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### Research Article

Implanted foreign bodies are highly susceptible to pyogenic infections and represent a major problem in modern medicine. In an effort to understand the pathogenesis of these infections, we studied the phagocytic function in the vicinity of a foreign body by using a recently developed guinea pig model of Teflon tissue cages subcutaneously implanted (Zimmerli, W., F.A. Waldvogel, P. Vaudaux, and U.E. Nydegger, 1982, *J. Infect. Dis.*, 146:487-497). Polymorphonuclear leukocytes (PMN) purified from tissue cage fluid had poor bactericidal activity against a catalase-positive microorganism. When compared with blood or exudate PMN, they exhibited a significant reduction in their ability to generate superoxide in response to a particulate or a soluble stimulus (72 and 57%, respectively,  $P$  less than 0.001). Not only their total contents in myeloperoxidase, beta-glucuronidase, lysozyme, and B12 binding protein were significantly reduced (by 62, 21, 47, and 63%, respectively,  $P$  less than 0.01), but also their capability for further secretion of residual B12 binding protein upon stimulation. Ingestion rates of endotoxin-coated opsonized oil particles were reduced by 25% ( $P$  less than 0.05). In an effort to reproduce these abnormalities in vitro, fresh peritoneal exudate PMN were incubated with Teflon fibers in the presence of plasma. Interaction of PMN with the fibers led to significant increases in hexose monophosphate shunt activity and exocytosis of secondary granules ( $P$  less than [...])

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## Pathogenesis of Foreign Body Infection Evidence for a Local Granulocyte Defect

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**A**bstract. Implanted foreign bodies are highly susceptible to pyogenic infections and represent a major problem in modern medicine. In an effort to understand the pathogenesis of these infections, we studied the phagocytic function in the vicinity of a foreign body by using a recently developed guinea pig model of Teflon tissue cages subcutaneously implanted (Zimmerli, W., F. A. Waldvogel, P. Vaudaux, and U. E. Nydegger, 1982, *J. Infect. Dis.*, 146:487-497). Polymorphonuclear leukocytes (PMN) purified from tissue cage fluid had poor bactericidal activity against a catalase-positive microorganism. When compared with blood or exudate PMN, they exhibited a significant reduction in their ability to generate superoxide in response to a particulate or a soluble stimulus (72 and 57%, respectively,  $P < 0.001$ ). Not only their total contents in myeloperoxidase,  $\beta$ -glucuronidase, lysozyme, and B<sub>12</sub> binding protein were significantly reduced (by 62, 21, 47, and 63%, respectively,  $P < 0.01$ ), but also their capability for further secretion of residual B<sub>12</sub> binding protein upon stimulation. Ingestion rates of endotoxin-coated opsonized oil particles were reduced by 25% ( $P < 0.05$ ).

In an effort to reproduce these abnormalities in vitro, fresh peritoneal exudate PMN were incubated with Teflon fibers in the presence of plasma. Interaction of PMN with the fibers led to significant increases in hexose mono-

phosphate shunt activity and exocytosis of secondary granules ( $P < 0.01$ ). PMN eluted after such interaction showed defective bactericidal activity, oxidative metabolism, and granular enzyme content similar to those observed in tissue cage PMN.

The local injection of fresh blood PMN into tissue cages at the time of, or 3 h after, inoculation with 100 microorganisms (*Staphylococcus aureus* Wood 46) reduced the infection rate from 50 of 56 cages to 1 of 21 ( $P < 0.001$ ) and 3 of 8 cages ( $P < 0.001$ ), respectively. These results suggest that the in vivo as well as in vitro interaction of PMN with a nonphagocytosable foreign body induces a complex PMN defect, which may be partly responsible for the high susceptibility to infection of foreign bodies.

### Introduction

During the past two decades, an increasing number of prosthetic devices have been implanted in orthopedic (1-3), plastic reconstructive (4), cardiovascular (5-8), and general surgery (9); foreign materials have also been used extensively for the treatment of various medical problems (10-12). Infection is one of the major complications of such implants: it usually persists until removal of the prosthesis (13), leads to loss of function (14), and is associated with a high mortality especially in patients with cardiovascular prostheses (15). Despite our considerable clinical knowledge regarding infections of prosthetic devices, little is known about their pathogenesis (16).

Infections of prosthetic devices are characterized by (a) the high infecting power of a low inoculum, mostly staphylococci (1, 17, 18); (b) their protracted evolution (18, 19); (c) their usually limited spread to the tissues in immediate contact with the implanted foreign body (20), and (d) their persistence without removal of the prosthesis (13, 16). Based on these observations, we have previously postulated that local host defense mechanisms might be unable to cope with the local invasion of microorganisms, and have developed to that effect a guinea pig model of foreign body infection, using tissue cages implanted subcutaneously (21). This model reproduced all the previously mentioned characteristics of the disease and allowed the collection and analysis of interstitial fluid and inflammatory cells,

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which had been in contact with the implant. We also showed that whereas opsonic activity of sterile tissue cage fluid was adequate for optimal opsonization of *Staphylococcus aureus* Wood 46, the local tissue cage polymorphonuclear leukocytes (PMN)<sup>1</sup> were unable to kill the same bacteria in an in vitro phagocytic assay (21).

In the present study we have extended these findings by delineating: (a) some of the steps responsible for the abnormal bactericidal activity of the PMN surrounding the implanted tissue cages; (b) the mechanisms leading to such an acquired granulocyte defect; and (c) its pathogenic significance by preventing infection with local infusion of peripheral PMN.

## Methods

**Reagents.** Cytochrome C type VI, phorbol myristate acetate (PMA), superoxide dismutase, nitroblue tetrazolium (NBT), Triton X-100, *o*-dianisidine-di HCl, *N*-formyl-methionyl-leucyl-phenylalanine (FMLP), egg-white lysozyme standard, phenolphthalein- $\beta$ -glucuronate, NADH, and zymosan were purchased from Sigma Chemical Co., St. Louis, MO; cytochalasin B from Aldrich Chemical Co., Milwaukee, WI; Dextran T-500 and Percoll from Pharmacia Fine Chemicals, Uppsala, Sweden; endotoxine lipopolysaccharide B *Escherichia coli* 026: B<sub>6</sub> and micrococcus lysodeikticus from Difco Laboratories, Detroit, MI; phosphate-buffered saline (PBS, Dulbecco's) from Gibco-Bio-Cult Co., Glasgow, Scotland; D(+)-glycogen purum from Fluka AG, Buchs SG, Switzerland; diisodecyl phthalate from Sigfried Zofingen, Switzerland; <sup>57</sup>Co-B<sub>12</sub>, hexadecan-<sup>14</sup>C, and [1-<sup>14</sup>C]glucose from Amersham Corp., Arlington Heights, IL; and <sup>111</sup>indium-oxine from Byk-Mallinckrodt, Pretten, The Netherlands.

**Tissue cage implantation.** Rigid perforated polytetrafluoroethylene (Teflon) tubes (internal and external diameters of 10 and 12 mm, respectively; length, 32 mm) were implanted subcutaneously into guinea pigs, as previously described (21). Animals were used for further experimentation one or several weeks after tissue cage implantation.

**Preparation of leukocytes.** Acute peritoneal exudates rich in PMN (>95%) were obtained in guinea pigs by two i.p. injections of sterile glycogen in 0.9% NaCl (0.1%, wt/vol), 15 h and 2 h before harvest, respectively (22). Persistent peritonitis as a control for a chronic exudate generated in the absence of a foreign body was produced by repeated glycogen injections over a period of 2 wk. These latter exudates showed the same leukocyte differential counts as tissue cage fluid, i.e., ~40% PMN and 60% mononuclear leukocytes (21). The exudates were collected as previously described (21). Blood PMN were obtained as a 60–80% pure suspension by the method described by Chenoweth et al. (23). PMN of sterile tissue cage fluid were aspirated percutaneously from tissue cages 1–2 wk after surgery and washed with the same procedure as blood and exudate PMN (21). Blood contamination in tissue cage fluid was 0.5–5% at this postoperative interval. When necessary (median number of PMN per milliliter of tissue cage fluid,  $8 \times 10^5$ ), PMN were concentrated by centrifugation and pooled by using cells from various tissue cages.

1. **Abbreviations used in this paper:** CFU, colony-forming units; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; HMS, hexose monophosphate shunt; MPO, myeloperoxidase; NBT, nitroblue tetrazolium; PMA, phorbol myristate acetate.

Whenever mentioned, the mixed leukocyte population was further purified on a discontinuous Percoll gradient, adapted to guinea pig leukocyte separation from the method of Hjorth et al. (24). Stock isotonic Percoll was prepared by dissolving solid NaCl in Percoll to a final concentration of 0.15 M. This mixture is referred to as 100% Percoll (density  $\rho = 1.136$  g/ml). The separations were performed in  $17 \times 100$ -mm Falcon tubes (Becton & Dickinson Co., Oxnard, CA). PMN were separated from anticoagulated blood (10 mM EDTA, final concentration) as follows: 3 ml of dextran-sedimented, leukocyte-rich plasma was layered onto a double-density Percoll gradient. The gradient consisted of 3 ml of 50% Percoll (diluted with EDTA anticoagulated guinea pig plasma) on top of 3 ml of 70% Percoll. The gradient tubes were centrifuged at 350 g for 20 min. The mononuclear cells and platelets accumulated at the interphase between the plasma and the 50% solution, the PMN accumulated at the interphase between the two different Percoll solutions, and the erythrocytes sedimented to the bottom. The PMN fraction was harvested through the upper gradient and washed twice in 15 ml 0.9% NaCl at 150 g for 10 min. In order to purify tissue cage and exudate PMN, an identical procedure was used, but on a 40% top gradient and a 65% bottom gradient. This allowed the sedimentation of the less dense peritoneal exudate and tissue cage PMN at the interphase between the two different Percoll solutions. After this procedure >98% of each type of PMN excluded Trypan Blue. The yield of each type of PMN was between 40 and 60% of the initial cell count.

**Bactericidal assay.** The bactericidal assay used in these experiments has been previously described by us in detail (21). We used two microorganisms as test strains: a catalase-negative species, i.e., *Streptococcus faecalis* (HC82, wild strain), and a catalase-positive species, i.e., a *S. aureus* (strain Wood 46). Each of these strains depended on complement for its opsonization, as shown by the total absence of PMN killing after opsonization with heat-inactivated serum (56°C for 30 min). The test incubation contained 0.1 ml of the washed and appropriately diluted bacterial suspension [ $2 \times 10^6$  colony-forming units (CFU)], 0.1 ml of pooled guinea pig serum, and 0.8 ml of PBS containing  $1 \times 10^6$  PMN. This suspension was incubated at 37°C for 2 h in a shaking (140 rpm) water bath. Counts of the viable bacteria were assessed at zero time, 1 h, and 2 h of incubation, according to the method previously described (21). The bacterial killing by exudate PMN and tissue cage PMN was calculated from the results of these repetitive cultures. Bacterial clumping, which can artifactually diminish the bacterial counts in the absence of PMN killing, was excluded by morphological controls with gram stains and by control experiments in which the bacteria were incubated in the absence of PMN. In these test tubes, growth of *S. aureus* Wood 46 reached  $380 \pm 43\%$ , and of *S. faecalis*  $170 \pm 19\%$  (mean  $\pm$  SD), respectively, at 2 h.

**Ingestion rate.** Ingestion was quantitated by the uptake rates of endotoxin-coated diisodecylphthalate particles as previously described (25) with the following modifications: diisodecylphthalate was labeled with hexadecane-<sup>14</sup>C (26) and a micromethod was used (27). Normal pooled guinea pig serum was used to opsonize the particles.

**Superoxide generation assay.** Superoxide ion production was measured as the superoxide dismutase-sensitive reduction of cytochrome *c* in a continuous assay as described by Newburger et al. (28). We used PMA (0.01–2  $\mu$ g/ml) and opsonized zymosan (1–4 mg/ml) as stimuli (28). The NBT slide test was performed according to the method of Newburger et al. (29) and scored as described by Lew et al. (30). For this assay Percoll-purified PMN were used.

**Enzyme assays.** All enzyme assays were performed with Percoll-purified PMN. Total enzymatic activity of PMN was determined by adding 0.025–0.1% (final concentration) Triton X-100 to the cell sus-

pensions in the following assays. Myeloperoxidase (MPO) activity was determined in a continuous assay, by following the change of absorbance at 450 nm which accompanies the oxidation of *o*-dianisidine-di HCl as described by Babior and Cohen (31). The rate of oxidation of dianisidine was calculated as follows: dianisidine oxidation in nanomoles per minute =  $50 \times A$  units (31). Lysozyme activity was determined by measuring the rate of lysis of *Micrococcus lysodeikticus* at pH 6.2 with a turbidometric method (31). Enzyme activity was expressed in terms of micrograms per milliliter egg-white lysozyme standard (31).  $\beta$ -Glucuronidase activity was assayed by measuring the release of phenolphthalein from its  $\beta$ -glucuronate after 2-h incubation at pH 4.5 (31). Activity was expressed as nanomoles of phenolphthalein hydrolyzed by  $10^6$  PMN per minute (activity nmol/ $10^6$  PMN =  $0.45 \times A$  units/2 h) (31).  $B_{12}$  binding protein was measured by its ability to take up radioactive cobalamin from the medium.  $B_{12}$  binding capacity was expressed as picograms per  $10^6$  cell equivalents (31). Lactic dehydrogenase was assayed by measuring the consumption of NADH (31).

MPO slide tests were performed according to Kaplow (32).

**Degranulation assay.** Degranulation was measured as the release of  $B_{12}$  binding protein into the extracellular fluid. Two different stimuli were used, namely FMLP in the presence of cytochalasin B and opsonized zymosan. PMN ( $2.5 \times 10^6$ /ml) were preincubated at 37°C for 5 min in the presence or absence of cytochalasin B (5  $\mu$ g/ml). At this time, FMLP ( $10^{-7}$  M) or opsonized zymosan (3 mg/ml), respectively, was added. The incubation was continued for another 5 min (FMLP) or 10 min (zymosan). The reaction was stopped in ice, the PMN were sedimented at 1,000 rpm/10 min, and  $B_{12}$  binding protein was measured as marker for secondary granule exocytosis (31). The absence of measurable lactic dehydrogenase activity excluded cell destruction (31). Results are given as percent of total cellular enzyme, determined after lysis with Triton-X (0.025%).

**PMN turnover assay.** In order to get an estimate of the time of exposure of PMN to the tissue cage surface, we studied their turnover time as previously described by Cohen et al. (33). Briefly,  $5 \times 10^6$ – $1 \times 10^7$  Percoll-purified homologous blood PMN were resuspended in 1 ml of PBS. 200–300  $\mu$ Ci  $^{111}$ indiumoxine was added, and the PMN were incubated for 20 min at 37°C. After washing twice in NaCl 0.9%/plasma 30%, the PMN-associated radioactivity, which usually exceeded 80% of the added value, was determined. These washed cells were suspended in 1 ml of PBS and administered by intracardiac injection to a guinea pig with two implanted tissue cages. Leukocyte differential counts were determined in blood and tissue cage fluid at 2 and 30 min, and 2, 4, 20, 24, and 27 h after injection. Total and cell-associated radioactivity were determined in aliquots of the samples with a gamma-radiation counter. The cell-associated radioactivity was usually 70–80% of the added value during the first 30 h after PMN administration into the cages. Turnover rate ( $k$ ) and turnover time ( $t_r$ ) were calculated as described by Cohen et al. (33).

**In vitro interaction between tissue cage material (Teflon) and PMN.** To test the interaction between tissue cage material and PMN, we used peritoneal exudate PMN. The PMN-foreign body interaction was studied with three types of experiments. First, we measured the oxidation of [ $^{14}$ C]glucose [hexose monophosphate shunt (HMS)] during the incubation with 200 mg of Teflon fibers (diameter 0.3 mm; Angst & Pfister Co., Zürich, Switzerland). 0.5 ml of a PMN suspension in PBS ( $2 \times 10^7$  PMN/ml) containing 0.2  $\mu$ Ci of [ $^{14}$ C]glucose and 0.5 ml guinea pig plasma were incubated with or without 200 mg Teflon fibers at 37°C in vials stoppered by caps fitted with cups (Kontes Glass Co., Vineland, NJ), and containing filter paper wicks impregnated with 200  $\mu$ l of NaOH 8%. The reaction was stopped at different incubation time

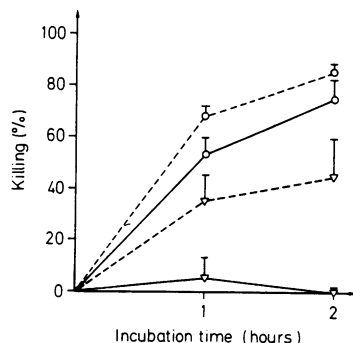
intervals after gentle shaking in a water bath by injection of 0.5 ml of 2 N H<sub>2</sub>SO<sub>4</sub> through the stopper into the incubation medium. After a 30-min equilibration period, the cups were removed and assayed for radioactivity. Positive controls were run in each assay using 1  $\mu$ g of PMA as stimulus. These values were 15–35 times higher than the activity of resting cells. Negative controls were run with 0.5 ml 2 N H<sub>2</sub>SO<sub>4</sub> in the incubation medium. These values were 10–15 times lower than the activity of resting cells (34). Second, we determined exocytosis during contact with Teflon fibers by measuring  $B_{12}$  binding protein and  $\beta$ -glucuronidase activity (31) released in the supernatant of a PMN suspension ( $5 \times 10^6$ /ml in PBS supplemented with 5% heated serum). Third, we measured the residual bactericidal function, the residual superoxide production capacity, and the residual enzymatic activity of PMN after their incubation with Teflon fibers as follows. 200 mg Teflon fibers were incubated during 1 h with  $10^7$  PMN in PBS supplemented with 10% plasma in polypropylene syringes of 5 ml (Becton & Dickinson Co.). Control PMN were incubated under the same conditions but without Teflon fibers. To remove PMN from the Teflon fibers, the syringes were rinsed with 5 vol NaCl 0.9% and 10 vol acid-citrate-dextrose, by gently tapping the syringe barrel against the palm of the hand (34, 35). This procedure enabled us to recover >50% of the PMN incubated with Teflon and >70% of the control PMN. PMN recovered from different syringes were pooled and resuspended in PBS at a concentration of  $1 \times 10^7$ /ml and tested for their residual bactericidal activity with *S. aureus* Wood 46, for their superoxide production by stimulation with PMA and opsonized zymosan, and for their residual MPO activity (see above).

**Experimental infections and protection experiments.** *S. aureus* Wood 46, stored in aliquots of the same initial culture at –70°C, was used as infecting microorganism for experimental tissue cage infections. Overnight cultures in Muller-Hinton broth were washed and resuspended in 0.9% NaCl. Tissue cages were inoculated with 0.2 ml of a  $10^{-6}$  dilution, which usually contained  $5 \times 10^2$ – $10^3$  CFU/ml, as routinely checked by plating on Muller-Hinton agar. PMN used for protection experiments were harvested according to the method described above. For each protection experiment,  $1 \times 10^7$  peripheral blood PMN or tissue cage PMN were injected in a volume of 0.2 ml of PBS into the tissue cage. The serum injected into tissue cages in some experiments was collected and pooled as previously described (21). Quantitative bacterial cultures with a minimal detection level of 10 CFU were performed by aspiration of tissue cage fluid for 1 wk. Tissue cage fluid showing no growth (<10 CFU/ml) 8 d after inoculation were recorded as sterile.

## Results

### Validation of the experimental model

1–3 wk after tissue cage implantation, fluid was aspirated under sterile conditions and analyzed as to sterility and PMN counts, which ranged from  $7 \times 10^4$  to  $8 \times 10^6$ /ml (median value  $8 \times 10^5$ /ml) for 39 cages tested. Injection of  $10^2$  CFU of *S. aureus* Wood 46 led to infection in 37 of 39 cages, as documented by macroscopic examination and quantitative bacterial cultures. The two only tissue cages that could not be infected had  $6 \times 10^5$  and  $3 \times 10^6$  PMN/ml, respectively, i.e., not the highest cell counts. We concluded that infection could be easily produced under standard conditions, irrespective of the number of PMN present in the cage fluid at the time of inoculation. We therefore undertook to analyze some phagocytic and metabolic functions of these PMN before experimental infection, taking PMN from



**Figure 1.** Bactericidal capacity of PMN obtained from sterile tissue cage fluid 14 d after implantation ( $\nabla$ ) compared with peritoneal exudate PMN obtained after stimulation with glycogen 0.1% ( $\circ$ ). The phagocytic-bactericidal assay contained in a final volume of 1 ml PBS,  $1 \times 10^6$  PMN,  $2 \times 10^6$  CFU of *S. aureus* Wood 46 (—),  $2 \times 10^6$  CFU of *S. faecalis* (---), respectively, and 0.1 ml pooled guinea pig serum. Each point represents the mean of six determinations. Bars represent  $\pm 1$  SD.

blood and from acute and chronic peritoneal exudates as controls. Viability of tissue cage PMN at the time of their *in vitro* testing was confirmed by a Trypan Blue exclusion of 95–97% as well as by their ability to reduce NBT (see below). In addition, we determined the turnover rate ( $k$ ) and time ( $t_k$ ) of homologous,  $^{111}\text{In}$ -labeled blood PMN in the tissue cage. After intracardiac injection, the homologous, labeled PMN disappeared from the blood with a half-life time of 5.4 h. The turnover rate of PMN in the tissue cages was  $0.17 \text{ h}^{-1}$ , and their turnover time, i.e., the average lifetime of a PMN in the tissue cage fluid, was 3.7 h. Thus, any functional or metabolic difference between blood and tissue cage PMN can not be ascribed to major age differences of the cell population tested.

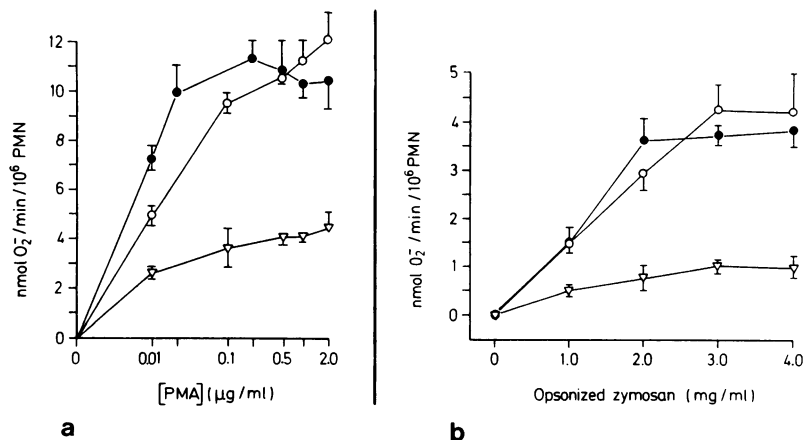
#### Evaluation of various phagocytic and metabolic activities of tissue cage PMN

**Bactericidal activity and ingestion rate.** Bactericidal activity of tissue cage PMN was tested on a catalase-positive, i.e., *S. aureus*

Wood 46, and a catalase-negative microorganism, i.e., *S. faecalis*. Fig. 1 shows that tissue cage PMN were unable to kill the catalase-positive microorganism, whereas they still possessed some bacterial activity against a catalase-negative strain. Particularly striking were the results obtained with the catalase-positive strain *S. aureus* Wood 46, where almost no killing could be demonstrated either at 60 or at 120 min despite optimal previous opsonization. These results led us to investigate several parameters important in the bactericidal activity of normal PMN (36, 37). Small, but significantly decreased, ingestion rates of endotoxin-coated oil particles were observed. We found an ingestion rate of  $75 \pm 27\%$  ( $508 \pm 184$  cpm, mean  $\pm$  SD,  $n = 5$ ) for tissue cage PMN compared with  $100 \pm 14\%$  ( $672 \pm 92$  cpm, mean  $\pm$  SD,  $n = 6$ ) for peritoneal exudate cells ( $P < 0.05$ ).

**Superoxide production.** Fig. 2 shows the superoxide production rate as a function of (a) the concentration of the soluble stimulus PMA, and (b) the particulate stimulus, opsonized zymosan. With suboptimal as well as with optimal soluble and particulate stimulation, tissue cage PMN produced markedly less superoxide than control PMN. The superoxide production rate of chronic peritoneal exudate cells was within the same range as for blood-derived PMN. NBT slide tests showed that nearly all tissue cage PMN reduced NBT, but to a lower degree than peritoneal control PMN (Table I).

**Total granular enzyme content.** Inasmuch as a nonphagocytosable surface may stimulate exocytosis under certain experimental conditions (38), we measured MPO, lysozyme,  $\beta$ -glucuronidase, and  $\text{B}_{12}$  binding protein activities of tissue cage PMN and compared them with peritoneal exudate PMN (Table II). Total MPO activity of tissue cage PMN was reduced to 38%, lysozyme to 53%,  $\beta$ -glucuronidase to 79%, and  $\text{B}_{12}$  binding protein to 37% of control values. MPO slide tests showed that most of the tissue cage PMN had a lower granule content than control PMN, as established by a scoring system showing that 5% (control 0%) of PMN were negative, 44% weakly positive (control 3%), and 51% strongly positive (control 97%). These results taken together suggested that partial degranulation had taken place during the previous contact with the implanted foreign body.



**Figure 2.** (a) The effect of PMA concentration on the rate of  $\text{O}_2^-$  generation by tissue cage PMN ( $\nabla$ ), chronic peritoneal exudate PMN ( $\circ$ ), and blood PMN ( $\bullet$ ). PMA was added at zero time and  $\text{O}_2^-$  production was measured under standard assay conditions (28). Each point represents the mean of triplicate determinations. Bars represent  $\pm 1$  SD. (b) Same assay as described above but with increasing concentrations of opsonized zymosan.

Table I. Reduction of NBT by PMN Purified from Tissue Cage Fluid and from a Peritoneal Exudate\*

Degree of NBT reduction‡	PMN reducing NBT	
	Tissue cage PMN	Peritoneal control PMN
	%	%
None	7	0
Weak	63	35
Strong	30	65

\* The slide test was performed as described by Newburger et al. (29), with PMN in suspension in the presence of PMA (1 µg/ml).

‡ Individual PMN were examined under direct microscopy by two examiners in a blind fashion and scored as previously described (30).

**Degranulation.** To test the potential of tissue cage and peritoneal exudate PMN to mobilize granule components, we incubated the cells with two different stimuli. The results are given in Table III. Tissue cage PMN had a decreased ability to degranulate when compared with peritoneal exudate PMN. The impaired degranulation was observed both with a soluble (FMLP in the presence of cytochalasin B), as well as with a particulate (zymosan) stimulus.

#### *In vitro interaction of foreign body material (Teflon fibers) with PMN*

In that these descriptive results did not allow us to determine how the PMN defects were acquired, we tried to reproduce them in vitro by adding intact PMN suspended in 10% guinea pig plasma to a nonphagocytosable surface: 10<sup>7</sup> peritoneal PMN were incubated in the presence and in the absence of 200 mg Teflon fibers for 2 h to assess HMS activity, for 1 h to assess endpoint PMN functions (phagocytic-bactericidal activity, oxidative metabolism, and total residual MPO activity), and for

30 min for exocytosis. Fig. 3 a shows that the interaction between PMN and a foreign body led to a modest but significantly increased metabolic activity during the 2 h of incubation as measured by the HMS. Furthermore, Fig. 3 b shows that the mentioned interaction led to selective release of the secondary granule marker B<sub>12</sub> binding protein, as shown by the higher release of B<sub>12</sub> binding protein in the Teflon fibers containing medium in the absence of a significant release of β-glucuronidase (<5% of total β-glucuronidase released for the whole period tested in both conditions). When these peritoneal PMN were eluted from the Teflon fibers with acid-citrate-dextrose and tested for their residual phagocytic-bactericidal activity, oxidative metabolism, and MPO content, the PMN showed a significant decrease in all these parameters when compared with the same PMN population incubated without Teflon fibers, but treated identically (Table IV). Thus, in vitro contact of exudate PMN with non-phagocytosable Teflon surfaces led to increased HMS activity and exocytosis, and produced a cell population with decreased functional and metabolic residual activities, as previously demonstrated in the animal model.

#### *Protective effect of PMN infusion into tissue cages*

Because tissue cages could be almost uniformly infected with 100 CFU of *S. aureus*, and because tissue cage PMN had been shown subsequently to be defective in overall killing, ingestion rate, oxidative metabolism, and various enzymatic activities, we investigated whether the very high infection rate obtained with an inoculum of 100 CFU of *S. aureus* could be reduced by the local administration of intact PMN. With the infusion of 1 × 10<sup>7</sup> PMN obtained from peripheral blood, the mean number of PMN in the tissue cage fluid was increased from 6.1 × 10<sup>5</sup> (5 × 10<sup>4</sup>–2 × 10<sup>6</sup>) to 2.3 × 10<sup>6</sup> (1 × 10<sup>5</sup>–7.2 × 10<sup>6</sup>) per ml of tissue cage fluid. With 1 × 10<sup>7</sup> tissue cage PMN the cell counts increased from 3 × 10<sup>5</sup> (5 × 10<sup>4</sup>–5 × 10<sup>5</sup>) to 1.6 × 10<sup>6</sup> (9 × 10<sup>5</sup>–3.3 × 10<sup>6</sup>) PMN per ml of tissue cage fluid. Fresh serum was added at time 20 h because opsonic activity of tissue cage fluid was decreased at this period of infection (21). Table V shows the results of different protection regimens. Without

Table II. Granular Enzymatic Activity of Tissue Cage PMN in Comparison with Peritoneal Exudate PMN

PMN type	Granular enzymatic activity released by Triton X-100			
	MPO	Lysozyme	β-Glucuronidase	B <sub>12</sub> binding protein
	nmol/min per 10 <sup>6</sup> PMN	µg	nmol/min per 10 <sup>6</sup> PMN	pg/10 <sup>6</sup> PMN
Tissue cage	55±7 (10)	0.9±0.13 (6)	1.1±0.07 (6)	20.1±1.1 (6)
Peritoneal	146±8 (5)	1.7±0.14 (6)	1.4±0.01 (6)	54.1±1.1 (6)
Significance P*	<0.001	<0.001	<0.01	<0.001

MPO activity was determined in a continuous assay following the change of absorbance at 450 nm that accompanies the oxidation of *o*-dianisidine (31). The lysozyme assay was based on the change in light absorption by a suspension of *Micrococcus lysodeikticus*, when lysozyme acts on it (31). β-Glucuronidase activity was assayed by measuring the release of *p*-nitrophenol from *p*-nitrophenyl β-D-glucuronide (31). B<sub>12</sub> binding protein was measured by its ability to take up radioactive cobalamin from the medium (31). Mean±SD (n). \* Statistical analysis with the unpaired Student's *t* test.

Table III. Extracellular Release of B<sub>12</sub> Binding Protein from Tissue Cage PMN and Peritoneal Exudate PMN Incubated under Different Conditions\*

Stimulus	B <sub>12</sub> binding protein released from ‡		Significance§ P
	Tissue cage PMN	Peritoneal exudated PMN	
	%		
PBS	3.5±1.13	3.2±0.25	>0.05
Cytochalasin B (5 µg/ml) + FMLP (10 <sup>-7</sup> M)	12.4±1.04	16.2±0.71	<0.01
Opsonized zymosan (3 mg/ml)	7.5±0.63	10.4±0.21	<0.001

\* 2.5 × 10<sup>6</sup> PMN/ml were preincubated at 37°C for 5 min in the presence or absence of cytochalasin B. At this time the mentioned stimulus (FMLP or zymosan) was added and the incubation continued for another 5 and 10 min, respectively. The reaction was stopped in ice, the PMN were sedimented at 1,000 rpm/10 min, and the B<sub>12</sub> binding protein was determined as described in Methods (31). Lactic dehydrogenase release was <3%.

‡ Percent of total cellular B<sub>12</sub> binding protein, as determined by lysis with Triton X-100 (0.025% final concentration). Mean±SD from three determinations.

§ Statistical analysis with the unpaired Student's *t* test.

any addition of PMN and serum, implanted tissue cages had an infection rate of 91%. The single local injection of 10<sup>7</sup> blood PMN into 2 ml of tissue cage fluid at the time of inoculation of 100 CFU of *S. aureus* did not afford any protection against infection. In contrast, two PMN infusions, one at the time of the bacterial inoculation and the other 20 h later were highly protective. The same regimen was significantly protective, when the first PMN infusion was performed 3 h after the bacterial

challenge, but was ineffective when delayed by 6 h. A control experiment with two injections of 10<sup>7</sup> tissue cage PMN provided no significant protection, despite a similar increase of cell counts (Table V). Thus, implanted tissue cages could be protected from infection by appropriately timed peripheral blood PMN infusions in the first hours after bacterial challenge.

## Discussion

Infections arising in close contact with a foreign body or with implanted prosthetic material are a vexing clinical problem, which to the present time has escaped adequate understanding and effective treatment strategies (16). Such infections are characterized microbiologically by the high prevalence of staphylococci (16, 39, 40), histologically by a cellular infiltrate rich in PMN, and clinically by the absence of spread beyond the foreign body in most cases, its protracted course, and its absence of cure without removal of the foreign material (13). These empirical observations have been well illustrated by the classic work of Elek and Conen (41), and by Noble (42) who demonstrated in a stitch abscess model that the initial bacterial inoculum could be as low as 100 CFU of *S. aureus*, whereas 10<sup>7</sup> microorganisms injected subcutaneously would not produce any lasting infection. Unfortunately, the model developed by these authors did not allow a quantitative evaluation of the microbiologic, immunologic, and cellular events preceding, or associated with, a foreign body infection.

We have recently developed an animal model which leads Elek's and Noble's observation one step further by allowing the quantitation of the microbiologic, of the fluid phase, and of the cellular events induced by a sterile or an infected foreign body (21). It also reproduces the main clinical characteristics of clinical prosthetic infection, including the occasional emergence of a resistant organism after protracted antibiotic treatment (43).

Inasmuch as it is generally accepted that staphylococcal infections are controlled by phagocytes, we postulated that one

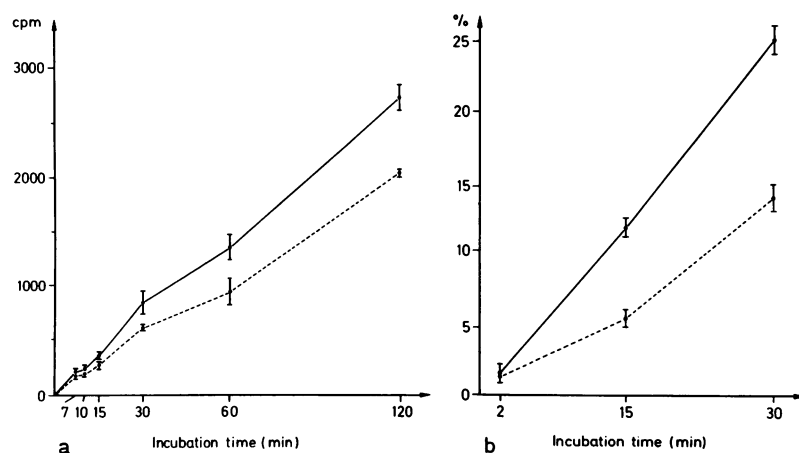


Figure 3. (a) Results of oxidation of [1-<sup>14</sup>C]glucose to <sup>14</sup>CO<sub>2</sub> by 10<sup>7</sup> PMN incubated in 50% plasma/PBS solution with (—) or without (---) 200 mg Teflon fibers, respectively, and 0.2 µCi of [1-<sup>14</sup>C]glucose. Figure shows counts per minute trapped as CO<sub>2</sub> vs. time. Each point is the mean of three determinations. Bars represent ± 1 SD. Significances of difference between <sup>14</sup>CO<sub>2</sub> production in absence and in presence, respectively, are *P* < 0.05 at 7 min, *P* < 0.025 at 10 and 30 min, and *P* < 0.01 at 15, 60, and 120 min (Student's related *t* test). (b) Extracellular release of B<sub>12</sub> binding protein from 5 × 10<sup>6</sup> peritoneal exudate PMN incubated with (—) or without (---) 200 mg Teflon fibers. Results are given as percent total enzyme released in the medium after 2, 15, and 30 min of incubation at 37°C. Each point represents the mean of three determinations. Bars represent ± 1 SD. The significance of differences was *P* < 0.01 at 15 min and *P* < 0.02 at 30 min (Student's related *t* test).

Table IV. Effect of Preincubation of PMN with Nonphagocytosable Teflon Fibers: Residual Functional and Metabolic Activities

Assay	Incubation of $1 \times 10^7$ peritoneal exudate PMN at 37°C for 1 h		Significance* P
	Without Teflon	With 200 mg Teflon	
Phagocytic-bactericidal test, %	67.1±3.6 (6)‡	12.1±9.9 (6)	<0.001
Superoxide production, nmol $O_2^-$ /min per $10^6$ PMN			
Phorbol myristate acetate	10.3±1.3 (3)	5.4±1.3 (6)	<0.001
Preopsonized zymosan	2.6±0.2 (3)	1.3±0.8 (3)	<0.05
Myeloperoxidase activity, nmol <i>o</i> -dianisidine oxidized/min per $10^6$ PMN	73.4±6.8 (3)	49.4±6.8 (5)	<0.001

The phagocytic-bactericidal activity was determined as described in Zimmerli et al. (21). The results are reported as percent killing of  $2 \times 10^6$  CFU *S. aureus* after 45-min incubation with  $2 \times 10^6$  PMN/ml. The superoxide production was measured with the method described by Newburger et al. (29). The results are reported as nmol  $O_2^-$ /min per  $10^6$  PMN after challenge with the mentioned stimulus. MPO activity was determined in a continuous assay following the change of absorbance at 450 nm that accompanies the oxidation of *o*-dianisidine (31). Results are reported as nmol *o*-dianisidine oxidized/min per  $10^6$  PMN.

\* Statistical analysis with the unpaired Student's *t* test  
‡ Mean±SD (*n*).

of the factors leading to foreign body infection could be a locally acquired phagocytic defect. In our previous study, we demonstrated that the fluid-phase bathing sterile tissue cages promoted adequate opsonization, but that the PMN isolated from sterile tissue cage fluid were unable to kill *S. aureus* Wood 46 adequately. In the present study, we have extended these results by showing that this phagocytic-bactericidal defect (a) involves multiple steps in the phagocytic events, including decreased ability to ingest particles, decreased granule content, and decreased capacity to mount a respiratory burst; (b) that it can be reproduced in vitro by incubation of peritoneal PMN with nonphagocytosable Teflon fibers; and (c) that it is of pathogenic importance, in that local infusion of PMN protects against tissue cage infection.

Several of our results deserve some comments. First, we considered that peripheral blood PMN were not fully adequate controls for our tissue cage studies, because peripheral blood and exudate PMN have been recently shown to differ in some of their characteristics (44). By repeated glycogen injections we were able to produce chronic peritoneal exudates in the absence of a foreign body, as determined by cytological criteria. Care

was taken to harvest these exudates at a time interval similar to the first aspiration of tissue cage fluid, and to purify the cells under the same conditions. We therefore believe that the chronic peritoneal PMN provided an adequate control for the tissue cage PMN. In addition, we tried to rule out that tissue cage PMN were defective merely because of aging or incipient cellular death. Indeed, our turnover studies showed short half-lives for tissue cage PMN. NBT slide tests and Trypan Blue exclusion tests confirmed the viability of the cells studied. Moreover, the NBT histochemical technique allowed us to demonstrate that the PMN defect involved the majority of the cells in the cage and did not involve two different subpopulations.

Second, the very poor bactericidal activity of tissue cage PMN demonstrated against *S. aureus*, but much less dramatically against catalase-negative *S. faecalis*, and the markedly decreased oxidative metabolism of tissue cage PMN upon stimulation with opsonized zymosan are reminiscent of early observations made on PMN from patients with chronic granulomatous disease (36, 37). However, Lew et al. (30) have recently shown that, if PMN from a patient with a variant of chronic granulomatous disease generate even small amounts of superoxide, the PMN can mount a significant bactericidal activity against catalase-positive organisms. Thus, the described defect, although striking, cannot be the sole explanation for the poor bactericidal activity of tissue cage PMN against *S. aureus*. We postulate that this defect is due to the combined synergistic effect of partial deficiencies at various steps of the phagocytic process, and also includes decreased ingestion rates and decreased granule contents as well as the ability to degranulate further. MPO and lysozyme were markedly decreased and these enzymes have been shown to potentiate the bactericidal activity of phagocytes (45). Because  $B_{12}$  binding protein, a secondary granule marker, was the most decreased function, our findings confirm the observation of Wright and Gallin (46) showing a selective release of secondary granules during adherence to foreign surfaces.

What is the cause of the defects found in tissue cage PMN? Several possibilities had to be investigated, the most obvious ones being a detrimental factor present in tissue cage fluid and toxic to incoming PMN, and a PMN-PMN interaction between residential and incoming phagocytic cells. Neither the preincubation of peritoneal PMN with 50% tissue cage fluid nor the coincubation of tissue cage PMN with peritoneal PMN decreased the phagocytic bactericidal activity of these cells (unpublished observations).

We therefore postulated that the tissue cage PMN population was damaged by a temporally limited contact with the foreign body to which they had been attracted. This damage could be due to frustrated phagocytosis along the nonphagocytosable surface, as proposed in another experimental situation by Klock and Stossel (34), Klock and Bainton (35), and Wright and Gallin (46). These authors showed that adherence of PMN to nylon fibers is associated with variable degrees of cell spreading (47) and activation of oxygen metabolism and exocytosis of granules (46) leading to membrane damage (34) and to defective bactericidal activity (35). In order to detect whether similar defects could be elicited in our system, we incubated peritoneal PMN



Table V. Local Treatment with Infusions of PMN into Tissue Cages

Local injection of PMN at time (h) of:*				Local injection of 0.2 ml serum at 20 h*	Infection rate	Significance	
0	3	6	20			$P_1$ ‡	$P_2$ ‡
					<i>n</i>	%	
<b>10<sup>7</sup> blood PMN</b>							
-	-	-	-	-	50/56	= 91	
+	-	-	-	-	5/5	= 100	NS
+	-	-	-	+	3/4	= 75	NS
+	-	-	+	+	1/21	= 5	<0.001
-	+	-	+	+	3/8	= 38	<0.001
-	-	+	+	+	6/6	= 100	NS
<b>10<sup>7</sup> tissue cage PMN</b>							
+	-	-	+	+	2/4	= 50§	NS <0.05

The peripheral blood PMN and the tissue cage PMN used in these experiments were purified as described in Methods. The serum was collected and pooled as previously described (21). For each protection experiment,  $1 \times 10^7$  peripheral blood PMN were injected in 0.2 ml of PBS. In the control experiment,  $1 \times 10^7$  tissue cage PMN from several tissue cages of one guinea pig were injected in 0.2 ml of PBS. \* Time after inoculation of 100 CFU of *S. aureus* Wood 46 into tissue cages. Zero time means that PMN were injected 2 min after the bacterial inoculation. ‡ Statistical analysis: Fisher's exact test.  $P_1$  = treatment vs. no treatment;  $P_2$  = treatment with tissue cage PMN vs. blood PMN treatment; NS = >0.05. § In this particular experiment, the simultaneous infection rate of four unprotected animals was 50%. Statistical comparison was done with pooled results of control animals.

with Teflon fibers in plasma and found a modest but significant increase in the HMS activity of PMN incubated with Teflon fibers in comparison with the controls. These results indicate a stimulation of metabolic activity by adherence to nonphagocytosable material (48). In a second type of experiments, we detected significant increases in the release of a secondary granule marker in presence of Teflon fibers. In a third series of experiments, we tested the phagocytic-bactericidal and metabolic activities of PMN after their incubation with Teflon fibers, which had been carefully washed to exclude the presence of phagocytosable material. The results showed that, in the absence of any phagocytosis, the various functional and metabolic parameters of fresh PMN were markedly reduced, an additional argument in favor of PMN-Teflon interaction and PMN stimulation. These results taken together suggest that this interaction leads to partial exhaustion of PMN function. Interestingly, incubation periods of only 1 h led to a population of cells with remarkably similar defects to those found in vivo, i.e., in the tissue cage PMN.

PMN infusions were used in order to demonstrate that the observed phagocytic defect was operational in the development of foreign body infection, and that its restoration would abrogate this high risk. Such techniques have been used previously by others in another experimental model to prevent pseudomonas pneumonia in neutropenic dogs (49). As the infusions led to PMN counts frequently found under standard conditions in sterile tissue cage fluids, and as the infusion of tissue cage fluid PMN provided no significant protection, we conclude that the protection afforded by fresh blood PMN must be due to a qual-

itative rather than quantitative improvement of the local phagocytic activity. However, the protection obtained with fresh PMN infusions was highly dependent on their timing, and delays beyond 6 h led invariably to infection. Although our findings provide an explanation for the high susceptibility of foreign material to infection, other factors must be operational in its persistence, one of these being late loss of opsonic activity of the infected tissue cage fluid (21).

In conclusion, our data show that in a subcutaneous foreign body animal model, local PMN have low ingestion and bactericidal activities associated with low levels of granular enzymes and decreased ability to mount a respiratory burst. We postulate, based on our data, that this defect is secondary to the prolonged stimulation of the PMN by the foreign, nonphagocytosable surface. Although this defect probably explains partially the high susceptibility of prosthetic material to infection, we do not want to imply that this is the sole pathogenic mechanism. Recent studies by two other groups have shown that foreign surfaces irreversibly attach some microorganisms which then form a glycocalyx (50-53). We postulate that the combination of a PMN defect and increased bacterial adherence act in concert to favor infection on prosthetic material.

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