Differentiation between endogenous pyrogen and leukocytic endogenous mediator

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MAPES, CAROL A., AND PHILIP Z. SOBOCINSKI. Differentiation between endogenous pyrogen and leukocytic endogenous mediator. Am. J. Physiol. 232(1): C15–C22, 1977 or Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 1(1): C15–C22, 1977. – The crude material released from glycogen-stimulated rabbit peritoneal polymorphonuclear leukocytes when administered to experimental animals elicits a number of metabolic and physiologic alterations characteristic of those observed in the host inflammatory response. Classically, the mediator of febrile response observed in rabbits and other species has been termed endogenous pyrogen (EP), whereas leukocytic endogenous mediator (LEM) has been used as a general term to denote the substance(s) mediating multiple inflammatory responses observed in rats. The latter substance, however, has not been previously demonstrated to differ from EP. This report presents evidence indicating that EP and LEM are different molecular species. Evidence supporting the differentiation between these entities includes: physical separation of EP from one or more mediators that induce metabolic alterations attributed to LEM; production of LEM activities by stimulated polymorphonuclear leukocytes in the absence of detectable pyrogenic activity; and differences in the release of EP and LEM from stimulated rabbit granulocytes in the presence of potassium ion.

rats; plasma zinc; neutrophils; hepatic amino acid uptake; potassium ion inhibition; affinity adsorbent; stimulated rabbit PMN

POLYMORPHONUCLEAR LEUKOCYTES (PMN) obtained from rabbit peritoneal exudate fluid release one or more substances that cause a multiplicity of metabolic and physiologic alterations when administered to experimental animals. The metabolic alterations observed in rats include: decreased serum zinc and iron concentrations (14, 15, 25); increased Zn and Fe concentrations within the liver (27); increased serum Cu and ceruloplasmin concentrations (26); increased release of neutrophils from bone marrow (13); a flux of amino acids into the liver (33); increased serum α1- and α2-acute-phase globulins (6); increased RNA synthesis (34); and decreased portal vein glucose concentrations accompanied by increased concentrations of glucagon and insulin (8). In addition, the crude material released from stimulated PMN causes a hypothermic (2) or hyperthermic (18) response in rats depending upon the route of administration; causes a hyperthermic response in rabbits (3, 12, 17); acts as a chemotactic agent in vitro (11); and appears to have in vitro bactericidal activity toward Escherichia coli (36).

The alterations in host metabolism are attributed to the presence of one or more endogenous mediators in the crude material released from PMN. Classically, the mediator of febrile response has been termed endogenous pyrogen (EP), whereas leukocytic endogenous mediator (LEM) has been used as a general term to denote the substance(s) that mediate the biological responses observed in rats. The use of these dissimilar terms suggest the possibility that EP and LEM are two different molecules. However, the previously ascribed similarities and differences between EP and LEM have not provided a definitive answer to the question of whether the molecules are identical because multiple activities in a given preparation have not been separated chemically or physically.

This report presents experimental data indicating that EP and LEM are not the same molecular species. The data supporting this conclusion are based on a) differences in the release of EP and LEM from stimulated rabbit PMN in the presence of K+; b) the physical separation of a substance that induces a pyrogenic response from one or more mediators that induce metabolic alterations attributed to LEM; and c) the production of LEM by stimulated PMN in the absence of detectable pyrogenic activity.

MATERIALS AND METHODS

Animals. Male Dunning-Fisher rats weighing 160–200 g were purchased from Microbiological Associates (Walkersville, Md.) or Charles River Laboratories, Inc. (Wilmington, Mass.). They were maintained at 22°C with 12-h intermittent periods of light and darkness. They were fed pelleted rat chow and water ad libitum. Locally purchased male New Zealand white rabbits were routinely used for pyrogen assays, whereas rabbits of either sex were used for production of stimulated PMN.

Production of mediators. The procedure for mediator production was adapted from the initial work of King and Wood (17) and Gillman et al. (9). All procedures were carried out aseptically to minimize endotoxin contamination. Peritoneal exudate cells were obtained from 2- to 4-kg New Zealand white rabbits after intra-peritoneal injection of 300–500 ml of 0.2% shellfish glyco-
gen (Schwarz/Mann, Orangeburg, N.Y.) in physiological saline. Sixteen hours after glycerogen infusion, the fluid was aspirated from the peritoneal cavity into a flask containing 800 U of Na heparin (Abbott Laboratories, North Chicago, Ill.) and subsequently passed through sterile gauze. The leukocytes, containing more than 95% viable PMN, were harvested and processed by a series of steps including: centrifugation at 900 × g for 30 min at 4°C in an IEC model PR-6 centrifuge; washing with Krcs-Ringer phosphate (KRP) buffer, pH 7.0, that contained 2,000 U Na penicillin (Upjohn, Kalamazoo, Mich.), 0.4 mg streptomycin (Charles Pfizer and Co., New York City), and 10 U Na heparin/ml (PSH); osmotic lysis of contaminating erythrocytes with sterile water; and repetition of the KRP wash. The washed PMN were resuspended in physiological saline-PSH at a final concentration of 10⁸ cells/ml. After 2 h incubation at 37°C in a shaking-water bath, the cells were removed by centrifugation at 62,000 × g for 30 min at 4°C in a Beckman model L2-65 ultracentrifuge. The supernatant contained 2,000 U Na penicillin (Upjohn, Kalamazoo, Mich.), 0.4 mg streptomycin (Charles Pfizer and Co., New York City), and 10 U Na heparin/ml (PSH); osmotic lysis of contaminating erythrocytes with sterile water; and repetition of the KRP wash. The washed PMN were resuspended in physiological saline-PSH at a final concentration of 10⁸ cells/ml. After 2 h incubation at 37°C in a shaking-water bath, the cells were removed by centrifugation at 62,000 × g for 30 min at 4°C in a Beckman model L2-65 ultracentrifuge. The supernatant solution was decanted and filtered through 0.45-μm Nalgé filters. The filtered solution represents the crude mediator preparation used for all experiments. Alternatively, mediators were produced by Merrell-National Laboratories (Swiftwater, Pa.) using the above procedure. These fevers return to basal levels within 60-90 min and remain there during the subsequent 2-3 h.

Inhibition of mediator release by K⁺. The inhibitory effect of potassium ion was studied using aliquots of a PMN preparation that were processed in a manner analogous to that for production of mediators as described above. Incubations to test the inhibitory effect of K⁺ consisted of 2.4 × 10⁸ cells suspended in 6 ml of saline-PSH plus 6 ml of KCl solution having twice the desired molarity. This mixture was incubated 2 h at 37°C in a shaking-water bath and then diluted with an equal volume of saline-PSH. Control incubations containing 2.4 × 10⁸ cells suspended in 12 ml of saline-PSH were incubated 2 h at 37°C and then diluted with 6 ml of the appropriate KCl solution and 6 ml of saline-PSH. An additional control consisted of 2.4 × 10⁸ cells incubated in 12 ml of saline-PSH for 2 h after which the suspension was diluted with an equal volume of saline. This preparation is referenced as normal LEM.

Pyrogen assays. A colony of 48 healthy, temperature-stable rabbits was selected for pyrogen assay by the following criteria: a) rabbits received 1 wk of veterinary care during which time they were monitored for signs of clinical illness and treated for coccidiosis; and b) healthy rabbits were monitored 4 consecutive days for temperature stability. Rabbits were accepted for routine assay if their temperature was between 101°F and 104°F and did not vary more than 0.3°F during the last 60 min of a 2-h selection period. Rabbits not meeting these criteria on 2 consecutive days were culled from the colony. The colony was divided into four groups of 12 rabbits for use on a rotating schedule. Rabbits were examined weekly by a veterinarian and were treated for respiratory disease with Coca Terramycin (Chas. Pfizer and Co.) as necessary.

The temperature response of nonendotoxin refractory rabbits was measured by rectal thermistors connected to a potentiometric Honeywell recorder. The basal temperature of each rabbit was established during a 1- to 2-h selection period prior to administration of a test sample. All samples were diluted to 1.0 ml with pyrogen-free, physiological saline prior to intravenous administration. The fever of each rabbit was monitored for 90-120 min after injection. Temperatures were printed on a moving chart every 5 min, automatically providing a graph of fever response vs. time. Fever height was read directly from the chart and was defined as the difference between the mean temperature in the 60 min preceding injection and the maximum temperature attained 45 min after administration of a sample. Results were analyzed by one-way analysis of variance (32).

Bioassays of LEM activity. Plasma Zn, total blood neutrophils, and hepatic amino acid uptake were measured in samples taken from rats 5 h after intraperitoneal administration of a 1-ml test sample. Plasma Zn concentrations (reported as µg/dl) were determined by atomic absorption spectroscopy as previously described (24). Blood leukocytes were counted in a Coulter counter after a 1:500 dilution of the blood with Isoton (Coulter Diagnostics, Inc., Hialeah, Fla.) and lysis of the erythrocytes with Hemastall LA-Hbg reagent (Fisher Scientific Co.). Total neutrophils followed from a 100-cell differential of a smear stained with a modified polychrome methylene blue stain (Hema-Tek Stain Pak, Ames Co., Elkhart, Ind.). Hepatic amino acid uptake was measured by the method of Wannemacher et al. (33), with the exception that [1-¹⁴C]α-aminoisobutyric acid (¹⁴C)AIB (New England Nuclear Corp.) was used as the nonmetabolizable amino acid. [¹⁴C]AIB uptake, expressed as counts/min per 50 mg wet wt, was measured in liver samples perfused clear of visible blood. (The phrase "amino acid fluxing-activity" is used to designate mediator activity measured by this method.) Bioassay controls consisted either of mediator samples that had been heated at 100°C for 30 min prior to administration or of pyrogen-free saline. Data were statistically analyzed by one-way analysis of variance. The significance of differences between means (P < 0.01) was determined using Fischer's protected least significant difference (32).

Protein and osmolality. Protein was measured by the method of Lowry et al. (19). Bovine serum albumin was used as a protein standard. Osmolality was measured...
utilizing a Fiske osmometer (1).

Binding of mediators to glass. Glass beads, 75-150 μm (Sigma Chemical Co., lot 104C-5049), were autoclaved for 2 h prior to use. The glass beads were added to a mediator preparation (1.0 mg/ml) and stirred slowly for 2 h at 4°C. The glass was either allowed to sediment or was removed by centrifugation at 3,000 rpm for 5 min at 4°C. The supernatant solution was decanted for bioassay. An attempt was made to recover mediators from the glass by consecutive elution with physiological saline, 0.5 and 0.75 M NaCl. In each case the beads were stirred in the eluting solvent for 30 min at 4°C. Controls consisted of: a) a pyrogen-free saline eluate of the glass beads prior to use and b) a mediator preparation stirred for 2 h at 4°C in the absence of glass beads.

RESULTS

Inhibition of protein release from stimulated PMN. The effect of K+ on protein release from stimulated leukocytes is shown in Fig. 1. An increase in the inhibition of protein release was observed between 0 and 15 mM added K+. The addition of more than 15 mM potassium ion changed the solution from isotonic to hypertonic. Although studies were not undertaken to elucidate any deleterious effects of hypertonicity on the cellular system, it appeared that inhibition of protein release might have been muted by hypertonic solutions.

Inhibition of EP release by potassium ion. Figure 2 shows the effects of K+ on release of pyrogenic activity from stimulated PMN. Pyrogenic activity released in the presence of 5-30 mM K+ did not induce a fever in rabbits statistically different from the fever produced by administration of an equivalent volume of the nonpyrogenic substance, saline, thereby indicating 100% inhibition of EP release. In contrast, the pyrogenic activity released in control incubations was not affected by the subsequent addition of 5-20 mM K+. Controls to which more than 20 mM K+ were added showed suppressed activity but still induced significant febrile responses.

Inhibition of LEM release by potassium ion. Utilizing aliquots of the same mediator preparation, the inhibitory effect of K+ on EP release was compared to that of a substance(s) mediating three metabolic responses attributed to LEM. Figure 3 compares the Zn-depressing activity released in the presence of specific K+ concentrations with that released in controls to which equivalent K+ concentrations were added subsequent to the release period. An inhibitory effect on this release was detected at a K+ concentration of 10 mM (P < 0.05); however, significant inhibition of release was obtained only with 15 mM K+ (P < 0.01) and nonphysiological concentrations of 20-30 mM K+ (P < 0.001).

The experimental data also were compared to bioassay controls that induced the following rat plasma Zn concentrations (means ± SE): saline, 128.7 ± 1.7 μg/dl; LEM prepared and bioassayed in the absence of K+, 59.2 ± 2.7; and heated LEM, 134.0 ± 3.9. Comparison of the data presented in Fig. 3 with these controls indicated the following: a) all of the Zn-depressing activity released in the presence of K+ was less potent than that released and bioassayed in the absence of K+, thereby indicating some perturbation of the system even in the absence of detectable inhibition; b) Zn-depressing activity released in the presence of physiological K+ concentrations caused a significant depression of plasma Zn as determined by comparison with saline or heated LEM; c) comparison of the activity released and assayed in the absence of K+ with that of control incubations showed that K+ had no significant effect on preformed mediator activity; and d) the consistent heat lability of mediator preparations indicated the absence of significant quantities of endotoxin.

Figure 4 shows the effect of potassium ion on release of an amino acid fluxing activity. Comparing the activity released in the presence of specific K+ concentrations to that released in controls to which equivalent K+ concentrations were added subsequent to the release period, one can see that release of activity was inhibited only by nonphysiological K+ concentrations of 20 (P < 0.01), 25, and 30 mM (P < 0.001). These data also were compared to bioassay controls that induced the following levels of hepatic [14C]AIB uptake (means ± SE): saline, 451.8 ± 33.9 counts/min per 50 mg liver; LEM released and bioassayed in the absence of K+, 1078.7 ± 65.3; and heated 1 K+M, 374.5 ± 47.6. The following information was gained through these comparisons: a) all of

FIG. 1 Typical effect of [K+] on release of protein from stimulated rabbit PMN after 2 h incubation at 37°C. Protein released into incubation medium in presence of specified K+ (●) concentrations and osmolality (○) is expressed as a percent of protein released in controls to which an equivalent amount of K+ was added at termination of incubation.

Dose-response studies in this laboratory have shown that 10 μg of endotoxin are required to elicit either neutrophil response, plasma Zn depression, or hepatic [14C]AIB uptake in 100-220 g male rats. Thus, it has been tentatively concluded that the LEM activities bioassayed in rats are not due to endotoxin if the same preparation does not induce a characteristic endotoxin response when administered to rabbits. In addition, the biological activity of endotoxin (10 μg/ml) is not heat labile. The reader is referred to the work of Pekarek et al. (28) for additional proof that endotoxin and LEM are different molecular entities.
Figure 2. Pyrogenic activity released in presence of various K⁺ concentrations (△) is compared to that of controls (●) to which an equivalent amount of K⁺ was added subsequent to release period. Each data point represents mean ± SD of febrile response in 4 rabbits. Shaded area represents mean ± SD of febrile response to 1.0 ml pyrogen-free saline.

Figure 3. Zn-depressing activity released into incubation medium in presence of a specific K⁺ concentration is compared to activity released in its individual control to which an equivalent amount of K⁺ was added subsequent to release period. Each bar represents bioassay mean ± SE of plasma zinc concentration in 8 rats 5 h after intraperitoneal injection of a 1.0-ml test sample.

Figure 4. Amino acid fluxing activity released into incubation medium in the presence of specific K⁺ concentrations is compared to activity released in individual controls to which equivalent amounts of K⁺ were added subsequent to release period. Each bar represents bioassay mean ± SE of [¹⁴C]AIB uptake in 8 rats 5 h after intraperitoneal injection of a 1.0-ml test sample.

Figure 5. Neutrophil-elevating activity released into incubation medium in presence of specific K⁺ concentrations is compared to activity released in individual controls to which equivalent amounts of K⁺ were added subsequent to release period. Each bar represents bioassay mean ± SE of total blood neutrophils in 8 rats 5 h after intraperitoneal administration of a 1.0-ml test sample.

The amino acid fluxing activity released in the presence of K⁺ caused significant metabolic responses in comparison to saline and heated LEM, thereby indicating less than 100% inhibition of release; b) the addition of K⁺ to preformed amino acid fluxing activity had no significant effect as determined by comparing the activity released and assayed in the absence of K⁺ with that of the control incubations; and c) all mediator activities were heat labile when compared to saline.

Figure 5 shows the effect of potassium ion on release of neutrophil-enhancing activity. Detectable inhibition of release of this activity occurred at K⁺ concentrations ranging from 15 to 30 mM when the activity released in

4 Several investigators have reported nonspecific neutrophilia after administration of either foreign protein (b) or heparin (7, 23) to experimental animals. However, it seems reasonable to conclude that mediator preparations contain a leukopoietic factor that induces this metabolic alteration in rats based on the following experimental observations: a) rabbit-leukocyte products induce neutrophil responses in rabbits analogous to those induced in rats; b) the maximal concentration of heparin that is present in any mediator preparation does not cause neutrophil or enhanced leukocyte responses in the rat; and c) mediators prepared using titrated saline as anticoagulant have induced neutrophil responses similar to those induced by mediators containing heparin.
**DIFFERENTIATION BETWEEN EP AND LEM**

Phil-elevating activity released in the presence of 5–15 mM K⁺ did not differ significantly from the activity released and assayed in the absence of K⁺; b) the addition of 15 or 25 mM K⁺ to preformed neutrophil-enhancing activity resulted in a significant difference between these controls and the activity released and assayed in the absence of K⁺. This peculiarity resulted in a statistical difference between the activity released in the presence of 15 mM K⁺ and its corresponding control; and c) all of the mediator activities were heat labile.

**Physical separation of EP and LEM activities.** A second and perhaps more convincing method of demonstrating that pyrogen and LEM are different molecular species comes from their physical separation. Reports in the literature indicate that pyrogen purification is plagued by continuous loss of activity due to its nonspecific binding to glass (21, 22). Therefore, 75- to 100-μm glass beads were tested as a possible affinity adsorbent for leukocyte mediators. The recovery of protein and pyrogenic activity in two representative studies is summarized in Table 1. Approximately 80% of the total protein in a mediator preparation bound to glass. There was no detectable pyrogenic activity in the unbound material in comparison with the untreated mediator preparation. Attempted elution of pyrogenic activity from the glass was unsuccessful as anticipated from a report that indicates irreversible binding of EP to surfaces (21) or perhaps denaturation of the molecule. Because pyrogenic activity could not be recovered, the experimental conditions were simulated to insure that they had no detrimental effect on activity. A statistically insignificant decrement of 0.2°C fever in this control indicates that pyrogenic activity was bound to the glass beads rather than inactivated by the experimental conditions.

The same mediator preparations also were bioassayed for LEM activities before and after treatment with glass beads. Based on statistical analyses of bioassay results obtained with three different dilutions of the mediator preparations (Fig. 6), there were no detectable differences in zinc-depressing, amino acid-fluxing, or neutrophil-elevating activities before and after treatment with glass. It should be noted, however, that a 10% loss of activity from the mediator preparations would not be detected due to the experimental error inherent in the rat bioassay.

**TABLE 1. Binding of leukocytic mediators to glass beads**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein</th>
<th>T₄</th>
<th>T₆</th>
<th>ΔT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEM</td>
<td>41.5</td>
<td>100.6 ± 0.2</td>
<td>102.4 ± 0.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Unbound</td>
<td>10.3</td>
<td>101.1 ± 0.1</td>
<td>101.3 ± 0.1</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Study 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEM</td>
<td>61.0</td>
<td>101.7 ± 0.2</td>
<td>100.8 ± 0.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Unbound</td>
<td>12.3</td>
<td>103.8 ± 0.2</td>
<td>103.8 ± 0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>0.75 M NaCl eluate</td>
<td>30.8</td>
<td>103.1 ± 0.1</td>
<td>103.1 ± 0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Stirred 5 h at 4°C</td>
<td>102.4 ± 0.2</td>
<td>104.2 ± 0.1</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

*Fever values are means ± SE.

Although numerous mediator preparations have yielded the same experimental results (dose-response curves are variable in shape), we cannot make more accurate statements concerning the percent recovery of LEM for the following reasons: a) LEM content of mediator preparations is inferred from the biological activities that they induce when administered to rats; b) dose-response curves (Fig. 6) are normally nonlinear, which prohibits the use of parallel line assays for assessing percent recovery; and c) there is neither consistency of LEM potency between preparations nor an international standard of LEM activity that enables calculation of meaningful specific activities.

**Preferential production of mediators.** During the weekly production and analysis of mediator preparations, it was fortuitously noted that not all preparations contained detectable pyrogenic activity although they were not unusual with respect to the LEM activities bioassayed. The arbitrary criteria for evaluating an active mediator preparation as assigned by this laboratory are: a) in comparison to the heat-inactivated preparations, a 1-ml test sample will cause a 40% depression of plasma Zn, a 1.5-fold increase in hepatic amino acid uptake, and a twofold elevation in peripheral blood neutrophils 5 h after intraperitoneal administration to rats; and b) a 50-μl aliquot, diluted to 1.0 ml with pyrogen-free saline, will induce 0.9°F fever in rabbits 45–60 min after intravenous administration.

Comparative analyses of several mediator preparations (Table 2) indicate that stimulated PMN do not consistently produce pyrogenic activity. Mediators prepared between late July and late September did not induce statistically significant rabbit fevers although they induced plasma Zn depression, hepatic amino acid uptake, and elevation of total neutrophils in the rat.

**DISCUSSION**

Characterization studies on partially purified fractions indicate that endogenous pyrogen is a protein with an apparent molecular weight of 10,000-30,000 (29-31) that is nondialyzable (31), heat and protease sensitive
TABLE 2. Multiple mediator activities elaborated by various stimulated rabbit PMN preparations

<table>
<thead>
<tr>
<th>Date Prepared</th>
<th>Plasma Zn$^{118}$, $\mu$/dl</th>
<th>Hepatic (14C)AIB, counts/min per 50 mg liver</th>
<th>Total neutrophil, $\times 10^6$ blood</th>
<th>Fever, $\Delta$F</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 25-Sept 25</td>
<td>55 ± 2</td>
<td>226 ± 23</td>
<td>244 ± 30</td>
<td>0.7 ± 0.3*</td>
</tr>
<tr>
<td>Oct 1-Nov 12</td>
<td>39 ± 2</td>
<td>321 + 36</td>
<td>252 ± 19</td>
<td>2.3 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Plasma Zn, hepatic (14C)AIB uptake, and total blood neutrophils were measured 5 h after intraperitoneal administration of a 1.0 ml test sample. Each preparation was tested in 8-10 rats. Fever responses normally were measured in 4 rabbits, 45 min after intravenous injection of a 50-µl test sample diluted to 1.0 ml with pyrogen-free saline. *Fever responses were obtained with 200 µl of a mediator preparation diluted to 1.0 ml with pyrogen-free saline.

In addition to these factors, it is important to consider that mediator preparations differ not only in their potency (Table 2) but also in the quantity of Na, K, Ca, Zn, and Cu. The concentrations of these metals are fairly consistent for preparations processed within a given laboratory; however, there are wide discrepancies in comparing mediator solutions prepared by different laboratories. Because it is known that most of these metal ions affect the release of one or more mediator activities (this laboratory, unpublished data), it is logical to assume that quantitative differences in the K+ concentration required for inhibition of LEM release may occur, although empirical differences between EP and LEM will be observed. This, in fact, was observed with one PMN preparation that required more than 10 mM K+ to inhibit release of Zn-depressing activity, whereas release of pyrogenic activity was inhibited by 5, 20, and 25 mM K+, respectively.
Differential production of LEM activities by stimulated PMN in the absence of detectable pyrogenic activity, provide evidence that PMN produce multiple factors that engender several metabolic alterations when administered to experimental animals.

The existence of multiple leukocyte mediators expands the need for experimentation to delineate the role of leukocyte physiology in the inflammatory response. It will be of interest to determine whether LEM itself is composed of multiple factors as subtly suggested by these experiments and, if so, to determine the mechanisms by which PMN are stimulated to produce and release multiple components. The knowledge of stimulated-leukocyte physiology gained from these studies will be of key significance in understanding the pathophysiology of infectious diseases.

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In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals, as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

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