Leukocyte Stimulation: Enhanced Phagocytosis of Staphylococcus (36446)

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Phagocytosis by polymorphonuclear leukocytes (PMN) has been recognized as an important means of defense against staphylococcus. Attempts to increase significantly the phagocytic ability of PMN for staphylococcus by active immunization have proven unsuccessful (1, 2) and there is little information on enhancement by nonspecific means. However, nonspecific enhancement of phagocytosis has been shown by mononuclear leukocytes derived from rabbits given injections of *Mycobacterium bovis* strain BCG (3). Not only was phagocytosis of the tubercle bacilli enhanced but the ingestion of unrelated particulate materials was greatly increased, suggesting a cellular type of response. Recently, increased phagocytosis of *Staphylococcus aureus* and other bacteria by leukocytes treated *in vitro* with C-reactive protein has been demonstrated and related to nonspecific immunity (4). This report presents data showing that an enhanced phagocytic response by PMN for staphylococci occurred following *in vitro* treatment with staphylococcal antigens.

Materials and Methods. A coagulase-positive strain of *S. aureus* isolated from a human being with furunculosis was used in these experiments. The antigens were prepared by suspending the washed bacteria in 24% (w/v) NaCl containing lysostaphin (Schwarz/Mann, 1 unit/ml) at 37° (5). After 60 min the resulting protoplast-like cocci (6, 7) were removed by centrifugation and the supernatant fluid containing antigens was dialyzed against cold distilled water. Following concentration by lyophilization the antigens were reconstituted in 0.15 *M* NaCl.

Blood was obtained from healthy adult volunteers of both sexes ages 20–40 and from umbilical cords of human neonates with heparin as an anticoagulant (143 USP units/7 ml). PMN collected from the buffy coats of the blood were washed once with 0.15 *M* NaCl. PMN were concentrated to 18,000/mm^3 and 0.5 ml aliquots were placed in 100 × 13 mm silicone-coated tubes. To one tube was added 25 μg of antigen protein (0.05 ml) (8) and to a second tube 0.05 ml of saline (control). The tubes were placed in a rocking incubator at 37°. After 5 min the PMN were washed in saline. Bacteria or polystyrene latex particles (0.8 μ diam) were added to the tubes at a ratio of 100 particles to 1 PMN. Two-tenths milliliter of homologous plasma was added to each tube and incubation continued for 30 min. Duplicate blood smears were then made and stained by Wright's method. The number of bacteria or particles engulfed by each of 50 randomly selected PMN per smear was recorded.

Results. Antigen treatment of PMN from healthy donors doubled the number of cells phagocytically active for staphylococci (Table I). Statistical analysis of the response by PMN from a single donor indicated that the staphylococcal antigens specifically stimulated a subpopulation of PMN to become phagocytically active (Fig. 1). In subsequent experiments the staphylococcal antigens were designated phagocytosis-promoting factor (PPF).

The observation that not all the PMN within a population showed increased phagocytosis following PPF treatment prompted the study of the ability of cord blood PMN to phagocytize staphylococci. The results comparing the phagocytic response to PMN from 5 cord blood specimens with the response of 5 adult blood specimens are presented in Fig. 2. The data were transformed to $(X + 0.5)^{1/2}$, and evaluated by analysis of variance. PPF failed to increase significantly at 95% confidence limits the phagocytic response of

TABLE I. Effect of Staphylococcal Antigen Treatment on the Phagocytic Ability of Human PMN for *S. aureus* After 30 min Incubation *in Vitro*.[a]

PMN specimen no.	Treatment	Percentage phagocytizing PMN	Mean no. of phagocytized staphylococci per PMN
1	Antigen	93	12
	Saline	41	8
2	Antigen	94	15
	Saline	43	8
3	Antigen	90	13
	Saline	40	9

[a] PMN concentration (8000 cells/0.5 ml) exposed to 0.05 ml staphylococcal antigen (25 μg antigen protein) or the same volume of saline for 5 min.

cord blood PMN for staphylococci; indeed, pretreatment with PPF resulted in a significant decrease in two specimens. In contrast, PPF significantly enhanced the response by PMN in each specimen from adult human beings. These results suggest that the enhanced phagocytic response of PMN following antigen treatment is an acquired characteristic.

The specificity of the phagocytic response following PPF activation was studied by comparing the ability of PMN from a single adult human donor to phagocytize different species of bacteria and polystyrene latex particles. The bacteria studied were *S. aureus, S. epidermidis, Escherichia coli, Diplococcus pneumoniae, Neisseria gonorrhoeae,* and alpha-hemolytic *Streptococcus*. PPF-stimulated PMN showed enhanced phagocytosis of *S. aureus, S. epidermidis* and *E. coli* but not for the other bacteria or latex particles. The degree of specificity of the phagocytic stimulation by PPF appears to be restricted.

Antiserum produced in rabbits by immunization with PPF neutralized the ability of PPF to activate enhanced phagocytosis of *S. aureus* by PMN. On the contrary, antiserums produced against intact staphylococci did not neutralize PPF activity which suggests that PPF from this particular isolate of *S. aureus* is a "buried" antigen. Repeated attempts to neutralize PPF activity with sera from healthy human donors have been unsuccessful, indicating the lack of circulating antibodies in these sera against this factor.

Discussion. To our knowledge, these findings provide the first evidence of an acquired phagocytic response by PMN following exposure to staphylococcal antigen *in vitro*. This response was characterized by en-

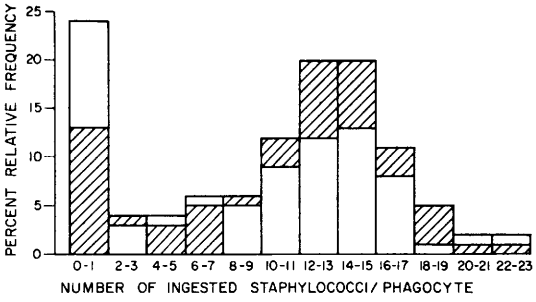

FIG. 1. The phagocytic response of PMN from a single adult donor for *S. aureus* following 5 min exposure to PPF *in vitro*. The blank areas of the bars indicate the frequency of PMN phagocytizing a given number of staphylococci after 30 min of incubation without exposure to staphylococcus antigens. The lined areas thus represent the phagocytic response of PMN following staphylococcus antigens exposure under the same conditions.

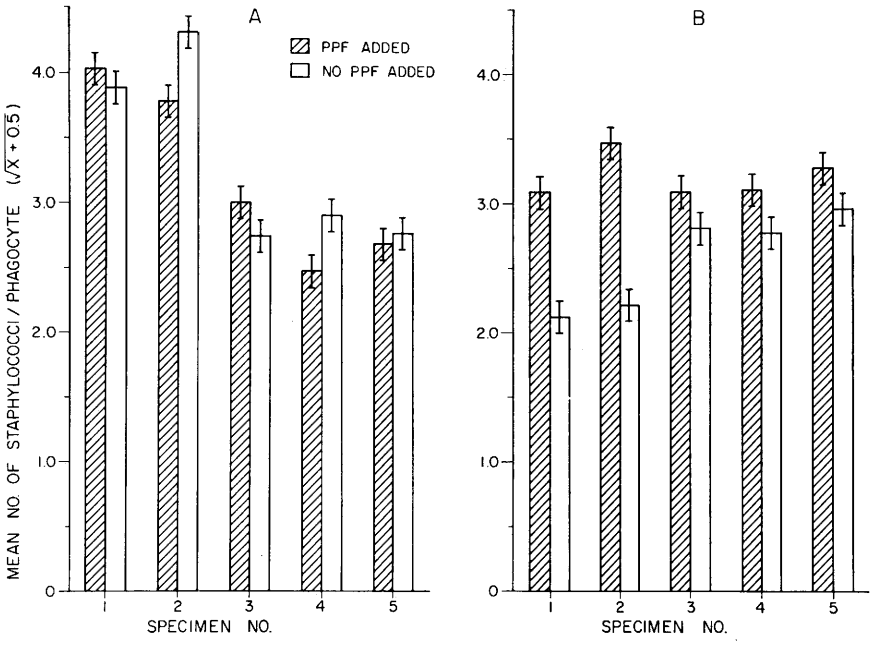

FIG. 2. The mean phagocytic response of cord blood (A) and adult blood (B) PMN for *S. aureus* following *in vitro* treatment with staphylococcal antigens (PPF). The blank area of the bars indicate the transformed phagocytic response of PMN not exposed to PPF whereas the lined areas represent the response of PMN following PPF exposure. Small vertical lines indicate the 95% confidence interval for each mean.

hanced ingestion of staphylcocci and *E. coli*. In addition, the response to PPF appears to be limited to a given number of cells within a population of PMN. Of particular interest to our findings are the experiments of Florman (9), who found increased resistance in mice and rabbits to challenge with *S. aureus* following administration of a substance isolated from culture supernatants of *S. aureus* strain Bartlett. This substance also protected mice against *E. coli* and herpes simplex virus. The resistance-enhancing factor was not effective *in vitro*, but did elicit quantitative and qualitative changes in phagocytes *in vivo*.

The questions of whether PPF enhances the bactericidal properties of PMN or activates a cellular response for macrophages have not been answered in our study. However, the findings reported in this study add a new dimension to the phagocytosis of staphylococci by PMN and perhaps offers a new approach to the understanding of resistance to staphylococcal disease.

Summary. Polymorphonuclear leukocytes (PMN) from adult human beings showed enhanced phagocytosis of *S. aureus* following *in vitro* exposure to staphylococcal antigens. The enhanced phagocytic response by stimulated PMN appeared to be an acquired characteristic.

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