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Comparative Superoxide-Generating System of Granulocytes from Blood and Peritoneal Exudates

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Blood polymorphonuclear leukocytes (PMN), upon interaction with specific chemotactic stimuli, leave the blood stream and migrate to tissues. At such a location, and upon contact with invading microorganisms, they generate superoxide (O_2^-) as a part of the respiratory burst of phagocytosis. We have compared the O_2^--generating system of guinea pig peritoneal exudate PMN with that of peripheral blood PMN from the same species or of human origin. The rate of O_2^- production by casein-induced guinea pig exudate cells in response to a particulate stimulus (opsonized zymosan) was significantly decreased when compared with peripheral blood PMN. Furthermore, the activation time of the O_2^--generating system was shorter in exudate than in peripheral blood PMN. Important differences in sensitivity to a metabolic inhibitor were found: (i) *N*-ethylmaleimide increased the activation time of the O_2^--generating system only in guinea pig exudate cells and not in blood cells; (ii) when *N*-ethylmaleimide was added after complete activation of the PMN, the O_2^- production rate was inhibited in guinea pig as well as human blood PMN, but not in guinea pig exudate PMN. We conclude that exudation markedly alters one of the most important antibacterial mechanisms of PMN, the superoxide-generating system.

Blood polymorphonuclear leukocytes (PMN), upon interaction with specific chemotactic stimuli, leave the bloodstream and migrate to tissues (7, 17, 19). At such a location, interaction with invading microorganisms and higher concentrations of chemotactic factors lead to the triggering of the superoxide (O_2^-)-generating system (13–15, 18, 22, 26, 30).

Using appropriate stimulatory conditions or inhibitors, we have defined in recent years two phases of the superoxide-generating system: activation of this system and maintenance of its activity (9, 10, 12, 24). Most of our previous studies were performed on either guinea pig exudate cells or human blood PMN. However, important differences in the characteristics of the O_2^--generating system in these two populations of cells were found: first, the O_2^- production rate, stimulated by opsonized zymosan, is lower in guinea pig exudate PMN than in human blood PMN (3, 12); second, *N*-ethylmaleimide (NEM) increases the activation time of phorbol myristate acetate (PMA)- or digitonin-stimulated cells in guinea pig exudate PMN but not in human blood PMN (10, 24); third, when NEM is added to PMA- or digitonin-stimulated cells after complete activation, the O_2^- production rate is inhibited in human blood PMN but not in guinea pig exudate PMN (10, 24).

The question therefore arose as to whether the differences observed were due to species differences or to the different sites of collection of phagocytic cells. The aim of this study was to compare the O_2^--generating system in guinea pig PMN obtained from blood or peritoneal exudate with that of human blood. Our results suggest that the O_2^--generating system of guinea pig blood PMN is similar to that of human blood PMN, whereas exudation of these cells results in altered characteristics of their oxidative burst.

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MATERIALS AND METHODS

PMA, cytochrome *c* type VI, superoxide dismutase, NEM, NitroBlue Tetrazolium (NBT), and zymosan were purchased from Sigma Chemical Co., St. Louis, Mo.; dextran T-500 was from Pharmacia Fine Chemicals, Uppsala, Sweden; casein and phosphate-buffered saline (PBS) were from GIBCO-Bio-Cult Co., Glasgow, Scotland; and D-(+)-glycogen purum was from Fluka AG, Buchs SG, Switzerland. Zymosan was washed in normal saline before use. PMA was stored as a stock solution of 2 mg/ml in dimethyl sulfoxide at −20°C in a desiccator. Each day a 50-μl aliquot was thawed and diluted to 20 μg/ml in PBS.

Human (2) and guinea pig blood (8) PMN were partially purified by dextran sedimentation. Guinea pig peritoneal exudate PMN were prepared by intraperitoneal injection of either 12% casein (in normal saline) or, for control experiments, 0.1% glycogen (in normal saline) as previously described (34). The cells were washed three times in physiological saline and suspended in PBS, pH 7.4, if not otherwise stated, generally at 5×10^6 to 1×10^7 PMN/ml. The viability of PMN was checked with the trypan blue exclusion test after the last washing. Over 94% of the exudate PMN and >96% of the peripheral blood PMN excluded the trypan blue.

Zymosan was opsonized with fresh human or guinea pig serum (20 mg/ml) by incubation at 37°C for 20 min. The serum was then removed, and the zymosan was washed twice in normal saline and suspended in PBS at 15 mg/ml. Aliquots were kept at −70°C. Opsonized zymosan was kept on ice until just before use, when it was warmed to 37°C. O_2^- generation induced by PMA and opsonized zymosan was determined in a double-beam spectrophotometer (model 35; Beckman Instruments, Inc., Fullerton, Calif.) with a temperature regulator set, as described previously (33). Control assays were run with zymosan preincubated with heated

TABLE 1. O_2^- production by different types of PMN stimulated by opsonized zymosan

PMN type	No. of expt	Initial linear rate of O_2^- production (nmol of O_2^-/min per 10^6 PMN, mean ± SD)	Statistical analysis (P)[a]	Lag time (s)	Statistical analysis (P)[a]
Human blood	6	5.7 ± 2.1		33 ± 4.2	
Guinea pig					
Blood	5	3 ± 0.4	<0.001	52 ± 5.5	<0.01
Casein-elicited exudate	7	1.4 ± 0.6	NS	41 ± 5.5	<0.05
Glycogen-elicited exudate	10	2.7 ± 0.3		42 ± 8.4	
Guinea pig blood, incubated in vitro in:[b]					
PBS	3	3.5 ± 0.3	<0.001	39 ± 3.1	<0.01
Casein suspension[c]	3	2.2 ± 0.3		25 ± 4.2	<0.01
Particle-free casein solution[d]	3	3.5 ± 0.3		27 ± 3.0	

[a] Statistical analysis with the unpaired Student t-text. NS, Not significant.
[b] Incubation at a concentration of 10^7 PMN/ml in a water bath at 37°C during 1 h; measurement of the O_2^- production without previous washing (33).
[c] Casein, 12%, in physiological saline (protein content, 66 mg/ml).
[d] Casein, 12%, in physiological saline, centrifuged at 20,000 × g for 20 min and filtered (diameter, 0.22 μm) (protein content, 57.2 mg/ml) as described by Van Epps and Garcia (30).

serum (56°C, 30 min), i.e., decomplemented guinea pig and human sera. This stimulus did not result in any detectable O_2^- production. The rate of O_2^- production was calculated from the linear rate of absorbance change and the molar extinction coefficient for this reduction (19,500 M^{-1} cm^{-1}) (3).

The NBT slide test was performed and scored as previously described (33). To study the effect of differences in the PMN preparation, we compared the zymosan-induced O_2^- production of exudate PMN washed by either the standard procedure with saline (33) or sedimentation with dextran T-500 (6%). These two cell preparations from the same animal produced O_2^- to the same extent, namely, 0.86 ± 0.2 (n = 3) and 1 ± 0.08 (n = 3) nmol/min per 10^6 PMN, respectively (P > 0.05).

In some experiments NEM, a substance which interferes with the oxidative metabolism of phagocytic cells, was added in the assay. The effect of NEM on PMA-induced activation of the O_2^--generating system was studied by adding this substance together with the PMN. The effect of NEM on the PMA-induced activity of the enzyme system itself was studied by adding NEM at the time of linear activity, i.e., 90 s after stimulation with PMA.

All data are reported as means ± standard deviation; statistical analyses were done with Student's t test for comparison of two means.

RESULTS

Activity and activation of PMA-stimulated O_2^- production. We compared three types of PMN, namely, human blood PMN, guinea pig blood, and exudate PMN. Human blood PMN, stimulated with PMA (2 μg/ml), produced 12.9 ± 1.3 nmol of O_2^-/min per 10^6 PMN with a lag time of 53.2 ± 10.4 s (n = 15). Guinea pig blood PMN superoxide production was 16.0 ± 3.8 nmol of O_2^- with a lag time of 53.5 ± 12.8 s (n = 13). The superoxide production by exudate PMN was somewhat lower, 11.1 ± 3.0 nmol of O_2^-/min per 10^6 PMN (P < 0.01), with a shorter lag time (45 ± 4.5 s; n = 9; P < 0.01) than that for guinea pig blood PMN.

Thus, the activity of the superoxide-generating system was somewhat higher in guinea pig blood PMN than in guinea pig exudate cells. Furthermore, the lag time in guinea pig exudate cells was shorter than that for guinea pig blood PMN. However, the lag time of guinea pig blood PMN was similar to that of human blood PMN.

NBT slide tests with guinea pig exudate PMN after stimulation with PMA showed 5% without, 30% with weak, and 65% with strong NBT reduction. Two percent guinea pig peripheral blood PMN failed to reduce NBT, 25% reduced at a weak degree, and 73% reduced NBT strongly. Thus, study of the individual PMN demonstrates that differences between exudate and peripheral blood PMN were not due to a subpopulation of injured cells.

O_2^- production rates by opsonized zymosan-stimulated PMN. The responses of the O_2^--generating system of different PMN types to opsonized zymosan were compared (Table 1). Exudate PMN elicited by the particulate stimulus casein showed a 53% decrease in the O_2^- production rate when compared with blood PMN of the same species. Glycogen-elicited PMN did not show this impaired O_2^- production in response to a particulate stimulus. The activation time,

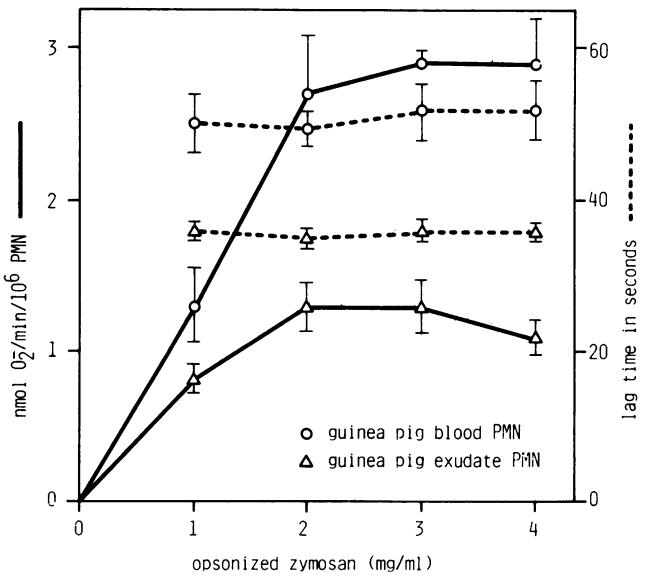

FIG. 1. Effect of opsonized zymosan concentration on rate of O_2^- production and lag time for activation. Guinea pig blood and exudate PMN were stimulated with opsonized zymosan at concentrations of 1 to 4 mg/ml. The effect of the dose on the rate of O_2^- production and the lag time is shown. Each point and bar represents the mean and standard deviation of triplicate determinations.

however, was shorter in both types of exudate. To test the casein particle effect in more detail, we compared the activation and activity of the superoxide-generating system of guinea pig blood PMN incubated in vitro with PBS, casein suspension, and particle free casein solution. Whereas incubation in the particulate casein suspension led to a cell population with decreased O_2^--generating activity in response to opsonized zymosan, the particle-free solution did not induce this effect. The activation time was significantly shorter in PMN preincubated with either particulate or soluble casein than in control cells.

Figure 1 shows a dose-response curve of zymosan-induced O_2^- generation. The difference between peripheral blood PMN and casein-elicited PMN was found with each zymosan concentration tested ($P < 0.01$). The lag time was independent of zymosan concentration, as previously noted (12).

Effect of temperature and pH on activity and activation of the superoxide-generating system. One of us has previously shown that the activity as well as the activation of the O_2^--generating system in digitonin-stimulated guinea pig exudate PMN is affected by changes in temperature (9). We now compared the effect of temperature on blood PMN of two different species and from two sites of collection in the same species. Table 2 shows that human as well as guinea pig blood PMN behaved somewhat differently from guinea pig exudate PMN. Guinea pig exudate PMN showed a marked decrease in O_2^- production at temperatures below 37°C, whereas in peripheral blood PMN of the two species the substantial decrease in activity occurred only at temperatures below 30°C.

Table 3 shows that human and guinea pig blood PMN as well as guinea pig exudate PMN behaved similarly in media of different pH values. The optimum for activity was at pH 7.5. pH variations had no effect on activation of the O_2^--generating system (data not shown).

Effect of NEM on activity and activation of the superoxide-generating system. (i) Addition of NEM before PMN activation. Figure 2 shows the behavior of guinea pig blood and exudate PMN in the presence of increasing concentrations of NEM. The activation time of the O_2^--generating system (Fig. 2, top) was significantly inhibited by NEM only in guinea pig exudate cells (comparison of no NEM versus 1 mM NEM, $P < 0.01$). NEM inhibited the superoxide-generating system of guinea pig exudate PMN only when added before complete activation by PMA. For complete inhibition of O_2^- production >1 mM NEM was required for guinea pig exudate or blood PMN (Fig. 2, bottom). We have previously reported that human blood PMN are completely inhibited by lower concentrations of NEM (30 μM) (24).

TABLE 3. Effect of pH on activity of PMA-stimulated O_2^- production[a]

pH[b]	Human blood PMN (nmol of O_2^-/min per 10^6 PMN)[c]	Guinea pig	
		Blood PMN (nmol of O_2^-/min per 10^6 PMN)	Exudate PMN (nmol of O_2^-/min per 10^6 PMN)
6.0	3.8 ± 0.1	8.5 ± 0.6	6.5 ± 0.6
7.0	11.8 ± 1.7	12.2 ± 0.6	7.1 ± 1.2
7.5	13.9 ± 1.1	12.8 ± 0.6	11.3 ± 0.7
8.0	6.4 ± 0.2	9.3 ± 0.9	9.1 ± 1.5
9.0	3.1 ± 1.0	3.3 ± 1.4	7.2 ± 0.4

[a] Mean ± standard deviation of triplicate determinations.
[b] pH of the reaction mixture. The assay was performed as described in the text.
[c] Initial linear rate of O_2^- production.

(ii) Addition of NEM after complete activation. Figure 3 shows the effect of NEM given only after complete activation of the PMN by PMA, i.e., after 90 s. There was a striking difference between guinea pig blood PMN and exudate PMN. Exudate PMN O_2^- production could not be blocked by NEM, whereas guinea pig blood PMN demonstrated a dose-dependent decrease in activity in the presence of NEM, similar but not identical to that of human blood cells. Inhibition of guinea pig blood PMN was not complete even at concentrations as high as 500 μM NEM.

DISCUSSION

PMN are altered by the passage from intravascular to extravascular sites (5, 15, 18, 22, 30–32). This passage is initiated when an inflammatory stimulus results in chemotaxis, a process which leads to the transformation of blood cells into exudate cells. Little is known about the difference between blood PMN and exudate PMN of the same species. This comparison has usually been made between exudate PMN from guinea pigs and blood PMN from human beings (3, 9, 10, 12, 24). In this study we compared PMN from different sites of the same species, namely, the guinea pig exudate and blood PMN, and PMN from identical sites in different species (human and guinea pig blood cells). The present findings demonstrate that some of the differences observed in the two cell types are in fact the differences between cells of the intravascular versus extravascular milieu rather than being species related.

We focused our studies on two parameters of the O_2^--generating system, namely its activation time (i.e., the lag time to detect O_2^-) and its activity. By using a continuous assay, this analysis allows the demonstration of various steps involved in the generation of O_2^-: the activation period

TABLE 2. Effect of temperature on activity and activation of PMA-stimulated O_2^- production[a]

Temp (°C)	Human blood PMN		Guinea pig			
			Blood PMN		Exudate PMN	
	nmol of O_2^-/min per 10^6 PMN[c]	Lag time (s)[d]	nmol of O_2^-/min per 10^6 PMN	Lag time (s)	nmol of O_2^-/min per 10^6 PMN	Lag time (s)
25	5.2 ± 0.6	201 ± 26	4.6 ± 1.2	119 ± 25	4.1 ± 0.6	76 ± 4
30	11.3 ± 1.5	120 ± 12	13.8 ± 1.0	89 ± 20	4.6 ± 0.3	62 ± 8
32	10.6 ± 0.7	88 ± 25	10.9 ± 0.6	73 ± 20	4.5 ± 0.5	61 ± 2
37	13.6 ± 0.9	62 ± 7	15.7 ± 0.4	50 ± 9	11.3 ± 0.7	35 ± 2
46	6.6 ± 0.6	66 ± 6	11.4 ± 1.0	45 ± 5	4.2 ± 0.5	35 ± 2

[a] Mean ± standard deviation of triplicate determinations.
[b] Temperature at time of PMA addition. The assay was performed as described in the text.
[c] Initial linear rate of O_2^- production.
[d] Time elapsing between addition of PMA and the initial linear rate of O_2^- production.

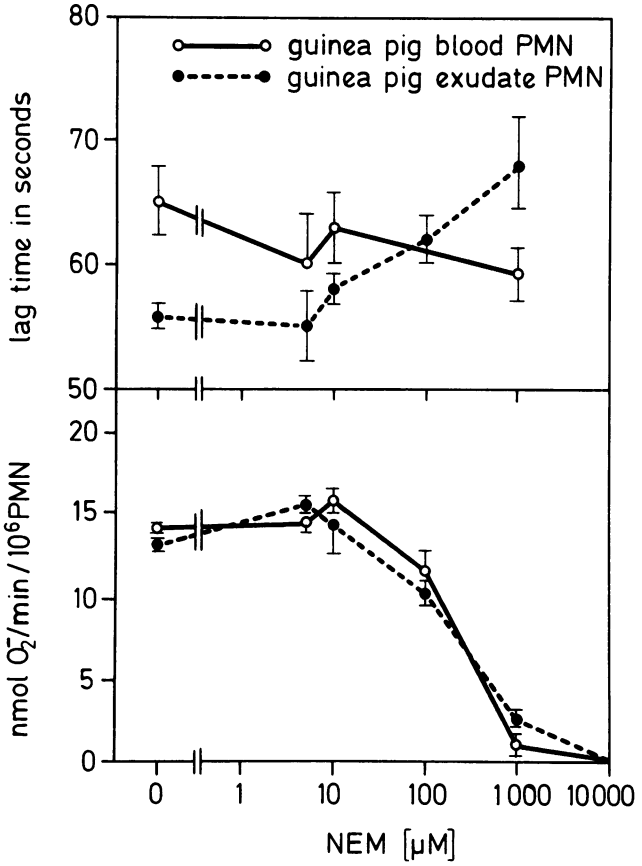

FIG. 2. Effect of NEM on activation (top) and activity (bottom) of PMA-stimulated O_2^- production by guinea pig blood and exudate PMN. The concentration of NEM in the standard assay was modified as indicated on the logarithmic abscissa. NEM was added before activation of the O_2^--generating system with PMA. Each point and bar represents the mean and standard deviation of triplicate determinations.

reflects stimulator binding to specific receptors and intracellular signaling as well as presumably insertion of oxidase into the plasma membrane, whereas the activity depends on the number of oxidase units activated and their intrinsic initial O_2^- production rate (1). This assay can be performed with soluble stimuli, such as PMA (24), as well as with particulate stimuli, such as zymosan (12). The O_2^- production rates in response to PMA led to a two to eight times higher activity than that to zymosan in each cell type tested. This higher O_2^- production rate conditioned by PMA may be more apparent than real since some O_2^- is made in zymosan-containing phagocytic vesicles and is therefore not available to cytochrome *c*. The O_2^- production of exudate cells in response to PMA was somewhat lower than that of blood PMN, but this difference was slight. However, if the particulate stimulus zymosan was used, the O_2^- production rate of exudate PMN was much lower than with blood PMN of the identical animal. A similar difference was found between human blood PMN and guinea pig exudate PMN by Badwey and al. and was attributed mainly to species differences (3). These differences in O_2^- production in response to the C_3b receptor-dependent stimulus zymosan could be due to at least three different mechanisms: (i) proteolytic cleavage of membrane receptors during degranulation (21), (ii) autooxidative damage of C_3b receptors (4–6, 16, 28, 30), and (iii) internalization of C_3 membrane receptors during phagocytosis (25). Our experiments argue in favor of the last possibility, because we found differences between exudate PMN elicited by the particulate stimulus casein, which is presumably ingested by the cells, and PMN elicited by the soluble stimulus glycogen (3). The O_2^- production in response to opsonized zymosan was significantly decreased only in casein-elicited PMN, whereas the activation time of both types of exudate PMN was shorter compared with the one in peripheral blood PMN. The in vitro experiments with incubation of blood PMN in PBS, casein suspension, and particle-free casein solution show that the decreased activity may indeed be due to previous phagocytosis or interaction of membrane surface components with casein, whereas the shortened activation time is due to previous chemotactic stimulation with either particulate or soluble casein.

No major differences among peripheral blood PMN of the two species and guinea pig exudate cells were found when the effect of temperature and pH was investigated. It is worth noting the dramatic changes in the rate of O_2^- production that occur at temperatures below 37°C in exudate PMN and below 30°C in blood PMN. This could represent either a decreased metabolism or a phase transition from a more fluid to a more constrained form in the plasma membrane at lower temperature.

To further characterize the oxidative metabolism of different types of PMN, we tested the effect of NEM, a sulfhydryl reagent and metabolic inhibitor, on the activation and activity of the superoxide-generating system. O_2^--generation by exudate cells was inhibited only if NEM was added before activation and this addition also slowed the activation proc-

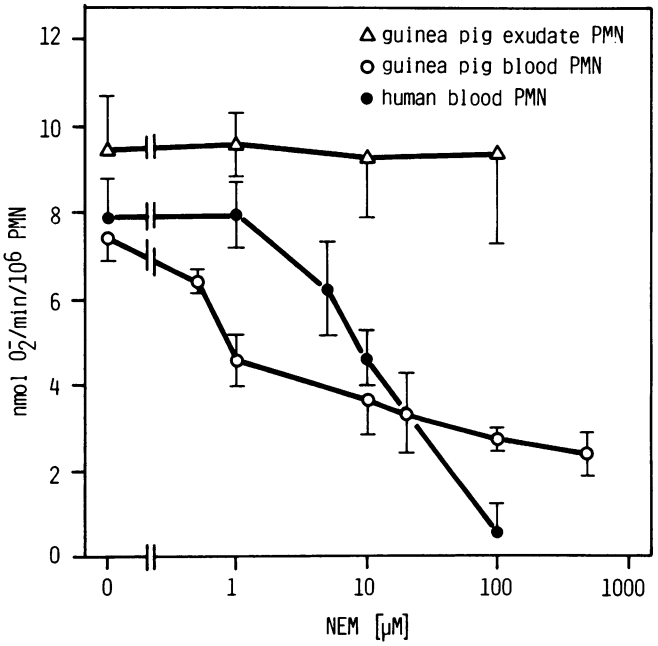

FIG. 3. Effect of NEM on rate of PMA-stimulated O_2^- production by human blood PMN, guinea pig blood, and guinea pig exudate PMN. The concentration of NEM in the standard assay was modified as indicated on the logarithmic abscissa. NEM was added after complete activation of the O_2^--generating system, i.e., 90 s after the addition of PMA. Each point and bar represents the mean and standard deviation of triplicate determinations.

ess. In contrast, NEM produced a concentration-dependent inhibition of the rate of O_2^- production in peripheral blood PMN whether added before or after the activation with no effect on the lag time.

Thus, the inhibitor experiments showed that peripheral blood PMN of two different species had similar behavior as opposed to PMN from different sites of the same species (10, 11, 24). The described differences between peripheral blood PMN and peritoneal exudate PMN could be due either to PMN heterogeneity with preferential mobilization of a subpopulation to exudates or to a transformation of cells during exudation (20).

Previous interaction with chemotactic factors or other activating agents in vitro has been shown to markedly alter PMN responses to subsequent stimulation (13–15, 18, 22, 30). It is possible that the levels of various intracellular messengers necessary to activate the oxidase, such as cytosolic free calcium or protein kinase C activity, are not back to resting or basal levels in previously stimulated or exudate cells (23, 27). Alternatively, a change in the intracellular metabolism could explain the differential response of blood and exudate PMN to the metabolic inhibitor NEM. The observation by Scott and Cooper of a striking rise in glycogen content in casein-elicited guinea pig exudate PMN argues in favor of this hypothesis (29).

In conclusion, our experiments show that differences in PMN metabolism, which were previously attributed to species differences, are (at least in guinea pigs) due to their different sites of origin. It seems that an initial inflammatory stimulus leads to important alterations of one of the most important antibacterial mechanism of granulocytes, i.e., the O_2^--generating system. We give some evidence that this difference depends on the nature of the chemotactic stimulus producing the exudate. It would be of interest to test whether this phenomenon also occurs with human PMN.

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LITERATURE CITED

1. **Babior, B. M.** 1984. The respiratory burst of phagocytes. J. Clin. Invest. **73**:599–601.
2. **Babior, B. M., and H. J. Cohen.** 1981. Measurement of neutrophil function: phagocytosis, degranulation, the respiratory burst and bacterial killing, p. 2. *In* M. J. Cline (ed.), Leukocyte function, 1st ed. Churchill Livingstone & Co., New York.
3. **Badwey, J. A., J. T. Curnutte, J. M. Robinson, J. K. Lazdins, R. T. Briggs, M. J. Karnovsky, and M. L. Karnovsky.** 1980. Comparative aspects of oxidative metabolism of neutrophils from human blood and guinea pig peritonea: magnitude of the respiratory burst, dependence upon stimulating agents, and localization of the oxidases. J. Cell. Physiol. **105**:541–551.
4. **Baehner, R. L., L. A. Boxer, J. M. Allen, and J. Davis.** 1977. Autooxidation as a basis for altered function by polymorphonuclear leukocytes. Blood **50**:327–335.
5. **Baehner, R. L., N. Gilman, and M. L. Karnovsky.** 1970. Respiration and glucose oxidation in human and guinea pig leukocytes: comparative studies. J. Clin. Invest. **49**:692–700.
6. **Boxer, L. A., R. L. Baehner, and J. Davis.** 1977. The effect of 2-deoxyglucose on guinea pig polymorphonuclear leukocyte phagocytosis. J. Cell. Physiol. **91**:89–102.
7. **Cartwright, G. E., J. W. Athens, and M. M. Wintrobe.** 1964. The kinetics of granulopoiesis in normal man. Blood **24**:780–803.
8. **Chenoweth, D. E., T. A. Lane, J. G. Rowe, and T. E. Hugli.** 1980. Quantitative comparisons of neutrophil chemotaxis in four animal species. Clin. Immunol. Immunpathol. **15**:525–535.
9. **Cohen, H. J., and M. E. Chovaniec.** 1978. Superoxide generations by digitonin-stimulated guinea pig granulocytes. A basis for a continuous assay for monitoring superoxide production and for the study of the activation of the generating system. J. Clin. Invest. **61**:1081–1087.
10. **Cohen, H. J., and M. E. Chovaniec.** 1978. Superoxide production by digitonin-stimulated guinea pig granulocytes. The effect of N-ethyl maleimide, divalent cations, and glycolytic and mitochondrial inhibitors on the activation of the superoxide generating system. J. Clin. Invest. **61**:1088–1096.
11. **Cohen, H. J., M. E. Chovaniec, M. K. Wilson, and P. E. Newburger.** 1982. Con-A stimulated production by granulocytes: reversible activation of NADPH oxidase. Blood **60**:1188–1194.
12. **Cohen, H. J., P. E. Newburger, M. E. Chovaniec, J. C. Whitin, and E. R. Simons.** 1981. Opsonized zymosan-stimulated granulocytes—activation and activity of the superoxide-generating system and membrane potential changes. Blood **58**:975–981.
13. **Cohen, H. J., J. C. Whitin, M. E. Chovaniec, E. H. Tape, and E. R. Simons.** 1984. Is activation of the granulocyte by concanavalin A a reversible process? Blood **63**:114–120.
14. **Curnutte, J. T., and B. M. Babior.** 1974. Biological defense mechanisms. The effect of bacteria and serum on superoxide production by granulocytes. J. Clin. Invest. **53**:1662–1672.
15. **English, D., J. S. Roloff, and J. N. Lukens.** 1981. Regulation of human polymorphonuclear leukocyte superoxide release by cellular responses to chemotactic peptides. J. Immunol. **126**:165–171.
16. **Fearon, D. T., I. Kaneko, and G. G. Thomson.** 1981. Membrane distribution and adsorptive endocytosis by C3b receptors on human polymorphonuclear leukocytes. J. Exp. Med. **153**:1615–1628.
17. **Gallin, J. I.** 1981. Abnormal phagocyte chemotaxis: pathophysiology, clinical manifestations, and management of patients. Rev. Infect. Dis. **3**:1196–1220.
18. **Gallin, J. I., J. R. Durocher, and A. P. Kaplan.** 1975. Interaction of leukocyte chemotactic factors with the cell surface. I. Chemotactic factor-induced changes in human granulocyte surface charge. J. Clin. Invest. **55**:967–974.
19. **Gallin, J. I., D. G. Wright, H. L. Malech, J. M. Davis, M. S. Klempner, and C. H. Kirkpatrick.** 1980. Disorders of phagocyte chemotaxis. Ann. Intern. Med. **92**:520–538.
20. **Klempner, M. S., and J. I. Gallin.** 1978. Separation and functional characterization of human neutrophil subpopulations. Blood **51**:659–669.
21. **Lane, T. A., G. C. Lamkin, and B. E. Windle.** 1981. Phagocytosis-induced modulation of human neutrophil chemotaxis receptors. Blood **58**:228–236.
22. **Lehmeyer, J. E., R. Snyderman, and R. B. Johnston, Jr.** 1979. Stimulation of neutrophil oxidative metabolism by chemotactic peptides: influence of calcium ion concentration and cytochalasin B and comparison with stimulation by phorbol myristate acetate. Blood **54**:35–45.
23. **McPhail, L. C., P. M. Henson, and R. B. Johnston, Jr.** 1981. Respiratory burst enzyme in human neutrophils. Evidence for multiple mechanisms of activation. J. Clin. Invest. **67**:710–716.
24. **Newburger, P. E., M. E. Chovaniec, and H. J. Cohen.** 1980. Activity and activation of the granulocyte superoxide-generating system. Blood **55**:85–92.
25. **Niedel, J. E., I. Kahane, and P. Cuatrecasas.** 1979. Receptor-mediated internalization of fluorescent chemotactic peptide by human neutrophils. Science **205**:1412–1414.
26. **O'Flaherty, J. T., and P. A. Ward.** 1979. Chemotactic factors and the neutrophil. Semin. Hematol. **16**:163–174.
27. **Pozzan, T., D. P. Lew, C. B. Wollheim, and R. Y. Tsien.** 1983. Is cytosolic ionized calcium regulating neutrophil activation? Science **221**:1413–1415.
28. **Root, R. K., J. Metcalf, N. Oshino, and B. Chance.** 1975. H_2O_2 release from human granulocytes during phagocytosis. I. Docu-

mentation, quantitation, and some regulation factors. J. Clin. Invest. **55**:945–955.
29. **Scott, R. B., and L. V. W. Cooper.** 1974. Leucocyte glycogen response in inflammatory exudates. Br. J. Haematol. **26**:485–496.
30. **Van Epps, D. E., and M. L. Garcia.** 1980. Enhancement of neutrophil function as a result of prior exposure to chemotactic factor. J. Clin. Invest. **66**:167–175.
31. **Wade, B. H., and G. L. Mandell.** 1983. Polymorphonuclear leukocytes: dedicated professional phagocytes. Am. J. Med. **74**:686–693.
32. **Wright, D. G., and J. I. Gallin.** 1979. Secretory responses of human neutrophils: exocytosis of specific (secondary) granules by human neutrophils during adherence *in vitro* and during exudation *in vivo*. J. Immunol. **123**:285–294.
33. **Zimmerli, W., P. D. Lew, and F. A. Waldvogel.** 1984. Pathogenesis of foreign body infection: evidence for a local granulocyte defect. J. Clin. Invest. **73**:1191–1200.
34. **Zimmerli, W., F. A. Waldvogel, P. Vaudaux, and U. E. Nydegger.** 1982. Pathogenesis of foreign body infection: description and characteristics of an animal model. J. Infect. Dis. **146**:487–497.