Pooled human immunoglobulins reduce adhesion of Pseudomonas aeruginosa in a parallel plate flow chamber

K. A. Poelstra,1,2† H. C. van der Mei,2 B. Gottenbos,2 D. W. Grainger,3* J. R. van Horn,4 H. J. Busscher2
1Anthony G. Gristina Institute for Biomedical Research, 520 Huntmar Park Drive, Herndon, Virginia 20170
2Department of Biomedical Engineering, University of Groningen, Bloemsingel 10, 9712 KZ
 Groningen, The Netherlands
3GAMMA-A Technologies, Inc., 520 Huntmar Park Drive, Suite 100, Herndon, Virginia 20170
4Department of Orthopaedic Surgery, University Hospital Groningen, AZG, Hanzeplein 1, Postbus 30.001, 9700 RD
 Groningen, The Netherlands

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Abstract: The influence of pooled polyclonal immunoglobulin (IgG) interactions with both bacteria and model substrates in altering Pseudomonas aeruginosa surface adhesion is reported. Opsonization of this pathogen by polyclonal human IgG and preadsorption of IgG to glass surfaces both effectively reduce initial deposition rates and surface growth of P. aeruginosa IFO3455 from dilute nutrient broth in a parallel plate flow chamber. Polyclonal IgG depleted of P. aeruginosa–specific antibodies reduces the initial deposition rate or surface growth to levels intermediate between exposed and nonexposed IgG conditions. Bacterial surface properties are changed in the presence of opsonizing IgG. Plateau contact angle analysis via sessile drop technique shows a drop in P. aeruginosa surface hydrophobicity after IgG exposure consistent with a more hydrophilic IgG surface coat. Zeta potential values for opsonized versus nonopsonized bacteria exhibit little change. X-ray photoelectron spectroscopy measurements provide surface compositional evidence for IgG attachment to bacterial surfaces. Surface elemental ratios attributed to IgG protein signals versus those attributed primarily to bacterial polysaccharide surface or lipid membrane change with IgG opsonization. Direct evidence for antibody-modified P. aeruginosa surface properties correlates both with reduction of bacterial adhesion to glass surfaces under flow in nutrient medium reported and previous reports of IgG efficacy against P. aeruginosa motility in vitro and infection in vivo. © 2000 John Wiley & Sons, Inc. J Biomed Mater Res, 51, 224–232, 2000.

Key words: flow chamber; Pseudomonas; antibodies; immunoglobulin; bacterial adhesion; infection; opsonization

INTRODUCTION

Advances in the development of bioengineered implants and devices provide clinicians with an enormous range of prostheses that improve the quality of life for millions of patients. Unfortunately, this progress has been accompanied by clinical challenges involving implant-associated infections and host foreign body responses that compromise healing and often resist conventional antibiotic treatment.1–4 Infectious organisms preferentially target synthetic implanted materials, eliciting serious and costly infections that frequently require removal of the colonized device.4–7 Pathogen colonization and maturation into biofilms have been documented on numerous biomedical devices used in orthopedics, urology, cardiology, and other fields of surgery, even those that survive treatments with first-line antibiotics.4,8 Initial microbial adhesion is a primary determinant of biomaterial-centered infection because initially adhering microorganisms often progress to a mature biofilm attached to the biomaterial surface and elicit device-centered infection.4–6,9

Prevention of microbial surface colonization on implants is one possible approach to reduce biomaterial-and implant-centered infection. For decades, many different strategies have attempted to redesign, surface-modify, or coat implant materials to block initiation of this process.5,10–17 Many bacteria–surface interactions have been reported. Correlations between bacterial surface hydrophobicity and surface adhesion

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have been well studied and are now well established. However, design of surfaces that resist colonization and reduce infection remains problematic. In addition to surface chemistry approaches, antibiotic-releasing coatings have not been fully successful in overcoming clinical presentation of biofilm formation and subsequent implant failure. Both systemic and local antibiotic therapies are frequently ineffective in reducing infection incidence on implants, are complicated by the array of possible pathogens involved in implant-centered infection, or encounter antibiotic resistant infections.

Because surgical sites are often immunocompromised as a result of surgical- and implant-induced trauma, the restoration, stimulation, and supplementation of endogenous immune responses are alternatives to bolster host defenses against infection. Both clinical presentations—acute, immediate postoperative infection of newly implanted biomaterials, and longer-term, latent implant site infection—could benefit from local potentiation of the host’s immune system to defeat contaminating indigenous and exogenous pathogens during and after surgical implantation procedures. Because infection often interferes with wound healing, local wound site immunostimulation may also improve subsequent healing processes and long-term tissue integration, but such benefits have not yet been reported.

As a predominant host immune component, human polyclonal immunoglobulin (IgG) is composed of millions of opsonizing antibodies specific to microorganism epitopes, facilitating endogenous pathogen killing by stimulating phagocytosis, complement lysis, and oxidative responses by macrophages and neutrophils. Of the array of immunoglobulins produced for host defense, IgG subclasses comprise the largest mass fraction of circulating antibodies. Direct elucidation of IgG opsonization on the adherence of bacteria to a substratum has not yet been reported although it is often asserted that opsonization hinders bacterial tissue interactions. In vitro assays have recently shown that pooled polyclonal human IgG limits motility of several flagellar pathogens, a factor linked to virulence. In vivo studies have used locally delivered pooled polyclonal human IgG to reduce Pseudomonas aeruginosa infection severity and incidence in several animal infection models. These data provide evidence for the protective benefit of exogenously administered polyclonal antibodies to overcome lethal infection from several different virulent bacteria strains.

To further understand aspects of the basic mechanisms surrounding bacteria–surface interactions and the influence of antibodies to moderate biomaterial-centered infection, the efficacy of pooled human polyclonal IgG in the direct prevention of bacterial adhesion and surface-growth was investigated. In this study, we compared the initial adhesion rates and surface-growth dynamics for a proven virulent IgG-opsonized and nonopsonized P. aeruginosa on glass surfaces in situ using a published parallel plate flow chamber system. Surface adhesion and growth characteristics of both IgG-opsonized and nonopsonized bacteria were correlated with bacteria surface properties determined via contact angle, ζ potential, and X-ray photoelectron surface analytical measurements.

MATERIALS AND METHODS

Bacteria and growth conditions

The flagellar pathogen, P. aeruginosa IFO3455, was used in this study. The strain was streaked and grown for 24 h from frozen stock on nutrient agar (lab-lemco powder, 1.0 g/L; yeast extract, 2.0 g/L; peptone, 5 g/L; sodium chloride, 5.0 g/L; pH 7.4; Oxoid). The plate was then kept at 4°C for not more than 1 week, at which time a new plate was streaked. A preculture was inoculated and incubated at 37°C in ambient air for 24 h. This preculture was used to inoculate a second culture in 100 mL 100% nutrient broth (100% NB) that was grown for 18 h. Bacteria from the second culture were harvested by centrifugation at 10,000 × g for 5 min at 10°C and washed twice with Millipore-Q water. Subsequently, bacteria were sonicated on ice for 10 s to separate cell clusters and then resuspended at a concentration of 3 × 10^8 cells/mL in sterile 2% NB in isotonic phosphate-buffered saline (PBS) (pH 7.0) with or without 0.2 wt % commercial pooled human polyclonal IgG (Baxter GammaS/D; Lot 98F03AB11).

Microbial cell-surface characterization

Bacterial cell surfaces were characterized by ζ potential and water contact angle measurements in the presence and absence of opsonizing IgG. Zeta potential measurements were conducted on P. aeruginosa cultures harvested and washed as described above. Bacteria were resuspended (3 × 10^7 cells/mL) in either sterile PBS or sterile PBS supplemented with 0.2 wt % pooled polyclonal IgG, both at pH 7.0. The electrophoretic mobility of each suspended bacterial sample was measured at 150 V using an automated Lazer Zee Meter 501 (PenKem, Brookhaven Instruments Worcestershire, UK) and converted into ζ potentials, assuming that the Helmholzt–von Smoluchowski equation is valid. Automation of the Lazer Zee Meter yielded extremely short measuring (“voltage on”) times of 1 min. Consequently, a high voltage of 150 V could be used without Joule heating of the suspension and bacterial mobility did not interfere with the measurements.

Sessile drop contact angle measurements on washed P. aeruginosa IFO3455 filter lawns were performed as described previously to determine the intrinsic cell-surface hydro-
phobicity in the absence and presence of adsorbed human polyclonal IgG. Briefly, *P. aeruginosa* harvested from an overnight culture in nutrient broth was suspended either in sterile PBS or sterile PBS supplemented with 0.2 wt % IgG. Bacteria were subsequently washed three times to remove soluble polysaccharides, proteins, and salts, resuspended in Millipore-Q water, and deposited on 0.45 μm pore-size cellulose acetate filters to produce an even, confluent bacterial lawn of approximately 50 stacked bacterial layers. The *P. aeruginosa* IFO3455 lawns were air-dried until so-called plateau contact angles could be measured using sessile water droplets, followed by analogous contact angle measurements with formamide, methylene iodide, and α-bromonaphthalene as described previously.38

X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy was used to determine the overall microbial cell-surface composition and performed as described previously.39 In short, freeze-dried, copiously rinsed freshly cultured bacteria in Millipore water, either preincubated with polyclonal IgG or untreated, were pressed into small-stainless steel cups and placed on the sample stage of the spectrometer (S-probe; Surface Science Instruments, East Sussex, UK) equipped with an aluminum anode (10 kV, 22 mA) and operating at 10−9 Torr. Survey spectra were collected using a pass energy of 150 eV and spot size of 250 × 1000 μm. High-resolution spectra were acquired at a pass energy of 50 eV for selected peaks in the sequence C 1s, O 1s, N 1s, P 2p, and again C 1s to account for contamination or deterioration of the samples under X-ray flux. The binding energy for all spectra were referenced to the Cls C–H peak at 284.8 eV. The area under each peak, after linear background subtraction, was used to calculate peak intensities, yielding elemental surface concentration ratios for oxygen/carbon, nitrogen/carbon, nitrogen/carbon, and phosphorus/carbon after correction for instrument sensitivity factors as supplied by the manufacturer. The O 1s peak was fit using a least-squares program into two Gaussian components assigned to 531.4 eV (O 1) and 532.8 eV (O 2), peak was fit using a least-squares program into two Gaussian components assigned to 531.4 eV (O 1) and 532.8 eV (O 2), and phosphorus/carbon after correction for instrument sensitivity factors as supplied by the manufacturer. The O 1s peak was fit using a least-squares program into two Gaussian components assigned to 531.4 eV (O 1) and 532.8 eV (O 2), and phosphorus/carbon after correction for instrument sensitivity factors as supplied by the manufacturer.

Parallel plate flow and adhesion chamber

The parallel plate flow chamber (internal dimensions: 76 × 38 × 0.6 mm), associated microscope, and image analysis system have been described previously.9,19,34 This system is composed of a phase-contrast microscope (Olympus BH equipped with Olympus ULW-CD Plan 40 PL long-working-distance objective) with a CCD-MXR camera (High Technology, Eindhoven, The Netherlands) coupled with a TEA (Difa, Breda, The Netherlands) image analyzer. Initial adhesion rates, surface growth, and detachment were directly observed and recorded on the bottom glass plate in situ under laminar flow (flow rate 1.5 mL/min) without additional shear forces acting on the adhering bacteria. Glass was chosen both for its optical transparency necessary for observation by phase-contrast microscopy, cleansing convenience, and ease of sterilization by autoclaving. Just before sterilization, glass plates and spacers were thoroughly washed using sponges, water, and surfactant, sonicated for 30 min, and thoroughly rinsed multiple times with Milli-Q water.

Bacterial adhesion experiments

Before each experiment, the entire flow system was autoclaved and subsequently filled with 2% NB in PBS (pH 7.0), ensuring that all air bubbles were removed from the system. Once filled, and before the addition of bacterial suspension with or without IgG, additional nutrient broth was flowed through the system for 60 min at a flow rate of 1.5 mL/min, and subsequently switched to the bacterial suspension (~3 × 108/mL) at the same flow rate. All experiments were performed at 37°C. In one experimental setup, this flow was changed to 1 wt % IgG for deposition onto the glass surface for 60 min before exposure to a bacterial suspension in 2% NB. Subsequently, bacteria were perfused through the system for 75 min without recirculation, and images were captured using phase-contrast microscopy. The number of initially adhering bacteria was expressed as the so-called initial deposition rate, i.e., the increase in the number of adhering *P. aeruginosa* per unit area per time, extrapolated to *t* = 0,9,34 according to:

$$j_0 = \frac{dn(t)}{dt} \bigg|_{t=0}$$

where *n(t)* is the number of bacteria adherent at a given time, *t*, extrapolated to *t* = 0.

Following the 75-min exposure to bacterial suspension, flow was switched to 100% NB and continued for 18 h to study bacterial surface growth (generation and doubling times) and biofilm development. Fifteen successive images were recorded every 3 min during this surface-growth mode, from which the number of adhering organisms, the generation time of individual adhering bacteria, and the bacterial surface coverage were determined under different conditions.9,34 Images were automatically tracked sequentially by computerized image analysis and subject, based on frame-by-frame optically discriminated differences, to computer algorithms that calculate doubling times of surface coverage9,34 according to:

$$n_t = n_0 \times 2^{(U/t_d)}$$

where *t_d* is the doubling time sought, *n_t* is the number of bacteria on the surface at time *t*, and *n_0* is the original number of bacteria observed.

Generation times in 100% NB were calculated by visually timing the division speed of 30 different adhering, duplicating bacteria per experimental setup during the growth phase. Bacterial desorption rate constants were calculated according to previous methods15,40 from flow cell experiments performed in both the absence and presence of IgG.

In two flow chamber adhesion experiments, *P. aeruginosa*
was first exposed to 0.2 wt % pooled but modified human IgG solutions for 60 min. This IgG had been previously depleted of antibodies specific for this *Pseudomonas* strain by incubation for 2 h with 10^{12} colony-forming units of *P. aeruginosa* IFO3455. After this incubation, bacteria were spun down for 15 min at 10,000 × g and discarded with adhering specific antibodies, while the remaining IgG-containing supernatant was collected and filtered (0.2 μm) to remove possible bacterial contamination and subsequently used in this flow chamber experiment as depleted IgG.

**RESULTS**

**Bacterial adhesion and surface growth under flow**

Table I shows results determined directly from the flow chamber image analysis for the deposition of *P. aeruginosa* IFO3455 suspended in either 2% nutrient broth (NB) in PBS or 2% NB in PBS supplemented with 0.2 wt % IgG, both on either bare glass surfaces in NB or on glass surfaces preadsorbed with 1 wt % IgG. As the first column reflects, initial deposition rate in the absence of IgG was 258/cm² per second. Initial adhesion rates observed both for bacteria preopsonized with IgG (120/cm² per second) and for nonopsonized bacteria on the preadsorbed IgG glass surface (106/cm² per second) were significant (Student t test) compared to the initial adhesion rate in the absence of IgG (both p < .01). The combination treatment of both bacterial opsonization and glass preadsorption with IgG did not further reduce the initial adhesion rate (136/cm² per second). Interestingly, the adhesion rate observed for bacteria incubated with 0.2 wt % depleted IgG was also lower (186/cm² per second) than the initial adhesion rate for bacteria in NB on bare glass lacking IgG (258/cm² per second).

*Pseudomonas* surface generation times for adhering bacteria in 100% NB, presented in Table I, were significantly longer in the presence of IgG (43–45 min) compared to generation times in the absence of IgG (both 39 min; p < .01, Student t test). The surface doubling time (i.e., time to double bacterial numbers adhering to the surface) is presented in Table I as well. No significant difference existed between the different experimental groups. On average, approximately 60 min was required to double the bacterial surface coverage in 100% NB in either the presence and absence of IgG when no planktonic bacteria deposition was present.

Finally, bacterial surface coverage after 2 and 5 h of 100% NB flow is reported in Table I for the different experimental conditions. The observed characteristic clustered bacterial surface coverage shown in Figure 1(A) after 2 h was a more direct reflection of initial adhesion rate and generation time than the 5-h point. After 5 h, however, bacterial desorption kinetics and reorganization of the newly adherent and surface-resident organisms resulted in a more homogeneous, evenly spaced surface coverage, shown in Figure 1(B). Bacterial desorption rate constants averaged 2.7 × 10^{-4} s^{-1} for all experiments in the absence of IgG and 1.5 × 10^{-4} s^{-1} for all experiments in the presence of IgG. The observed surface redistribution of bacteria occurred in both the absence and presence of IgG (data not shown). For both the 2- and 5-h points the slowest surface growth was observed with preadsorbed IgG on the glass surface (11% and 65% coverage, respectively). Under other conditions, full surface coverage was nearly achieved after 5 h.

**Bacteria ζ potential**

*Pseudomonas aeruginosa* IFO3455 exhibited a narrow ζ potential distribution for freshly cultured bacteria in buffer with a mean value of −21 ± 4 mV. The negative potential was attributed to the ubiquitous, slightly anionic glycocalix (polysaccharides and peptidoglycans) that comprised the surface coats of these bacteria. After incubation with 0.2 wt % IgG in buffer, the bacterial ζ potential became slightly more negative with a wider distribution (−24 ± 9 mV). This difference lacked statistical significance.

| **TABLE I** | **Deposition Rates, Generation Times of Bacteria Adhered to Surface, Time to Double Surface Coverage, and Percent Surface Covered after 2 and 5 h as a Function of Flow Cell Conditions for *Pseudomonas aeruginosa* IFO3455 in Parallel Plate Flow Chamber in 2% Nutrient Broth at 37°C** |
|---|---|---|---|---|
| **Bacterial Adhesion Treatment** | **Mean Deposition Rate (SD)** | **Generation Time (SD)** | **Doubling Time (SD)** | **% Surface Coverage** |
| | (cells cm^{-2} s^{-1}) | (min) | (min) | 2h | 5h |
| Control (bare glass) | 258 (12) | 39 (6) | 61 (4) | 33 | 92 |
| PA preopsonized with IgG* | 120 (36) | 45 (3) | 56 (4) | 15 | 85 |
| Glass surface preadsorbed with IgG | 106 (3) | 44 (6) | 60 (2) | 11 | 65 |
| PA preopsonized + glass preadsorbed with IgG | 136 (22) | 45 (10) | 60 (3) | 25 | 92 |
| PA preopsonized with PA-depleted IgG* | 186 (7) | 43 (4) | 58 (4) | 20 | 90 |

All experiments were performed in triplicate.

*Represents the mean of two separate experiments.
Contact angle analysis

Bacterial contact angles are summarized in Table II and demonstrate that *P. aeruginosa* IFO3455 is an unusually hydrophobic organism. Water contact angles on lawns of this organism approximate 120°, indicative of high nonwetting. Measured water contact angles were probably artifactually high owing to the inevitable roughness of microbial lawns. Scanning electron micrographs of such lawns have been published and show a distinct roughness that may increase contact angles on these hydrophobic surfaces. Consequently, the contact angles presented give an indication of the cell surface hydrophobicity and how it changes with IgG adsorption, but do not lend themselves accurately to further surface thermodynamic analyses. Exposure of bacteria to 0.2 wt % IgG in PBS, followed by copious rinsing as described above, produced a remarkable decrease in bacterial cell-surface hydrophobicity, exhibiting water contact angles of 64°, a trend also reflected in the reduced formamide bipolar probe liquid contact angles. Contact angles measured with lower surface apolar energy methylene iodide and α-bromonaphthalene probe liquids, probing dipolar and apolar cell-surface characteristics were also significantly reduced after treatment with IgG.

XPS

Results for XPS analysis of freeze-dried *P. aeruginosa* samples with or without IgG exposure are shown in Table II. XPS elemental ratios shown for nitrogen, oxygen, and phosphorus each compared with carbon supported significant surface compositional changes after, rather than before, IgG exposure. Specifically, nitrogen contributions increased substantially after IgG exposure, consistent with the adsorption of antibodies (e.g., amide groups) to the bacterial cell wall surfaces. Reductions in oxygen and phosphorus elemental contributions supported these data. Oxygen was generally composed of a higher atomic compositional percent of polysaccharides and peptidoglycans in bacterial cell walls than in proteins. IgG opsonization contributed new oxygen signals unique to the IgG protein layer but attenuated those signals derived from the buried bacterial cell wall components, leading to a net oxygen signal reduction (Table II). Closer examination of the high-resolution O-1s XPS signal at 532 eV produced two distinct oxygen signals, representing two different oxygen species distributions before and after IgG exposure, at 532.2 and 532.8 eV. Table II shows a quantitative breakdown of these contributions attributed to carbonyl oxygens (i.e., from amides, aldehydes, ketones, and esters) at the lower O-1s binding energy, and the ester and alcohol oxygens at higher O-1s binding energies. A significant shift in the relative contributions of each oxygen species to lower binding energy (e.g., to protein carbonyl oxygens) was observed after IgG adsorption. This is consistent with both addition of an overlayer of protein (IgG) and a masking of underlying bacterial cell wall saccharide chemistry. Phosphorus signals, attributed to phospholipids in the bacterial cell membrane,
dropped nearly 50% after IgG exposure, also attenuated by the overlying IgG protein layer.

**DISCUSSION**

In this work, the adhesion of *P. aeruginosa* IFO3455 to glass was studied in a parallel plate flow chamber in the absence and presence of pooled polyclonal human IgG antibodies. This bacterial strain was previously studied with regard to its in vivo virulence and pathogenicity. In addition, recent in vitro data indicate that commercial pooled human polyclonal IgG preparations contain measurable titers and binding activity to slow its flagellar motility and growth. In vitro flow conditions reported here used a documented adhesion buffer (2% NB in PBS) that maintains bacterial metabolic activity while promoting minimal growth. Subsequent bacteria surface-growth dynamics were assessed in full medium (100% NB) that supports bacterial proliferation.

Flow chamber bacterial deposition kinetics observed under different conditions in this study demonstrated that *P. aeruginosa* IFO3455 adherence to glass was repeatedly, significantly reduced in the presence of IgG preadsorbed to the glass surface and by the presence of IgG opsonization of bacteria in the adhesion buffer. Combinations of both IgG exposures did not further decrease initial deposition rates. IgG depleted of *P. aeruginosa*-specific fractions reduced bacteria deposition rates, although this decrease over the control was not nearly as significant as the adhesion reduction observed with undepleted polyclonal IgG. However, the *P. aeruginosa*-specific depleted polyclonal IgG exhibited a drastically reduced, yet small, measurable binding titer against IFO3455 (data not shown). This could explain the reduced but still measurable decrease in surface deposition of bacteria observed with depleted IgG. In addition, nonspecific bacterial binding activity to adsorbed IgG on glass should contribute to this effect. Selected components from nutrient broth likely adsorbed at some undetermined surface composition to form a heterogeneous conditioning film composed of small molecule salts and organics, peptides, proteins, and polysaccharides on the glass surface. The presence of IgG adsorbed to the flow chamber glass surface also reduced the initial deposition rates of intrinsically hydrophobic, nonopsonized *P. aeruginosa*. Because excess nonopsonized IgG was immediately removed from the flow chamber as soon as adhesion buffer flushed it away, *P. aeruginosa* IFO3455 was less able to adhere actively to glass surfaces in the presence of IgG, whether it was presence of IgG, whether it was preadsorbed on the glass surface or opsonized on the cell surface.

Generation times of adhering bacteria in 100% NB were significantly longer for *P. aeruginosa* adhering on glass in the presence of IgG, whether preadsorbed to the flow chamber glass surface or first directly opsonized onto planktonic bacteria before adhesion. Similar to complete IgG, *P. aeruginosa*-depleted IgG extended the generation time of adherent bacteria significantly compared to nutrient broth lacking IgG. These results suggest that some IgG influence on adhesion may be nonspecific. Bacterial adhesion to polyclonal IgG-adsorbed polymer surfaces has been shown to be nonspecific. Visual observations of individual bacteria to determine the generation time suggest that bacteria were physically inhibited from stretching or cell body expansion before separation after their division in the presence of adsorbed immunoglobulins. The time necessary for adhering bacteria to double their surface coverage was comparable between all experiments performed in this study in the absence and presence of IgG (~60 min). These data indicate that whereas initial adhesion of bacteria was influenced by IgG, eventually the log-order proliferation rates of adhering bacteria compensated to equalize all initial conditions over time.

Inhibitory influences of IgG on bacteria postadhesion (i.e., surface generation or doubling times) should have been limited because only the original bacterial population in the flow cell interacted with IgG in solution before rinsing. IgG initially bound to the glass surface remained after rinsing the flow cell, but was expected to undergo time-dependent changes (i.e., denaturation, desorption, exchange with NB). Fractions of IgG initially capable of adhering bacteria may have

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**TABLE II**

**Surface Hydrophobicity and XPS-Determined Elemental Surface Composition Ratios for Bacterial Lawns Composed of *P. aeruginosa* IFO3455 Alone or *P. aeruginosa* IFO3455 Preadsorbed with Polyclonal Human IgG**

<table>
<thead>
<tr>
<th>P. aeruginosa Incubated With</th>
<th>Contact Angle* (degrees)</th>
<th>XPS Elemental Ratio</th>
<th>Oxygen Species (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water Formamide Methylene iodide α-Bromonaphthalene</td>
<td>N/C O/C P/C</td>
<td>O₁ (531.4 eV) O₂ (532.8 eV)</td>
</tr>
<tr>
<td>No IgG</td>
<td>120 55 58 40</td>
<td>0.096 0.347 0.015</td>
<td>36.7 63.3</td>
</tr>
<tr>
<td>Polyclonal IgG</td>
<td>64 38 48 23</td>
<td>0.130 0.280 0.009</td>
<td>49.7 50.3</td>
</tr>
</tbody>
</table>

*Percentage of total XPS O₁s peak centered at 532.2 eV; two Gaussian contributions for two oxygen binding environments.

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29Inhibitory influences of IgG on bacteria postadhesion (i.e., surface generation or doubling times) should have been limited because only the original bacterial population in the flow cell interacted with IgG in solution before rinsing. IgG initially bound to the glass surface remained after rinsing the flow cell, but was expected to undergo time-dependent changes (i.e., denaturation, desorption, exchange with NB). Fractions of IgG initially capable of adhering bacteria may have...
lost this capability over time on the glass surface. Because generation and doubling times were measured in IgG-free 100% NB, new dividing populations of adherent bacteria produced did not necessarily undergo opsonization or IgG-associated effects. Hence, eventual proliferation rates could readily mask initial IgG influences on bacteria over time. Because initial rates of adhesion were higher and generation times were shorter for bacteria in the control, IgG-free condition (more rapid proliferation) but doubling time was comparable to IgG-containing experiments, the desorption rate for generations of proliferating *P. aeruginosa IFO3455* must have been more rapid in the absence of IgG over time. Indeed, desorption rate constants calculated from experimental data supported slightly higher desorption kinetics in the absence of IgG, supporting this contention.

Bacterial ζ potentials remained approximately the same value before and after exposure to IgG, leading to the conclusion that electrostatic properties probably did not contribute significantly to differences observed in the bacteria deposition and surface growth in the different experiments. The pH for immunoglobulin was 6.3–7.3, similar enough to the buffered pH used in these flow experiments to ensure little new surface charge by IgG opsonization. Opsonization of *P. aeruginosa* presumably neutralized, masked or physically covered considerable glyocalix surface area of each bacterium. Antibodies are known to occupy surface areas of approximately 2200 Å²/molecule under close-packed conditions, sufficient area to cover many individual hydrophobic or charged bacterial surface residues. Estimates of 10³ to 10⁴ IgG molecules per opsonized bacterium indicate that substantial surface modification of bacteria can occur by exposure to IgG. Zeta potential measurements indicate that surface charge remained generally unaffected, however.

Contact angle data showing extreme bacterial hydrophobicity before IgG exposure supported an absence of high surface charge density for this strain: Despite roughness, relatively few polar or charged residues must be surface resident to produce these high contact angles. Treatment of data in Table II using known methods provides further insight regarding surface energy. The apolar surface tension component, γ LW, for *P. aeruginosa* is calculated to be approximately 29.7 mJ/m² from the 58° diiodomethane contact angle. This value, combined with the observed aqueous contact angle of 120° for untreated bacterial lawns, leads to an unreasonably extreme surface hydrophobicity (assuming γ H2O = 72.8 mJ/m² at 20°C) for this microbe. Given pronounced surface roughness observed for these lawns, the use of high surface tension probe liquids (e.g., water) is known to produce exaggerated high contact angles on hydrophobic surfaces, precluding more rigorous analysis. By contrast, contact angle data for IgG-coated *P. aeruginosa* are more consistent. Data for water, diiodomethane, and formamide produce values of γ LW = 35.4 mJ/m², γ H2O = 2.4 mJ/m², and γ F = 11.1 mJ/m² for the apolar, Lewis acid, and Lewis base surface tension components, respectively. The appreciably large γ⁺ value suggests that the bacterial lawns are still substantially hydrated when measured, a result seen previously with other bacterial lawns. Other bacteria exhibit more wettable surfaces, although *P. aeruginosa* as a Gram-negative species is generally more hydrophobic.

Two interpretations therefore appear to be likely: (a) opsonization by IgG is sparse, leaving the majority of charge on the original glyocalix and its double layer intact, and the ζ potential, therefore, largely unaffected; or (b) the IgG binding effectively substitutes equivalent charge and double-layer contributions to compensate for masked bacterial surface charge, while contributing more polar protein chemistry producing higher wettability. Based on contact angle and XPS data, plus previous in *vitro* motility and in *vivo* infection efficacy data known for this virulent pathogen strain in IgG-binding scenarios, it appears likely that the latter explanation is more appropriate. Because pooled IgG is known to exhibit considerable measurable in *vitro* and in *vivo* influence on *P. aeruginosa*, IgG opsonization capacity must be great. This effect is consistent with contact angle and XPS data (Table II) showing significant quantitative changes in bacterial physical and chemical compositional properties after IgG exposure, consistent with IgG binding.

As described above, *P. aeruginosa* IFO3455-specific IgG buries glyocalix surface chemistry under the IgG hydrophilic protein hydration shell, leading to improved opsonized aqueous wettability. However, IgG-specific binding cannot be solely responsible for the entire observed reduction in water contact angles because specific opsonizing IgG populations are a relatively miniscule fraction of the complete IgG pool. The large observed reductions in bacterial surface hydrophobicity for *P. aeruginosa* IFO3455 (Table II) incubated with IgG could be more logically be attributed to nonspecific adherence of hydrophilic IgG components to the bacterial surface, ultimately leading to reduced water contact angles. Substantial nonspecific opsonization is consistent with long-standing analysis in the literature for IgG.

In summary, this study demonstrates that human polyclonal IgG influences the deposition and surface growth dynamics of *P. aeruginosa* IFO3455 in a parallel plate flow chamber in several conditions of relevance to device-centered infection. Implant-associated or biomaterial-centered infection is phenomenologically linked to bacteria–surface interactions. Although molecular aspects of adhesion mechanisms and methods to influence them are not well documented, technolo-
gies directed at reducing initial bacterial surface adhesion events continue to develop. The use of selected antibodies against adhesive bacterial components has allowed new insight into both mechanisms of surface-mediated virulence as well as development of new antimicrobial approaches useful for fighting infection in implant sites. However, aside from such customized or designer antibody approaches, the ability to address more generic issues surrounding biomaterial-centered infection effectively will rely on broader understanding of bacterial–implant surface interactions in serum, plasma, and physiological milieu. The results reported here attempt to address the interactions of bacteria and surfaces with pooled polyclonal IgG as a step toward understanding more complex aspects of biomaterial-centered infection.

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