INFECTION AND IMMUNITY, July 1979, p. 328-336
0019-9567/79/07-0328/09$02.00/0

Vol. 25, No. 1

Analysis of the Effects of Lipopolysaccharide on Macrophages: Differential Phagocytic Responses of C3H/HeN and C3H/HeJ Macrophages In Vitro

STEFANIE N. VOGEL,* SANDRA T. MARSHALL, AND DAVID L. ROSENSTREICH

Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205

Received for publication 28 March 1979

The phenomenon of lipopolysaccharide (LPS)-induced in vitro macrophage cytotoxicity has been reported by a number of investigators but has often been difficult to reproduce and to quantitate. In this report, we have examined the effect of LPS on the ability of macrophages to ingest ^{51}Cr-labeled, opsonized sheep erythrocytes as a method for examining the direct toxic effects of LPS on macrophages in vitro. By using this assay, we can clearly discriminate between LPS responder C3H/HeN macrophages and LPS nonresponder C3H/HeJ macrophages and demonstrate that LPS induces a profound inhibition of Fc-mediated phagocytosis in LPS responsive macrophages. Furthermore, low concentrations of LPS stimulate phagocytosis in macrophages derived for C3H/HeJ mice. The lipid A moiety of the LPS is responsible for the observed enhancement or inhibition of Fc-mediated phagocytosis. This assay was more sensitive than LPS-induced cytotoxicity, since inhibition of phagocytosis was detectable in cultures of LPS-sensitive macrophages even when cytotoxicity, assessed by trypan blue exclusion, was not. Thus, this assay represents an extremely sensitive method for analyzing the direct effects of LPS on macrophages.

Endotoxin, the lipopolysaccharide (LPS) derived from gram-negative bacteria, induces a broad range of biological alterations in vivo (reviewed in ref. 2). The mechanisms involved in endotoxicity are not well understood, but much evidence is available to support the hypothesis that lymphoreticular cells are involved as important targets of the LPS molecule (5, 14, 26). Macrophages constitute one cell type that is thought to be important in the mediation of certain LPS-triggered events in vivo (5, 22), and they have also been shown to respond to LPS in vitro as well. In 1965, Heilman (9) and Kessel and Braun (11) reported that LPS was cytotoxic for macrophages in vitro. Since these initial reports, this phenomenon has been utilized in a number of studies as a convenient measure of macrophage LPS responsiveness (5, 18, 23-25), and it has been clearly demonstrated that macrophages derived from LPS-responsive mouse strains are sensitive to the cytotoxic effect of LPS in vitro, whereas macrophages from LPS-resistant mouse strains are intrinsically unresponsive to this effect (5, 18). However, the utility of this effect and the ability to analyze it further have been hampered by problems of reproducibility, variation in sensitivity of different macrophage populations, and variations in the potency of different LPS preparations. Therefore, we sought a new, more sensitive measure of the direct toxic activity of LPS on macrophages.

In the present study we compared the cytotoxic effect of LPS with its effect on the ability of macrophages to ingest ^{51}Cr-labeled, opsonized sheep erythrocytes. We have found inhibition of phagocytosis to be a quantitative, reliable, and sensitive assay. Furthermore, our findings demonstrate that macrophages derived from the LPS "nonresponder" C3H/HeJ mouse strain exhibit altered phagocytic responses when exposed to LPS. Finally, data presented in this report indicate that the observed phagocytic alterations are mediated by the lipid A moiety of LPS, and that prostaglandins do not seem to play a role.

MATERIALS AND METHODS

Mice. C3H/HeN female mice were obtained from the Division of Research Resources, National Institutes of Health, Bethesda, Md. C3H/HeJ female mice were obtained from Jackson Laboratories (Bar Harbor, Maine). All animals used were 8 to 12 weeks old. Mice were fed standard lab chow and water ad libitum.

Reagents. RPMI 1640 medium (GIBCO, Grand Island, N.Y.) was supplemented with penicillin (100

μg/ml) and streptomycin (100 μg/ml) and 2 mM glutamine. Fetal calf serum (FCS; Flow Laboratories, Rockville, Md.) was heat inactivated at 56°C for 30 min. Phenol-water-extracted LPS [LPS(Ph)] was prepared from *Escherichia coli* K-235 by the extraction procedure of McIntire et al. (12). The O-polysaccharide-deficient glycoprotein derived from *Salmonella minnesota* R595, extracted by chloroform-methanol treatment, was kindly donated by David Morrison (Scripps Clinic and Research Foundation, La Jolla, Calif.). Purified polysaccharide (FP) derived from *S. typhimurium* was prepared by the method Freeman (3) and was obtained from C. Bona (National Institutes of Health). Lipid A was prepared by acid hydrolysis of phenol-extracted K-235 LPS(Ph) by the method of Nowotny et al. (17). Polymyxin B, indomethacin, and prostaglandin E_2 $(PGE)_2$ were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Macrophage collection and cell culture. Macrophages were induced by injecting 3 ml of 3% sterile thioglycolate broth intraperitoneally (i.p.). Five or 6 days later, cells were collected by peritoneal lavage with 5 to 6 ml of pyrogen-free saline (Abbott Laboratories, Chicago, Ill.), washed, and suspended in RPMI + 20% FCS. Cells were incubated for 2.5 to 3 h at 37°C in a humidified atmosphere containing 5% CO_2, and subsequently the macrophage monolayers were washed three times with RPMI + 10% FCS to remove nonadherent cells. The macrophage cultures were then reincubated for the duration of the assay in RPMI + 2% FCS with or without LPS.

Assay of in vitro LPS-induced macrophage cytotoxicity. This technique for measuring macrophage cell death has been previously reported (4). Thioglycolate-induced peritoneal cells were collected, washed, and suspended to a final concentration of 2×10^6/ml, and 1-ml portions were cultured as described above in flat-bottomed glass vials (Bellco Glass Co., Vineland, N.J.). Cell death was evaluated by exclusion of trypan blue dye (GIBCO, Grand Island, N.Y.). Duplicate vials were cultured for each treatment and, in general, at least 200 cells per vial were scored for viability.

LPS-induced inhibition of phagocytosis. LPS pretreatment of macrophages in vitro inhibits their phagocytosis of ^{51}Cr-labeled, opsonized sheep erythrocytes. The details of the phagocytosis assay have been previously described by Snyderman et al. (27) and modified by Vogel et al. (28). Briefly, 400,000 macrophages were plated per well in TC-24 culture dishes (Costar, Cambridge, Mass.) in the presence of 20% FCS. After the 2.5-h incubation, nonadherent cells were washed from the wells. Various doses of LPS were added to the wells in RPMI containing 2% FCS. After overnight incubation, the culture supernatants were removed and replaced with 0.5 ml of a suspension of hemolysin-coated, ^{51}Cr-labeled sheep erythrocytes per well. The hemolysin was obtained from Flow Laboratories, Rockville, Md. Cultures were reincubated for 1 h to permit phagocytosis, washed once with Veronal saline containing gelatin (GVB^{++}), and subsequently washed with an ammonium chloride erythrocyte-lysing solution (ACK-lyse; National Institutes of Health Media Unit) to remove noninternalized erythrocytes. Two additional GVB^{++} washes followed. Finally, the monolayers were dissolved in 0.6 ml of 0.5% sodium dodecyl sulfate (Sigma Chemical Co., St. Louis, Mo.), and 0.5 ml/well was sampled for counting in a Beckman gamma counter. Triplicate wells were cultured for each LPS dose tested. The results are expressed as percentage of phagocytosis of control cultures, i.e., % phagocytosis = cpm LPS-treated cultures × 100%)/(cpm control cultures). In some studies, polymyxin B (5.0 μg/ml), indomethacin (10.0 μg/ml), or prostaglandin E_2 (10^{-4} to 10^{-8} mg/ml) was added to the cultures at the same time as the LPS(Ph). Indomethacin was initially prepared in 0.1 M Na_2CO_3 at a final concentration of 10 mg/ml and subsequently diluted in RPMI + 2% FCS. PGE_2 was dissolved in absolute ethanol at a concentration of 1.0 mg/ml and was also subsequently diluted in RPMI + 2% FCS.

Assay of LPS-induced prostaglandin production by macrophages. Before opsonized erythrocytes were added, 24-h supernatants from the phagocytosis cultures were collected and analyzed for their concentration of LPS-induced PGE_2. PGE_2 levels were determined by a previously described radioimmunoassay technique (1).

RESULTS

LPS-induced macrophage cytotoxicity versus LPS-induced alterations in macrophage phagocytosis. It has been reported previously that LPS exerts a cytotoxic effect on C3H/HeN macrophages, whereas C3H/HeJ macrophages are not killed by LPS in vitro (4). We first compared the sensitivity of LPS-induced cytotoxicity and phagocytosis inhibition of these two strains of mice. LPS(Ph) was cytotoxic for thioglycolate-induced C3H/HeN macrophages over a concentration range of 1 to 50 μg, whereas C3H/HeJ macrophages were not killed under these conditions (Fig. 1A). The results with phagocytosis inhibition were somewhat different. This assay was more sensitive with C3H/HeN macrophages than the cytotoxic assay, since comparable effects were produced by approximately one-fifth the concentration of LPS at each point (Fig. 1B). Macrophages from C3H/HeJ mice exhibited an increase in phagocytosis at LPS concentrations of 1 to 5 μg/ml. Higher concentrations of LPS (10 to 50 μg/ml) significantly inhibited phagocytosis by C3H/HeJ macrophages; nevertheless, at every concentration of LPS there was a highly significant difference in the sensitivity of C3H/HeN and C3H/HeJ macrophages. These findings illustrate two points. First, phagocytosis inhibition is a more sensitive measure of the effects of LPS on macrophages in vitro than is measurement of cell death. Second, phagocytosis inhibition can occur in the absence of detectable cell death. This effect is seen most strikingly in the C3H/HeJ macrophages.

In preliminary studies we had noted that cy-

totoxicity could be markedly decreased by heating of the LPS preparation. To better characterize this effect, LPS was heated at 100°C for 60 min, and the cytotoxic and inhibitory effects of the heated LPS were determined. In comparison to the unheated preparation, heat-treated LPS was less cytotoxic for C3H/HeN macrophages at every concentration (Fig. 2A). Thus, at a concentration of 50 μg/ml, heat-treated LPS killed only 13% of the macrophages in comparison to 66% for the unheated preparation.

A similar pattern was observed with phagocytosis inhibition, although again the enhanced sensitivity of this assay was observed. Even though the heat-treated LPS was less inhibitory than the unheated preparation, significant phagocytosis inhibition was observed at concentrations of boiled LPS as low as 1 μg/ml (Fig. 2B). Since the boiled LPS preparation was not significantly cytotoxic at concentrations up to 10

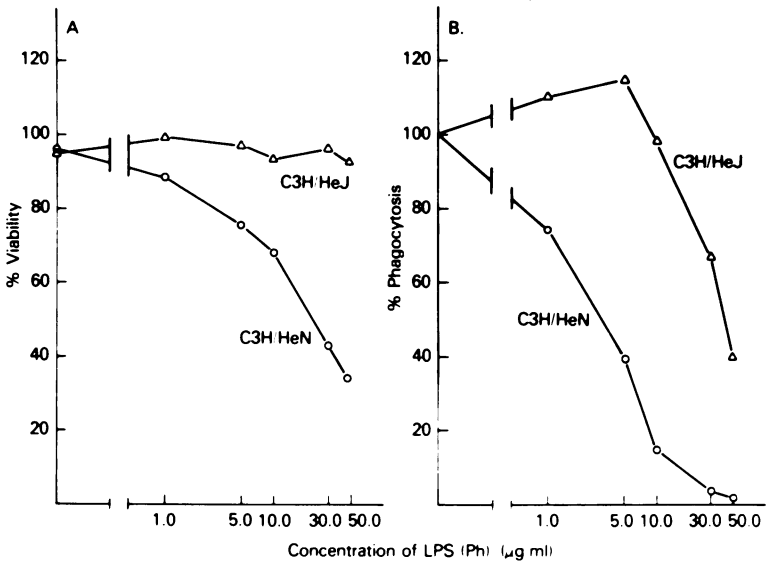

FIG. 1. *Comparison of LPS(Ph)-induced cytotoxicity and inhibition of Fc-mediated phagocytosis in C3H/HeN and C3H/HeJ macrophage cultures. The results for cytotoxicity represent the mean viabilities of duplicate cultures, and the results for inhibition of phagocytosis represent the mean values for triplicate wells. These data were derived from a single representative experiment in which macrophages were pooled from four mice for each strain. All standard errors were within 10% of the mean.*

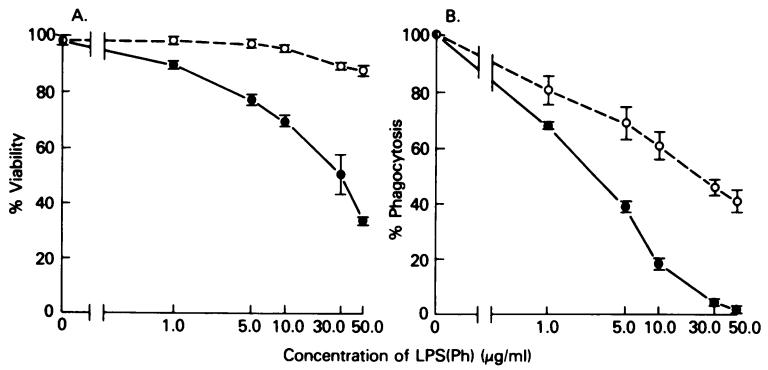

FIG. 2. *Comparison of LPS(Ph)-induced cytotoxicity and inhibition of Fc-mediated phagocytosis in C3H/HeN macrophage cultures, using untreated LPS(Ph)* (●——●) *and LPS(Ph) heated at 100°C for 1 h* (○-----○). *The results for cytotoxicity represent the mean viabilities ± standard error of the mean for two separate experiments in which duplicate cultures were scored for viability for each dosage tested. The results for inhibition of phagocytosis represent the mean values ± standard error of the mean for five to eight separate experiments in which triplicate cultures were assayed for each dosage tested. Macrophages from five mice were pooled for each experiment.*

μg/ml, these experiments confirm the fact that phagocytosis inhibition can occur in the absence of detectable cytotoxicity.

We next tested the effects of boiled LPS on C3H/HeJ macrophages (Fig. 3). As was seen in Fig. 1, unheated LPS has two effects on C3H/HeJ macrophages. At low concentrations, LPS enhanced phagocytosis by C3H/HeJ macrophages, whereas at high concentrations it was inhibitory. Interestingly, heat treatment of LPS only reduced its inhibitory effects, since the phagocytosis-enhancing effects of LPS were retained by the boiled preparation.

Identification of the LPS moiety responsible for inhibition of Fc-mediated phagocytosis. We next sought to determine which component of the LPS molecule was responsible for inhibition of phagocytosis. Various lipid A or polysaccharide preparations were obtained and tested for their ability to inhibit phagocytosis by macrophages (Fig. 4). Two lipid A-containing preparations, the glycolipid extracted from *S. minnesota* R595 (Fig. 4B) and the lipid A produced by acid hydrolysis of *E. coli* LPS(Ph) (Fig. 4C), produced significant inhibition of phagocytosis. In contrast, the Freeman polysaccharide preparation was much less inhibitory (Fig. 4D). The glycolipid preparation also enhanced phagocytosis by C3H/HeJ macrophages as well as the intact LPS, whereas the polysaccharide preparation was less active in this regard. With all preparations tested, the differences between C3H/HeN and C3H/HeJ macrophages were retained. Thus, these findings strongly suggest that both types of phagocytic alterations induced by LPS are mediated by the lipid A moiety.

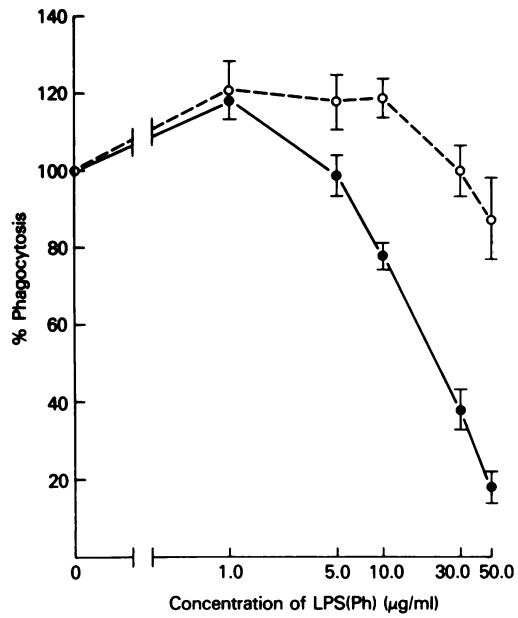

FIG. 3. *Comparison of the effects of unheated versus heated LPS(Ph) on phagocytosis by C3H/HeJ macrophages. Unheated LPS(Ph)* (●——●) *and LPS(Ph) heated at 100°C for 1 h* (○-----○) *are shown. The results represent the mean values ± standard error of the mean for five to eight separate experiments in which triplicate cultures were assayed for each dosage tested. Macrophages from five mice were pooled for each experiment.*

The cyclic peptide antibiotic, polymyxin B, has been previously demonstrated to prevent the lethal endotoxic activity of LPS in vivo (21), as well as inhibit the in vitro mitogenic activity of LPS (15), by forming a stable molecular complex with the lipid A of LPS (16). We therefore tested the effect of polymyxin B on the ability of LPS(Ph) to inhibit Fc-mediated phagocytosis to confirm the role of lipid A in this event. Polymyxin B completely abolished the inhibitory effect of LPS on C3H/HeN macrophages at concentrations of LPS up to 10 μg/ml (Fig. 5A). At 30 μg of LPS per ml, significant inhibition was observed in the presence of polymyxin B. Polymyxin B also blocked the inhibitory effects of LPS on C3H/HeJ macrophages (Fig. 5B). Interestingly, polymyxin B also completely blocked LPS-induced phagocytosis enhancement as well. These data support the conclusion that LPS-induced alterations of phagocytosis are mediated by lipid A.

Role of prostaglandins in LPS-induced phagocytosis inhibition. Prostaglandins are thought to play an important role in endotoxicity in vivo (reviewed in ref. 4). LPS induces prostaglandin synthesis by macrophages in vitro, and prostaglandins have also been shown to act on macrophages by enhancing their ability to produce the enzyme collagenase (22, 29). In addition, exogenously added prostaglandins have been reported to modulate Fc-mediated phagocytosis in vitro (19). We therefore sought to determine whether or not LPS-induced inhibition of phagocytosis was prostaglandin mediated. The levels of prostaglandin E_2 produced by LPS-stimulated macrophages are illustrated in Fig. 6. C3H/HeN macrophages secrete detectable amounts of PGE_2 (415 pg/ml) at LPS concentrations as low as 0.1 μg/ml, and are maximal at LPS concentrations of 30 μg/ml (2,339 pg/ml). PGE_2 levels in C3H/HeJ macrophage cultures remained low (<40 pg/ml) even at the highest LPS concentrations.

We next investigated whether exogenous prostaglandin E_2 added at physiological concentrations would effect LPS-induced phagocytosis inhibition. C3H/HeN or C3H/HeJ macrophages

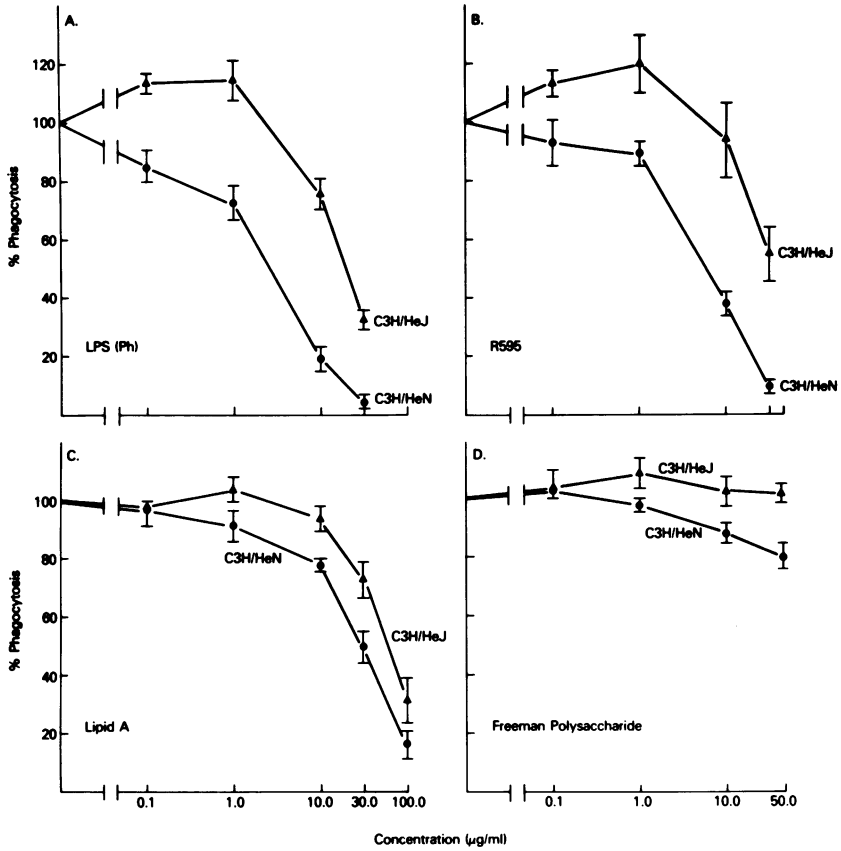

FIG. 4. *Comparison of the effects on different LPS-derived preparations on Fc-mediated phagocytosis by C3H/HeN and C3H/HeJ macrophages. The results represent the arithmetic means ± standard error of the mean for four to seven separate experiments in which macrophages from five mice per strain were pooled for each experiment.*

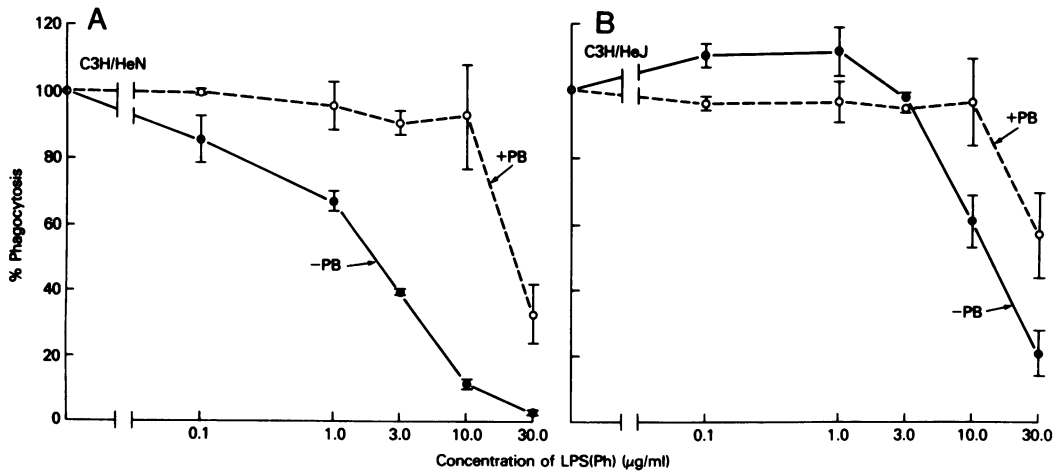

FIG. 5. *Effect of 5 μg of polymyxin B per ml on LPS-induced inhibition of phagocytosis in C3H/HeN (A) and C3H/HeJ (B) macrophages. The results represent the arithmetic means ± standard error of the mean for two experiments in which triplicate cultures were assayed for each concentration tested. Macrophages from five mice per strain were pooled for each experiment.*

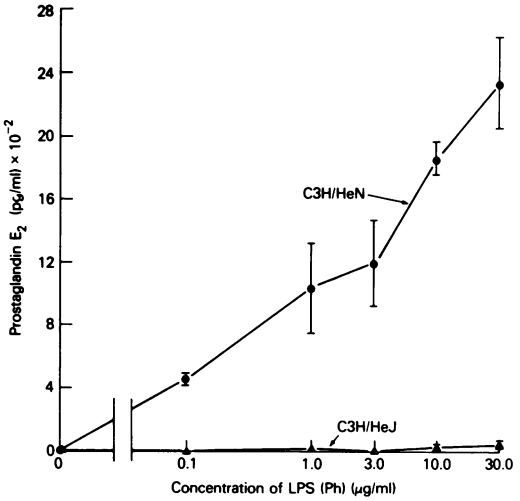

FIG. 6. *PGE_2 production in response to LPS(Ph) stimulation. These results represent the arithmetic means ± standard error of the mean for triplicate cultures in which macrophages from five mice per strain were pooled.*

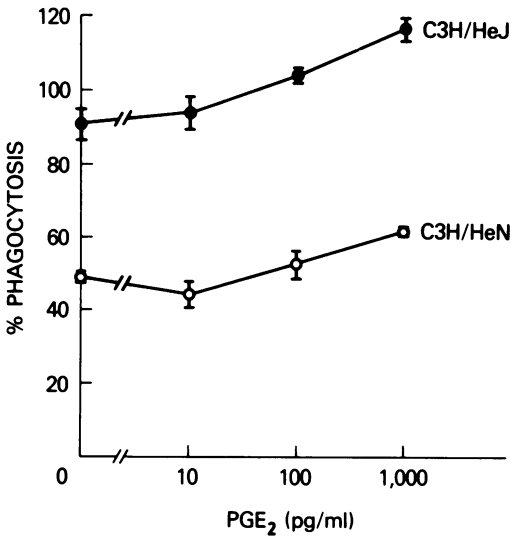

FIG. 7. *Effect of exogenously added PGE_2 on LPS-induced inhibition of phagocytosis. Results represent the arithmetic means ± standard error of the mean for two separate experiments in which triplicate cultures were tested for each treatment. Macrophages obtained from five mice per strain were pooled for each experiment. All cultures were treated with 3.0 μg of LPS(Ph) per ml, a dose at which either stimulatory or inhibitory PGE_2-mediated effects could be detected in both the C3H/HeN and C3H/HeJ macrophage cultures simultaneously. The effect of 1,000 pg of PGE_2 per ml was found to be highly significant for both C3H/HeN macrophages ($P < 0.005$) and C3H/HeJ macrophages ($P < 0.025$) as determined by Student's t test.*

were treated with a concentration of LPS (3 μg/ml) that inhibited C3H/HeN macrophage phagocytosis by approximately 50%. The effect of different concentrations of added PGE_2 was subsequently assessed. The addition of PGE_2 to either C3H/HeN or C3H/HeJ macrophages resulted in an increase in their phagocytic activity (Fig. 7). No concentration of PGE_2 produced any more phagocytosis inhibition in comparison to LPS alone. PGE_2 had no effect on macrophages cultured in the absence of LPS (data not shown). It should be noted that the highest concentration of PGE_2 tested (1,000 pg/ml) is similar to the level produced by C3H/HeN macrophages stimulated with 3 μg of LPS per ml (see Fig. 6). These data suggest that phagocytosis inhibition is not mediated by prostaglandins.

To confirm this finding, the effect of the prostaglandin synthetase inhibitor, indomethacin, was also assessed. Indomethacin at 10 μg/ml will prevent the production of PGE_2 by LPS-stimulated macrophages (29). No difference in the degree of LPS-induced phagocytosis inhibition was observed in either C3H/HeN or C3H/HeJ macrophage cultures in the presence of 10 μg of indomethacin per ml (Fig. 8). Thus, these data support the conclusion that LPS-induced phagocytosis inhibition is not mediated by prostaglandins.

DISCUSSION

In this report we have assessed LPS-induced inhibition of phagocytosis as an alternative method to LPS-induced cytotoxicity for quantitatively analyzing the direct in vitro effects of LPS on macrophages. By measuring the effects of LPS on the ingestion of ^{51}Cr-labeled, opsonized sheep erythrocytes, we have found that macrophages derived from LPS-sensitive, C3H/HeN mice exhibit a significant decrease in their ability to phagocytize when exposed to as little as 0.1 μg of LPS per ml in vitro. Thus, this assay is approximately five times more sensitive than measuring the cytotoxic effect of LPS on macrophages.

The increased sensitivity of this assay is reflected by our data demonstrating that macrophages derived from LPS-resistant, C3H/HeJ mice are not totally refractory to the effects of LPS, a conclusion which might well be drawn from cytotoxicity data, but rather exhibit a significant and reproducible enhancement of phagocytosis at low concentrations of LPS and significant inhibition of phagocytosis at higher concentrations of LPS. However, although macrophages from C3H/HeJ mice have been shown in this study not to be totally unresponsive to the effects of LPS, C3H/HeN macrophages are

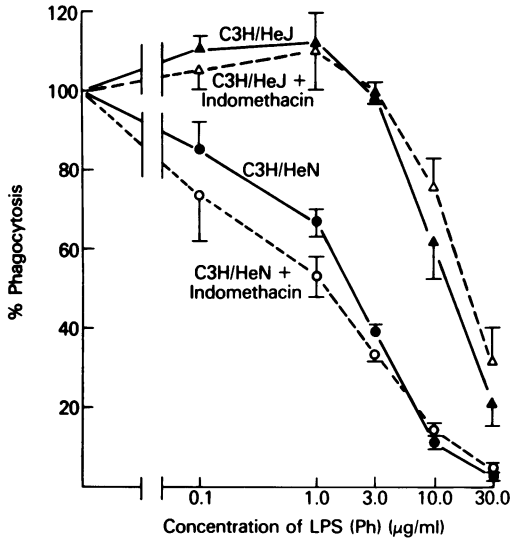

FIG. 8. *Effect of indomethacin on LPS-induced inhibition of phagocytosis on C3H/HeN and C3H/HeJ macrophage cultures. These results represent the arithmetic means ± standard error of the mean for two separate experiments in which triplicate cultures were assayed for each dosage of LPS(Ph) tested. Macrophages from five mice per strain were pooled for each experiment.*

much more sensitive to LPS-induced inhibition of Fc-mediated phagocytosis. Other LPS-sensitive and -resistant mouse strains, such as the C57Bl/10Sn versus the C57Bl/10ScCr or C57Bl/10ScN strains, exhibit comparable differential sensitivity to LPS in this assay (28), and we have previously reported that resident peritoneal macrophages derived from LPS-sensitive and -resistant strains exhibit responsiveness to LPS(Ph) similar to thioglycolate-induced cells (23). Thus, we have found LPS-induced inhibition of phagocytosis to be a highly sensitive, quantitative measure of macrophage LPS responsiveness, detecting subtle LPS-induced modifications not detectable by observing cell death.

The inhibition of phagocytosis by LPS(Ph), as well as the enhancement of phagocytosis observed in LPS-resistant macrophages, both appear to be mediated by the lipid A moiety of the LPS molecule. Two lines of evidence have been presented to support this statement. First, only LPS derivatives rich in the lipid A moiety induce LPS-mediated inhibition of phagocytosis. The kinetics of inhibition observed with the R595 preparation strongly parallel that observed with the LPS(Ph), suggesting that it is the lipid A moiety, and not the associated polysaccharide moiety, which is responsible for the action of the intact LPS molecule. The K-235 lipid A preparation tested clearly induced inhibition of phagocytosis, although, it was not as potent an agent as the intact LPS(Ph) preparation. There are two possible reasons for this diminished activity. The finding of decreased in vitro biological activity in lipid A preparations has also been reported by Nowotny et al. (17) and Wahl et al. (30) and is most likely due to a general loss of potency observed in LPS preparations having undergone acid hydrolysis (8, 20). In addition, we have found that heat treatment markedly decreases the cytotoxic properties of LPS, and since production of lipid A involves boiling for 45 min, this might also explain its loss of potency. Finally, using a relatively pure polysaccharide preparation (FP), little or no inhibition of phagocytosis was observed.

Further confirmation for the involvement of the lipid A moiety in this response resides in the fact that the incorporation of polymyxin B in the culture medium during incubation with LPS(Ph) strongly counteracts the inhibition of phagocytosis normally observed in C3H/HeN macrophages as well as the enhancement (at low concentrations of LPS) and inhibition of phagocytosis (at higher concentrations of LPS) observed in C3H/HeJ macrophage cultures. Since it has been previously demonstrated that polymyxin B inhibits other in vitro activities of LPS (15) by acting though the lipid A moiety (16), our results suggest that lipid A must therefore be the key chemical moiety involved in the LPS-mediated alterations of phagocytosis presented in this report.

Although we have established a strong case for the fact that lipid A is the portion of the LPS molecule responsible for the effects of intact LPS(Ph) on Fc-mediated phagocytosis, the precise mechanism involved remains to be determined. It is clear from this study, however, that blocking prostaglandin production by LPS-sensitive macrophages with indomethacin does not lessen LPS inhibition of phagocytosis, nor does the addition of PGE_2 to LPS-resistant cultures lead to an inhibition of phagocytosis, strongly suggesting that prostaglandin production is not an essential element in the inhibition mechanism. Furthermore, we have shown that cell death is not an essential mechanism underlying LPS-induced inhibition of phagocytosis. This statement is supported by the observations that (i) phagocytosis by C3H/HeJ macrophages is significantly reduced at 50 μg of LPS per ml in the absence of cell death, and (ii) heated LPS, which is diminished in its cytotoxic capacity, still retains the ability to significantly reduce Fc-mediated phagocytosis in C3H/HeN macrophages. Therefore, it is clear that the LPS preparation need not be cytotoxic to cause an alter-

ation in Fc-mediated phagocytosis. To verify this finding another way, we tested a second *E. coli* K-235 LPS(Ph) preparation, which was found to be less than 5% cytotoxic for LPS-sensitive macrophages but known to be biologically active in other in vitro assays (D. Morrison, personal communication), and have found the pattern of inhibition of phagocytosis to be comparable to that obtained with our heated LPS(Ph) preparation (data not shown). Therefore, an extremely useful advantage, coincident with the increased sensitivity of this assay in detecting LPS-induced alterations in C3H/HeJ macrophages, is that it can be used as an indicator of LPS sensitivity or resistance under conditions which preclude LPS-induced cell death.

The observations that C3H/HeJ macrophages exhibit slight enhancement of phagocytosis at low concentrations of unboiled LPS(Ph) and that the inhibition of phagocytosis observed at high concentrations of LPS(Ph) can be eliminated by boiling the LPS(Ph) for 1 h, suggest the possibility that untreated LPS may be providing the macrophages with two different signals, i.e., one stimulating phagocytosis and one inhibiting phagocytosis. The C3H/HeN macrophages may be so much more sensitive to the inhibitory effects of LPS(Ph) that any enhancing activity is overridden. Some support for this hypothesis rests in the observation that, at a low dose of LPS(Ph) (0.1 μg/ml), C3H/HeN macrophages occasionally exhibit a slight enhancement of phagocytosis. Interestingly, LPS stimulates phagocytosis of labeled latex particles in certain macrophage cell lines (M. Ito and P. Ralph, Fed. Proc. **36**:1277, 1978), suggesting that these lines may be metabolically similar to C3H/HeJ macrophages. Although the enhancement of phagocytosis in C3H/HeJ macrophages was small, it was highly reproducible. This finding is also consistent with the observation in vivo that C3H/HeJ mice respond to an intraperitoneal injection of LPS with a rapid influx of inflammatory cells into the peritoneal cavity, whereas the response of C3H/HeN mice to LPS occurs considerably later and is significantly reduced (13). The response of this "LPS-unresponsive" strain to lipid A is consistent with previous observations and with the hypothesis that these mice are actually not LPS unresponsive, but instead respond differently to LPS (6, 7).

The measurement of LPS-induced inhibition of Fc-mediated phagocytosis provides a quantitative and extremely sensitive assay for detecting LPS-induced changes in a major macrophage function. The mechanisms underlying inhibition of phagocytosis and the relationship between the in vitro phenomenon and the effects of LPS on Fc-mediated phagocytosis in vivo still remain to be determined; nevertheless, this assay may prove to be extremely valuable in analyzing the metabolic and biochemical alterations induced by LPS in one of the primary cell types involved in the host response to endotoxin.

ACKNOWLEDGMENTS

We thank Lynda Weedon for her technical assistance, L. M. Wahl for determining the prostaglandin levels in this study, David Morrison and C. Bona for their generous gifts of endotoxin preparations, and Sybil Ceja for her secretarial assistance in preparing this manuscript. We also thank Robert N. Moore and Anita Corman Weinblatt for reviewing this manuscript.

LITERATURE CITED

1. **Auletta, F. J., R. M. Zusman, and B. V. Caldwell.** 1974. Development and standardization of radioimmunoassays for prostaglandins E, F, and A. Clin. Chem **20**: 1580–1587.
2. **Berry, L. J.** 1977. Bacterial Toxins. Crit. Rev. Toxicol. **5**: 239–318.
3. **Freeman, G. G.** 1942. The preparations and properties of a specific polysaccharide from bacteria typhimurium Ty_2. Biochem. J. **36**:340–356.
4. **Glode, L. M., A. Jaques, S. E. Mergenhagen, and D. L. Rosenstreich** 1977. Resistance of macrophages from C3H/HeJ mice to the *in vitro* cytotoxic effects of endotoxin. J. Immunol. **119**:162–166.
5. **Glode, L. M., S. E. Mergenhagen, and D. L. Rosenstreich.** 1976. Significant contribution of spleen cells in mediating the lethal effects of endotoxin in vivo. Infect. Immun. **14**:626–630.
6. **Glode, L. M., I. Scher, B. Osborne, and D. L. Rosenstreich.** 1976. Cellular mechanism of endotoxin unresponsiveness in C3H/HeJ mice. J. Immunol. **116**:454–461.
7. **Haas, G. P., A. G. Johnson, and A. Nowotny.** 1978. Suppression of the immune response in C3H/HeJ mice by protein free lipopolysaccharide. J. Exp. Med. **148**: 1081–1086.
8. **Haskins, W. T., M. Landy, K. C. Milner, and E. Ribi.** 1961. Biological properties of parent endotoxins and lipoid fractions, with study of acid hydrolyzed endotoxin. J. Exp. Med. **114**:665–684.
9. **Heilman, D. H.** 1965. The selective toxicity of endotoxin for phagocytic cells of the reticuloendothelial system. Int. Arch. Allergy **26**:63–79.
10. **Jacobs, D. M., and D. C. Morrison.** 1971. Dissociation between mitogenicity and immunogenicty of TNP-lipopolysaccharide, a T-independent antigen. J. Exp. Med. **141**:1453–1458.
11. **Kessel, R. W. I., and W. Braun.** 1965. Cytotoxicity of endotoxin *in vitro*. Effects of macrophage from normal guinea pigs. Aust. J. Exp. Med. Sci. **43**:511–527.
12. **McIntire, F. C., H. W., Sievert, G. H. Barlow, R. A. Finley, and A. Y. Lee.** 1967. Chemical, physical, and biological properties of a lipopolysaccharide from *Escherichia coli* K-235. Biochemistry **6**:2363–2372.
13. **Moeller, G. R., L. Terry, and R. Snyderman.** 1978. The inflammatory response and resistance to endotoxin in mice. J. Immunol. **120**:116–123.
14. **Moore, R. N., K. J. Goodrum, and L. J. Berry.** 1976. Mediation of an endotoxin effect of macrophages. RES J. Reticuloendothel. Soc. **19**:187–197.
15. **Morrison, D. C., S. J. Betz, and D. M. Jacobs.** 1976. Isolation of a lipid A bound polypeptide responsible for "LPS-initiated" mitogenesis C3H/HeJ spleen cells. J. Exp. Med. **144**:840–846.

16. **Morrision, D. C., and D. M. Jacobs.** 1976. Binding polymyxin B to the lipid A protein of bacterial lipopolysaccharide. Immunochemistry **133**:813–818.
17. **Nowotny, A., U. H. Behling, and H. L. Chang.** 1975. Relation of structure to function in bacterial endotoxins. VII. Biological activities in a polysaccharide-rich fraction. J. Immunol. **115**:199–203.
18. **Peavy, D. L., R. E. Baughn, and D. M. Musher.** 1978. Strain-dependent cytotoxic effects of endotoxin for mouse peritoneal macrophages. Infect. Immun. **21**:310–319.
19. **Razin, E., S. Bauminger, and A. Globerson.** 1978. Effect of prostaglandins on phagocytosis of sheep erythrocytes by mouse peritoneal macrophages. RES J. Reticuloendothel. Soc. **23**:237–242.
20. **Ribi, E., W. T. Haskins, K. C. Milner, R. L. Anacker, D. B. Ritter, G. Good, R.-J. Trapani, and M. Landy.** 1962. Physiochemical changes in endotoxin associated with loss of biological potency. J. Bacteriol. **84**:803–814.
21. **Rifkind, D.** 1967. Prevention by polymyxin B of endotoxin lethality in mice. J. Bacteriol. **93**:1463–1464.
22. **Rosenstreich, D. L., L. M. Glode, L. M. Wahl, A. L. Sandberg, and S. E. Mergenhagen.** 1977. Analysis of the cellular defects of endotoxin-unresponsive C3H/HeJ mice. P. 314–320. *In* D. Schlessinger (ed.), Microbiology—1977. American Society for Microbiology, Washington, D.C.
23. **Rosenstreich, D. L., S. N. Vogel, A. R. Jaques, L. M. Wahl, and J. J. Oppenheim.** 1978. Macrophage sensitivity to endotoxin: genetic control by a single codominant gene. J. Immunol. **121**:1664–1670.
24. **Rosenstreich, D. L., S. N. Vogel, A. Jaques, L. M. Wahl, I. Scher, and S. E. Mergenhagen.** 1978. Differential endotoxin sensitivity of lymphocytes and macrophages from mice with an X-linked defect in B cell maturation. J. Immunol. **121**:685–690.
25. **Shands, J. W., Jr., D. L. Peavy, B. J. Gormus, and J. McGraw.** 1974. In vitro and in vivo effects of endotoxin on mouse peritoneal cells. Infect. Immun. **9**:106–112.
26. **Skidmore, B. J., J. M. Chiller, W. O. Weigle, R. Riblet, and T. Watson.** 1976. Immunologic properties of bacterial lipopolysaccharide (LPS). III Genetic linkage between *in vitro* mitogenic and *in vivo* adjuvant properties of LPS. J. Exp. Med. **143**:143–150.
27. **Snyderman, R., M. C. Pike, D. G. Fischer, and H. S. Koren.** 1977. Biologic and biochemical activities of continuous macrophage cell lines P388D_1 and J774. 1. J. Immunol. **119**:2060–2066.
28. **Vogel, S. N., C. T. Hansen, and D. L. Rosenstreich.** 1979. Characterization of congenitally LPS-resistant, athymic mouse strain. J. Immunol. **122**:619–622.
29. **Wahl, L. M., C. E. Olsen, A. L. Sandberg, and S. E. Mergenhagen.** 1977. Prostaglandin regulation of macrophage collagenase production. Proc. Natl. Acad. Sci. U.S.A. **74**:4955–4958.
30. **Wahl, L. M., D. L. Rosenstreich, L. M. Glode, A. L. Sandberg, and S. E. Mergenhagen.** 1979. Defective prostaglandin synthesis by C3H/HeJ mouse macrophages stimulated with endotoxin preparations. Infect. Immun. **23**:8–13.