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Attachment of *Staphylococcus aureus* to Polymethylmethacrylate Increases Its Resistance to Phagocytosis in Foreign Body Infection

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The mechanisms responsible for the development of a pyogenic infection (most commonly due to staphylococci) in the vicinity of an implanted foreign body have been studied recently by several investigators. Thus, we have been able to demonstrate that the phagocytic function of residential polymorphonuclear leukocytes (PMN) is deficient in the presence of a foreign body. Others have shown that in the presence of foreign surfaces, microorganisms produce extracellular amorphous material, the pathogenic role of which is still to be defined. In the present study we use a novel assay system to demonstrate that *Staphylococcus aureus* Wood 46, after attachment to polymethylmethacrylate (PMMA), shows increased resistance to the phagocytic-bactericidal action of normal PMN. The first step of this assay involves the reproducible attachment of [^{3}H]thymidine-labeled bacteria to PMMA cover slips. During the second step, attached bacteria were exposed to guinea pig peritoneal exudate PMN. In the third and final step, attached *S. aureus* cells were removed from the cover slips using a procedure harmless to the bacteria. The extent of bacterial detachment was estimated by radioactive counts and their viability by standard colony counts. Whereas bacteria that were attached artificially and rapidly by centrifugation and immediately exposed to PMN were killed in the phagocytic assay, bacteria adhering spontaneously to the cover slips for a prolonged period of time were more resistant to the killing action of the phagocytes. The spontaneous adherence of *S. aureus* to PMMA renders it poorly susceptible to the killing action of PMN.

Infections of prosthetic devices are usually due to staphylococci and are characterized by (i) the high infective power of a low inoculum, (ii) the poor response to antibiotic treatments considered effective in the absence of foreign material, (iii) their limited spread to the tissues in immediate contact with the implanted foreign body, and (iv) the spontaneous cure of such infections after the removal of the prosthetic materials. These observations (6, 11, 14, 15) suggested that host defense mechanisms were locally ineffective against infective microorganisms colonizing the implants. Recent observations have given support to such a hypothesis. In a recently developed experimental model of foreign body infection using polymethylmethacrylate (PMMA) tissue cages implanted subcutaneously into guinea pigs, we showed that local tissue cage polymorphonuclear leukocytes (PMN) were unable to kill *Staphylococcus aureus* Wood 46 used as the infecting organism (23, 24). In this model, experimental infections could be produced by 10^3 CFU of *S. aureus* Wood 46, a strain devoid of protein A (12), whereas in the absence of foreign material even 10^8 CFU were noninfective (24).

Another important characteristic of foreign body infections is the extensive growth of bacterial microcolonies on the surface of such implants. These bacteria are highly adherent to the synthetic materials and are often embedded in a thick matrix of polysaccharides or glycoproteins (20), designated as either slime (1, 2, 16–18) or glycocalyx (3–5). It has been postulated based on morphological evidence that adherent bacteria embedded in such polysaccharide-containing components (3–5, 19) were protected from efficient phagocytosis and killing by the PMN. Such a hypothesis has yet to be confirmed by appropriate functional assays. This paper describes the adaptation of a phagocytic assay to evaluate the susceptibility to PMN of *S. aureus* cells after their adherence to PMMA standardized surfaces. The reliability of such an assay depends on the following requirements: (i) the development of a well-defined adherence assay and (ii) a harmless extraction procedure to remove *S. aureus* from PMMA at the end of the phagocytic assay, allowing accurate estimates of bacterial susceptibility to phagocytosis. Using this new assay, we demonstrate that *S. aureus* attaching spontaneously to PMMA is poorly susceptible to the PMN bactericidal action, as opposed to fluid phase staphylococci.

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MATERIALS AND METHODS

Chemicals and materials. Phosphate-buffered saline (PBS) solutions with 1 mM Ca^{2+} and 0.5 mM Mg^{2+} were purchased from GIBCO Ltd., Paisley, Scotland. Partially purified trypsin, showing 1% contamination with chymotrypsin, was purchased from Serva-Feinbiochemica, Heidelberg, Federal Republic of Germany, and Triton X-100 was purchased from E. Merck AG, Darmstadt, Federal Republic of Germany.

Cover slips (1 by 1 cm) made of PMMA were cleaned with 100% ethanol and sterilized by heating at 120°C for 30 min (21). [^{3}H]thymidine and [^{35}S]methionine were purchased from Amersham, Buckinghamshire, England.

Bacterial strain. *S. aureus* Wood 46, which is devoid of protein A (12), was used for this study. A total of 8×10^7 CFU from an overnight culture in Mueller-Hinton broth was incubated with 200 μCi of [*methyl*-^{3}H]thymidine in 4 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 4 ml of 0.15 M NaCl.

Preparation of guinea pig serum and PMN. Serum was prepared from native blood obtained by cardiac puncture and stored at −70°C as previously described (24). PMN were

separated from glycogen-induced acute peritoneal exudates as previously described (24).

Attachment of *S. aureus* to PMMA cover slips. All manipulations were performed in 16-ml Falcon polyethylene tubes (17 by 100 mm; Becton Dickinson, Grenoble, France). In the first group of experiments, *S. aureus* Wood 46 was attached to both sides of the PMMA cover slips. A sample (4×10^6 CFU) of the radiolabeled bacteria suspended in 1 ml of PBS was incubated with each PMMA cover slip for 60 min at 37°C with agitation. At the end of the attachment period, the fluids containing unbound bacteria were drained, and the cover slips were transferred into new tubes containing 1 ml of 0.15 M NaCl. This transfer procedure minimized the carryover of fluid contaminated by unbound bacteria. After incubation for 5 min at 20°C, a second wash was performed with 1 ml of ice-cold 0.15 M NaCl for 30 min. This last procedure released less than 20% of the bacteria from the cover slips. Ice-cold conditions were selected to reduce the subsequent multiplication of the adherent bacteria during the phagocytic bactericidal assay. The average number of bacteria attached to each PMMA cover slip, as determined by radioactive counts, was 4.91×10^4 cells (± 1.40, mean $\pm$ the standard error of the mean [SEM]) in experiments performed on 5 different days.

In the second group of experiments, *S. aureus* Wood 46 was attached on a single side of each PMMA cover slip. The unexposed side of each cover slip was made identifiable by scratching, whereas the unscratched side was gently put into contact with the top of a 1-ml solution of PBS containing 3×10^7 CFU of labeled *S. aureus* and incubated for 60 min at 37°C on the top of the undisturbed liquid layer. At the end of the attachment period, the fluids containing unbound bacteria were drained, and the cover slips were rinsed twice with NaCl as described for the two-sided bacterial attachment. The average number of labeled bacteria attached on a single side of each PMMA cover slip amounted to 2.88×10^4 cells (± 0.59, mean $\pm$ SEM) in experiments performed on 3 different days.

In the third group of experiments, *S. aureus* Wood 46 was allowed to bind rapidly by centrifugation to a single side of each PMMA cover slip. Before attachment, 10^8 CFU/ml were suspended for 30 min at 37°C in 10% guinea pig serum in PBS. Thereafter, opsonized bacteria were washed by three centrifugations performed at $3,000 \times g$ for 10 min and finally suspended in 0.15 M NaCl at a concentration of 10^8 CFU/ml. Radiolabeled bacteria were diluted to 2×10^5 CFU/ml of PBS and sedimented onto the unscratched side of PMMA cover slips which had been immersed at the bottom of the polyethylene tubes. After the centrifugation, performed at $3,000 \times g$ for 10 min, the fluids containing the unbound bacteria were removed. The average number of labeled bacteria attached on a single side of each PMMA cover slip by centrifugation amounted to 1.98×10^4 cells (± 0.51, mean $\pm$ SEM) in experiments performed on 4 different days.

Phagocytic-bactericidal assay. After double-sided attachment of *S. aureus* to PMMA, each cover slip was immersed in a 1-ml solution of PBS supplemented in each case with 10% guinea pig serum. Triplicate tubes containing 5×10^6 guinea pig PMN and triplicate tubes with no PMN were run in parallel for 60 min at 37°C with agitation. Additional triplicate tubes, each containing a sterile PMMA cover slip, were run with the complete phagocytic mixture, namely, PBS, serum, PMN, and 5×10^4 CFU of *S. aureus* in suspension. These control tubes were run to verify that the phagocytic-bactericidal function of the PMN in suspension was not altered by the presence of the shaken PMMA cover slips.

After single-sided attachment of *S. aureus* to PMMA, each cover slip was deposited on the bottom of the plastic tube and overlaid with 1 ml of the phagocytic mixture. Care was taken to orient attached bacteria upward in the direction of sedimenting PMN. Five replicate tubes containing 5×10^6 PMN, 10% serum, and PBS and five tubes with no PMN, but with serum and PBS were run in parallel for 60 min at 37°C. The immersed cover slips were left unshaken to allow rapid attachment by sedimentation of the PMN to the cover slips. As a control of the phagocytic-bactericidal activity of the PMN in suspension, 3×10^6 CFU of *S. aureus* Wood 46 in suspension was mixed with 5×10^6 PMN in 1 ml of PBS supplemented with 10% serum and incubated at 37°C for 60 min with agitation.

Extraction of the attached bacteria from PMMA cover slips. At the end of the phagocytic incubation, the fluids containing the phagocytic mixtures were drained and analyzed for their radioactive content by scintillation counting and for their viability by CFU counts on Mueller-Hinton agar plates, using standard techniques and NaCl as a diluting solution. *S. aureus* still attached to PMMA was removed from cover slips by a harmless two-step procedure: (i) cover slips were first incubated with 0.1% Triton X-100 in 0.9% NaCl for 10 min at 20°C, allowing detachment of a fraction of attached *S. aureus*; (ii) cover slips were then incubated with 1 mg/ml of trypsin solution in PBS for 10 min at 37°C with agitation. The proteolytic reaction was stopped by adding a twofold excess of trypsin inhibitor for 10 min at 37°C. *S. aureus* cells detached by either Triton X-100 or trypsin were analyzed for viability by CFU counts. The radioactive content of each extract was estimated by determining counts per minute (cpm) of 400-μl portions mixed with 5 ml of an emulsion system selected for scintillation counting.

Evaluation of the susceptibility of attached *S. aureus* to the phagocytic-bactericidal action of PMN. Susceptibility of *S. aureus* incubated in suspension with PMN (control assays) and exposed to their phagocytic-bactericidal action was evaluated by the following survival formula: % survival = (CFU with PMN/CFU without PMN) × 100. Survival of attached *S. aureus* is the ratio of CFU counted in the presence versus that in the absence of PMN after extraction of the bacteria from PMMA. This ratio is corrected by the amount of cell associated radioactivity recovered in the fractions: % survival = [(CFU with PMN/CFU without PMN) × (cpm without PMN/cpm with PMN)] × 100.

Attachment of PMN to PMMA cover slips. PMN (10^7/ml) were labeled for 60 min in sterile siliconized glass tubes containing 20 μCi/ml of [^{35}S]methionine in PBS supplemented with 10% serum and 0.5% glucose at 37°C with agitation. After removal of the unbound radioactivity by three centrifugations performed at $300 \times g$ for 5 min, the labeled PMN were suspended in PBS supplemented with 10% serum. PMN were then incubated with cover slips in conditions identical to those described for the phagocytic-bactericidal assays, except that adherent bacteria were not labeled with [^{3}H]thymidine for this assay. To assess PMN attachment to PMMA cover slips after 0, 15, 30, or 60 min of incubation at 37°C, cells were fixed by adding 1/10 volume of 25% glutaraldehyde for 60 min at 20°C. After the fixative was rinsed in cacodylate buffer, acid-soluble radioactivity was extracted from the cover slips by incubation with 5% trichloracetic acid for 18 h at 4°C and filtration on Whatman GFC filters (Whatman, Inc., Clifton, N.J.). The filters were washed with 5% trichloroacetic acid and then dried, and the

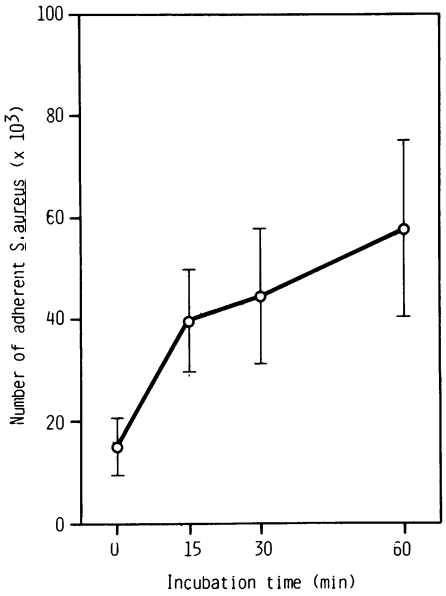

FIG. 1. Time course of *S. aureus* Wood 46 attachment to a single side of PMMA cover slips. Bacteria (3×10^7 CFU/ml) were incubated with cover slips for periods of 30 s to 60 min in 1 ml of PBS at 37°C and then washed as described in Materials and Methods. The values shown are means ± SEM obtained in three experiments.

cpm values were determined. The radioactivity still associated with PMMA cover slips was also evaluated by scintillation counting and added to the counts recovered on the GFC filters.

Statistics. One-way variance analysis and paired and unpaired *t* tests were performed by using specific programs adapted to a computer HP-41C (Hewlett-Packard Co., Geneva, Switzerland).

RESULTS

Characteristics and validation of the modified phagocytic-bactericidal assay of attached *S. aureus*. Optimal conditions for the two-sided attachment of *S. aureus* to PMMA cover slips have been described in a previous report (21). Single-sided attachment of *S. aureus* to PMMA cover slips was studied using various bacterial concentrations and incubation periods. Figure 1 shows the average values of three experiments recording the time course of attachment to the cover slips, starting with a suspension of 3×10^7 CFU/ml of PBS. Single-sided attachment of *S. aureus* to PMMA took place essentially during the first 15 min of incubation.

The second requirement of the modified phagocytic-bactericidal assay was the development of a harmless extraction technique to remove a high proportion of attached *S. aureus* from PMMA cover slips. Tables 1 and 2 demonstrate that more than 80% of the cell-associated radioactivity, initially attached to PMMA, was released from the cover slips, either during the phagocytic incubation or during the two-step extraction. Thus, less than 20% of the initially attached bacteria were not evaluable for their susceptibility to the phagocytic-bactericidal action of PMN (Tables 1 and

TABLE 1. Effects of PMN on the release and survival of *S. aureus* attached on both sides of PMMA cover slips[a]

Fraction	% Cell-associated radioactivity:		% Survival of *S. aureus* incubated with PMN
	Without PMN	With PMN	
S. aureus suspended with PMN and PMMA (control)	>95	>95	8 ± 3
S. aureus initially attached to PMMA			
Released during incubation	36 ± 5[b]	30 ± 6	10 ± 4
Extracted after incubation[c]	56 ± 4	54 ± 6	70 ± 4[d]
Unextracted (residual)	8 ± 1	16 ± 3	NA[e]

[a] See Materials and Methods for the experimental procedures of double-sided *S. aureus* attachment, exposure to PMN, and removal from PMMA cover slips and for the evaluation of survival.
[b] Mean ± SEM of six experiments with triplicate determinations.
[c] Data from the Triton-extracted and trypsin-extracted bacteria are pooled.
[d] Significantly different ($P < 0.001$) from the survival of other fractions.
[e] NA, Not analyzable.

TABLE 2. Percentage of labeled *S. aureus* Wood 46 that was either released from single-sided attachment to PMMA during the phagocytic incubation or extracted from PMMA after the phagocytic incubation[a]

Fractions of *S. aureus* initially attached to PMMA	% Cell-associated radioactivity (mean ± SEM) attached to PMMA:			
	Spontaneously:		By centrifugation:	
	Without PMN	With PMN	Without PMN	With PMN
Released	9 ± 3	9 ± 3	15 ± 3	14 ± 3
Extracted by Triton X-100	41 ± 1	44 ± 4	11 ± 3	39 ± 8[b]
Extracted by trypsin	41 ± 2	38 ± 8	67 ± 4	40 ± 5[b]
Unextracted (residual)	9 ± 1	9 ± 1	7 ± 2	7 ± 2

[a] See Materials and Methods for the experimental procedures for bacterial attachment, exposure to PMN, and removal from PMMA cover slips.
[b] $P < 0.05$ for experiments in the presence versus that in the absence of PMN (paired *t* test).

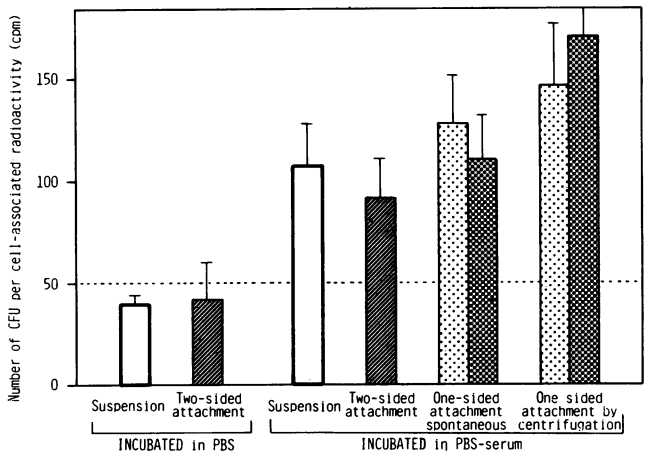

FIG. 2. Viability of *S. aureus* Wood 46 attached to PMMA cover slips and removed by the extraction procedure described in Materials and Methods. The number of CFU per cell-associated radioactivity in the extracts or in a suspension of *S. aureus* is shown under nongrowing incubation conditions (PBS) or growing conditions (serum-PBS). Symbols: ☐, suspension; ⁙, Triton extracted; ▦, trypsin extracted; ▨, pooled extracts.

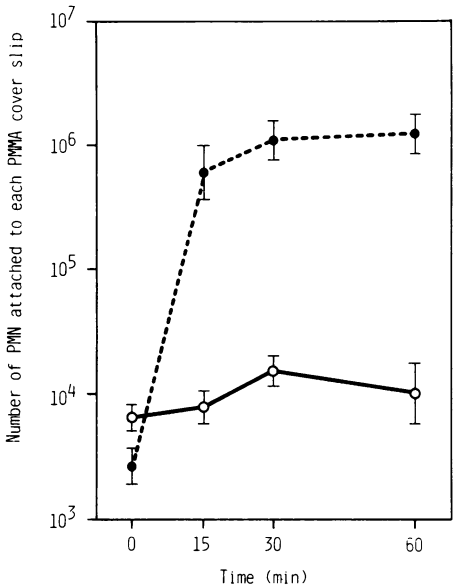

FIG. 3. Time course of attachment of [^{35}S]methionine-labeled PMN to PMMA cover slips. PMN (5×10^6/ml) were incubated with cover slips having attached unlabeled bacteria for periods of 30 s to 60 min in 1 ml of PBS plus 10% serum at 37°C. (See Materials and Methods for the experimental procedure for bacterial attachment.) The values shown are means ± SEM obtained in three experiments involving triplicate determinations. Symbols: ○, double-sided *S. aureus* attachment followed by PMN binding under shaking conditions; ●, single-sided *S. aureus* attachment followed by PMN binding to cover slips by gravity sedimentation.

2). The harmless effect of the extraction procedure was assessed under two different conditions of incubation: (i) After double-sided attachment of *S. aureus* and incubation in a nongrowing medium (PBS), bacteria extracted from the cover slips showed an identical survival to fluid phase bacteria incubated under identical conditions (Fig. 2). In other words, attached or fluid phase bacteria showed identical CFU counts at equivalent amounts of cell-associated radioactivity. (ii) When extracted *S. aureus* was obtained from cover slips incubated in serum-PBS for 60 min at 37°C, CFU counts of the extracts showed a significant increase over that of the initial culture (Fig. 2). A similar increase in CFU per cell-associated radioactivity was observed for *S. aureus* in suspension (Fig. 2), indicating similar growth rates for both attached and suspended bacteria. Thus, the viability of *S. aureus* attached to PMMA and removed from the cover slips appeared to have been preserved throughout the extraction procedure, thus demonstrating its harmless effect.

Susceptibility of *S. aureus* to PMN after double-sided attachment of the bacteria to PMMA. PMN prepared from acute peritoneal exudates showed a good phagocytic-bactericidal activity on *S. aureus* Wood 46 in suspension, since more than 90% of the bacteria were killed within 60 min, whether in the absence (data not shown) or in the presence of sterile PMMA cover slips (Table 1, control). Thus, the presence of PMMA had no adverse effects on the phagocytic-bactericidal function of PMN in suspension. With regard to *S. aureus* cells attached to PMMA, Table 1 shows that the presence of PMN did not promote the release of *S. aureus* from the PMMA cover slips when compared with incubations run in the absence of PMN. At the end of the phagocytic incubation, attached *S. aureus* were extracted from the cover slips in a similar proportion, whether in the presence or in the absence of PMN (Table 1). Survival of the bacteria removed from the cover slips by extraction was excellent, averaging 70%, and thus was much higher ($P < 0.001$) than that of *S. aureus* incubated with PMN in suspension or initially attached but released in the presence of PMN (Table 1). Thus, the susceptibility of *S. aureus* to competent PMN in suspension appeared severely decreased after double-sided attachment of the bacteria to PMMA cover slips.

Attachment of PMN to PMMA cover slips during the phagocytic-bactericidal assay. The ability of PMN to contact the PMMA cover slips during the phagocytic-bactericidal assay was evaluated. Figure 3 shows the time course of PMN attachment to PMMA cover slips during the phagocytic-bactericidal experiments. The average numbers of PMN attached to cover slips after double-sided bacterial attachment and recorded after either 15, 30, or 60 min of incubation at 37°C with agitation ranged from 8×10^3 to 1.6×10^4 PMN ($n = 3$). In contrast, much higher numbers of PMN attached to PMMA during the single-sided phagocytosis experiments presented below, where the phagocytic cells adhered by sedimentation in the absence of shaking (Fig. 3).

Susceptibility of *S. aureus* to PMN after single-sided attachment of the bacteria to PMMA. Results of the phagocytic assay with *S. aureus* Wood 46 attached on both sides of PMMA cover slips showed their decreased susceptibility to PMN when compared to the same bacteria in the fluid phase. Such results could be explained either by alterations in bacterial characteristics or by the reduced ability of PMN to attach to the surface of PMMA cover slips. Poor attachment

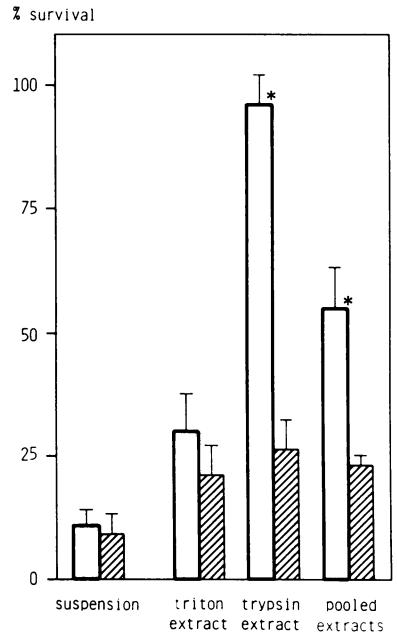

FIG. 4. Survival of *S. aureus* Wood 46 exposed to PMN after single-sided bacterial attachment to PMMA cover slips either spontaneously or by centrifugation. (See Materials and Methods for the experimental procedure for attachment, phagocytosis, and the determination of survival estimates.) Symbols: □, spontaneous attachment; ▨, attachment by centrifugation of preopsonized *S. aureus* (*, significantly different from other groups, $P < 0.01$).

of PMN to PMMA under shaking conditions was therefore overcome by establishing a stationary phagocytic-bactericidal assay in which PMN attached in high numbers to the cover slips by gravity sedimentation (Fig. 3). In addition, a single-sided bacterial attachment system was set up which could be modulated in two ways, *S. aureus* attachment being either spontaneous (60 min, Fig. 1) or accelerated by centrifugation (10 min). Rapid attachment of *S. aureus* by centrifugation was expected to minimize the time-consuming processes needed for exopolysaccharide synthesis and deposition on the surface of the bacteria.

Table 2 and Fig. 4 present the overall results of the experiments of single-sided phagocytosis. In each experiment, PMN prepared from peritoneal exudates showed a good phagocytic-bactericidal activity on *S. aureus* in suspension (Fig. 4). The percentage of cell-associated radioactivity released during incubation with or without PMN was low (<15%) and was not affected by the presence or absence of the phagocytes or by the mode of attachment of *S. aureus* to PMMA (Table 2). In contrast, the percentage of cell-associated radioactivity extracted after incubation with PMN by Triton X-100 followed by trypsin was influenced by the mode of attachment of *S. aureus* to PMMA: bacteria attached by centrifugation were better extracted ($P \leq 0.05$) by Triton X-100 after exposure to PMN than without PMN (Table 2); however, bacteria attached spontaneously were similarly extracted by Triton X-100 and trypsin regardless of prior exposure to PMN (Table 2). The most interesting effect of the modified phagocytic-bactericidal assay was the influence of bacterial attachment on their survival (Fig. 4). Whether attached spontaneously or by centrifugation, the fraction of attached *S. aureus* extracted by Triton X-100 appeared to have been killed during incubation with PMN, since their survival was reduced to less than 30%. In contrast, the fraction of attached *S. aureus* extracted by trypsin showed almost 100% survival following spontaneous attachment, but less than 30% survival following rapid attachment by centrifugation ($P < 0.001$). Thus, the trypsin-extracted subpopulation of *S. aureus* attached spontaneously to PMMA cover slips fully escaped the phagocytic-bactericidal action of a high local concentration of PMN.

DISCUSSION

Foreign body infections are characterized by the persistence of highly adherent bacteria on the surface of the synthetic prosthetic materials. Such bacteria have been proposed to escape host-dependent phagocytic and bactericidal mechanisms. This study has described the development of new techniques for evaluating the susceptibility or resistance of adherent bacteria to the phagocytic-bactericidal activity of PMN. Whereas phagocytic-bactericidal assays can be easily performed on *S. aureus* in suspension using multiple sampling of well-controlled volumes of PMN-bacteria mixtures, a similar approach is not applicable to bacteria attached to solid substrates such as PMMA cover slips: (i) intimate contact between phagocytes and attached bacteria must be induced, and (ii) the majority of the attached bacteria (>80%) must be extracted from the cover slips by a harmless technique at the end of the test.

The choice of the extracting agents Triton X-100 and trypsin was purely empirical, and no single agent tested could remove more than 50% of the attached bacteria from the PMMA cover slips. The strain *S. aureus* Wood 46 was the most convenient for our study since it has well-established opsonizing properties in phagocytic assays (13, 23, 24) and was extensively used in our previously developed model of foreign body infection (2, 23, 24).

Our results show that of the two attachment systems of *S. aureus* to PMMA cover slips used, the single-sided one offers a significant advantage over the double-sided one. In the former system, *S. aureus* Wood 46 remained more firmly attached to the cover slips during incubation with either serum-PBS medium or with the complete phagocytic system. Furthermore, intimate contact between PMN and adherent *S. aureus* was favored by the very high attachment of the phagocytes to the cover slips.

An additional advantage of the single-sided adherence system was the possibility to attach *S. aureus* Wood 46 to PMMA cover slips in two different ways and to analyze in an identical phagocytic assay the susceptibility to PMN of variously attached bacteria. This selective approach was used to differentiate factors affecting in a general way the phagocytic function of PMN, such as the interaction of the phagocytes with the hydrophobic surface of PMMA, from factors related to the bacteria, involving possible surface changes induced by their mode of attachment to PMMA.

A short-term adverse effect of PMMA on the main functions of attached phagocytes was ruled out by the fact that *S. aureus* attached by centrifugation to the cover slips was killed normally by the PMN monolayer in the phagocytic-bactericidal assay. Although this observation may appear to stand in contrast to a prior report (23) showing PMN defects in contact with a nonphagocytosable foreign surface, we think that these defects were unlikely to be fully expressed during the short time of incubation of our phagocytic assay and that minor defects could be compensated for by the very high number of phagocytes coming into contact with the PMMA surface. The reason for using preopsonized bacteria for single-sided attachment experiments was to facilitate attached *S. aureus*-PMN interactions. This control system has definitely assessed that PMN can, under selected conditions, kill bacteria directly attached to a smooth plastic surface. In contrast to bacteria attached by centrifugation, *S. aureus* Wood 46 adherent to the PMMA cover slips by spontaneous attachment showed an improved survival in the presence of the PMN monolayer. Surprisingly, bacterial survival on the PMMA cover slips was very different for the two fractions of *S. aureus* that were sequentially extracted by Triton X-100 and trypsin. Whereas trypsin-extracted bacteria showed nearly 100% survival in the presence of the PMN monolayer, the Triton-extracted bacteria appeared to have been killed by the phagocytes. Although the reasons for such a difference in the survival of the Triton-extracted versus the trypsin-extracted fractions of attached *S. aureus* remain unknown, our results suggest the possibility that *S. aureus* attached spontaneously to PMMA has been modified by the production of slime or glycocalyx, which is likely to affect their susceptibility to phagocytes. Another explanation for such an altered susceptibility of attached bacteria to PMN could be the presence of intrinsic irregularities on the surface of PMMA cover slips, which would provide convenient sites for organism implantation (16–18). Both explanations, although not mutually exclusive, are still merely hypothetical, since the low numbers of spontaneously attached *S. aureus* (4×10^4 cells per cm^2) to PMMA cover slips precluded ultrastructural studies of the bacteria by electron microscopy. Attempts to increase the number of spontaneously attached bacteria per unit area of PMMA, using highly concentrated suspensions of *S. aureus* Wood 46, were unsuccessful: the maximum number of bacteria attached to each cover slip was only 4×10^5 when incubated

with 4×10^9 CFU of *S. aureus* Wood 46 per ml (unpublished results). Thus, other techniques of spontaneous *S. aureus* attachment must be developed to perform ultrastructural studies.

Although recent studies from our laboratory (21, 22) have documented the role of fibronectin deposited on the surface of PMMA cover slips as a ligand to *S. aureus* adherence, we do not know how fibronectin may modulate the susceptibility of attached bacteria to phagocytic cells. In vivo, the direct attachment of *S. aureus* to the smooth surface of implanted biomaterials may be limited to cases of accidental contamination of the foreign bodies occurring at the time of their implantation into human patients.

Results from this study combined with previous reports from our group (21–24) and from others (1, 7–11, 14–19) reinforce the concept that multiple factors are involved in favoring bacterial infections around biomaterial implants. Decreased susceptibility to phagocytosis following bacterial attachment to foreign bodies and inadequate PMN response may act in concert to favor infection on prosthetic material.

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