Comparison of Endotoxin Detoxification by Leukocytes and Macrophages[1] (35797)

JAMES P. FILKINS[2]

Department of Physiology and Biophysics, University of Tennessee Medical Units, Memphis, Tennessee 38103

Endotoxin administered intravenously interacts with leukocytes and other buffy coat cells prior to its rapid clearance from the circulation and sequestration by the macrophages of the hepatic and splenic sinusoids (1). Since rampant endotoxemia has deleterious effects on the host and may induce cardiovascular collapse and shock, it is generally assumed that the leukocyte and/or the macrophage interactions with endotoxin modulate potential host damage. Obviously, a primary mechanism of host survival requires eventual inactivation of the toxic potential of the lipopolysaccharide.

Recent studies of this laboratory have confirmed the endotoxin detoxifying ability of the liver and spleen (2), and presented presumptive evidence for the hepatic macrophage lysosomes as an intracellular locus of the endotoxin inactivation process (3). Since a more ready source of lysosomal material was required to pursue this line of investigation, the present study was undertaken to compare the ability of leukocytes vis-à-vis macrophages to detoxify endotoxin *in vitro*. The detoxification end point used was the inability of minute amounts of endotoxin to induce shock in the lead-sensitized rat (2).

Materials and Methods. Male rats of the Sprague-Dawley strain, 300 ± 20 g, were obtained from the Holtzman Company, Madison, Wisconsin. The general procedures for animal handling and the bioassay of endotoxin detoxification were performed as described in detail previously (2, 3). In essence, the materials to be evaluated for endotoxin detoxifying potential were incubated at either 37 or 4° with 3 μg/ml of *Salmonella enteritidis* lipopolysaccharide (Difco Laboratories, Detroit, Michigan); then, 1 ml of the incubation media was injected intravenously along with 5 mg of lead acetate. At this endotoxin dosage, *i.e.*, 1 μg/100 g, samples incubated at 4° which did not undergo detoxification were usually 100% lethal. Death occurred 14–24 hr later and was characterized by the typical postmortem signs of endotoxin shock.

Leukocytes were obtained by two methods: (a) from fresh heparinized rat blood using a dextran sedimentation technique (4); and (b) from peritoneal exudates induced 3 hr prior to harvest by intraperitoneal injection of 16 ml/100 g of 1% casein (5). Routine differential examinations verified the exudate yields as 75–90% polymorphonuclear leukocytes and 10–15% small mononuclear or lymphoid cells. Hepatic macrophages or Kupffer cells were obtained by the method described previously (2), which is based on the magnetic isolation of iron-laden macrophages from an enzymatic dispersion of liver cells. Alveolar macrophages were obtained by the tracheal lavage method as adapted to rats by Pavillard (6). Peritoneal macrophages were harvested 96 hr after induction of a casein exudate. Cell counts and morphology were ascertained using routine hemocytometry. All cells were suspended and washed in 0.02 *M* phosphate buffered saline, pH 7.4, for the endotoxin inactivation studies. Cellular sonicates were prepared by treatment of the suspensions for 2 min with the macroprobe of a Bronwill Biosonik III sonifier (Bronwill Scientific, Rochester, New York). Except as noted in the Results section, all sonicates were diluted to a protein concentration of 1 to 2 mg/ml as determined by the

[1] Supported by Grant HE 11499 from the National Heart and Lungs Institute, U.S. Public Health Services and the Tennessee Heart Association.

[2] Present address: Department of Physiology, Loyola University, Stritch School of Medicine, Maywood, Illinois 60153.

TABLE I. Comparison of Endotoxin Inactivation by Leukocyte and Macrophage Sonicates.[a]

Cell sonicate	Incubation temp (°C)	Mortality (deaths/no. of rats in trial)				Totals	
		Expt. 1	Expt. 2	Expt. 3	Expt. 4	Mortality	%
Leukocytes							
Blood	4	8/8	8/8	8/8	8/8	32/32	100
	37	6/8	6/8	8/8	6/8	26/32	81
3-hr peritoneal exudate	4	8/8	8/8	8/8	—	24/24	100
	37	8/8	7/8	8/8	—	23/24	96
Macrophages							
96-hr peritoneal exudate	4	7/8	8/8	8/8	—	23/24	96
	37	0/8	1/8	0/8	—	1/24[b]	4
Kupffer cells	4	10/12	8/8	8/8	—	26/28	93
	37	1/12	2/9	0/8	—	3/29[b]	10
Alveolar	4	7/10	6/10	7/10	9/13	29/43	67
	37	3/12	2/12	2/12	2/12	9/47[b]	19

[a] Sonicates were prepared in phosphate-buffered saline at a protein concentration of 1 to 2 mg/ml. Incubations were performed with 3 μg/ml of endotoxin for 180 min. One ml of media plus 5 mg of lead acetate were then injected into assay rats.

[b] $p < .05$.

method of Lowry *et al.* (7), using bovine albumin as the protein standard. Large granule fractions consisting of the 15,000*g* sediment of peritoneal macrophage homogenates were prepared by a slight modification of the method of Cohn and Weiner (8). These granule preparations were also sonified prior to use in the endotoxin-inactivation assay.

Lethality data were tested for statistical significance at a confidence level of 95% using the chi-square test corrected for continuity with the Yates' factor.

Results. Comparison of in vitro *endotoxin detoxification by leukocytes vs macrophages.* As indicated in Table I, neither blood leukocytes nor peritoneal leukocytes manifested ability to detoxify endotoxin as evaluated by shock mortality in the lead-sensitized assay recipients. In analogous control studies, leukocyte sonicates without endotoxin were completely nonlethal upon injection. In contrast to the leukocyte sonicates, the macrophage preparations, *i.e.*, hepatic, alveolar, and peritoneal, displayed ability to detoxify endotoxin. In these studies the lethality to the sonicates incubated with 3 μg/ml of endotoxin at 4° served as controls. In addition, macrophage sonicates without endotoxin were nonlethal when injected iv.

Figure 1 depicts a comparison of endotoxin detoxification in peritoneal leukocyte vs peritoneal macrophage sonicates at varying protein concentrations. Macrophage sonicates were potent in inactivating endotoxin at protein concentrations as low as 0.5 mg/ml. In contrast, leukocytes were not active even up to 5.3 mg/ml.

Endotoxin detoxification during exudate development. Endotoxin inactivation was evaluated in cells harvested from peritoneal exudates at 3, 6, 12, 24, 48, 72, and 96 hr (Fig. 2). The cellular profile was restricted to three general categories: polymorphonuclear leukocytes, primarily neutrophils; small mononuclear cells, <15 μ; and large mononuclear cells, >15 μ, *i.e.*, typical macrophage types. In confirmation of numerous studies (9), the early and predominant neutrophilia waned within 24 hr and was followed by a mononuclear population with an increasing percentage of large mononuclear cells. As shown in Fig. 2, the development of endotoxin inactivation by the exudate sonicates corresponded to the presence and ultimate preponderance, at 96 hr, of macrophages.

Endotoxin inactivation by large granule fractions of peritoneal macrophages. In order to extend our recent study on endotoxin detoxification by hepatic macrophage lysosomes, the ability of large granule fractions

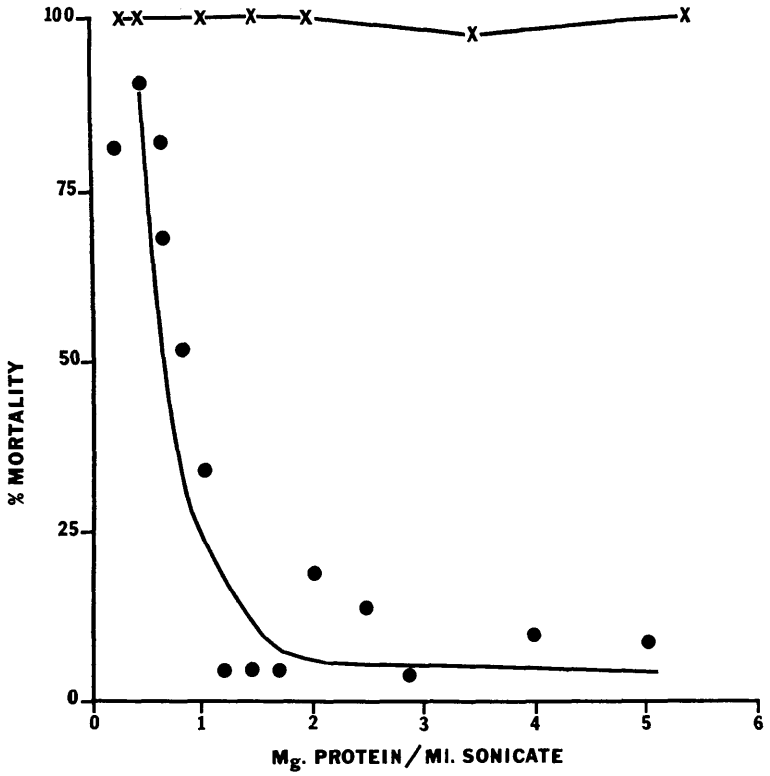

FIG. 1. Effect of varying protein content on peritoneal leukocyte and macrophage inactivation of endotoxin: (X—) leukocyte; (●—) macrophage. Each point represents the average of 10–12 rats. Test sonicates were prepared in phosphate-buffered saline and incubated at 37° for 180 min with 3 μg/ml of endotoxin; after incubation, each test rat received 1 ml of the incubation media plus 5 mg of lead acetate iv.

of peritoneal macrophages to inactivate endotoxin was studied. As shown in Fig. 3, endotoxin detoxification was manifest in the sonicates of macrophage large granule fractions at protein concentrations as low as 12–20 μg/ml. Both the increase in specific detoxifying activity and localization in the granule fraction which is rich in mitochondria and lysosomes support the notion of a unique role for macrophage lysosomes in the detoxification of endotoxin. An attempt to separate the mitochondrial fraction from the lysosomes of the macrophage granules, using the method of Ragab *et al.* (10), was unsuccessful. Further work is needed to develop a method to achieve this separation and test endotoxin inactivation.

Discussion. Endotoxin enters the blood vascular system under diverse circumstances, *i.e.*, during a gram-negative infection, accompanying either intestinal perforation or a defect in the mucosal barrier, following administration of contaminated parenterals, or experimentally. Among the characteristic acute host responses to endotoxemia are fever, a transient leukopenia followed by a prolonged leukocytosis, coagulation disturbances, and a deterioration of microcirculatory homeostasis which may eventuate in the state of "endotoxin shock." Since leukocytes are intimately involved in host response to local infection and systemic endotoxemia, numerous studies have dealt with various aspects of the leukocyte—endotoxin interaction (11, 12). While numerous data exist relative to the effects of endotoxin on leukocytes, relatively few studies have focused on the converse problem, *i.e.*, the effects of leu-

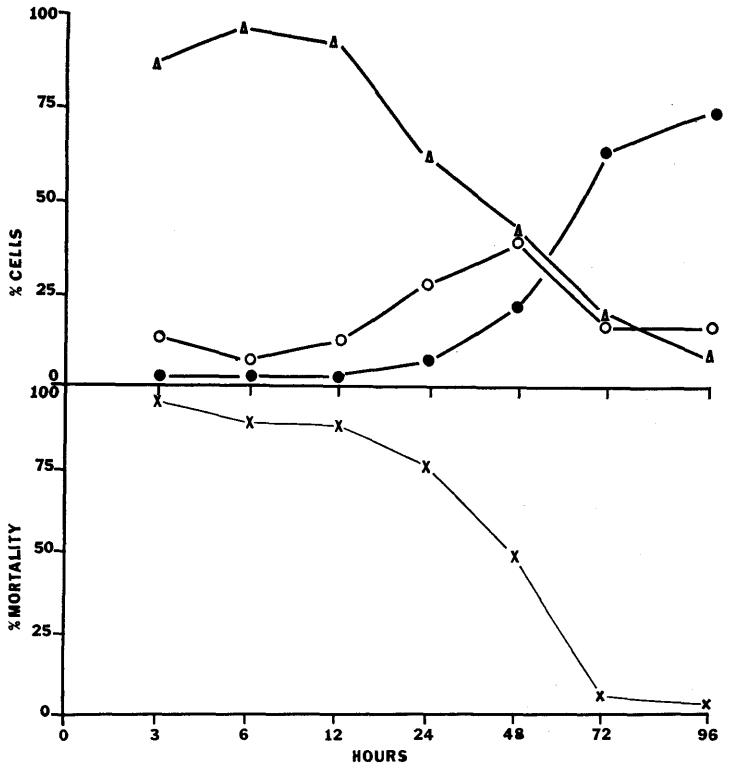

FIG. 2. Time course of development of endotoxin detoxification in peritoneal exudates: (△—) polymorphonuclear leukocytes—mainly neutrophiles; (○—) small mononuclear cells of <15 μ in diameter; (●—) large mononuclear cells, *i.e.*, macrophages, of >15 μ in diameter. For cell distributions, each point represents the average of 3–10 exudate preparations of 8–10 rats/pool. For the mortality, each × represents the average of 10–12 rats/assay test.

kocytes on endotoxin.

In the current study, leukocyte sonicates were found unable to cause any alteration of endotoxin's characteristic ability to induce cardiovascular collapse and shock. These findings, therefore, agree with an earlier report, by Rutenburg *et al.* (13), on the role of the leukocytes in shock. In contrast to the shock aspect, leukocytes and their lysosomal system have been reported to reduce endotoxin's Shwartzman eliciting potential, pyrogenicity, and tumor necrotizing potential (14). However, leukocyte lysosomes were not effective in altering the immunogenic ability of the lipopolysaccharide (14). Since numerous studies suggest endotoxin shock is a hypersensitivity-type phenomenon (15), this separation of the leukocyte's potential in dealing with the various biologically active sites of endotoxin is worthy of note.

Macrophages and especially the fixed component of the reticuloendothelial system have been associated with endotoxin detoxification (13). This study definitely affirmed this function in three types of macrophages—hepatic Kupffer cells, alveolar macrophages, and exudate peritoneal macrophages. Furthermore, the time course of development of macrophages in the exudate correlated with the appearance of endotoxin inactivation. This relationship raises interesting possibilities regarding the role of endotoxin as either a potential chemotactic or macrophage activating substance in gram-negative lesions.

Furthermore, in confirmation of recent work on hepatic lysosomes in endotoxin inactivation (2), this study demonstrated endotoxin detoxification in the large granule fraction of peritoneal macrophages. It is anticipated that further analysis of this fraction will allow explicit characterization of the macrophage endotoxin detoxifying system.

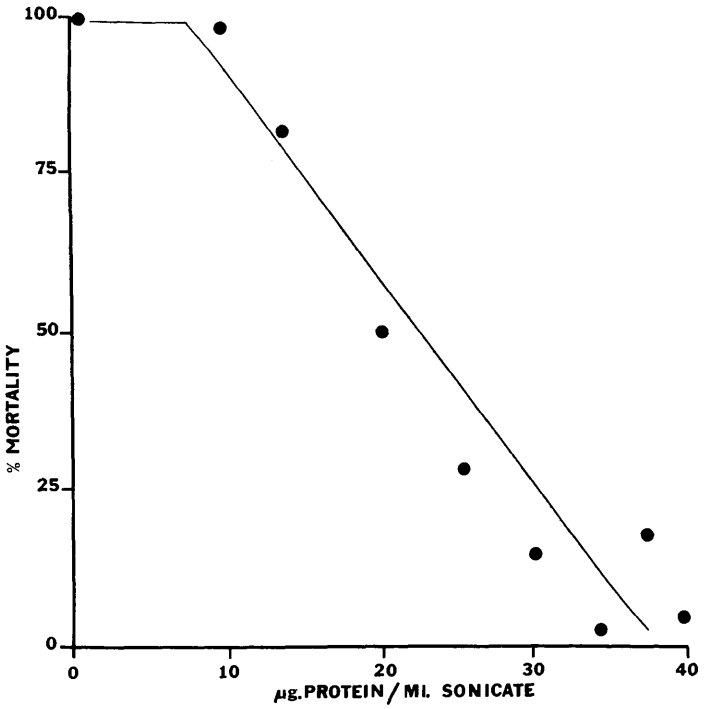

FIG. 3. Endotoxin detoxification by peritoneal macrophage large granule fractions: Each point represents the average of 10–12 rats.

Summary. Endotoxin detoxification by leukocyte and macrophage sonicates was evaluated using the lead-sensitized rat shock bioassay. Leukocyte sonicates from both blood and peritoneal exudates did not inactivate endotoxin. In contrast, sonicates of macrophages from liver, lung, or peritoneal exudates displayed marked endotoxin detoxifying ability. During the development of peritoneal exudates to casein ip, the cell progression from a neutrophilia to a macrophage population was associated with the development of exudate endotoxin detoxifying ability. Endotoxin inactivation was demonstrated in the large granule fraction of peritoneal macrophages. The probable specificity of macrophage lysosomes in certain aspects of endotoxin detoxification is discussed.

1. Braude, A. I., "Bacterial Endotoxins," p. 98. Rutgers Univ. Press, New Brunswick, N.J. (1964).

2. Filkins, J. P., Proc. Soc. Exp. Biol. Med. **134**, 610 (1970).

3. Filkins, J. P., J. Reticuloendothel. Soc. **9**, 480 (1971).

4. Malawista, S. E., and Bodel, P. T., J. Clin. Invest. **46**, 786 (1967).

5. Reed, P. W., and Tepperman, J., Amer. J. Physiol. **216**, 223 (1969).

6. Pavillard, E. R. J., Aust. J. Exp. Biol. Med. Sci. **41**, 265 (1963).

7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

8. Cohn, Z. A., and Weiner, E., J. Exp. Med. **118**, 991 (1963).

9. Spector, W. G., Int. Rev. Exp. Pathol. **8**, 1 (1969).

10. Ragab, H., Beck, C., Dillard, C., and Tappel, A. L., Biochim. Biophys. Acta **148**, 501 (1967).

11. Cline, M. J., Melmon, K. L., Dans, W. C., and Williams, H. E., Brit. J. Haematol. **15**, 539 (1968).

12. Collins, R. D., and Wood, W. B., J. Exp. Med. **110**, 1005 (1959).

13. Rutenburg, S. H., Schweinburg, F. B., and Fine, J., J. Exp. Med. **112**, 801 (1960).

14. Mesrobeanu, I., Mesrobeanu, L., Bona, C., Vranialici, D., and Petrovici, A., Z. Immunitaetsforsch. Allerg. Klin. Immunol. **139**, 301 (1970).

15. Stetson, C. A., "Bacterial Endotoxins," p. 658. Rutgers Univ. Press, New Brunswick, N.J. (1964).

Received Apr. 13, 1971. P.S.E.B.M., 1971, Vol. 137.