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Adsorption of Fibronectin onto Polymethylmethacrylate and Promotion of *Staphylococcus aureus* Adherence

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Recent data suggest that fibronectin may favor *Staphylococcus aureus* infection by promoting attachment to either injured tissues or implanted foreign bodies. We studied the quantitative adsorption of fibronectin onto polymethylmethacrylate (PMMA) cover slips by using a ^{125}I-labeled preparation of the purified plasma glycoprotein. Fibronectin in buffer solutions showed a high affinity to PMMA coverslips. Adherence of *S. aureus* Wood 46 was studied on PMMA pre-exposed to fibronectin, using an assay specifically adapted to the cover slip model. Whereas *S. aureus* adherence in an albumin-containing buffer was $\leq 10^3$ CFU on control uncoated cover slips, adherence in the same medium increased up to maximum of 7.7×10^4 CFU on cover slips preincubated in a solution of fibronectin (125-μg/ml). At intermediate fibronectin concentrations, bacterial adherence was a linear function of both the quantity in solution and of the quantity adsorbed on the PMMA cover slips. The presence of human serum proteins, as represented by a fibronectin-depleted pool, essentially prevented adsorption of radiolabeled fibronectin on PMMA and subsequent bacterial adherence on the cover slips. Precoating of PMMA with denatured collagen resulted in increased fibronectin adsorption on PMMA, even in the presence of serum proteins, and *S. aureus* adherence was optimal on such surfaces. Collagen may therefore play a role as a cofactor contributing to *S. aureus* adherence onto fibronectin-coated substrata or foreign bodies.

Fibronectin (FN) is a large glycoprotein found in plasma, other body fluids, on the surface of various cell types, and as a constituent of the extracellular matrix of connective tissues (16, 31, 32, 41). FN is involved in a number of essential cell functions such as cell to cell interactions, cell attachment to surfaces, and cell shape control (11, 16, 32, 33, 41). Unrelated to these functions, the occurrence of FN-binding reactions with gram-positive bacteria suggested a role for this glycoprotein in some instances of microbial infections, in particular those involving *Staphylococcus aureus* (4, 8, 24, 26, 33, 37–40, 42–44). Whereas *S. aureus* binding to FN may promote infection in tissues (37, 44), this protein may alternatively function as an opsonin in plasma. Modulation of this opsonic activity by FN bound to *S. aureus* is not clearly understood at the present time (4, 26, 39, 42, 43).

Several clinical (9, 21, 45) and experimental (5, 17, 34) observations made on implanted foreign bodies, such as shunts, valves, or sutures, or on inserted catheters (27) have documented their high susceptibility to staphylococcal infections. To analyze the host factors favoring infections of implants, we have developed an experimental model of foreign body infection, using polymethylmethacrylate (PMMA) tissue cages subcutaneously implanted into guinea pigs (48). This model allowed us to analyze both the fluid phase and the cellular phase (47, 48) of sterile or infected interstitial fluid bathing the foreign body. Experimental infections could be provoked by 10^3 CFU of *S. aureus* Wood 46, a strain which is devoid of protein A (23), whereas in the absence of foreign material, even 10^8 CFU were noninfective.

To study the adhesive properties of the foreign material, we have inserted PMMA cover slips to the interior of tissue cages implanted for a period of 4 weeks into the guinea pig (P. E. Vaudaux, R. Susuki, F. A. Waldvogel, J. J. Morgenthaler, and U. E. Nydegger, J. Infect. Dis., in press). Using an in vitro assay measuring bacterial adherence to PMMA cover slips in the presence of serum proteins, we have found that explanted cover slips were more adhesive for *S. aureus* Wood 46 than unimplanted ones. Enhanced bacterial adherence on explanted cover slips was shown to be caused by fibronectin deposited on the foreign body surface, since it was inhibited in a dose-related way by specific antifibronectin antibodies (Vaudaux et al., in press). The present report shows an alternative approach for characterizing the interaction of *S. aureus* with purified FN adsorbed on PMMA, using a simplified, well-controlled in vitro assay.

(This study was presented in part previously [P. Vaudaux, J. J. Morgenthaler, U. Nydegger, F. A. Waldvogel, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 1013, 1983].)

MATERIALS AND METHODS

Chemicals and materials. Phosphate-buffered saline solutions without divalent cations (PBS) or with 1 mM Ca^{2+} and 0.5 mM Mg^{2+} were purchased from GIBCO Bio-Cult, Glasgow, Scotland. Purified trypsin was purchased from Sigma Chemical Co., St. Louis, Mo.

FN was purified from pooled human plasma by affinity chromatography on gelatin-Sepharose 4B (6); plasma proteins which bound unspecifically to the matrix were first removed with a column packed with unmodified Sepharose (46). Plasma proteins weakly bound to the gelatin-Sepharose were washed off with 1 M urea. Thereafter, FN was eluted with 4 M urea, concentrated by ultrafiltration and dialyzed against PBS. Purified FN was shown to be free of contaminants by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (25). The concentration of purified FN was measured spectrophotometrically by using an absorbance value at 280 nm of 1.28 (32).

FN was labeled with ^{125}I by the lactoperoxidase method

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(28) and reisolated from the incubation mixture by chromatography on gelatin-Sepharose. Radiolabeled FN (specific activity, 3×10^6 to 6×10^6 cpm/μg) was stored at −70°C at a concentration of 10 μg/ml in Tris-HCl buffer, pH 7.2, supplemented with 1 mg of human serum albumin per ml and was used within a month. FN-depleted serum (dFN-serum) was prepared by the method of Zardi et al. (46). The removal of FN was checked by laser nephelometry (30). Fibrinogen was purified from human plasma as described in detail elsewhere (Vaudaux et al., in press). Fibrinogen showed <0.05% contamination by FN, as checked by laser nephelometry (30). Collagen purified from rat tail tendon was kindly provided by J. M. Dayer, Immunology Division, Geneva University Hospital, Geneva, Switzerland. Denatured collagen was prepared by heating at 70°C for 20 min.

Cover slips (1 by 1 cm) made of PMMA were cleaned with 100% ethanol and sterilized by heating at 120°C for 30 min. Depending on the experimental protocol, they were either used directly in the FN-binding assay, or alternatively, they were precoated with heat-denatured collagen by incubation for 60 min at 20°C in a 1-ml solution of 10 mg of collagen per ml in 0.5 M acetic acid. After being rinsed with PBS, cover slips were ready for use in the FN-binding assay. The quantity of denatured collagen coating the PMMA cover slips was estimated to 1.92 μg (±0.12, mean + standard error of the mean of nine determinations), using a preparation of collagen which was radiolabeled with ^{125}I by the lactoperoxidase method (28).

Bacterial strain. *S. aureus* Wood 46, which is devoid of protein A (23), was used for this study. A total of 2×10^7 CFU from an overnight culture in Mueller-Hinton broth was incubated with 100 μCi [*methyl*-^3H] thymidine in 1 ml of Mueller-Hinton broth and grown for 3 h at 37°C up to 1×10^8 to 2×10^8 CFU/ml. After removal of the unbound radioactivity by two centrifugations, performed at 3,000 × *g* for 10 min, the labeled strain was suspended in 1 ml of 0.15 M NaCl.

Adsorption of FN on PMMA. Each PMMA cover slip, whether coated with collagen or uncoated, was immersed for 60 min at 37°C in a 1-ml solution of FN. The 60-min period of incubation was selected after time-course studies had shown FN adsorption to be completed at any time exceeding 30 min. Decreasing concentrations of FN (from 500 to 16 μg/ml) were prepared from a stock solution (1,000 μg/ml) containing 5×10^5 to 5×10^6 cpm of ^{125}I-labeled FN as a tracer. FN concentrations ranging from 16 to 1 μg/ml were prepared from a stock solution (16 μg/ml) also containing 5×10^5 to 5×10^6 cpm of ^{125}I-labeled FN as a tracer. At the end of the adsorption period, the fluids containing unbound FN were drained and counted for radioactivity. Cover slips were then rinsed with 2 ml of PBS for 10 min at 20°C. Rinsing fluids contained consistently ≤3% of the radioactivity introduced at the beginning of the FN-binding assay, which made further rinsing unnecessary. After removal of the rinsing fluids, carefully drained cover slips were transferred into clean polystyrene tubes and counted for radioactivity. For each FN concentration tested, the recovery of the total radioactivity introduced was controlled by summing up radioactivity of unbound FN with that of the rinsing fluid, the PMMA cover slip, and FN adsorbed on the wall of the drained polypropylene tube. Total recovery differed by no more than ±10% from the expected value.

FN adsorption from serum solutions, either on PMMA or collagen-PMMA cover slips, was tested by using dFN-serum reconstituted with known amounts (500 to 1 μg/ml) of purified FN, including 5×10^5 to 5×10^6 cpm of ^{125}I-labeled FN as a tracer. The next part of the binding assay was processed as described above.

FN-promoted adherence of *S. aureus* Wood 46. Promotion of *S. aureus* Wood 46 adherence by FN adsorbed on either PMMA or collagen-PMMA was studied after an adsorption period of 60 min at 37°C with decreasing concentrations (500 to 1 μg/ml) of unlabeled FN. After being rinsed, FN-coated PMMA cover slips were incubated with 4×10^6 CFU/ml of labeled *S. aureus* Wood 46 for 60 min at 37°C. The adherence medium was PBS with 1 mM Ca^{2+} and 0.5 mM Mg^{2+} supplemented with 5 mg of human serum albumin per ml which prevented nonspecific adherence of *S. aureus* to PMMA cover slips (Vaudaux et al., in press). At the end of the attachment period, the fluids containing unbound bacteria were drained and the PMMA cover slips were transferred into new tubes containing 1 ml of 0.15 M NaCl (saline). This transfer procedure minimized the carry over of fluid contaminated by unbound bacteria. After 5 min at 20°C, a second wash was performed with 1 ml of fresh saline for 30 min at 20°C. Finally, PMMA cover slips were transferred into new tubes containing 1 ml of PBS and incubated for another 30 min at 37°C with agitation. This last procedure released <20% of the bacteria from the cover slips. Thereafter, drained slides were immersed into scintillation fluid (Dimilume, Packard Instrument Co., Inc., Rockville, Md.), and their radioactivity was counted in a liquid scintillation counter (Beckman LS-3145 T). The counts per minute were then multiplied by the CFU/counts per minute ratio of the radiolabeled culture of *S. aureus* Wood 46 (average ratio, 43 ± 14 CFU/cpm, $n = 40$). This allowed quantification of the number of bacteria adherent to PMMA cover slips.

Statistics. Linear regression, one-way variance analysis and unpaired *t*-test were performed by using specific programs adapted to a computer HP-41C (Hewlett-Packard, Geneva, Switzerland).

RESULTS

Adsorption of FN on uncoated PMMA cover slips and its effects on *S. aureus* Wood 46 adherence. Adsorption isotherms (37°C) of purified FN on uncoated PMMA are shown in Fig. 1A. The presence of dFN-serum markedly influenced the adsorption process. Whereas FN binding to PMMA was a dose-dependent, saturable process in unsupplemented PBS, addition of dFN-serum to the adsorption medium prevented such FN binding to the cover slips (Fig. 1A).

In Fig. 1B, quantitative adherence of *S. aureus* Wood 46 is shown as a function of prior FN adsorption on PMMA. Whereas *S. aureus* adherence was $<10^3$ CFU on cover slips unexposed to FN, adherence was significantly promoted by FN adsorbed on PMMA from unsupplemented PBS solutions. The dose-response curve of bacterial adherence followed rather closely that of FN adsorption to PMMA. Control experiments verified that FN adsorbed on PMMA remained firmly bound to the substrate with 10% release occurring during the *S. aureus* adherence assay. When FN adsorption on PMMA had been performed in PBS–dFN-serum, adherence of *S. aureus* Wood 46 was barely detectable (Fig. 1B). This was related to the poor adsorbance properties of soluble FN onto the uncoated PMMA in the presence of dFN-serum (Fig. 1A).

The specificity of *S. aureus* Wood 46 binding to FN adsorbed on PMMA was studied by three different approaches. (i) Purified FN was heated at 90°C for 5 min before adsorption on PMMA at a concentration of 125 μg/ml for 60 min. The promoting effect of adsorbed FN on subsequent *S. aureus* adherence was found to be destroyed by heating,

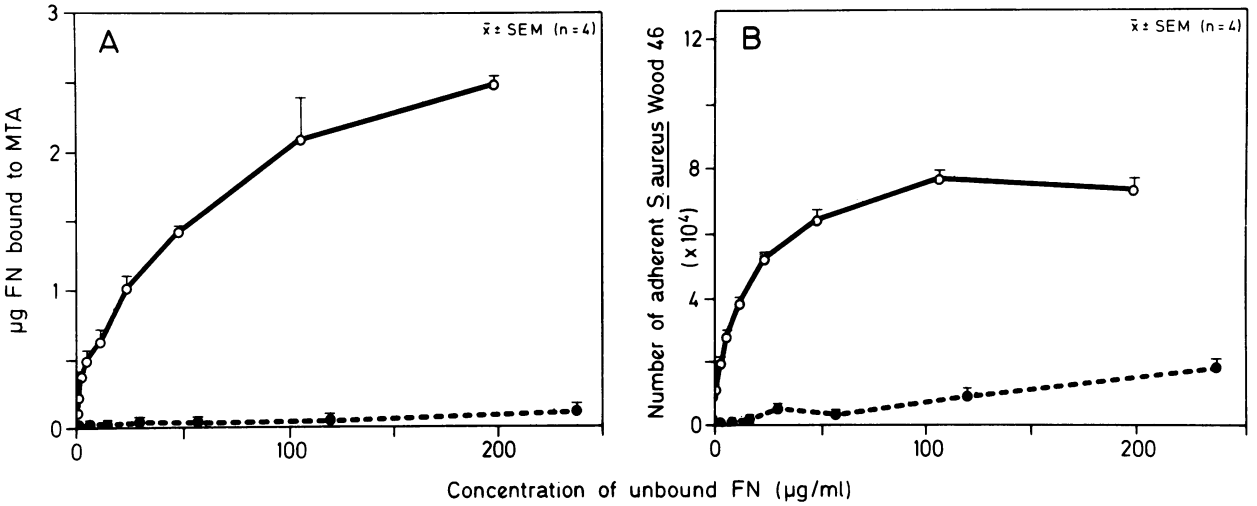

FIG. 1. FN adsorption (A) to uncoated PMMA cover slips and the number of adherent *S. aureus* Wood 46 (B) as a function of unbound FN. Symbols: ○, PBS medium; ●, dFN-serum–PBS medium.

since bacterial adherence was 10^3 CFU per cover slip. (ii) FN adsorbed on PMMA cover slips from a solution containing 125 µg of protein per ml was susceptible to the proteolytic action of purified trypsin. After digestion for 20 min at 37°C with 10 µg of trypsin per ml, 74% of radiolabeled FN was released into the medium. Residual FN on PMMA slightly promoted adherence of *S. aureus* Wood 46, which was only 16% of that promoted by control untrypsinized FN-coated cover slips. (iii) FN interaction *S. aureus* Wood 46 was unaffected by the presence in the incubation medium of gelatin (1 mg/ml), heparin (500 µg/ml), or fibrinogen (500 µg/ml) during the bacterial adherence assay (Table 1). Furthermore, control cover slips not coated with FN bound $<10^3$ CFU of *S. aureus* Wood 46, whether in the presence or absence of either gelatin or heparin (Table 1). In contrast, fibrinogen added to the incubation mixture slightly stimulated *S. aureus* Wood 46 adherence onto uncoated cover slips ($P < 0.05$; Table 1). Yet, such adherence was only 12% of that recorded on the FN-coated cover slips.

Effects of precoating PMMA cover slips with denatured collagen. In Fig. 2A adsorption isotherms are shown for the binding of FN to collagen-coated PMMA cover slips in either PBS or PBS–dFN-serum. Heat-denatured collagen was preferred to native collagen that has varying, type-specific binding properties for FN (7, 19). Collagen PMMA cover slips bound FN in a dose-related way, both in the absence or presence of FN serum (Fig. 2A). However, FN adsorption from PBS–dFN-serum on collagen-PMMA was reduced to ca. 20 to 30% that recorded in unsupplemented PBS (Fig. 2A). Yet FN adsorption in the presence of dFN-serum was nevertheless 10 times higher on collagen-PMMA than it was on uncoated PMMA (Fig. 1A and 2A). In Fig. 2B quantitative adherence of *S. aureus* Wood 46 is shown as a function of prior FN adsorption on collagen-PMMA in either unsupplemented PBS or PBS–dFN-serum. Irrespective of the adsorption medium used, collagen-PMMA cover slips exposed to FN bound equivalent numbers of bacteria for equivalent concentrations of unbound FN in either adsorption medium (Fig. 2B). Collagen-coated PMMA cover slips unexposed to FN bound a low number of bacteria (2×10^3 to 4×10^3 CFU, $n = 4$).

Langmuir adsorption isotherms of FN bound to PMMA/collagen-PMMA. To analyze in further detail the data of FN adsorption on either uncoated or collagen-coated PMMA, we used a model of saturation kinetics (10, 22) derived from the Langmuir equation. In this model, the ratio of unbound/bound molecules is plotted versus the concentration of unbound molecules. In a monolayer situation, this plot should yield a straight line if all binding sites are independent and have identical affinities for the adsorbate molecules. Furthermore, there should be no interaction between the adsorbate molecules either in the solution or on the surface of the adsorbent. The values on the slope and intercept of the straight line provide means of estimating the maximal number of adsorption sites and the affinity of the binding process. At the lower concentrations of unbound FN, the Langmuir-type adsorption model fitted the experimental results of FN adsorption performed in PBS, on either uncoated or collagen-coated PMMA, as shown in Fig. 1 and 2. The values of the derived adsorption parameters were 1.55×10^{-6} µM on uncoated PMMA and 1.21×10^{-6} µM on collagen-coated PMMA for the maximum number of binding sites on either substrate. These values imply that the maximal amount of FN bound as a monolayer to the cover slips is 0.68 µg on uncoated PMMA and 0.53 µg on collagen-MTA. The affinity constants were 2.37×10^5 ml/µM on uncoated PMMA and 7.44×10^4 ml/µM on collagen-PMMA.

Effectiveness of FN bound under various conditions in promoting bacterial adherence. Results of bacterial adherence to FN adsorbed on either collagen-coated or uncoated

TABLE 1. Absence of competition of *S. aureus* Wood 46 binding to FN-PMMA cover slips by various site specific FN ligands[a]

Incubation mixture addition[b]	No. of adherent[c] *S. aureus* Wood 46 (CFU × 10^4)	
	FN-coated cover slips	Control cover slips not coated with FN
None	5.61 (±0.20)	<0.03
Gelatin (1,000 µg/ml)	7.04 (±0.61)	<0.03
Heparin (500 U/ml)	6.95 (±0.36)	<0.03
Fibrinogen (500 µg/ml)	7.80 (±0.36)	0.90 (±0.35)

[a] PMMA cover slips were coated with FN by preincubating for 60 min at 37°C with 125 µg of native FN per ml in PBS.

[b] For the adherence assay each incubation mixture contained 4×10^6 CFU of *S. aureus* Wood 46 in 1 ml of PBS with 1 mM Ca^{2+} and 0.5 mM Mg^{2+}-human serum albumin supplemented with the indicated components. See the text for the experimental procedure of bacterial adherence.

[c] Mean ± standard error of the mean of five determinations.

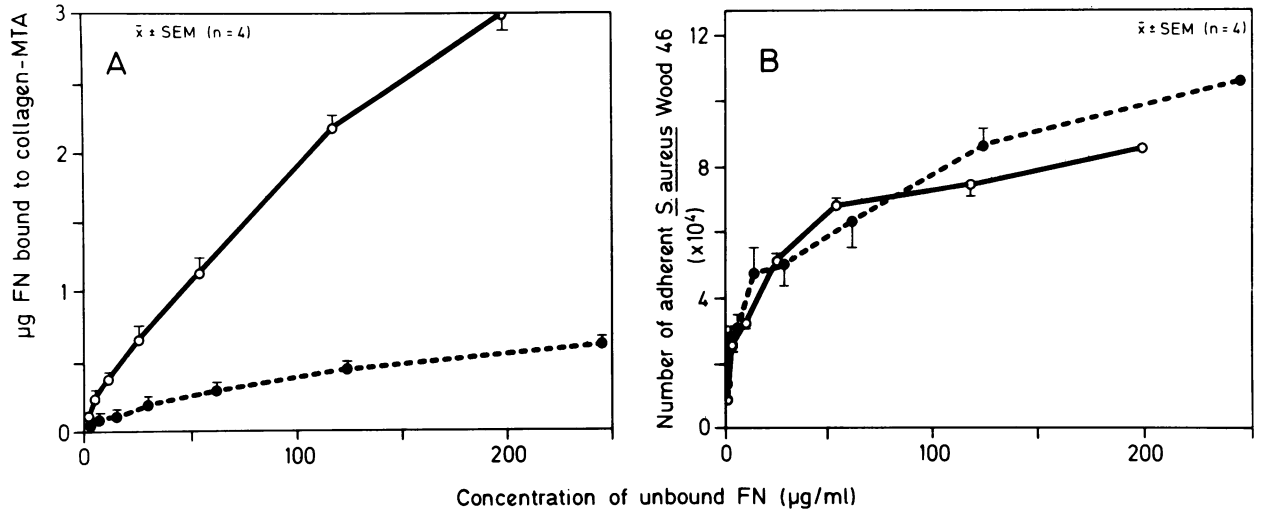

FIG. 2. FN adsorption (A) to collagen-coated PMMA cover slips and number of adherent *S. aureus* Wood 46 (B) as a function of unbound FN. Symbols: ○, PBS medium; ●, dFN-serum–PBS medium.

PMMA in the presence or absence of dFN-serum suggested varying degrees of biological activity for FN adsorbed under each condition. Accordingly, data from Fig. 1 and 2 were plotted to analyze the direct relationship between the quantity of FN adsorbed on the cover slips under various conditions and the number of adherent bacteria promoted by each kind of adsorption. Three different dose-response curves were thus obtained (Fig. 3). (i) FN bound to PMMA in unsupplemented PBS promoted bacterial adherence in a strictly proportional way, from 0.10 to 0.62 µg of adsorbed FN. (ii) A parallel dose-response curve was obtained with FN adsorbed on collagen-PMMA in unsupplemented PBS, in a range of 0.06 to 0.65 µg of bound FN; however, FN bound to collagen-PMMA was more efficient than equivalent amounts on uncoated PMMA ($P < 0.01$, $n = 8$). (iii) Finally, bacterial adherence to FN adsorbed on collagen-PMMA in the presence of dFN-serum was two to three times higher than adherence recorded under the previously described conditions of FN adsorption ($P < 0.01$, $n = 8$).

DISCUSSION

Infection of prosthetic devices are characterized by the high infective power of a low amount of inoculum, mostly staphylococci (5, 17, 34, 48). Foreign body infections are not cured by antimicrobial treatments considered to be effective in the absence of foreign material (9, 21, 27, 45). In a previous study, we have postulated that local host defense mechanisms were ineffective. Using an experimental model of foreign body infection, we could demonstrate that the local tissue cage polymorphonuclear leukocytes were unable to kill *S. aureus* Wood 46 used as the infecting organism (48). These findings were confirmed in a more recent study which showed multiple defects in the polymorphonuclear leukocytes drawn from the tissue cage fluid (47).

Another important characteristic of foreign body infections is the extensive growth of bacterial microcolonies on the surface of such implants: these bacteria are highly adhesive to the synthetic materials and are often embedded in a thick matrix of exopolysaccharides (2, 3, 35). Such elegant observations were, however, not adequate for understanding the early steps of bacterial adherence leading to their permanent colonization on the implants. Therefore, we have developed an in vitro adherence assay measuring *S. aureus* Wood 46 adherence using cover slips made of PMMA (Vaudaux et al., in press). An important finding of our adherence studies was the inhibition by blood proteins of staphylococcal adherence to PMMA cover slips when uncoated with fibronectin. *S. aureus* Wood 46 adherence to PMMA was prevented by the presence of blood proteins of various origins and composition, namely human or guinea pig serum and plasma, guinea pig tissue cage fluid, or purified human albumin. Cover slips explanted from the uninfected animal model showed enhanced adherence for *S. aureus* Wood 46 when adherence was tested in the presence of either serum or purified albumin (Vaudaux et al., in press). Fibronectin was shown to be deposited on such explanted cover slips by immunofluorescent studies. Furthermore, the causal relationship between the increased adhesive properties of explanted cover slips and the presence on their surface of fibronectin was demonstrated by

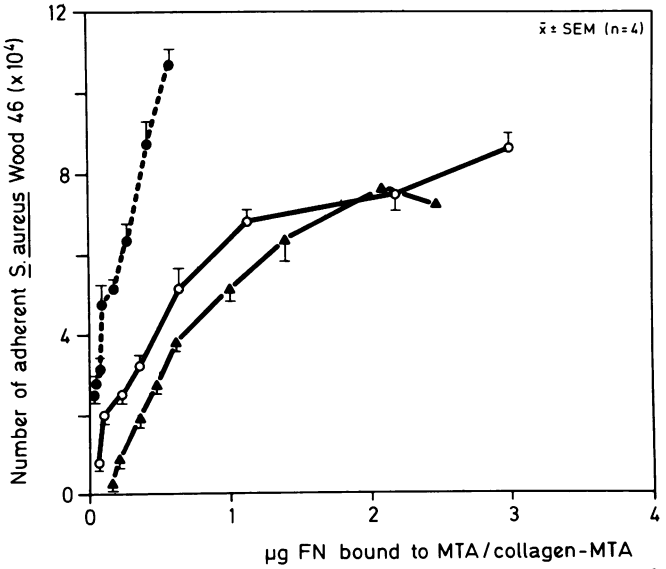

FIG. 3. Number of adherent *S. aureus* Wood 46 as a function of the FN bound to either uncoated PMMA cover slips (▲) or to collagen-coated PMMA cover slips (○,●). Symbols: ▲ and ○, PBS medium; ●, dFN-serum–PBS medium.

inhibition studies: affinity-purified anti-fibronectin antibodies applied to the explanted cover slips inhibited *S. aureus* Wood 46 adherence to such cover slips in a dose-related way. This inhibition reached 80% at antibody concentrations of 32 μg of protein per ml (Vaudaux et al., in press).

Comparative results of *S. aureus* adherence to either unimplanted or explanted cover slips afforded an apparent contradiction: plasma fibronectin, which was present in the human or guinea pig serum samples tested at an approximate concentration of 30 μg per ml of 10-fold diluted serum, was nevertheless unable to promote *S. aureus* Wood 46 adherence onto control PMMA cover slips not coated with FN. To solve this paradox, we found it necessary to study separately adsorption of FN from serum solutions onto PMMA and the subsequent biological effects of adsorbed FN on *S. aureus* Wood 46 adherence. FN adsorption from complex protein mixtures such as serum was performed after sequential depletion of endogenous plasma FN and serum reconstitution with purified radiolabeled FN. Under the latter conditions, FN adsorption on PMMA cover slips was inhibited by 98%, when compared to FN adsorption on PMMA cover slips in the absence of serum proteins. The quantity of FN adsorbed on PMMA in the presence of serum proteins was indeed too low to promote a significant adherence of *S. aureus* Wood 46. Previous studies had shown also the decreased affinity of FN for various hydrophobic surfaces, in the presence of serum proteins (12, 13, 18). Such a serum-mediated inhibition of FN binding to a wide range of plastic materials may be due to their common hydrophobic surface properties (18).

When serum proteins were omitted from the reaction mixture, solutions of purified plasma FN showed a considerable affinity for PMMA cover slips. FN adsorption and promoted bacterial adherence were clearly dose-related processes. Furthermore, FN adsorption onto PMMA was shown to be a saturable process, and as far as the concentration of unbound FN in unsupplemented PBS did not exceed 16 μg/ml, FN adsorption followed a Langmuir type of adsorption isotherm. Despite the apparent complexity of our adsorption assay, our estimates of maximal binding of FN as a monolayer, either on PMMA (0.34 $\mu g/cm^2$) or on collagen-PMMA (0.27 $\mu g/cm^2$), are in close agreement with a previous report (12), showing a maximum level of 0.32 μg of FN per cm^2 of a nonwettable polystyrene petri dish. Our data are also compatible with a close-packing model of 4.4×10^{11} FN molecules per cm^2 as computed by Grinnell and Feld (12).

Therefore, we believe that when FN was adsorbed in quantities exceeding 0.6 μg on each cover slip, this glycoprotein was packed as a multilayer on either uncoated or collagen-coated PMMA. Another characteristic of our experimental system was the strictly quantitative relationship observed between adherence of *S. aureus* Wood 46 and the amount of FN deposited on PMMA, although each of three different incubating conditions produced a specifically different dose response (Fig. 3). Bacterial adherence may be an additional sensitive, quantitative assay of one of the multiple biological activities of FN. The binding activity of FN adsorbed on PMMA was destroyed by either preheating of the purified protein or proteolytic digestion with trypsin. A previous report has shown that *S. aureus* adherence occurred on the amino-terminal region of the FN molecule (33), which is quite distinct from either the gelatin or heparin-binding sites as well as from the attachment site for eucaryotic cells (11, 14–16, 19, 20, 24, 29, 31–33, 36, 38, 40–42). Under our assay conditions, pretreatment of FN-coated PMMA cover slips with high concentrations of gelatin, heparin, or fibrinogen did not reduce the subsequent adherence of *S. aureus* to FN-PMMA cover slips, confirming the specific site of adhesion for *S. aureus* Wood 46.

Another important finding of our study was the improved biological activity of FN adsorbed onto cover slips after collagen pretreatment of the PMMA surfaces. The most significant differences in the biological activity of adsorbed FN were recorded when the lowest amounts of FN covered the cover slips (Fig. 3). Whereas a minimal amount of 100 ng of FN adsorbed from PBS solutions was required on uncoated PMMA cover slips to promote a significant increase in bacterial adherence, the same amount adsorbed on collagen-PMMA promoted bacterial adherence that was already five times larger than that on uncoated PMMA. The suboptimal activity of the FN deposited on the uncoated PMMA, in contrast to that of FN adsorbed on collagen-PMMA, may be explained by the adsorption studies of Grinnel and Feld, who suggested the occurrence of significant changes in the conformation and biological activity of FN after its adsorption on hydrophobic surfaces at a low concentration in buffer (12, 13). These changes were suggested by the reduced binding of antifibronectin antibodies (13) by adsorbed FN and also by a reduced promoting effect of the adsorbed product on the fibroblast spreading (12, 13). These observations suggest that for adsorption studies performed in unsupplemented PBS and in the absence of collagen a significant proportion of FN molecules have been inactivated by adsorbing on PMMA as a monolayer. Further increase in the attachment of *S. aureus* cells in situations of multilayer formation may be explained by the deposition of FN molecules devoid of direct contact with PMMA and thus less sensitive to inactivation by the hydrophobic surface. In contrast to nonphysiological substrata, FN binds to collagen in a specific way (18–20). Such binding involves on one side a specific sequence within the collagen molecule, which contains the mammalian collagenase cleavage site (19, 20), and on the other side one specific collagen-binding site in the FN molecule (29).

Thus, our data are in agreement with the concept that FN molecules adsorbed on collagen-coated PMMA are in some way protected from inactivation due to the hydrophobic surface of the synthetic material (18). Detailed understanding of FN interaction with either uncoated or collagen-coated PMMA will require the use of appropriate physicochemical techniques. A further complexity of the biological activity of adsorbed FN with regard to *S. aureus* attachment concerns the multiple interactions which are likely to occur for a single bacterium with several adsorbed FN molecules. A recent study has shown the number of FN-binding sites on *S. aureus* to exceed 100 per bacterium (40). We do not know how many of these multiple bonds were required to protect bacteria from detachment during the washing steps of the adherence assay.

The in vivo impact of our in vitro study may be summarized as follows. We have found experimental conditions for FN adsorption on a synthetic material, as represented by PMMA, which are either nonpermissive or permissive with regard to *S. aureus* attachment and subsequent colonization of the implant. (i) In the absence of collagen on PMMA, FN is essentially prevented from adsorbing on such a surface by competing effects of other serum proteins such as albumin (12). (ii) In contrast, the presence of collagen on the surface of PMMA allows for the selective adsorption of FN from the complex mixture of serum proteins. Our in vitro model with denatured collagen adsorbed on PMMA in a random configuration gives an oversimplified view of the in vivo environment of foreign bodies. Microscopic observations of PMMA

cover slips explanted from the guinea pigs have shown on their surface cellular and fibrillar material organized as an extracellular matrix (Vaudaux et al., in press). That such material may include sulfated proteoglycans in addition to collagen and fibronectin is suggested by other reports on the pericellular matrix of the connective tissues (15). Furthermore, fibroblasts may contribute by their own protein synthesis machinery to the deposition of fibronectin on the extracellular matrix coating the foreign body (13, 15).

Thus, at least two independent pathways may account for FN adsorption on collagen-coated foreign bodies. One involves the trapping of exogenous plasma FN from the extracellular fluid bathing the implant; the other pathway implies endogenous synthesis and deposition of a cellular type of FN by cells colonizing the foreign bodies. Use of monoclonal antibodies which bind preferentially to cellular rather than plasma FN (1) might be helpful for understanding the contribution of either type of FN to foreign body infection.

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