molecules such as Fg, fibronectin, collagen, and IgG through surface protein adhesins. Clumping factor A (ClfA) is an adhesin that mediates *S. aureus* binding to Fg [2] and promotes the attachment of *S. aureus* to biomaterial surfaces [1], blood clots [3], and damaged endothelial surfaces [3]. The Fg-binding domain of ClfA is located within region A of the full-length protein [4] (figure 1*A*). Between region A and the C-terminal cell wall–associated region of ClfA is a dipeptide repeat region (region R) composed predominantly of Asp and Ser residues [2]. Region R is postulated to span the peptidoglycan layer of the cell wall and to act as a stalk to expose the Fg-binding domain of the protein to the extracellular environment. The *clfA* R region varies in size from 193 to 440 aa among different *S. aureus* strains [4]. Although no correlation between the length of the

repeat and the ability of the strains to clump in soluble Fg has been observed [5], a longer region R may allow region A of ClfA to project beyond the extracellular capsule layer [6].

ClfA plays an important role in *S. aureus* binding to platelets—an interaction that is critical to the induction of staphylococcal endocarditis [7]. Damage to cardiac valves results in exposure of the subendothelial matrix and deposition of fibrin and platelets at the site of endovascular injury. Adherent staphylococci may cause further platelet aggregation, leading to an increase in the size of the endocardial vegetation. A *clfA* mutant produced ∼50% less endocarditis than the parental strain in a rat model of *S. aureus* endocarditis [3], and infectivity was restored by complementing the mutant with a wild-type (WT) copy of *clfA*.

S. aureus associates with human platelets by multiple mechanisms [8]. ClfA is the dominant proaggregatory component on the surface of cells from stationary phase [9]. It binds platelets by an Fg bridge to the platelet integrin GPIIb/IIIa and requires IgG to engage the FcγRIIa receptor to stimulate activation. The fibronectin-binding proteins are the major *S. aureus* components from the exponential phase of growth that stimulate platelet activation by Fg and fibronectin bridges to the platelet integrin, as well as the IgG-FcγRIIa interaction [10]. Protein A can bind to platelets through its interaction with von Willebrand factor, which binds to its receptor GPIb [11], or by interacting with the complement receptor gC1qR/p33 [12]. The *S. aureus* extracellular Fg-binding protein is a secreted product that binds Fg and inhibits platelet aggregation in vitro and in vivo [13, 14].

There is conflicting evidence in the literature regarding the effect of capsular polysaccharide (CP) production on the adherence of *S. aureus* to extracellular matrix proteins or to eukaryotic cells. Surface-associated CP prevents the interaction between complement components deposited on the bacterial cell wall and receptors on the neutrophil membrane [15, 16], thereby rendering *S. aureus* resistant to complement-mediated opsonophagocytic killing [16–18]. Snodgrass et al. [19] showed that CP5 or CP8 production did not significantly influence the ability of *S. aureus* collagen-binding adhesin to bind to immobilized collagen. By contrast, CP has been shown to impede adherence of *S. aureus* to endothelial cells [20] and staphylococcal invasion of epithelial cells [21].

Previous studies that examined the interaction between *S. aureus* and Fg or platelets tested acapsular strains or strains cultivated under conditions that supported minimal capsule expression [6, 12, 22, 23]. The goals of our study were to determine whether CP production affected staphylococcal binding to Fg and platelets and to determine whether the repeat region of ClfA was required for functional binding of the ClfA Fg-binding region A. Plasmid-expressed *clfA* variants with repeat regions of differing lengths were introduced into isogenic

S. aureus strains that produced CP5 or CP8 or were CP negative. These strains were then tested for adherence to immobilized Fg and human platelets.

MATERIALS AND METHODS

Bacterial strains. The *S. aureus* strains and plasmids used in the present study are listed in table 1. Plasmid pJH1, carrying *clfA* with a frameshift mutation, was moved into *S. aureus* by transduction. Chromosomal *clfA* mutants of each strain were derived by allelic replacement mutagenesis [16] and verified by polymerase chain reaction analysis and loss of ClfA-mediated *S. aureus* clumping in rabbit plasma [24]. Recombinant plasmids (table 1) were introduced into the *clfA* mutants of *S. aureus* by electroporation [25], and ClfA expression was eval-
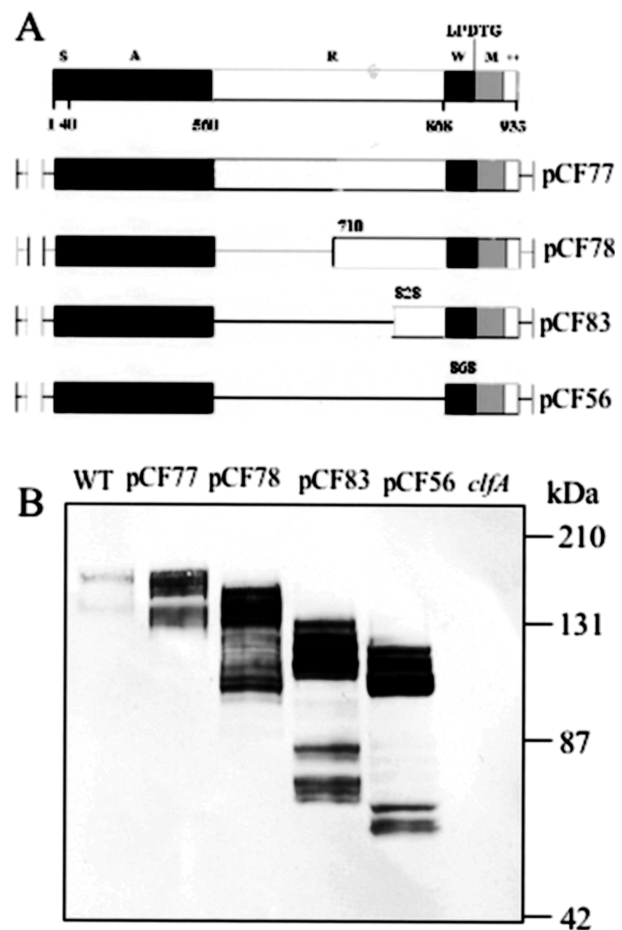
Figure 1. *A,* Schematic representation of the clumping factor A (ClfA) protein repeat region (aa 560–876; region R) derivatives of pCF77, which carries the full-length *clfA* gene. pCF56 lacks the repeat region. S, signal sequence; A, Fg-binding region (aa 40–559); W, wall-spanning region; M, membrane-spanning region; ++, positively charged hydrophobic C terminus. The position of the LPDTG motif is indicated (adapted from [6]). *B,* Identification of recombinant ClfA proteins produced by *Staphylococcus aureus* JLO22 by Western immunoblotting. WT, wild type.

Table 1. Bacterial strains and plasmids.

Strain	Relevant properties	Reference
Staphylococcus aureus		
Reynolds (CP5)	Serotype 5 capsule	[16]
Reynolds (CP8)	Serotype 8 capsule	[16]
JLO22	Reynolds Δcap5O	[26]
Reynolds (CP5) *clfA5*	CP5⁺, ClfA⁻	Present study
Reynolds (CP8) *clfA5*	CP8⁺, ClfA⁻	Present study
JLO22 *clfA5*	CP⁻, ClfA⁻	Present study
Plasmids		
pJH1	Emʳ temperature-sensitive *Escherichia coli–S. aureus* shuttle vector carrying *clfA* with a *Hin*dIII insertion site and resultant frameshift mutation at bp 114 *(clfA5)*	[10]
pCF56	pCU1 shuttle vector carrying *clfA* with region R deleted	[6]
pCF83	pCU1 shuttle vector carrying *clfA* with 40 aa residues in region R	[6]
pCF78	pCU1 shuttle vector carrying *clfA* with 158 aa residues in region R	[6]
pCF77	pCU1 shuttle vector carrying *clfA* with 308 aa residues in region R	[6]

NOTE. ClfA, clumping factor A; CP, capsular polysaccharide.

uated by SDS-PAGE and Western immunoblots by probing with antibodies to ClfA [6].

S. aureus were cultivated with aeration at 37°C in brain-heart infusion (BHI) broth or Columbia broth with 2% NaCl (CSB) for 24 h. Chloramphenicol (Cm; 10 μg/mL) was added to the culture medium when indicated. The bacterial cells were pelleted by centrifugation at 12,000 *g* and suspended in PBS for *S. aureus* binding to immobilized Fg. For some experiments, the bacteria were subjected to one additional PBS wash. For platelet-binding experiments, the bacteria were suspended in PBS, 1% bovine serum albumin (BSA), and 0.1% glucose (PBS-AG). CP production was quantified by an ELISA inhibition method [16].

Plasma clumping factor assay. Dense suspensions of *S. aureus* cells harvested from 24-h BHI broth or CSB cultures were prepared in PBS. A 10-μL aliquot of each suspension (∼10⁸ cfu) was added to a glass microscope slide. The suspensions were mixed with 20 μL of rabbit plasma or PBS for 1 min, and macroscopic agglutination was scored from 0 to 4+.

***S. aureus* binding to immobilized Fg.** Microtiter plates were coated overnight at 4°C with 5 μg/mL of Fg or BSA (both from Sigma Chemical). The plates were washed with PBS and blocked with 0.05% skim milk for 1 h. *S. aureus* (∼10⁹ cfu/200 μL) cells were added to duplicate or triplicate wells of the microtiter plates and incubated for 1 h at room temperature. The plates were washed twice and stained with 0.5% (wt/vol) crystal violet. After the microtiter plates were washed again, 50 μL of ethanol was added to each well to solubilize the dye. The absorbance readings at 550 nm of the BSA-coated plate were subtracted from the readings of the Fg-coated plate. Each experiment was performed at least twice, and the results were pooled and averaged.

Platelet isolation and staining. To minimize their activation, resting platelets were isolated by differential centrifugation in buffers warmed to 37°C. Five volumes of blood obtained from a healthy volunteer were added to 1 vol of 150 mmol/L of sodium citrate and 75 mmol/L citric acid (pH 5.2) in a polypropylene tube, and the mixture was centrifuged at 100 *g* for 15 min at 25°C. The platelet-rich plasma was separated and centrifuged again at 1000 *g* for 10 min. The pellet was washed twice in wash buffer (20 mmol/L HEPES, 140 mmol/L NaCl, and 1 mmol/L EDTA [pH 6.6]) and resuspended in PBS-AG. The platelets were counted in a hemacytometer (∼3 × 10⁷ platelets/mL) and stained with 0.5 μmol/L chloromethylfluoroscein diacetate (CMFDA; Molecular Probes) for 30 min. The platelets were pelleted, incubated at room temperature in wash buffer for 30 min, and washed twice more before resuspension in PBS-AG.

***S. aureus* interactions with platelets.** *S. aureus* strains were cultivated overnight in CSB or CSB with Cm. The bacteria were harvested by centrifugation, suspended in PBS-AG, and stained for 15 min in 10 μg/mL hexidium iodide (HI; Molecular Probes). After one wash in PBS-AG, the bacterial cells were diluted to achieve a ratio of bacteria to platelets of 100:1. The platelet and bacterial suspensions (0.5 mL each) were mixed for 1 min before analysis by flow cytometry (DakoCytomation). Bacterial adherence to platelets was estimated by measuring the percentage of particles that were dually stained (HI-labeled bacteria adherent to CMFDA-labeled platelets). The mean HI fluorescence intensity of the platelet population correlated with the number of adherent bacteria per platelet. For some experiments, the platelet suspensions with adherent bacteria were separated on a MoFlo High Speed Cell Sorter (DakoCytomation). Confocal microscopy was performed with a Zeiss

Table 2. *S. aureus* growth medium influence on capsule production and bacterial clumping in rabbit plasma.

Staphylococcus aureus strain[a]	BHI		CSB	
	CP, μg/10^{10} cfu	Clumping reaction	CP, μg/10^{10} cfu	Clumping reaction
Reynolds (CP5)	17	3+	516	0
Reynolds (CP8)	16	3+	700	0
JLO22	None	3+	None	4+

NOTE. BHI, brain-heart infusion broth; CP, capsular polysaccharide; CSB, Columbia salt broth.

[a] *clfA* mutants of each strain failed to clump in rabbit plasma, regardless of the growth medium.

LSM5 Pascal instrument equipped with a krypton/argon laser, and images were analyzed with PASCAL software (version 5; Zeiss). Platelet aggregation experiments were performed as described elsewhere [9].

RESULTS

The interaction of WT S. aureus with Fg. The ClfA protein is composed of an N-terminus Fg-binding domain (region A) followed by a region R domain with alternating Ser and Asp residues. Hartford et al. [6] constructed recombinant *S. aureus* plasmids that carried variants of the *clfA* gene with truncated region R domains (figure 1*A*). They characterized the functional activity of the variant *clfA* gene products in the *S. aureus* host strain DU5941, a protein A–negative mutant of the acapsular strain 8325-4. Compared with the *S. aureus* strain carrying the full-length ClfA protein, *S. aureus* strains expressing ClfA with ≤40 region R residues showed diminished adherence to immobilized Fg and decreased binding of ClfA antibodies. Deletion of the entire region R resulted in a 500-fold reduction in the *S. aureus* clumping titer in soluble Fg [6].

We sought to examine whether CP production influenced ClfA-mediated binding of the serotype 5 *S. aureus* strain Newman and the serotype 8 strain P1 to immobilized Fg. The results of those studies suggested that CP8 production by strain P1 inhibited Fg binding more than did CP5 production by strain Newman (data not shown). However, because the genetic backgrounds of the 2 strains differed, the differences could not be attributed to CP alone.

To circumvent these issues, we used isogenic strains of *S. aureus* strain Reynolds that produced equivalent amounts of either CP5 or CP8 [16] and the acapsular Reynolds mutant JLO22 [26]. To create ClfA-negative mutants of *S. aureus* Reynolds (CP5), Reynolds (CP8), and JLO22, we introduced a chromosomal *clfA* mutation into each strain (table 1). When *S. aureus* was cultivated in BHI, all of the WT strains clumped in rabbit plasma (table 2), whereas none of the *clfA* mutants clumped. Similarly, each of the WT strains grown in BHI bound to immobilized Fg (figure 2). However, if the strains were cul-

tivated in CSB to maximize CP production, only the acapsular mutant JLO22 bound to immobilized Fg (figure 2) and clumped in rabbit plasma (table 2). Reynolds (CP5) and Reynolds (CP8) harvested from CSB cultures, as well as the *clfA* mutants of all 3 isogenic strains, failed to agglutinate in plasma and showed little binding to Fg-coated microtiter plates (figure 2). ELISA inhibition experiments revealed that CP production by *S. aureus* was 30–40-fold less in BHI broth than in CSB (table 2), which suggests that CP5 and CP8 (optimally expressed in CSB cultures) inhibited ClfA-mediated binding to Fg.

Influence of the ClfA R region on staphylococcal binding to Fg. To determine whether the length of the ClfA R region influenced the interaction between *S. aureus* and Fg, plasmid-expressed *clfA* variants with differing length repeat regions (figure 1*A*) were introduced into *S. aureus* Reynolds (CP5), Reynolds (CP8), and JLO22. pCF77 carries a full-length ClfA region R (308 aa residues), pCF78 carries an R region of 158 aa residues, pCF83 carries an R region of 40 aa residues, and pCF56 lacks the entire R region. ClfA expression was evaluated by Western immunoblotting of cell wall–associated proteins from each strain (carrying the recombinant *clfA* plasmids). As shown in figure 1*B*, the immunoreactive proteins varied in size according to the length of the R region. Each construct in *S.*
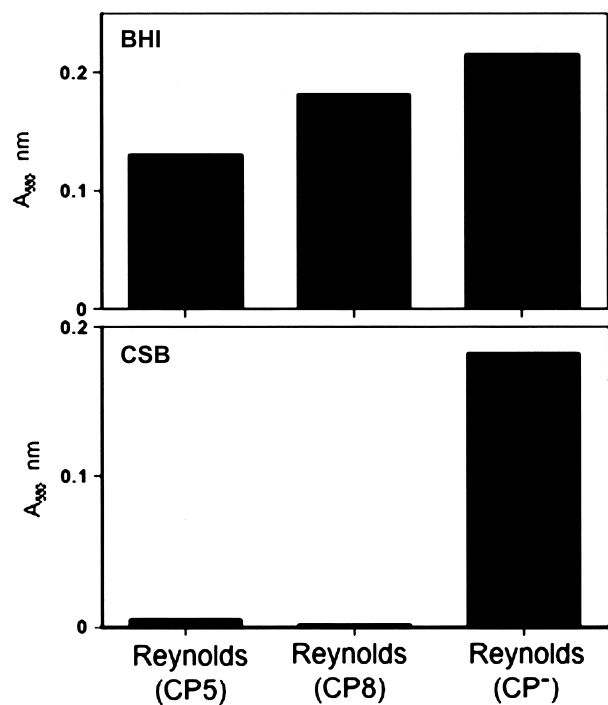

Figure 2. Binding of wild-type *Staphylococcus aureus* to fibrinogen (Fg)–coated microtiter plates. The strains were cultivated in brain-heart infusion broth (BHI; low capsular polysaccharide [CP] production) or Columbia salt broth (CSB; high CP production). Although the isogenic strains showed similar Fg binding after growth in BHI, the encapsulated strains showed minimal binding to Fg after growth in CSB.

aureus JLO22 expressed a number of immunoreactive bands that are typically observed in extracts prepared from strain Newman [5, 6]. The smaller bands are predicted to be ClfA N-terminal degradation products [6]. Protein expression levels were similar for each *clfA* construct expressed by strain JLO22 (figure 1*B*) and for constructs expressed by the encapsulated Reynolds strains (not shown).

Reynolds (CP5), Reynolds (CP8), and JLO22 cultivated in BHI (low CP production) showed a stepwise increase in ClfA-mediated binding to Fg-coated microtiter plates as the length of the ClfA R region increased (figure 3*A*). Likewise, when the 3 strains carrying the full-length ClfA construct were cultivated in BHI, each adhered to Fg in a concentration-dependent manner (figure 3*B*). When the staphylococci were cultivated in CSB, however, Reynolds (CP5) and Reynolds (CP8) bound poorly to Fg even when they expressed ClfA with a full-length R region (figures 3*B* and 4*A*). Thus, under conditions of optimal CP expression, only the acapsular mutant JLO22 adhered to immobilized Fg, and the length of the R region did not influence Fg binding of the encapsulated strains (note ordinates in figure 4). When strains Reynolds (CP5) and Reynolds (CP8) were washed in PBS, which has been shown to remove cell-associated CP [27], reversal of the CP-mediated inhibition of *S. aureus* binding to Fg was observed (figure 4*A* vs. 4*B*). Although Fg binding of Reynolds (CP5) and Reynolds (CP8) was still markedly diminished, compared with that of strain JLO22, a stepwise increase in binding to Fg was observed that correlated with the length of the ClfA R regions.

S. aureus *interactions with human platelets.* To determine whether the capsule inhibited binding of *S. aureus* to resting platelets, *clfA* mutants of Reynolds (CP5), Reynolds (CP8), and JLO22, each carrying pCF77 expressing full-length ClfA, were cultivated in CSB. Suspensions of HI-labeled bacteria were mixed with human platelets at a ratio of 100:1, and the suspensions were analyzed by flow cytometry. Each of the *S. aureus* strains attached to human platelets, although JLO22 showed greater adherence to platelets than did the isogenic encapsulated strains. As shown in figure 5, ~26% of the JLO22-platelet mixture showed dual fluorescence (HI-labeled *S. aureus* bound to CMFDA-labeled platelets), compared with 19% and 17% of the Reynolds (CP5) and Reynolds (CP8) mixtures, respectively ($P < .05$, analysis of variance [ANOVA]). The mean fluorescence index (MFI) of the samples provided an estimate of the mean number of labeled bacteria per platelet. The MFI of platelet samples incubated with acapsular strain JLO22 was almost 2-fold higher than that of platelet mixtures containing either of the encapsulated strains (figure 5*B*) ($P < .05$, ANOVA). Similar differences in MFIs were obtained when the parental isogenic strains (without *clfA* or pCF77) were mixed with platelets and assessed by flow cytometry (data not shown). The MFI of strain JLO22 *clfA* (pCF77) mixed with platelets was twice that of the JLO22 *clfA* mutant (data not shown).

Samples of the staphylococci-platelet mixtures were sorted by flow cytometry to enrich for platelets with adherent bacteria, and these samples were viewed by confocal microscopy. Large numbers of the acapsular strain JLO22 *clfA* (pCF77) were ad-
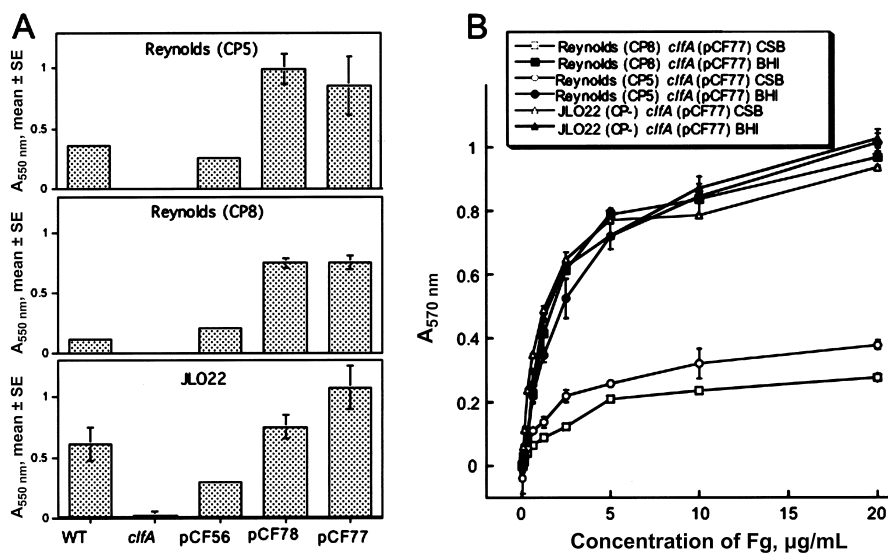

Figure 3. Influence of culture conditions on binding of *Staphylococcus aureus* Reynolds (capsular polysaccharide [CP] 5), Reynolds (CP8), and acapsular mutant JLO22 to fibrinogen (Fg)–coated microtiter plates. The cells were washed once before addition to the Fg-coated microtiter plates. *A, S. aureus* cultivated for 24 h in brain-heart infusion broth (BHI). Fg binding of the wild-type (WT) strain was compared with that of the *clfA* mutant and the mutant carrying plasmids encoding clumping factor A (ClfA) with repeat regions of different lengths. *B,* Acapsular *S. aureus* JLO22 cultivated in BHI or Columbia salt broth (CSB) showing dose-dependent adherence to immobilized Fg. Encapsulated strains grown in CSB showed reduced adherence, compared with the same strains grown in BHI.
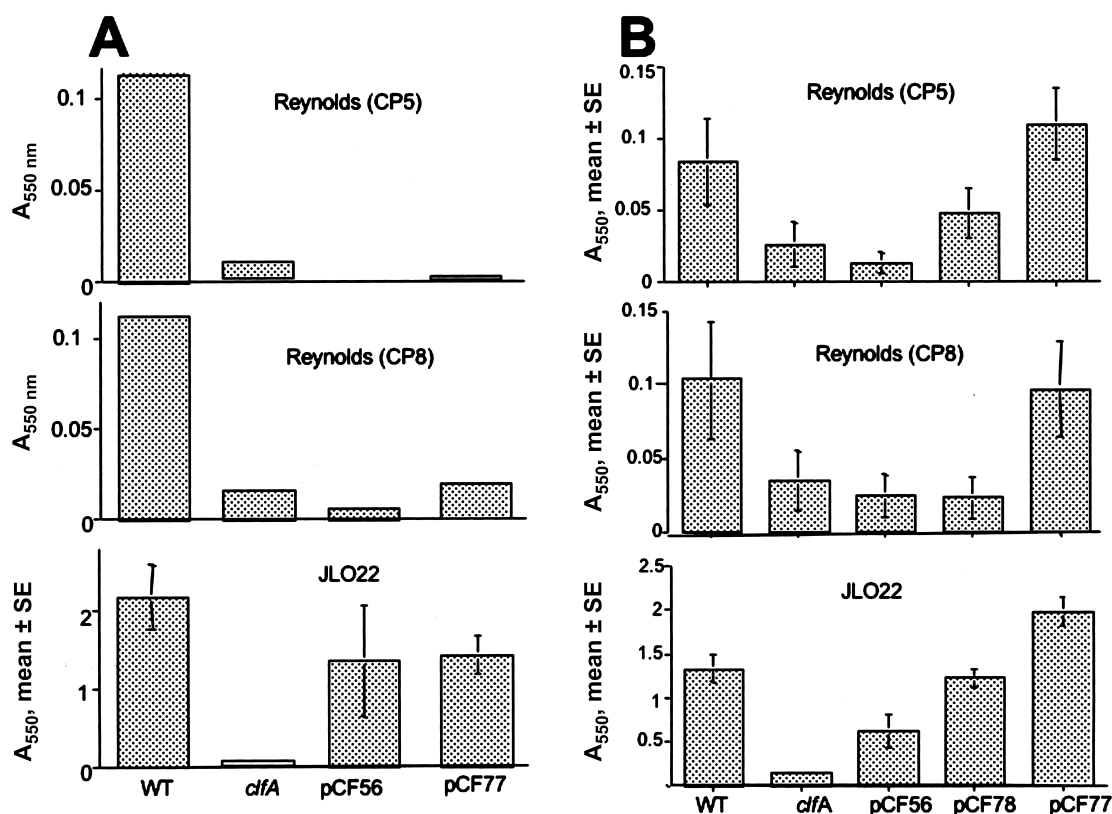
Figure 4. Binding of *Staphylococcus aureus* Reynolds (capsular polysaccharide [CP] 5), Reynolds (CP8), and acapsular mutant JLO22 to fibrinogen (Fg)–coated microtiter plates. Each strain was cultivated for 24 h in Columbia salt broth (CSB) and then washed once *(A)* or twice *(B)* before addition to the Fg-coated microtiter plates. Fg binding of the wild-type (WT) strains was compared with that of the *clfA* mutant and of the mutant carrying plasmids encoding ClfA with repeat regions of different lengths. Note the different scales on the *y*-axes.

herent to the human platelets, resulting in platelet aggregation (figure 5*C*). Small clumps of platelets with adherent bacteria were present in the samples containing Reynolds (CP5) or Reynolds (CP8).

To determine whether optimal CP production masks the functional ability of ClfA to mediate platelet activation, *clfA* mutants of Reynolds (CP5), Reynolds (CP8), and JLO22, each carrying pCF77, were added to platelet-rich plasma. All 3 *S. aureus* strains, cultivated in BHI or CSB, triggered platelet aggregation to a similar degree, with no significant differences in lag time or percentage aggregation (not shown).

DISCUSSION

ClfA is the major Fg-binding protein of *S. aureus* and an important virulence factor in the pathogenesis of staphylococcal endocarditis and septic arthritis [3, 28, 29]. In the present study, we have demonstrated that *S. aureus* CP production can inhibit ClfA-mediated binding to Fg. As the length of the ClfA repeat region increased, staphylococcal attachment to Fg was augmented, but only under conditions of suboptimal CP production (figure 3) or after the capsule was removed by washing (figure 4*B*). A full-length *clfA* R region promoted attachment

of the acapsular strain JLO22 to Fg (figures 3 and 4) and to human platelets (figure 5), whereas CP expression diminished *S. aureus* binding to both substrates. Likewise, Sullam et al. [30] reported that exopolysaccharide production inhibited *Streptococcus salivarius* binding to platelets.

S. aureus adherence to resting platelets is mediated by ClfA, and Loughman et al. [9] showed that both Fg and IgG are critical for triggering platelet activation and aggregation. However, in our studies, rapid ClfA-dependent binding of *S. aureus* to washed platelets occurred in the absence of added Fg or IgG. The *S. aureus*–platelet interaction that we observed may have resulted from partial activation of the washed platelets and concomitant release of Fg, creating a bridge between *S. aureus* and the GPIIb/IIIa platelet receptor. Alternatively, at a ratio of 100 bacteria:platelet, a direct interaction between surface-exposed ClfA and the platelets may have occurred. CP expression had no effect on the ability of our isogenic *S. aureus* strains to activate and subsequently aggregate human platelets in vitro, which suggests that small numbers of exposed ClfA molecules were capable of triggering platelet activation or that the agitation occurring during aggregometry resulted in CP removal and exposure of ClfA molecules.
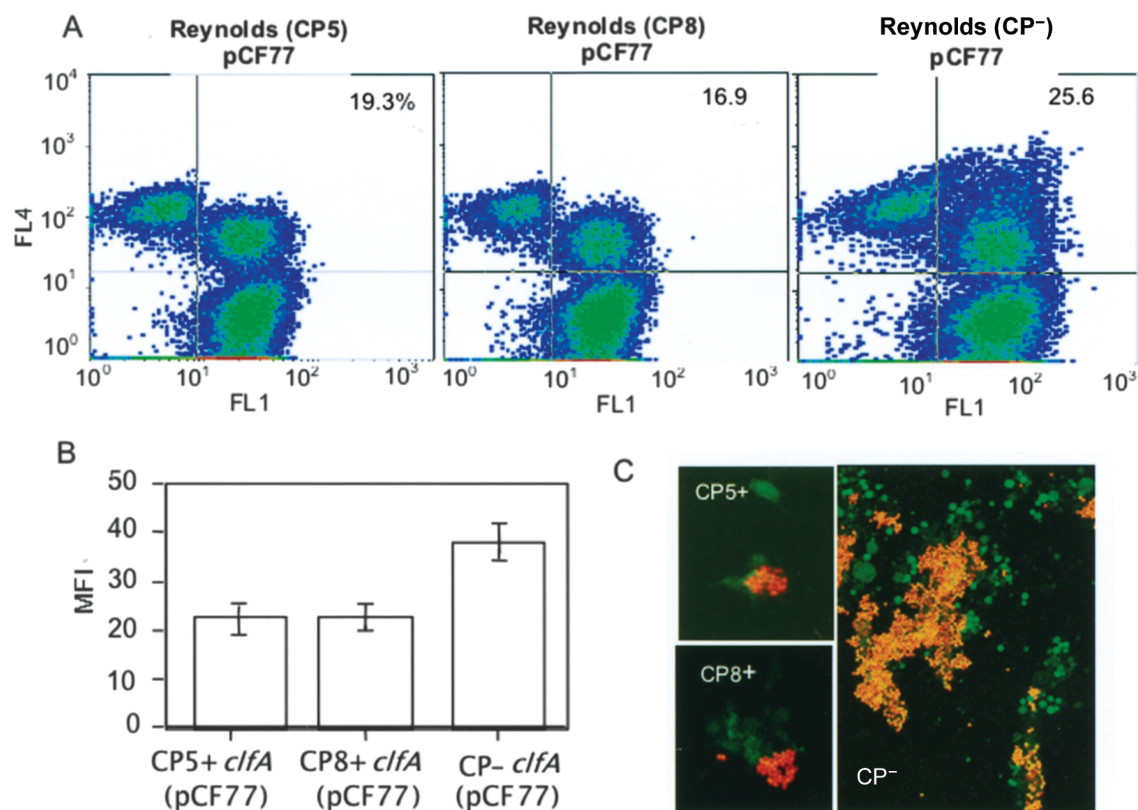
Figure 5. *Staphylococcus aureus* JLO22 adherence to human platelets in greater numbers than the capsular polysaccharide (CP) 5– or CP8-producing strains. *A*, Flow cytometry measurement of the interaction of labeled bacteria (shown on the FL1 axis) with platelets (on the FL4 axis). *B*, Mean fluorescence intensity (MFI) of platelets mixed with the CP-negative mutant JLO22 was significantly ($P<.05$) higher than that of the CP5- or CP8-producing strains. *C*, Confocal microscopic analysis of *S. aureus (red)* adherent to human platelets *(green)*. The encapsulated strains bound to the platelets in similar numbers, whereas large numbers of the acapsular strain JLO22 bound, resulting in marked platelet aggregation.

Niemann et al. [31] reported that soluble fibrin and not fibrinogen was the main mediator of *S. aureus* adhesion to platelets. They used a bacterium to platelet ratio of 10:1 and a 15-min incubation period. Our shorter 1-min assay focused on the initial interaction of bacteria with resting platelets before fibrin dimers form. A comparison of our studies with those of Loughman et al. [9] and Niemann et al. [31] suggests that in vitro assay conditions influence the interpretation of mechanisms by which *S. aureus* interacts with human platelets. Our experimental results indicate that the initial binding of *S. aureus* to platelets is modulated by the CP phenotype of the strain but that platelet activation may be unaffected.

Surface-associated CP prevents the interaction between complement components deposited on the bacterial cell wall and receptors on the neutrophil membrane [15, 16], thereby rendering *S. aureus* resistant to complement-mediated opsonophagocytic killing [16–18]. Although CP production was shown to enhance staphylococcal virulence in rodent models of bacteremia, abscess formation, and septic arthritis [16, 17, 32, 33], there was an inverse correlation between CP production and infectivity in the staphylococcal endocarditis model [34, 35].

These findings suggest that CP masks adhesins that are critical determinants of virulence in endocarditis [3, 36] and confirm our previous experiments showing that CP hindered the in vitro interactions between *S. aureus* and mammalian cells [20, 21].

Although our data indicate that ClfA can be masked by CP, ~20%–25% of *S. aureus* clinical isolates are acapsular because of mutations within the *cap5(8)* locus or within genes that regulate CP production [37]. Furthermore, CP is not expressed during the exponential phase of bacterial growth [27, 38]. CP expression is highly sensitive to environmental signals [27, 39], and our experiments indicate that *S. aureus* cultivated in CSB produce ~30-fold more CP than organisms grown in BHI. Similarly, in vivo CP expression is variable—CP5 was detected in the serum of infected rats [40, 41], on *S. aureus* cells recovered from endocardial vegetations [27], from mice colonized intranasally [42], and from cows with experimental mastitis [43]. By contrast, CP was not detected within the lung or nasal polyps of 2 patients with cystic fibrosis [44] or on *S. aureus* harvested from the granuloma pouch model [39].

Although *clfA* is not regulated by *agr* [45] or ArlRS [46],

clfA gene expression by strains Reynolds and Newman was maximal in postexponential-phase cultures [45, 47]. In a guinea pig tissue-cage model of infection, *clfA* transcripts peaked between 96 and 144 h after inoculation with *S. aureus* Reynolds and were more abundant from samples of sessile bacteria than those from planktonic bacteria [47].

The accessibility of *S. aureus* surface antigens has important implications for vaccine development, given that optimal targets for immunization should be surface exposed. Both ClfA and CP are expressed by *S. aureus* in postexponential growth, and we have shown that CP production at least partially masks cell wall–associated ClfA. A vaccine based solely on serotype 5 and 8 capsules failed to protect hemodialysis patients in phase III clinical trials [48] (Nabi Biopharmaceuticals; http://www.nabi.com/pipeline/clinicaltrials.php#1). Likewise, passive immunotherapy with a pooled human immunoglobulin preparation from donors selected for high antibody titers against ClfA showed no clinical benefit in premature neonates (Inhibitex; http://phx.corporate-ir.net/phoenix.zhtml?c=176944&p=irol-newsArticle&ID=877465&highlight). A vaccine that includes ClfA, CP5, and CP8 may be more effective than a single-component vaccine. Nonetheless, our findings argue for the development of a multicomponent staphylococcal vaccine that includes at least 1 surface antigen (such as fibronectin-binding protein A) that is expressed during the exponential phase of bacterial growth. Toxoid antigens derived from α-hemolysin or Panton-Valentine leukocidin are also under consideration for inclusion in a multicomponent *S. aureus* vaccine. Because of the prevalence of antibiotic-resistant *S. aureus* strains in hospitals and in the community, additional strategies to control this important bacterial pathogen are sorely needed.

Acknowledgments

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