INFECTION AND IMMUNITY, Apr. 1987, p. 974–978
0019-9567/87/040974-05$02.00/0

Vol. 55, No. 4

Dephosphorylation of the Lipid A Moiety of *Escherichia coli* Lipopolysaccharide by Mouse Macrophages

ARNOLD A. PETERSON† AND ROBERT S. MUNFORD*

Departments of Internal Medicine and Microbiology, The University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235

Received 15 August 1986/Accepted 7 January 1987

An *Escherichia coli* deep rough lipopolysaccharide (LPS), biosynthetically labeled with $^{32}PO_4$ and [^{3}H]glucosamine, was used to study dephosphorylation of the lipid A moiety by murine macrophages. Over a 48-h incubation period, the macrophages removed approximately two-thirds of the ^{32}P from [$^3H^{32}P$]LPS that was added to the culture medium. The LPS-derived phosphate was incorporated into cell components (e.g., phospholipids), as well as released from the cells. Cell lysates were also able to remove phosphate from [$^3H^{32}P$]LPS. The phosphatase activity was optimal at acidic pH and was greatly reduced by 10 mM sodium fluoride or heating at 80°C. There was no evident difference in the LPS-dephosphorylating ability of macrophages from LPS-responsive and -hyporesponsive mice. The results indicate that murine macrophages dephosphorylate the lipid A moiety of deep rough *E. coli* LPS and raise the possibility that enzymatic dephosphorylation may modify LPS bioactivity.

Lipid A, the bioactive region of gram-negative bacterial lipopolysaccharide (LPS), is a glucosamine disaccharide that is substituted with phosphates and ester- and amide-linked acyl chains. Little is known about the catabolic pathways for LPS or lipid A in eucaryotes. Studies of LPS deacylation have found LPS acyl amidases in slime molds (18), acyloxyacyl hydrolases in human neutrophils (10) and mouse macrophages (MACs) (13), and lipid A-deacylating activity in rats (6, 11). In contrast, dephosphorylation of LPS by eucaryotic cells or serum (19) has not been characterized in detail. Since recent reports (7, 12, 15, 22) indicate that the phosphates attached to lipid A may play an important role in determining the bioactivity of the molecules, we examined the ability of mouse MACs to dephosphorylate the lipid A moiety of LPS.

Enterobacterial LPS has approximately five to seven phosphates esterified to the core polysaccharide and lipid A regions. Some of these phosphates may be further esterified with functional groups (e.g., arabinosamine or ethanolamine). Loss of phosphate from LPS might thus occur differentially, depending on the molecular site. Accordingly, for these studies we used radiolabeled LPS from a deep rough (Re) mutant of *Escherichia coli* K-12 that is known to have unsubstituted phosphates that are attached only at the 1 and 4′ positions of the glucosamine disaccharide backbone (4, 15, 16, 19–21). Our goals were to learn whether thioglycolate-elicited mouse peritoneal MACs were capable of dephosphorylating lipid A, to determine whether phosphate was removed from one or both sites, and to test the hypothesis that the difference between LPS-responsive and -hyporesponsive mice is reflected in the ability of their MACs to dephosphorylate LPS.

MATERIALS AND METHODS

LPS preparation. *E. coli* D21f2 (provided by H. Boman, Umea, Sweden [2]) was grown in low-phosphate medium containing X21 salts (9) as previously described (17) but with 0.4% glucose and supplemented with either 0.5 mCi of *N*-acetyl-1,6-[^{3}H]glucosamine and 2 to 3 mCi of $^{32}P_i$ ([$^3H^{32}P$]LPS; labels in the lipid A backbone and phosphate, respectively) or 17 mCi of [2-^{3}H]acetate and 90 μCi of *N*-acetyl-[1-^{14}C]glucosamine ([$^3H^{14}C$]LPS; labels in acyl chains and lipid A backbone, respectively). All radiochemicals were purchased from New England Nuclear Corp., Boston, Mass. LPS was isolated by the phenol-chloroform-petroleum ether method (8), suspended in 0.1% (vol/vol) triethylamine–1 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), pH 8.2, and stored at −20°C until used. [$^3H^{32}P$]LPS had an activity of 14,000 cpm of ^{3}H and 4,000 cpm of ^{32}P per μg of LPS (preparation 1) or 57,000 cpm of ^{3}H and 140,000 cpm of ^{32}P per μg of LPS (preparation 2), and [$^3H^{14}C$]LPS had an activity of 36,000 dpm of ^{3}H and 1,500 dpm of ^{14}C per μg of LPS. Less than 5% of the labeled LPS was extractable into chloroform (1); after hydrolysis with acid and then base (10), none of the ^{32}P counts per minute and 10% of the ^{3}H counts per minute in [$^3H^{32}P$]LPS were chloroform extractable, as were 24% of the ^{14}C counts per minute and 91% of the ^{3}H counts per minute in [$^3H^{14}C$]LPS.

Macrophages. C3H/HeN mice (LPS responsive) were obtained from Charles River Breeding Laboratories, Wilmington, Mass., and C3H/HeJ mice (LPS hyporesponsive) were obtained from Jackson Laboratory, Bar Harbor, Maine. Peritoneal MACs, elicited with thioglycolate as previously described (13), were plated at 1.4×10^6 MACs per well (11 μg of DNA per well) in a phosphate-based medium (RPMI 1640; KC Biologicals, Lenexa, Kans.) with 1% (vol/vol) fetal bovine serum, 2 mM L-glutamine, 200 U of penicillin G per ml, and 50 μg of streptomycin per ml. MACs were allowed to adhere to the culture wells for 3 h, washed twice to remove nonadherent cells, and then covered with 2 ml of medium containing 1.5 μg of LPS per ml. After the desired incubation period, the culture supernatants were removed and the cells were washed once with 1 ml of Hanks balanced salt solution (KC Biologicals), scraped from the wells in 1 ml of 0.9% NaCl–50 mM EDTA (pH 7), and stored at −20°C. Visual examination of the culture wells before cell harvest indicated a small increase in free-floating cells with the longer LPS

* Corresponding author.
† Present address: Department of Bioengineering, University of Utah, Salt Lake City, UT 84112.

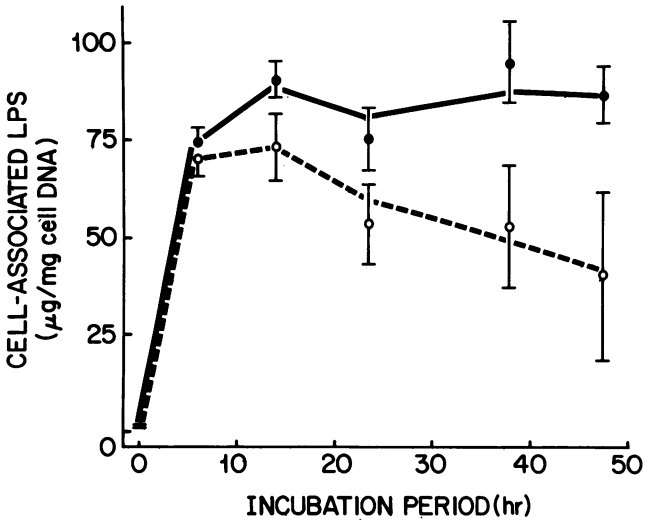

FIG. 1. Uptake of [^{3}H^{32}P]LPS by MACs from LPS-responsive mice. The amount of cell-associated LPS was determined by measuring ^{3}H (closed circles) and ^{32}P (open circles) in washed cells. Values represent the average (± the standard deviation) of two experiments (n = 5). The amount of LPS that bound to plates in the absence of cells was subtracted from each value.

incubations. The cell supernatants were not centrifuged to remove these cells.

Lipid analysis. Lipids were isolated from cell and supernatant fractions by chloroform-methanol extraction (1) in the presence of 0.005% butylated hydroxytoluene. The lipids in the chloroform phase were separated by thin-layer chromatography (TLC) on silica gel G plates (Analtech, Newark, Del.) with $CHCl_3$-CH_3OH-H_2O (90:52.5:12). Unsaturated lipids were visualized by iodine vapors, and radioactive compounds were located by fluorography after spraying with En^{3}Hance (New England Nuclear). Radioactive spots were scraped off the plates, added to 10 ml of scintillation cocktail (Safety-solve; Research Products International, Mount Prospect, Ill.), and counted.

Assays. ^{32}P released from LPS was measured after removal of [^{3}H^{32}P]LPS from solution by activated charcoal (5). Samples were mixed with activated charcoal (40 mg/ml, final concentration; Sigma Chemical Co., St. Louis, Mo.), the charcoal was sedimented by centrifugation, and a sample of the supernatant was counted. The total amount of ^{32}P present was determined from samples to which no charcoal was added. Approximately 90% of $^{32}P_i$ added to control solutions was found in the charcoal supernatant, whereas when [^{3}H^{32}P]LPS was used, less than 3% of the ^{32}P counts per minute and less than 1% of the ^{3}H counts per minute remained in the charcoal supernatant. The amount of LPS-derived phosphate (in picomoles) was determined from the ^{32}P counts per minute per μg of LPS at the time of the assay; the calculation assumed a molecular weight of 2,700 for the LPS and 2.4 phosphates per LPS (mole/mole). Each measurement was performed in triplicate.

To measure phosphatase activity, we incubated 25-μl samples of cell homogenates or cell supernatants with 0.5 μg of [^{3}H^{32}P]LPS in 110 μl (final volume) of 0.04% Triton X-100–70 mM NaCl–80 mM sodium acetate, pH 5.5, at 37°C for 1 h or more. The reaction mixture was then adjusted to 2 mM $MgCl_2$–40 mg of activated charcoal per ml–40 mM sodium citrate (pH 5), vortexed well, centrifuged, and counted as described above.

DNA was measured by the diphenylamine method (3) with calf thymus DNA as a standard.

Radioactivity measurements. Samples were counted in a model 2425 scintillation counter (Packard Instrument Co., Inc., Rockville, Md.). Determination of the ^{3}H radioactivity in samples included corrections for background events, as well as channel spillover (typically, there was about 3% spillover from the ^{32}P to the ^{3}H channels and 39% spillover from the ^{14}C to the ^{3}H channels).

RESULTS

We monitored the course of uptake of radiolabeled LPS into MACs by measuring the glucosamine radiolabel, as glucosamine would presumably be the last component of LPS to be degraded. We then monitored the fate of the LPS phosphates or acyl chains by measuring their respective radiolabels. Uptake of [^{3}H^{32}P]LPS into MACs was studied in two experiments, each done with a different preparation of [^{3}H^{32}P]LPS.

[^{3}H^{32}P]LPS that was incubated with C3H/HeN MACs was taken up by the cells; as measured by uptake of the [^{3}H]glucosamine label, uptake was nearly complete after 8 h and reached a plateau after about 14 h (Fig. 1). The maximum amount of cell-associated LPS was approximately 35% of the amount added to the culture medium. However, the amount of cell-associated ^{32}P increased for the first 14 h and then decreased steadily for the remainder of the incubation period (Fig. 1). The amount of cell-associated [^{3}H]glucosamine was very reproducible between experiments and is probably a good measure of the amount of cell-associated LPS, whereas the magnitude of the decrease in cell-associated ^{32}P after 14 h was variable between experiments and may reflect dephosphorylation of the LPS and release of phosphate from the cells. After 48 h of incubation, the ^{32}P/^{3}H counts per minute ratio in the cell fraction had decreased to only one-half that of the original added LPS, suggesting that approximately 50% of the phosphate had been removed from the cell-associated LPS and released into the medium. A transfer in LPS-derived phosphate from the cells to the medium was also indicated by an increase in the ^{32}P/^{3}H counts per minute ratio in cell supernatants.

Uptake of LPS by MACs was also measured with [^{3}H^{14}C]LPS. Both the [^{3}H]acyl chain label and the [^{14}C]glucosamine label were taken up and found in the cell fraction in nearly identical ratios at all incubation times as was previously reported for an Rc LPS from *Salmonella typhimurium* (12; data not shown).

Uptake of [^{3}H^{32}P]LPS into C3H/HeJ (LPS-hyporesponsive) MACs followed a time course similar to that found for cells from LPS-responsive mice (data not shown). The amount of cell-associated LPS, as indicated by the [^{3}H]glucosamine label, increased to approximately 80 μg of LPS per mg of cell DNA, whereas cell-associated ^{32}P reached a maximum at 14 h of incubation and then decreased in a manner similar to that seen with MACs from LPS-responsive mice.

Throughout the incubation period, there was a steady increase in the amount of [^{3}H^{32}P]LPS-derived phosphate that did not absorb to charcoal (soluble phosphate) (Fig. 2A). Although the soluble ^{32}P-labeled compounds were not identified, the charcoal adsorbed nucleotides, phospholipids, and LPS from solution. The amount of [^{3}H^{32}P]LPS-derived soluble phosphate increased over time from 0 to over 600 pmol of phosphate per culture well in the cell supernatant fractions. After incubation with MACs for 48 h, over 60% of the

total ^{32}P in the cell supernatant fractions did not adsorb to charcoal. In contrast, only 30 to 50 pmol of soluble phosphate per culture well was found in cell fractions throughout the incubation period (Fig. 2B); this accounted for only 7 to 9% of the total ^{32}P in the cells. A small fraction of cell-associated ^{32}P was recovered in phospholipids (see below), indicating that the cells reutilized some of the ^{32}P; the relatively constant amount of cell-associated soluble ^{32}P is probably in the intracellular free-phosphate pool. Similar results were obtained with MACs from C3H/HeJ mice (Fig. 2A and B). When C3H/HeN MACs were incubated with $^{32}P_i$ added to the culture medium, the cells took up approximately 5% of the $^{32}P_i$, suggesting that only a small fraction of the LPS-derived P_i that was released from the cells was reabsorbed.

Lipids were extracted from the cells and cell supernatants with chloroform-methanol and separated by TLC. The amount of cell-associated ^{32}P that was extractable into chloroform increased over time; after incubation for 48 h, 10% of the cell-associated ^{32}P was chloroform soluble. As visualized by exposure of the TLC plates to iodine vapors (to indicate the location of unsaturated lipids), the lipids extracted from MACs that were harvested after different incubation periods with [$^3H^{32}P$]LPS did not change detectably in either quantity or migration pattern, whereas the amount of iodine-detectable lipid in the cell supernatant fractions did increase. Possibly the lipids detected in the supernatants were thus from cells which detached from the culture plates. As visualized by fluorography, the level of radioactivity associated with the extracted lipids, from both the cells and cell supernatants, increased with incubation time (Fig. 3). The two fastest-migrating spots visualized by fluorography comigrated with iodine-stainable MAC lipids and had high levels of ^{32}P, suggesting that phosphate hydrolyzed from LPS was incorporated into these lipids. These two spots comigrated with phosphatidylglycerol and phosphatidylcholine, respectively, and ^{32}P was found in these spots when MACs were incubated with $^{32}P_i$ in the absence of LPS (data not shown). Material that remained near the origin on TLC did not comigrate with iodine-stainable spots and had a $^{32}P/^3H$ ratio (0.8) similar to that of intact [$^3H^{32}P$]LPS, suggesting that this material may be LPS (90% of intact LPS remained at or near the origin in this solvent system). The intermediate-migrating spots visualized by fluorography (Fig. 3, bracket) had very low $^{32}P/^3H$ ratios (0.1) when extracted from MACs that had been incubated with [$^3H^{32}P$]LPS, but when extracted from MACs that were incubated with [$^3H^{14}C$]LPS, these spots had $^3H/^{14}C$ ratios similar to the $^3H/^{14}C$ ratio of intact [$^3H^{14}C$]LPS. Possibly these spots represent partially degraded LPS that has lost phosphate groups but retained the acyl chains.

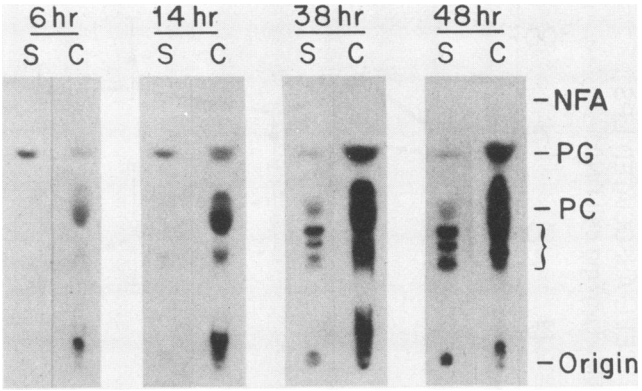

FIG. 3. TLC of extracted MAC lipids. Cells (C) and cell supernatants (S) with which [$^3H^{32}P$]LPS had been incubated for 6, 14, 38, or 48 h were extracted with chloroform-methanol, and the lipids were separated by TLC. Spots were visualized by fluorography; supernatant samples were exposed to the film four times longer than were cell samples. Standards: NFA, normal fatty acids (oleic acid); PG, phosphatidylglycerol; and PC, phosphatidylcholine. The bracket indicates spots that may represent dephosphorylated LPS.

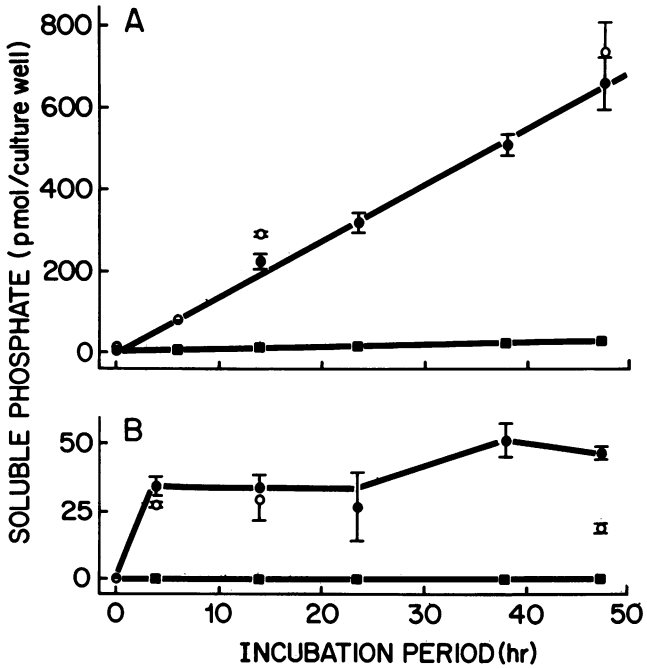

FIG. 2. LPS-derived soluble phosphate after adsorption of [$^3H^{32}P$]LPS to charcoal. After incubation with [$^3H^{32}P$]LPS for various times, cell culture supernatants (A) and Triton X-100-lysed cells (B) were mixed with charcoal and centrifuged. The average amount (± the standard deviation) of ^{32}P in the resulting supernatant was measured. Symbols: ●, C3H/HeN cells; ○, C3H/HeJ cells; ■, wells that contained [$^3H^{32}P$]LPS but no cells (n = 6).

Both Triton X-100-solubilized cell lysates and cell supernatants were assayed for phosphatase activity. Under the conditions used, the cell lysates hydrolyzed approximately 25 pmol of $^{32}P_i$ per μg of DNA per h from [$^3H^{32}P$]LPS (Fig. 4A), regardless of the age of the cell culture. The phosphatase activity in the cell supernatant fractions increased with longer culture periods, yet the maximum level of $^{32}P_i$ hydrolysis from LPS by the supernatants was only about 0.02% of the hydrolysis found for the cell fractions (per culture well). This rate of $^{32}P_i$ hydrolysis from LPS by the supernatant fractions in vitro was insufficient to account for the level of LPS-derived soluble phosphate detected in the cell supernatants. Thus, the LPS-derived soluble phosphate found in the cell supernatant fractions during incubation of cells with [$^3H^{32}P$]LPS (Fig. 2) was probably hydrolyzed by intracellular phosphatases and then released into the medium. Similar phosphatase activities were observed in MACs from LPS-hyporesponsive mice.

Phosphatase activity toward LPS was maximal in the acidic range (pHs 4.5 to 5.5; Fig. 4B). At pH 5.5, the phosphatase activity in cell lysates was inhibited 80% by 10 mM NaF, whereas 10 mM sodium tartrate had no effect.

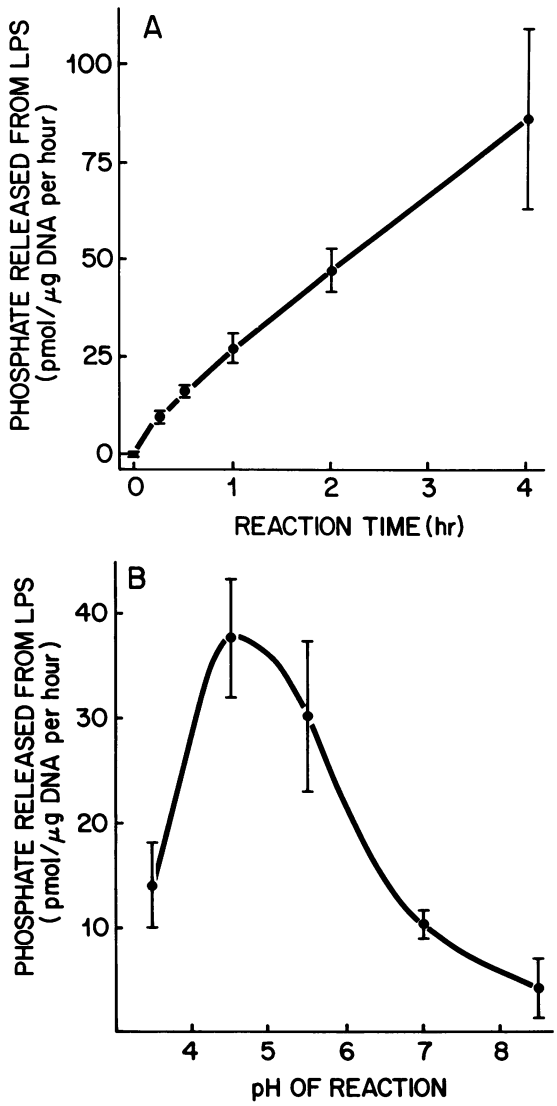

FIG. 4. (A) Time course of release of ^{32}P from [$^{3}H^{32}P$]LPS by cell lysates at pH 5.5. (B) pH curve of the reaction. The buffers contained 0.04% Triton X-100, 70 mM NaCl, and either 80 mM sodium acetate adjusted with HCl to obtain pH values of 5.5 and below, or 80 mM sodium HEPES adjusted with NaOH to pH 7 or 8.5. The amount of ^{32}P released from LPS in the absence of lysate (<5%) was subtracted from the plotted values (means ± one standard deviation; $n = 6$).

Heating the cell lysates at 80°C for 15 min also decreased the phosphatase activity by more than 80%.

DISCUSSION

Previous studies have been shown that murine MACs can enzymatically remove both hydroxylated and nonhydroxylated acyl chains from the lipid A moiety of LPS (13). The present results suggest that enzymes in these cells can also remove phosphates from the lipid A region of LPS.

The conclusion that MACs dephosphorylate cell-associated LPS is supported by (i) the increase over time in $^{32}P/^{3}H$ ratios in cell supernatants and the concomitant decrease in $^{32}P/^{3}H$ ratios in cells, (ii) the separation of the ^{32}P and ^{3}H LPS labels with charcoal adsorption after incubation of [$^{3}H^{32}P$]LPS with cells, and (iii) the phosphatase activity of cell lysates toward [$^{3}H^{32}P$]LPS. After incubation of [$^{3}H^{32}P$]LPS with MACs for 48 h, the ratio of ^{32}P to ^{3}H in the cell fraction had fallen from 0.96 to 0.46, reflecting a loss of approximately 50% of the ^{32}P from the cell-associated LPS to the supernatant. In addition, 9% of the cell-associated ^{32}P did not adsorb to charcoal (soluble phosphate), and another 10% was found in phospholipids. Thus, a minimal estimate is that at least 65 to 70% of the ^{32}P was released from cell-associated [$^{3}H^{32}P$]LPS. Additional ^{32}P was probably released from LPS and incorporated into other cell components such as nucleic acids and proteins (not studied).

Previous studies have indicated that LPS from *E. coli* D21f2 contains an average of 2.4 phosphates per molecule, of which 73% is monophosphate and 27% is pyrophosphate (4). These phosphates are distributed to two sites on LPS; the 4′ position of the glucosamine disaccharide is esterified entirely with monophosphate, and the 1 position is esterified with mono- or pyrophosphate. Loss of 70% of the ^{32}P from [$^{3}H^{32}P$]LPS would represent hydrolysis of an average of 1.7 phosphates per molecule, suggesting that phosphates are removed from both sites, although whether both mono- and pyrophosphates are removed is not known. Removal of all the phosphates from some molecules of LPS is suggested by the intermediate-migrating TLC spots, which contained the glucosamine and acyl chain radiolabels but very little ^{32}P.

After 48 h of incubation, more than 60% of the ^{32}P in each well (cell and cell supernatant combined) did not adsorb to charcoal. This amount of soluble ^{32}P could be produced if 60% of the LPS were cell associated and underwent complete dephosphorylation. The LPS uptake curves, however, indicate that at most only 35% of the added LPS was associated with the cells at any particular time. Thus, it appears that the plateau in the amount of cell-associated LPS represents a steady state between uptake of LPS and export of LPS and LPS degradation products (13).

There was no significant difference detected in the dephosphorylation of [$^{3}H^{32}P$]LPS by cells from either LPS-responsive or -hyporesponsive mice. MACs from both strains took up LPS, cleaved off phosphate, incorporated the phosphate into phospholipids, and released the phosphates and phosphorylated compounds into the overlying medium. In addition, MACs from both strains produced compounds which appeared by TLC analysis to be dephosphorylated LPS metabolites. A previous study found no apparent differences in LPS fatty acid deacylation between LPS-responsive and -hyporesponsive mice (13). Thus, a difference in the deacylation or dephosphorylation of the lipid A moiety of LPS by MACs from these strains does not seem to account for their striking difference in LPS responsiveness.

Purified potato acid phosphatase and bacterial alkaline phosphatase can remove both of the lipid A phosphates from alkali-treated LPS (in which the ester-linked acyl chains have been removed), though they seem to have little or no effect on intact LPS (17; A. Peterson, unpublished data). In our experiments, hydrolysis of phosphate from intact LPS by MAC lysates appeared to be mediated by an acid phosphatase, as removal of ^{32}P from LPS was greatest at acidic pHs, was inhibited by NaF, and was greatly reduced by heating of the cell lysate at 80°C. Within intact MACs or in cell lysates, some of the acyl chains may be removed from LPS before hydrolysis of the phosphates, or possibly the LPS is bound to cellular components in such a way as to expose the phosphates to the phosphatase(s); further studies are required to test these hypotheses. Further studies are also needed to determine whether animal cells can dephosphorylate LPS molecules in which the lipid A phos-

phates are esterified to groups such as ethanolamine. In addition, it should be noted that most gram-negative bacteria produce LPS that have a much larger polysaccharide component than is present in the Re LPS used for these studies. The overall shape of LPS aggregates and the accessibility of the lipid A region to various probes are significantly influenced by the size of the polysaccharide region (4, 14). Thus, dephosphorylation of the lipid A moiety might be affected by the length of the polysaccharide.

We speculate that dephosphorylation may alter the bioactivity of LPS. Lipid A analogs that lack the 1 or 4′ phosphates have reduced toxicity (as measured in the dermal Shwartzman reaction) and pyrogenicity, yet they retain immunostimulating activity (7, 12, 13, 15, 22). In contrast, analogs that lack both phosphates are essentially inactive, at least in part because they are insoluble (12). The present studies indicate that mouse MACs can dephosphorylate the lipid A moiety of *E. coli* LPS and thus raise the possibility that dephosphorylation may play a role in modulating the biological activities of LPS in animals.

ACKNOWLEDGMENTS

We thank Catherine L. Hall and Marva Griffith for their assistance and Donald A. Kennerly for advice.

This work was supported by grant AI18188 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Ames, G. F.** 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli*: structure and metabolism. J. Bacteriol. **95:** 833–843.
2. **Boman, H. G., and D. A. Monner.** 1975. Characterization of lipopolysaccharides from *Escherichia coli* K-12 mutants. J. Bacteriol. **121:**455–464.
3. **Cookson, S. L., and D. O. Adams.** 1978. A simple, sensitive assay for determining DNA in mononuclear phagocytes and other leukocytes. J. Immunol. Methods **23:**169–173.
4. **Coughlin, R. T., A. A. Peterson, A. Haug, H. J. Pownall, and E. J. McGroarty.** 1985. A pH titration study on the ionic bridging within lipopolysaccharide aggregates. Biochim. Biophys. Acta **821:**404–412.
5. **Crane, R. K., and F. Lipmann.** 1953. The effect of arsenate on aerobic phosphorylation. J. Biol. Chem. **201:**235–243.
6. **Freudenberg, M. A., and C. Galanos.** 1985. Alterations in rats *in vivo* of the chemical structures of lipopolysaccharide from *Salmonella abortus equi*. Eur. J. Biochem. **152:**353–359.
7. **Galanos, C., V. Lehmann, O. Lüderitz, E. T. Rietschel, O. Westphal, H. Brade, L. Brade, M. A. Freudenberg, T. Hansen-Hagge, T. Lüderitz, G. McKenzie, U. Schade, W. Strittmatter, K. Tanamoto, U. Zähringer, M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusunoto, and T. Shiba.** 1984. Endotoxic properties of chemically synthesized lipid A part structures. Comparison of synthetic lipid A precursor and synthetic analogues with biosynthetic lipid A precursor and free lipid A. Eur. J. Biochem. **140:**221–227.
8. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. **9:**245–249.
9. **Garen, A., and C. Levinthal.** 1960. A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli*. I. Purification and characterization of alkaline phosphatase. Biochim. Biophys. Acta **38:**470–483.
10. **Hall, C. L., and R. S. Munford.** 1983. Enzymatic deacylation of the lipid A moiety of *Salmonella typhimurium* lipopolysaccharides by human neutrophils. Proc. Natl. Acad. Sci. USA **80:** 6671–6675.
11. **Kleine, B., M. A. Freudenberg, and C. Galanos.** 1985. Excretion of radioactivity in faeces and urine of rats injected with ^{3}H,^{14}C-lipopolysaccharide. Br. J. Exp. Pathol. **66:**303–308.
12. **Kotani, S., H. Takada, M. Tsujimoto, T. Ogawa, I. Takahashi, T. Ikeda, K. Otsuka, H. Shimauchi, N. Kasai, J. Mashimo, S. Nagao, A. Tanaka, S. Tanaka, K. Harada, K. Nagaki, H. Kitamura, T. Shiba, S. Kusumoto, M. Imoto, and H. Yoshimura.** 1985. Synthetic lipid A with endotoxic and related biological activities comparable to those of a natural lipid A from an *Escherichia coli* Re-mutant. Infect. Immun. **49:**225–237.
13. **Munford, R. S., and C. L. Hall.** 1985. Uptake and deacylation of bacterial lipopolysaccharides by macrophages from normal and endotoxin-hyporesponsive mice. Infect. Immun. **48:**464–473.
14. **Peterson, A. A., A. Haug, and E. J. McGroarty.** 1986. Physical properties of short- and long-O-antigen-containing fractions of lipopolysaccharide from *Escherichia coli* 0111:B4. J. Bacteriol. **165:**116–122.
15. **Qureshi, N., and K. Takayama.** 1982. Purification and structural determination of nontoxic lipid A obtained from the lipopolysaccharide of *Salmonella typhimurium*. J. Biol. Chem. **257:** 11808–11815.
16. **Rosner, M. R., H. G. Khorana, and A. C. Satterthwait.** 1979. The structure of lipopolysaccharide from a heptose-less mutant of *Escherichia coli* K-12. II. The application of ^{31}P NMR spectroscopy. J. Biol. Chem. **254:**5918–5925.
17. **Rosner, M. R., J. Tang, I. Barzilay, and H. G. Khorana.** 1979. Structure of the lipopolysaccharide from an *Escherichia coli* heptose-less mutant. I. Chemical degradations and identification of products. J. Biol. Chem. **254:**5906–5917.
18. **Rosner, M. R., R. C. Verret, and H. G. Khorana.** 1979. The structure of lipopolysaccharide from an *Escherichia coli* heptoseless mutant. III. Two fatty acyl amidases from *Dictyostelium discoideum* and their action on lipopolysaccharide derivatives. J. Biol. Chem. **254:**5926–5933.
19. **Rowley, D.** 1956. The fate of ^{32}P-labelled bacterial lipopolysaccharide in laboratory animals. Lancet **i:**366–367.
20. **Strain, S. M., S. W. Fesik, and I. M. Armitage.** 1983. Characterization of lipopolysaccharide from a heptoseless mutant of *Escherichia coli* by carbon 13 nuclear magnetic resonance. J. Biol. Chem. **258:**2906–2910.
21. **Strain, S. M., S. W. Fesik, and I. M. Armitage.** 1983. Structure and metal-binding properties of lipopolysaccharides from heptoseless mutants of *Escherichia coli* studied by ^{13}C and ^{31}P nuclear magnetic resonance. J. Biol. Chem. **258:**13466–13477.
22. **Takayama, K., N. Qureshi, C. R. H. Raetz, E. Ribi, J. Peterson, J. L. Cantrell, F. C. Pearson, J. Wiggins, and A. G. Johnson.** 1984. Influence of fine structure of lipid A on *Limulus* amebocyte lysate clotting and toxic activities. Infect. Immun. **45:**350–355.