Pathogenesis of Foreign Body Infection: Description and Characteristics of an Animal Model

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An animal model involving the subcutaneous implantation of tissue cages into guinea pigs and subsequent infection with *Staphylococcus aureus* was used to study factors pertinent to foreign body infection. Whereas 10^8 colony-forming units (cfu) of *S. aureus* strain Wood 46 did not produce any abscesses in the absence of foreign material, 10^2 cfu was sufficient to infect 95% of the tissue cages despite the presence of polymorphonuclear leukocytes (PMNLs) in sterile tissue cage fluid. Opsonization of *S. aureus* by tissue cage fluid was adequate during the first hour of infection, but opsonic coating of the organisms decreased at 20 hr after the induction of infection. PMNLs from sterile tissue cage fluid showed decreased phagocytic and bactericidal activities when compared with PMNLs from either blood or peritoneal exudate obtained after short- or long-term stimulation ($P < 0.001$).

The enhanced risk of bacterial infections in the vicinity of a foreign body—such as sutures and metallic or polymeric implants—has been repeatedly documented in cardiovascular [1-3], orthopedic [4-6], plastic reconstructive [7], and general [8] surgery. *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli* are most frequently implicated as the etiologic agents [9]. Foreign body infection proceeds by two possible routes: early infection, due to local bacterial contamination during surgery [4], and late infection, after occasional seeding of microorganisms by the hematogenous route [6]. Both types of infections, once established, rarely heal, and excision of the foreign body remains the only effective treatment.

The factors responsible for the enhanced risk of bacterial infection of foreign bodies are not well understood. Ineffectiveness of local host defense mechanisms against pyogenic organisms is suggested by the classical experiments of Elek and Conen [10], James and MacLeod [11], and Noble [12], which showed that infection could be produced with as few as 100 cfu of *S. aureus* in the vicinity of silk sutures. Although polymorphonuclear leukocytes (PMNLs) accumulating around a foreign body appear microscopically normal, their functional status as well as the opsonizing capacity of the surrounding fluid have never been explored because of the lack of an appropriate animal model. In the present communication we describe the development of an animal model that reproduces the clinical characteristics of a foreign body infection and thus allows the in vitro evaluation of the various phagocytic steps in its neighborhood [13-16].

Materials and Methods

Tissue cages, animals, and implantation of tissue cages. Rigid polymethacrylate and polytetrafluoroethylene tubes (internal and external diameters, 10 and 12 mm, respectively; length, 32 mm) were perforated by ~250 regularly spaced holes (diameter, 1 mm) and sealed at each end with a cap of identical material.

Tricolor guinea pigs weighing 500-600 g each which were allowed free access to food and which

were housed individually were used throughout the experiments. After im induction of a neuroleptanalgesia (0.1 ml/100 g of body weight; Hypnorm®; Duphar, Amsterdam), a 4-cm incision was made with an aseptic technique, and the sc space was dissected bluntly. One gas-sterilized tissue cage was implanted in each flank [17-20], and the skin was closed with metal clips, which were removed five days after surgery. The animals were used for experimentation two or more weeks after implantation of the tissue cages, after full healing of the incision.

Cytologic, microbiologic, and protein determinations. Erythrocytes and leukocytes from freshly obtained tissue cage fluids were counted in a hemacytometer with trypan blue and Turk's stain, respectively. Cell viability was evaluated by exclusion of trypan blue. Quantitative bacterial cultures were performed on Mueller-Hinton agar, after lysis of PMNLs and appropriate serial dilutions in sterile water. Possible clumping of bacteria was checked for regularly and found to be minimal under these experimental conditions. The absence of spontaneous bacterial contamination of tissue cage fluids was confirmed by subculturing undiluted tissue cage fluid on blood agar and incubating the plates for 48 hr. Protein was quantitated by the Bio-Rad® protein assay (Bio-Rad Laboratories, Richmond, Calif.).

Collection and storage of serum and tissue cage fluid. Blood was obtained by cardiac puncture and allowed to clot at 37 C for 30 min. Serum was separated thereafter by centrifugation at 1,500 g for 10 min at 4 C, stored at −70 C, and tested within three months. Tissue cage fluid was collected by percutaneous aspiration, added to ice-cold siliconized glass tubes, centrifuged, and stored under the same conditions as serum. Pools of either serum or tissue cage fluid were obtained by mixing such samples from at least five healthy guinea pigs.

Experimental infections. S. aureus strain Wood 46, stored in aliquots of the same initial culture at −70 C, was used for all experiments. Overnight cultures in Mueller-Hinton broth were washed and resuspended in 0.9% NaCl; 0.1 ml of an appropriate dilution was injected into tissue cages, sc tissue, or the peritoneal cavity. The number of viable bacteria injected was checked by plating 0.1 ml of a 10^{-6} dilution of the washed overnight culture, which contained 7-15 × 10^8 cfu/ml.

In addition, the following infections were attempted under identical experimental conditions: (*1*) inoculation of sc cavities created by surgical extraction of tissue cages implanted four weeks previously; (*2*) inoculation of sc, longitudinally-halved tissue cages, thus allowing granulation tissue to grow on either side of the cage wall; or (*3*) before inoculation *S. aureus* strain Wood 46 was preopsonized by incubation with 10% serum for 30 min at 37 C, followed by two washes with 0.9% NaCl. The efficacy of the preopsonization procedure was assessed in an in vitro phagocytic assay without further addition of opsonins (serum or tissue cage fluid).

Preparation of leukocytes. Acute peritoneal exudates rich in PMNLs (>95%) were obtained from guinea pigs by ip injections of either 12% (wt/vol) sterile caseinate in 0.9% NaCl [21] or 0.1% (wt/vol) sterile glycogen in 0.9% NaCl [22]. Persistent peritonitis as a paradigm for a chronic exudate in the absence of a foreign body was produced by repeated injections of glycogen over a period of two weeks. After such a sustained stimulus, these exudates showed the same leukocyte differential count as tissue cage fluid—40% PMNLs and 60% mononuclear leukocytes; histopathologic examination of sections confirmed the presence of a chronic inflammation. The exudates were collected by abdominal lavage with an 18-gauge needle and were filtered through four layers of gauze (pore size, 1 × 2 mm) into ice-cold siliconized glass tubes. The cell suspensions were washed three times in cold 0.9% NaCl by centrifugation at 150 g for 10 min and resuspended in 5 ml of a solution of phosphate-buffered saline (Dulbecco's Gibco-Bio-Cult Co., Glasgow, Scotland). The total yield of PMNLs harvested by these techniques usually exceeded 10^8 cells (>95% PMNLs) per animal with acute peritonitis and 6 × 10^7 cells (~40% PMNLs) per animal with persistent peritonitis. PMNLs from blood were obtained as a 60%-80% pure suspension by the method described by Chenoweth et al. [23]. PMNLs from sterile tissue cage fluid were aspirated percutaneously from tissue cages (two to five weeks after surgery) and washed as described above. When necessary, PMNLs were concentrated by centrifugation or by pooling cells from two tissue cages from the same animal. PMNLs from infected tissue cages were aspirated with the same technique, washed, and resuspended in

phosphate-buffered saline. This procedure as well as the dilution of the cell suspension to an appropriate concentration of PMNLs for the phagocytic assay decreased the number of bacteria present in infected tissue cage fluid from 10^6 to 10^4 cfu/ml—that is, to 0.3% of the number of indicator bacteria used in the assay.

Phagocytic bactericidal assay. The same phagocytic bactericidal assay previously described by our laboratory [13, 24], was used throughout the present study to measure opsonic activities of tissue cage fluid and serum, the opsonic coating of *S. aureus* in the animal model, and the overall killing by as well as the ingestion rate of PMNLs.

Opsonic activity. An overnight culture of *S. aureus* strain Wood 46 was washed three times in distilled water by centrifugation at 3,000 g for 10 min and finally resuspended in 1 ml of 0.9% NaCl. Under such conditions, most bacteria appeared as single cocci on Gram stain, thus minimizing errors in cfu enumeration due to clumping, as we have shown previously [13, 24, 25]. Guinea pig PMNLs, bacteria, and the opsonic source to be tested were suspended in phosphate-buffered saline in siliconized glass tubes in a final volume of 1 ml. Each 1 ml of incubation mixture contained 5×10^6 PMNLs, 1.5-3 $\times 10^6$ cfu of *S. aureus*, and 0.1 ml of a serially diluted source of opsonins. The incubation and plating conditions have been described [13, 24, 25]. The reciprocal value of the dilution of serum or tissue cage fluid that gave 50% killing of *S. aureus* was defined as the opsonic titer [13, 24]. Controls included (*1*) heat-inactivated (at 56 C for 30 min) pooled serum or tissue cage fluid with PMNLs; (*2*) PMNLs without serum or tissue cage fluid; and (*3*) 10% pooled serum without PMNLs. None of these controls exhibited any bactericidal activity against the test strain. The lack of bacterial killing with heat-inactivated serum as the opsonic source confirmed the previously defined opsonic requirements of *S. aureus* strain Wood 46 [13, 24, 26].

Opsonic coating of bacteria. Experimentally infected tissue cage fluids were aspirated 1 and 20 hr after inoculation of bacteria, and the opsonic coating of the bacteria was assessed as follows. The microorganisms were washed three times in 0.9% NaCl and resuspended in phosphate-buffered saline. For each assay 0.5 ml of this bacterial suspension was added to a suspension of 0.5 ml of peritoneal PMNLs (5×10^6) in phosphate-buf-

fered saline and incubated without addition of any other opsonic source at 37 C in a shaking water bath. Samples of the test suspension were taken at zero-time and after 30 min of incubation, and bacterial survival was determined as described above. Simultaneous controls were obtained by preopsonizing an aliquot of the same bacterial suspension with either 10% heat-inactivated (at 56 C for 30 min) or 10% freshly obtained serum. Preopsonization of the bacteria with heat-inactivated serum did not increase the bacterial killing measured in the phagocytic bactericidal assay.

Phagocytic bactericidal capacity of PMNLs. The testing of the phagocytic bactericidal potential of PMNLs obtained from tissue cage fluids or peritoneal exudates presupposed that the opsonic source used in the phagocytic assay was not rate-limiting. This requirement was met by using $\geq 2.5\%$ pooled serum or $\geq 10\%$ sterile tissue cage fluid as the opsonic source [27].

Because PMNLs in tissue cages were unable to eliminate inocula as small as 10^2 cfu of *S. aureus* (see below), we assessed the in vitro bactericidal performance of 2×10^6 PMNLs/ml not only with the conventional inoculum (4×10^6 cfu), but also with a smaller inoculum of 8×10^3 cfu. After incubation of this suspension with 0.2 ml of pooled, sterile tissue cage fluid at 37 C in a shaking water bath, samples were drawn at zero-time and 30, 120, and 240 min, diluted, mixed with an equal volume of 0.2% Triton X-100 [25], and plated on Mueller-Hinton agar. Each test was accompanied by the following controls: (*1*) 20% heat-inactivated tissue cage fluid, which did not promote any bacterial killing, and peritoneal PMNLs, and (*2*) 20% freshly obtained tissue cage fluid without PMNLs, which led to bacterial growth after 2 and 4 hr of incubation in all tubes. In this modified assay, control PMNLs exhibited killing capacity which was within the ranges of the standard assay. Control experiments with either 10% serum or 40% tissue cage fluid as an opsonic source were also performed and showed identical killing rates in the presence of PMNLs. Moreover, preincubation of 40% tissue cage fluid with PMNLs from blood, tissue cages, or exudates did not inhibit the cells' phagocytic bactericidal function. Finally, because for technical reasons PMNLs from tissue cages could not be separated from other leukocytes from tissue cages before the in vitro assays, an inhibitory effect of the latter cells on

PMNL function was excluded by appropriate mixing experiments with tissue cage cells and control PMNLs from peripheral blood.

Ingestion rate of PMNLs. Ingestion rates of PMNLs were tested in a similar phagocytic assay with use of lysostaphin [25, 28, 29]. In brief, this assay consisted of incubating 5×10^6 PMNLs with 1×10^7 cfu of *S. aureus* strain Wood 46 and determining the overall killing in an aliquot after 30 min of incubation. Intracellular survival was assessed by incubation for another 30 min in the presence of 20 units of lysostaphin (Sigma Chemical Co., St. Louis), followed by treatment with 0.25% trypsin (Serva-Feinbiochemica, Heidelberg, Federal Republic of Germany) and neutralization of the proteolytic activity with 10% serum.

Complement studies. Total hemolytic complement activity was measured in a manual assay [30] and expressed as the reciprocal dilution that gave 50% hemolysis of sensitized red blood cells (CH_{50}). C3 levels were quantitated by single radial immunodiffusion with goat monospecific antibody to guinea pig C3 (Cappel Laboratories, Cochranville, Pa.) [31]. Values were expressed as percentages of the level in pooled normal guinea pig serum.

Statistical analysis. The significance of differences between mean values was determined by Student's t-test; the method of paired comparisons was used when appropriate (that is, comparison of opsonic coating at different conditions). For correlation analysis, the Spearman's correlation coefficient was used. Results were expressed as means $\pm$ SEM.

Results

Composition of normal, sterile tissue cage fluid and histopathology of the inflammatory reaction. After implantation of tissue cages, aspirated fluid showed the following characteristics. Erythrocyte counts decreased from 1.5×10^8/ml (range, $0.3-3.6 \times 10^8$) at two weeks after surgery to 1×10^7/ml (range, $0.6-9 \times 10^7$) at 12 weeks after surgery. Leukocyte counts decreased from 2.4×10^6/ml (range, $0.3-8.4 \times 10^6$) at two weeks after surgery to 1×10^5/ml (range, $0.5-8 \times 10^5$) at 12 weeks after surgery. Differential cell counts performed two weeks after implantation of tissue cages showed a slight predominance of PMNLs (60% PMNLs and 40% mononuclear leukocytes),

as opposed to two months later (30% PMNLs and 70% mononuclear leukocytes).

Histologic sections examined four weeks after implantation of tissue cages and stained with hematoxylin and eosin showed a richly vascularized granulation tissue containing lymphocytes, fibroblasts, and collagen fibers in close contact with the foreign body. There was heavy PMNL infiltration in the vicinities of infected tissue cages. Biochemical determinations of levels of calcium, magnesium, phosphorus, and zinc, pH, pO_2, and pCO_2 confirmed the results obtained in other animal species [19, 32]. The protein concentration in tissue cage fluid decreased from 33.0 ± 1.4 g/liter at two weeks to 22.8 ± 1.1 g/liter at 11 weeks. These values were significantly lower in tissue cage fluid than in serum (52.3 ± 0.9 g/liter) at any postoperative interval tested ($P < 0.001$).

Induction of infection in tissue cages and determination of the minimal infecting dose. To determine whether implantation of tissue cages is a representative model of foreign body infection, it had to be shown that the minimal infecting dose was close to the values observed in other systems [10-12]. For this purpose, the minimal infecting dose—defined as the number of cfu of *S. aureus* strain Wood 46 leading to a 95% infection rate—was determined for tissue cage fluid, as well as for sc tissue in the absence of a foreign body. Washed bacterial cultures were injected into tissue cage fluids or sc between weeks 2 and 13 after surgery. Inocula as small as 10^2 cfu (range, 46-210 cfu) produced infections in 44 (95.6%) of 46 of the tissue cages at 24 hr, as determined macroscopically and by bacterial cultures, and inoculation of $\geq 10^3$ cfu of *S. aureus* resulted in an infection in 40 of 40 tissue cages. The minimal infecting dose was identical whether polymethacrylate or polytetrafluoroethylene tissue cages were used. Autopsies of six animals showed the infection to be confined to the space within and around the tissue cage, without any evidence of spread to other organs; blood cultures were negative. In contrast, no infection could be produced in 30 animals by sc injection of 10^2-10^8 cfu of *S. aureus* strain Wood 46 in the absence of tissue cages. Ten animals were used as microbiologic controls for the sterility of sc tissue. For this purpose, the area of diffusion of the inoculated bacteria was determined in preliminary experiments with Evan's blue (Hoffmann–La Roche, Basel, Switzerland). After injection of the

various bacterial inocula, the area corresponding to the previously determined diffusion zone was biopsied, homogenized, and checked for bacterial growth. All the biopsy specimens were sterile at 72 hr after injection of inocula as large as 10^8 cfu.

Evaluation of the role of tissue cage dead space. Because the dead space present within tissue cages could create unfavorable conditions for surface phagocytosis, the following control experiments were carried out. Tissue cages were halved longitudinally and implanted as described. Histologic examination confirmed that granulation tissue had gained access to either side of the foreign body. Five of five of these modified tissue cages could still be infected with $<10^3$ organisms. The negligible role of the dead space in promoting infection was further demonstrated by removing implanted, sterile tissue cages after four weeks and inoculating the healed sc pouches after removal of the clips with *S. aureus* strain Wood 46 suspended in 4 ml of 0.9% NaCl. In seven animals no infection could be produced with 10^9 cfu, and in six animals the peritoneal cavity (a natural dead space) could not be infected with 10^9 cfu.

Bacterial growth and leukocyte accumulation during experimental infections of tissue cages. To assess the development of infection in the experimental model, quantitative cultures of *S. aureus* strain Wood 46 were performed in tissue cages over seven days with various initial inocula. At 22 hr after inoculation of from 10^2 to 10^8 cfu, bacteria reached concentrations ranging from 1.3×10^5 cfu/ml to 5.2×10^7 cfu/ml, respectively. Further observations over two to seven days showed that bacterial counts fluctuated between 10^5 cfu/ml and 10^8 cfu/ml, regardless of the initial inoculum. These values were lower than those obtained under in vitro conditions in Mueller-Hinton broth ($7-15 \times 10^8$ cfu/ml after 24 hr).

Quantitation of viable (trypan blue–negative) leukocytes in tissue cage fluid at induction and during establishment of infection with various inocula is shown in figure 1. The observed increase in leukocyte counts depended markedly on the inoculum size and on the time elapsed after inoculation, with a lag phase of 2–8 hr. At 22 hr, leukocyte counts had increased by factors of 5.1- and 40.4-fold from initial inocula of 10^2 and 10^8 bacteria, respectively.

Phagocytic bactericidal activity of PMNLs from tissue cage fluid in the absence or presence of
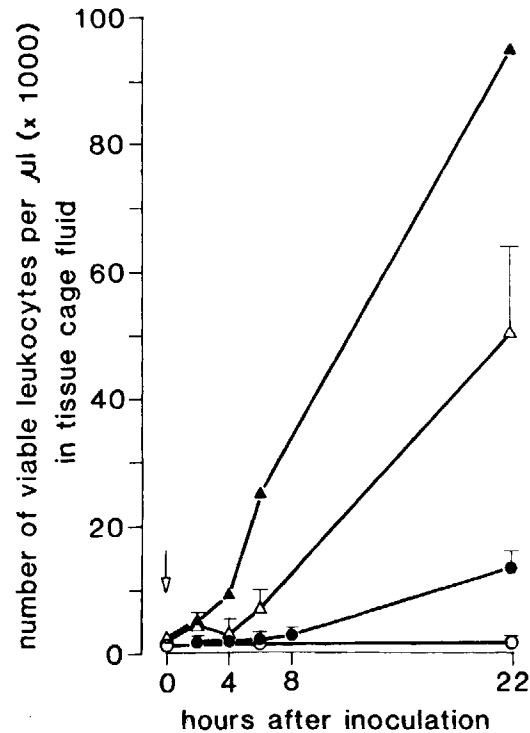
Figure 1. Viable leukocyte counts in tissue cage fluid during experimental infection of guinea pigs with *Staphylococcus aureus* strain Wood 46. At zero-time (arrow), the indicated inocula were given: (O) 10^2 cfu (seven animals), (●) 10^4 cfu (four animals), (△) 10^6 cfu (four animals), and (▲) 10^8 cfu (two animals). Data are mean + SEM (bars) numbers of leukocytes × 1,000/μl at intervals after infection.

infection. Bactericidal activity of PMNLs. Because tissue cages could be infected with as few as 10^2 cfu of *S. aureus* despite the presence of $1 \times 10^4-5 \times 10^6$ PMNLs/ml, we concluded that the presence of a tissue cage promoted infection even when the bacteria : PMNL ratio varied from $1 : 100$ to $1 : 50,000$. We therefore examined PMNLs from sterile tissue cages as to their bactericidal function over various incubation times with various bacterial inocula and compared the data with the bactericidal activity of PMNLs from either peripheral blood or peritoneal exudates — that is, unstimulated or attracted PMNLs. Finally, to compare exudates of similar age, the function of PMNLs from tissue cages was compared for each experimental condition with that of peritoneal PMNLs obtained after two weeks of stimulation.

The bactericidal activity of tissue case-derived
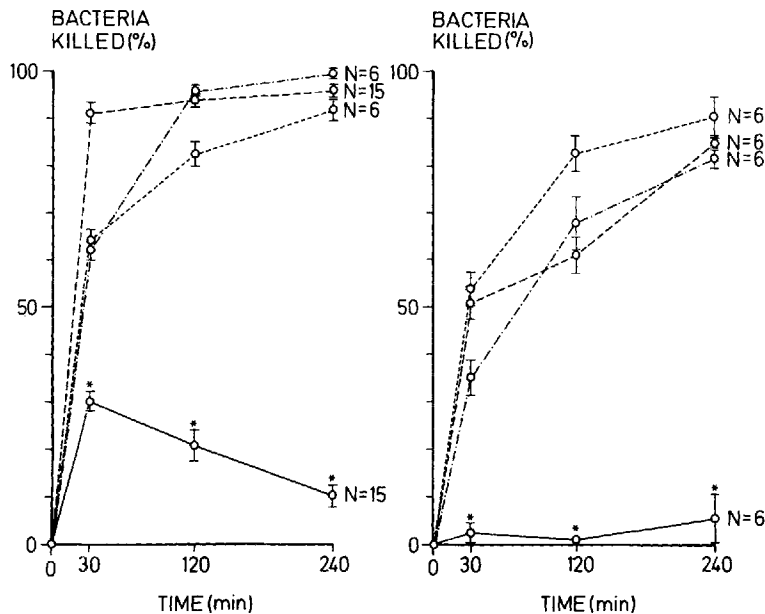
Figure 2. Bactericidal capacity of pol
morphonuclear leukocytes (PMNL
from sterile tissue cage fluid at 1
days after implantation in guinea pig
(o———o) compared with that c
peritoneal exudate PMNLs after
sterile acute (o- -o) or protracte
soluble (o— · —o) stimulation c
PMNLs from peripheral blood (o- -o)
The phagocytic bactericidal assa
contained, in a final volume of 1 m
of phosphate-buffered saline, 0.2 m
of pooled tissue cage fluid, 2 × 10
PMNLs, and (*left*) 8 × 10³ cfu (bac
teria : PMNL ratio, 1 : 250) or (*right*
4 × 10⁶ cfu (bacteria : PMNL ratio.
2 : 1) of washed *Staphylococcus
aureus*. Data are means ± SEM (bars).
Asterisks indicate significant differ-
ence (*P* < 0.001) in comparison with
each of the control PMNL prepara-
tions.

PMNLs was considerably lower than activities observed with PMNLs from acute and chronic peritoneal exudates and from peripheral blood (figure 2). This difference was significant with each bacterial inoculum and at each incubation interval tested, a result suggesting that the residential PMNLs in tissue cages were unable to cope with *S. aureus* strain Wood 46, even at minimal inocula. The viabilities of PMNLs from tissue cages, blood, and peritoneal exudate before and after incubation were comparable. Differential counts of leukocytes from tissue cages showed 40%-70% PMNLs and 30%-60% mononuclear leukocytes; these numbers were comparable to those obtained from persistent peritoneal exudates. In contrast to these results, in 10 assays the overall bactericidal activity of PMNLs accumulating in experimentally infected tissue cages at 20 hr after inoculation was 91.3% ± 1.9% when tested by standard bactericidal procedure. Similar results were recorded with control PMNLs from peritoneal exudates produced with glycogen or caseinate (bactericidal activity, 86.1% ± 2.4% [16 animals] and 79.6% ± 3.9% [10 animals], respectively).

Ingestion rates of PMNLs from sterile tissue cages. To define further the overall bactericidal defect observed with PMNLs from sterile tissue cage fluid, we measured their ingestion rate as well as their intracellular killing rate. In five animals,

PMNLs from tissue cages ingested 49.6% ± 2.3% and killed 39.1% ± 1.6% of the *S. aureus*, whereas peritoneal PMNLs ingested 87.2% ± 2.2% and killed 83.1% ± 2.5% of the bacteria. After determination of the number of ingested bacteria, it could be calculated that 21.1% ± 0.5% of the ingested organisms remained viable in PMNLs from tissue cages, whereas only 4.7% ± 0.5% of the bacteria ingested by control PMNLs survived intracellularly after 30 min of incubation.

Opsonic activity and complement levels of tissue cage fluid in the absence or presence of infection. After having identified a phagocytic and bactericidal defect in PMNLs from tissue cages, we analyzed another component of the phagocytic system — opsonization of the infecting organism. Figure 3 shows complement-mediated opsonic activity and total hemolytic complement levels in sterile tissue cage fluids, expressed as 50% killing and CH₅₀ units, measured at various intervals after surgery. Both values in tissue cage fluid decreased progressively with time, the opsonic activity being 14.4% and the complement level being 14.8% of the serum values at 75 days after surgery. When expressed per gram of protein, the values of the tissue cage fluids corresponded to 33.1% and 34.0% of the serum values, respectively. The correlation in 30 animals between the total hemolytic complement level and the opsonic titer was highly

Figure 3. Opsonic activity and total hemolytic complement level in tissue cage fluid from guinea pigs at intervals after implantation and in pooled normal guinea pig serum. Data are means ± SEM (bars) of the reciprocal dilutions that gave either 50% killing of bacteria in a phagocytic assay (K50) or 50% hemolysis in a hemolytic system (CH50). One asterisk indicates $P < 0.01$, and two asterisks indicate $P < 0.001$ in comparison with values in tissue cage fluid at 15 days after surgery.
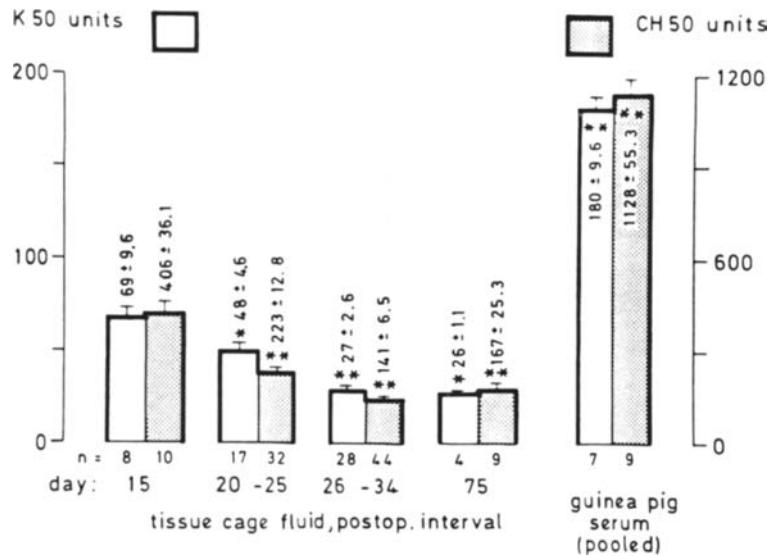
significant ($r = 0.81$; $P < 0.001$). In 43 animals, the mean ± SEM C3 level in tissue cage fluid was 18.6% ± 0.4% of the serum level.

In view of these low levels of 50% killing, CH_{50}, and C3 in tissue cage fluid, we considered the possibility that this fluid might be unable to promote adequate opsonization, as has been described for empyema [13]. To test this hypothesis, *S. aureus* strain Wood 46 was preopsonized in vitro with pooled guinea pig serum before inoculation into tissue cages. Preopsonization of bacteria was confirmed by incubating an aliquot with peritoneal exudate PMNLs for 30 min in a phagocytic assay which led to a killing rate of 80%. Despite optimal preopsonization, aliquots corresponding to 1.2×10^2 cfu injected into sterile tissue cages led to infection in four of four animals. Although the kinetics of bacterial growth and death were not formally tested in these experiments, low opsonic activity in tissue cage fluid could not be considered as a rate-limiting factor favoring the development of infection. During the development of infection, opsonic activity and the complement level were measured at 1 and 20 hr after inoculation of 10^7 cfu. The value of CH_{50} in six animals decreased moderately, from 267 ± 35.2 units to 172 ± 41.3 units ($P < 0.025$). Opsonic activity bound to *S. aureus*, however, showed a striking change during the course of the infection. In 20 animals, the opsonic coating of bacteria (figure 4, left) promoted 75.3% ± 2.9%

killing at 1 hr after infection, whereas killing was decreased to 46.7% ± 7.4% at 20 hr ($P < 0.001$). The opsonic coating of *S. aureus* could be improved (figure 4, middle) at 20 hr after infection by injection of 0.5 ml of fresh autologous serum (final concentration, 25%) into infected tissue cages, as demonstrated by aspirating the infecting bacteria 60 min after injection of fresh serum into tissue cages and testing the staphylococci in a phagocytic assay against peritoneal exudate PMNLs. In five assays, killing of these reopsonized bacteria improved from 35.3% ± 12.4% to 71.6% ± 4.1% ($P < 0.01$). Finally, the opsonic coating of *S. aureus* at 20 hr was poor (figure 4, right) regardless of the size of the initial inoculum, which varied from 10^3 cfu to 10^8 cfu. In 12 assays, killing of aspirated *S. aureus* could be improved from 38.2% ± 5.3% to 88.8% ± 2.0% ($P < 0.001$) by in vitro reopsonization with 10% pooled guinea pig serum. In summary, these experiments suggest that opsonic coating of *S. aureus* strain Wood 46 is incomplete at 20 hr after infection and that this defect can be corrected by addition of fresh guinea pig serum either into the tissue cage or to the phagocytic assay.

Discussion

The factors promoting foreign body infection are not well understood. In this report we have described an experimental guinea pig model that
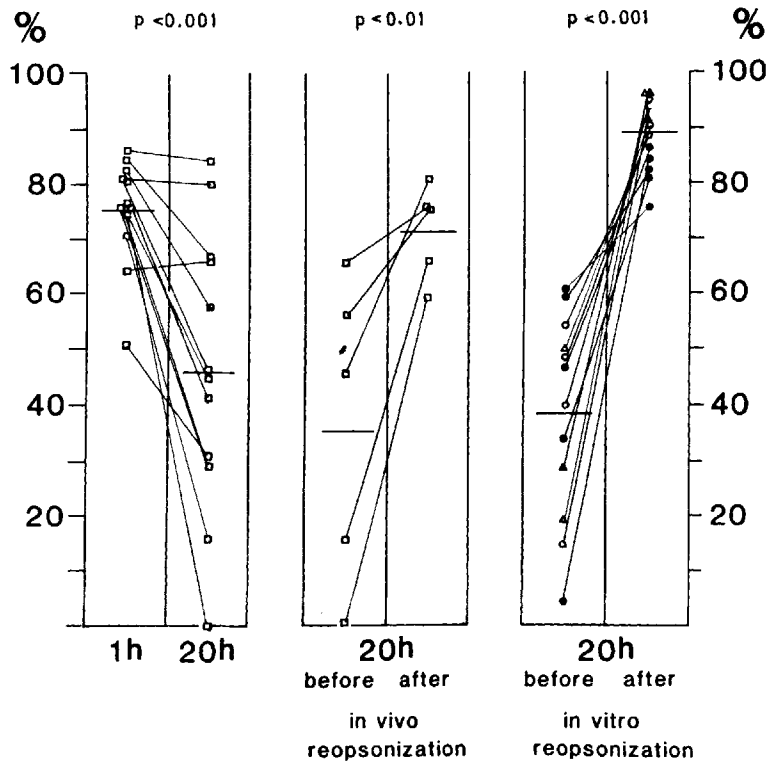
Figure 4. Opsonic activity bound to *Staphylococcus aureus* strain Wood 46 at intervals after experimental infection of tissue cages in guinea pigs: (*left*) 1 and 20 hr after infection with 10^7 cfu; (*middle*) 20 hr after infection with 10^7 cfu before and after in vivo supplementation of tissue cage fluid with 0.5 ml of fresh autologous serum (final concentration, 25%); and (*right*) 20 hr after infection with (O) 10^3 cfu, (●) 10^4 cfu, (△) 10^6 cfu, and (▲) 10^8 cfu before and after in vitro reopsonization with 10% pooled serum for a 30-min period. Data are percentages of killing of *S. aureus* isolated from infected tissue cages after incubation for 30 min in a phagocytic assay with 5×10^6 polymorphonuclear leukocytes from peritoneal exudate.

permits exploration of these factors and reproduces the main characteristics observed in its clinical counterpart. After sc implantation of polymer tissue cages, injection of 10^2 cfu of an organism of low virulence such as *S. aureus* strain Wood 46 led uniformly to local infection in <20 hr. Infection did not spread to any other organ and healed after elimination of the foreign body. These observations, as well as the generally accepted concept that the main host defense mechanisms against local infections with *S. aureus* include opsonization and phagocytosis [13, 33], led us to focus our analysis of these host factors only over the first 20 hr of infection. *S. aureus* strain Wood 46 was used in all experiments to avoid interference of staphylococcal protein A with IgG; this strain has been repeatedly shown to contain negligible amounts of protein A [26, 34] and to require complement only for optimal opsonization [13, 24, 26]. Although several biochemical determinations in tissue cage fluid differed from values in serum (data not shown), their variations were not sufficient to inhibit phagocytosis [35–39]. Such an effect, as well as a deleterious effect of

tissue cage fluid on PMNLs, was further ruled out by appropriate mixing experiments involving preincubation of tissue cage fluid with competent blood PMNLs.

Our observations have identified two host defense defects in the neighborhood of the foreign body that are manifested at distinct stages in an infection—namely, poor PMNL activity at the beginning and a steadily decreasing efficiency of opsonization during the course of the disease.

Initially, opsonization of strain Wood 46 was adequate and could therefore not account for the high susceptibility to infection in our model. Moreover, in vitro preopsonization of the infecting strain did not prevent the development of infection after inoculation into tissue cages. Our data further suggest that at this early stage, the PMNLs residing in sterile tissue cage fluid were deficient in their phagocytic bactericidal function when compared with control PMNLs from either blood—the milieu from which they are originating—or peritoneal exudates obtained by a single soluble stimulus. This defect could be demonstrated under the standard conditions of a conven-

tional phagocytic assay, as well as at a bacteria/ PMNL ratio of 1 : 250, which was the condition encountered in tissue cage fluid. Finally, this phagocytic bactericidal deficit in PMNLs from tissue cages tested two weeks after implantation was also evident when these cells were compared with PMNLs from an exudate of the same age, produced by soluble stimuli administered over 14 days.

Several aspects of this phagocytic bactericidal defect in PMNLs from tissue cages merit comment. (*1*) The development of a control system reproducing the conditions of a chronic exudate in the absence of a foreign body was of importance to compare the phagocytic function of PMNLs of similar ages in the presence and the absence of a foreign body. The identical time elapsed between the onset of these protracted stimuli (tissue cages or glycogen), the cytology of the two exudates, and the results of histopathologic examination of the two surrounding tissues suggest the presence of cell populations of the same age. This issue, however, can be resolved unambiguously only by future experiments involving comparison of pulse-labeled PMNLs; for the time being, comparison of PMNLs from blood and exudate has been considered as an adequate procedure in similar experiments [40]. (*2*) The observed phagocytic bactericidal defect in PMNLs from tissue cages is all the more striking because Van Epps and Garcia have demonstrated that PMNLs from peritoneal exudates of guinea pigs elicited by a soluble stimulus actually show increased, rather than decreased, metabolic activity [40].

In keeping with our results obtained with PMNLs from tissue cages, Klock and Bainton [41] and Klock and Stossel [42] have demonstrated that intimate contact of PMNLs with glass or nylon wool will lead not only to activation of their oxidative metabolism and to degranulation, but also to a decrease in the cells' phagocytic bactericidal function. Electron microscopic data obtained with our model have confirmed that PMNLs in close contact with tissue cages show decreased granule contents.[1] Degranulation and metabolic alterations have been described for other systems in-

volving PMNLs in contact with foreign, non-phagocytosable surfaces [43-45]. Taken together, these in vitro results suggest that intimate contact of PMNLs with foreign, nonphagocytosable surfaces might be detrimental to these cells, as suggested by the results of our experimental model. Finally, the deficit observed in PMNLs from tissue cages affected ingestion as well as bactericidal function, an observation suggesting that several subcellular mechanisms are altered. The mechanisms underlying these various alterations remain to be defined.

In contrast to these observations for the early stage of infection, opsonic coating of the infecting microorganism *S. aureus* strain Wood 46 — presumably by functional C3 [46] — was markedly decreased during the course of the infection. This observation is open to at least two interpretations. Either decreased opsonization of *S. aureus* might be a consequence of the imbalance between the rate of coating of bacteria with functionally active C3 and the logarithmic growth of the organisms, or cleavage of the opsonically active factor by β-1-H-globulin, C3b inactivator, and proteases [47-51] could have occurred during the development of a purulent infection. Loss of opsonic activity, as well as proteolytic cleavage of C3, has been recently demonstrated in several exudates rich in PMNLs [13, 15, 24]. Finally, our experiments showed that PMNLs attracted into the infected tissue cages displayed normal phagocytic bactericidal function, a finding which is apparently in contradiction with our observations in sterile tissue cages. In the case of a sterile tissue cage, however, the foreign body itself is probably responsible for the attraction of PMNLs; in the case of an abscess, additional chemoattractants such as bacterial polypeptides [52] are liberated, thus allowing PMNLs to accumulate in the fluid phase without being in contact with the foreign body. Intimate contact of PMNLs with foreign surfaces has indeed been shown to be necessary to alter the cells' metabolism and phagocytic functions [41, 42].

In conclusion, the phagocytic bactericidal defect in PMNLs from sterile tissue cage fluid probably represents one of the several determinants of foreign body infection and allows other pathogenic factors to progress during the first hours of the disease. Some of these factors

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[1] I. Joris, W. Zimmerli, G. Majno, and F. A. Waldvogel, "Foreign Body Reactions: Morphology of Inflammatory and Phagocytic Cells," manuscript in preparation.

may include organic substances coating the foreign body, which may protect bacteria from phagocytosis [53]; bacterial adhesion to foreign material [54]; and formation of a bacterial "glycocalyx" [55], which reduces bacterial accessibility to phagocytes and/or antibiotics. The animal model described here should permit the investigation of these and other possible factors that predispose foreign bodies to infection.

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