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Escherichia coli Lipopolysaccharides Diminish and Enhance Cell Function of Human Polymorphonuclear Leukocytes

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The effects of the lipopolysaccharide (LPS) of *Escherichia coli* J5 and 0111B4 on the function of human polymorphonuclear leukocytes (PMN) were tested. *E. coli* J5 is a UDP-galactose-4-epimerase-deficient mutant of *E. coli* 0111B4, and its LPS, therefore, contains mainly lipid A, as it lacks the polysaccharide side chains. PMN which had been incubated with J5 LPS showed decreased phagocytic, chemotactic, and metabolic activities as compared with control PMN. In contrast, incubation of PMN with 0111B4 LPS had no effect or even an enhancing effect on PMN function. When lipid A and the polysaccharide fraction were isolated from 0111B4 LPS, it was shown that lipid A had the same deleterious effect on PMN function as did J5 LPS and that the LPS fraction had no effect. When PMN were incubated with J5 LPS or lipid A, it could be shown that these structures were able to induce PMN to generate superoxide and chemiluminescence. 0111B4 LPS and the polysaccharide component were able to generate a metabolic burst by the PMN to a lesser extent. The induced defects in PMN function by J5 LPS could be prevented when polymyxin B or an oxygen-radical scavenger was present. We hypothesize that the lipid A portion of LPS is toxic for PMN due to the induction of toxic oxygen species by the PMN. These toxic oxygen species destroy the phagocytic, chemotactic, and metabolic activities of the PMN.

During phagocytosis of opsonized particles by polymorphonuclear leukocytes (PMN), oxygen is consumed, hexose monophosphate shunt activity increases, and hydrogen peroxide and activated toxic oxygen species are produced (for reviews, see references 2 and 24). The metabolic burst is accompanied by the generation of chemiluminescence (1).

The function of PMN can be influenced by a variety of substances. The rate of particle uptake depends on the extent of opsonization of the particles (29). By treating PMN with enzymes (neuraminidase [14] or trypsin [13, 27]), phagocytosis and leukocyte metabolism can be affected. Also, bacterial components can influence phagocytic cell function. Prior incubation with bacterial cell wall peptidoglycan inhibits chemotaxis and phagocytosis of PMN (21). There is considerable information available concerning the effects of endotoxin (lipopolysaccharide [LPS]) on phagocytes (7, 19, 20).

However, the results of various studies on the effects of endotoxin on PMN function are often contradictory. In different studies, PMN from different species and different endotoxin preparations were used. Also, the methods used to measure PMN function in the various studies are often difficult to compare. This prompted us to systematically investigate the effect of LPS preparations on the phagocytic, metabolic, and chemotactic activities of human PMN. LPS was isolated from mutant (containing core glycolipid only) and parent *Escherichia coli* strains. Chemotactic and phagocytic functions of PMN, incubated with the lipid A region of LPS, were diminished, as compared with control PMN, possibly due to the production of toxic oxygen radicals by PMN upon stimulation by the lipid A fraction. In contrast, the polysaccharide fraction of LPS preserved the function of the PMN.

MATERIALS AND METHODS

Bacteria. *E. coli* J5, a UDP-galactose-4-epimerase-deficient mutant (with LPS that contains only core glycolipid), and the parent strain, *E. coli* 0111B4 (with complete LPS), were kindly supplied by B. Lugtenberg (Department of Microbiology and Molecular Biology, University of Utrecht). These bacteria were grown to the late logarithmic phase in yeast broth at 37°C under vigorous aeration and then harvested, washed, and dried for LPS preparations. *Staphylococcus aureus* Ev, a clinical isolate (26), was used for the quantitation of phagocytosis, the induction of superoxide production, oxygen consumption, and chemiluminescence generation by PMN. *S. aureus* was grown by

overnight incubation in a shaker at 37°C in 5 ml of Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.). Desired concentrations were obtained spectrophotometrically after the bacteria had been washed three times and suspended in phosphate-buffered saline. For studies of phagocytosis, bacteria were radiolabeled by growing them in Mueller-Hinton broth to which 0.02 mCi of [^{3}H]thymidine (specific activity, 5 Ci/mmol; Radiochemical Centre, Amersham, England) had been added. Opsonization was achieved by incubating bacteria on a rotator for 30 min at 37°C in a dilution of serum in Hanks balanced salt solution with 0.1% gelatin (Gel-HBSS; GIBCO Bio-Cult Ltd., Paisley, Scotland).

Serum. Serum obtained from the blood of 10 normal, healthy adults was pooled and stored in 1-ml portions at −70°C (human pooled serum [HPS]).

Preparation of *E. coli* cell wall fractions. LPS of *E. coli* 0111B4 was prepared by phenol-water extractions as described by Westphal and Jann (33). Washed and acetone-dried *E. coli* cells were extracted with 45% phenol at 70°C. The water phase containing the LPS was dialyzed against water, purified by ultracentrifugation (100,000 × *g*), and lyophilized.

LPS from *E. coli* J5 was prepared by the phenol-chloroform-petroleum ether method described by Galanos et al. (11). Washed and dried bacteria were extracted with 90% phenol–chloroform–petroleum ether (2:5:8). LPS was precipitated with water, washed twice with 90% phenol and three times with anhydrous ether, suspended in water (60°C), extracted with ether, precipitated with 90% ethanol, and washed again with ether. To test the purity of LPS, the preparations were scanned at 260 nm (for RNA impurities), and ketodeoxyoctonate was measured (31). The protein content of J5 and 0111B4 LPS was less than 5% as determined by the method of Lowry et al. (15).

Lipid A and the polysaccharide part of LPS were prepared from 0111B4 LPS by hydrolysis in 1% acetic acid at 100°C for 4 h (12). The precipitate (lipid A) was washed three times with water, and the supernatant (polysaccharide) was dialyzed against water. Both preparations were lyophilized.

Preparation of leukocyte suspensions. PMN were isolated by a method modified from that of Böyum (6) as described previously (14, 28). Venous blood from healthy donors was drawn in heparinized syringes (10 U of heparin per ml of blood), and leukocytes were isolated by dextran sedimentation of erythrocytes, differential density centrifugation on Ficoll-Hypaque, and NH_4Cl lysis of contaminating erythrocytes. The PMN were sedimented at 160 × *g* for 5 min and washed twice in Gel-HBSS, and total and differential counts were performed. The final leukocyte pellets were adjusted to a concentration of 5×10^6 or 1×10^7 PMN/ml of Gel-HBSS or RPMI 1640 medium (IX) (GIBCO Bio-Cult Ltd.). Contamination of the PMN preparation by monocytes was evaluated by Wright-stained smears and never exceeded 1%.

Incubation of PMN with LPS and LPS derivatives. PMN (10^7) in 1 ml of RPMI were incubated with and without 0111B4 LPS, J5 LPS, polysaccharide, or lipid A in various concentrations (0 to 500 μg/ml). All of the compounds were added to polypropylene biovials (10 by 55 mm; Biovials, Beckman, Instruments, Inc., Chicago, Ill.), mixed, and incubated for various periods of time in a humidified atmosphere containing 5% CO_2 in air at 37°C without shaking. After incubation, the PMN were washed and suspended in Gel-HBSS to a final concentration of 5×10^6/ml for quantitation of phagocytosis; to a concentration of 10^7/ml for the measurement of superoxide production, oxygen consumption, and the generation of chemiluminescence; or in Eagle minimal medium to a concentration of 5×10^7/ml for measurement of chemotactic activity. Incubation of PMN with LPS had no effect on cell viability as determined by trypan blue exclusion (over 90% of the cells remained viable).

Quantitation of phagocytosis. Phagocytosis was studied with [^{3}H]thymidine-radiolabeled *S. aureus* Ev (28). Opsonized (5% HPS) bacterial suspension (0.2 ml) and 0.2 ml of PMN suspension (5×10^6 cells per ml) were incubated at 37°C in biovials for 2, 6, and 12 min. The final ratio of bacteria to leukocytes was 10:1. The phagocytosis reaction was stopped by the addition of 2.5 ml of ice-cold phosphate-buffered saline. The PMN, together with cell-associated *S. aureus*, were separated from unassociated staphylococci by centrifugation at 160 × *g* for 5 min and by washing three times in cold phosphate-buffered saline. The amount of cell-associated radioactivity at each of the three time periods was expressed as the percentage of the total radioactivity added. In previous studies, it was shown that this assay merely measured uptake and that the leukocyte membrane-associated radioactivity was less than 10% of the total leukocyte-associated radioactivity (28).

Superoxide production by PMN. Production of superoxide was assayed by measuring the reduction of ferricytochrome *c* (horse heart type VI; Sigma Chemical Co., St. Louis, Mo.) by using a modification of the method described by Babior et al. (3). The standard reaction mixture contained 0.15 ml of ferricytochrome *c* (1.2 mM), 1.0 ml of 1×10^7 PMN/ml, and 5×10^8 CFU of opsonized (1% HPS) *S. aureus* or 0 to 500 μg of LPS or LPS derivatives in enough Gel-HBSS to make a final volume of 2.15 ml. Paired reactions with and without superoxide dismutase (EC 1.15.1.1; bovine blood type I; Sigma; 200 μg per reaction mixture) were employed. All of the components were added to biovials and shaken at 150 rpm at an angle of 30° in a water bath at 37°C for 30 min. At the end of the experiment, the biovials were centrifuged at 4°C and 160 × *g* for 10 min, the supernatant fractions were removed and centrifuged at 1,600 × *g* for 15 min, and the difference in absorbance of the supernatant solutions was determined at 550 nm in a double-beam spectrophotometer (Perkin-Elmer model 124; Charles Goffin Instruments, de Bilt, The Netherlands). The number of nanomoles of cytochrome *c* reduced was determined from the increase in the absorbance at 550 nm by using the extinction coefficient $E_{550} = 2.10 \times 10^4\ M^{-1}\ cm^{-1}$.

Oxygen consumption by PMN. Oxygen consumption was measured with a Clark electrode (model YSI 5331; Yellow Springs Instrument Co., Yellow Springs, Ohio) as described by Weening et al. (30). PMN (5×10^6) were stimulated with 250 μg of J5 or 0111B4 LPS in a final volume of 1 ml of Gel-HBSS at 37°C.

Generation of chemiluminescence by PMN. Chemiluminescence was measured in a liquid scintillation counter (Mark II; Nuclear Chicago, Chicago, Ill.) in the out-of-coincidence mode, adjusted as previously described (1, 25). Briefly, PMN (5×10^6) were incu-

bated with 2.5×10^8 CFU of *S. aureus* (opsonized in 1% HPS) or with 0 to 250 μg of J5 or 0111B4 LPS and with 0.1 ml of a 0.3 μM luminol solution (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma) in a total volume of 1 ml of Gel-HBSS. For measuring resting values, no bacteria or LPS was added to the PMN, and these values never exceeded 5,000 cpm. The vials were placed inside glass vials in a liquid scintillation counter. Chemiluminescence was determined for 0.1 min every 60 s over a 20-min period. Between measurements, the biovials were incubated and shaken at 37°C.

Chemotactic activity of PMN. Chemotaxis of PMN under agarose was determined by the method of Nelson et al. (22). Leukocyte suspension (7 μl; 5×10^7 PMN/ml) was placed in one well (diameter, 2.4 mm) that had been cut into agarose; another well (diameter, 2.7 mm) contained 7 μl of zymosan-activated human serum. The plates were incubated at 37°C in a humidified atmosphere of 5% CO_2–95% air for 18 h. Migration distance was measured microscopically, in millimeters, and directed migration was calculated as migration toward zymosan-activated serum minus spontaneous migration in the opposite direction.

Statistical analysis. Results are expressed as the mean of three or more independent observations ± standard deviation. For significance analysis, Student's *t* test was performed. *P* values exceeding 0.05 were considered not significant.

RESULTS

Effects of LPS on phagocytosis of *S. aureus*. PMN were incubated with various concentrations of LPS (0 to 500 μg/ml) for various durations of time (0 to 18 h). Although LPS was shown to have an effect on PMN function within 2 h of incubation, results were most pronounced when PMN were incubated overnight with LPS. Therefore, results of 18-h incubations are shown. In Fig. 1, the results are given for uptake of *S. aureus* opsonized in 5% HPS by PMN (10^7/ml) incubated with J5 or 0111B4 LPS. Control PMN phagocytized 75% of the bacteria, whereas PMN incubated with 31 or 125 μg of J5 LPS per ml phagocytized 65 and 47% of the bacteria, respectively. In constrast, when PMN were incubated overnight with 0111B4 LPS, an increase in the uptake of bacteria was observed. Control PMN took up 75% of the bacteria, and PMN incubated with 31 μg of 0111B4 LPS per ml took up 90% of the bacteria. Similar differences were observed when PMN, incubated with LPS, were stimulated with staphylococci opsonized in serum from a patient with agammaglobulinemia or in hyperimmune serum. This indicates that the effect of LPS on PMN phagocytosis was not specific for one of the membrane receptors (complement or Fc receptors; data not shown).

Effects of LPS on chemotactic activity. The effect of various concentrations of J5 and 0111B4 LPS on PMN chemotactic activity was measured. After 2 h of incubation with 250 μg of the LPS preparations, clear differences between

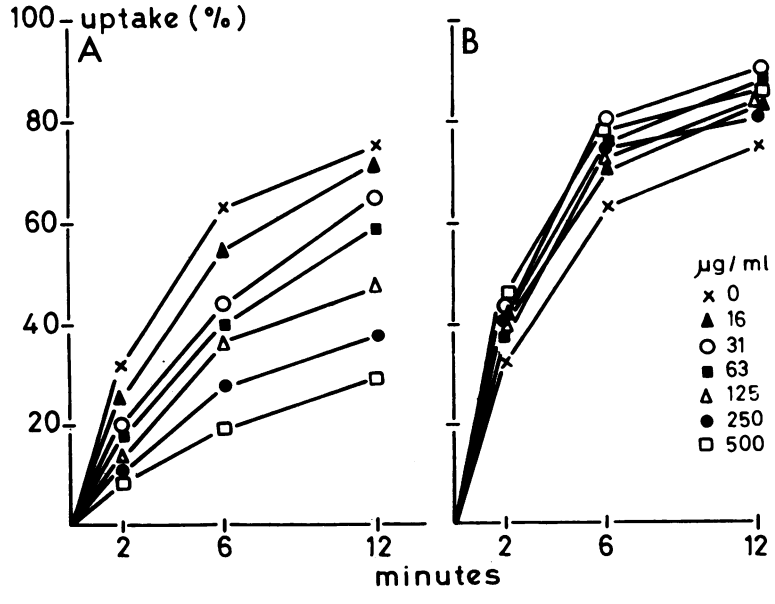

FIG. 1. Phagocytosis of *S. aureus*, opsonized in 5% HPS by PMN incubated with J5 LPS (0 to 500 μg/ml) (A) or 0111B4 LPS (0 to 500 μg/ml) (B) for 18 h at 37°C. PMN (10^6) were mixed with 10^7 CFU of opsonized radiolabeled bacteria and incubated for 2, 6, and 12 min at 37°C. Unassociated staphylococci were removed by washing and centrifugation (160 × *g* for 5 min). The uptake of *S. aureus* was calculated from the amount of cell-associated radioactivity.

TABLE 1. Chemotaxis of PMN incubated with J5 and 0111B4 LPS

LPS	Migration toward attractant (mm) after an incubation of[a]:	
	18 h	2 h
Control	0.17 ± 0.12	0.86 ± 0.11
0111B4	0.16 ± 0.09	0.64 ± 0.09
J5	0.00[b]	0.11 ± 0.10[b]

[a] Chemotaxis of PMN was performed under agarose toward zymosan-activated serum at 37°C for 18 h in a humidified atmosphere of 5% CO_2–95% air. Migration distance was measured microscopically in millimeters, and directed migration was calculated as migration toward zymosan-activated serum minus spontaneous migration.

[b] $P < 0.001$ compared with control or 0111B4 LPS values.

the chemotactic activity of neutrophils incubated with J5 LPS and cells incubated with 0111B4 LPS were seen (Table 1). Cells which had been incubated with J5 LPS migrated only over a small distance, whereas control PMN or cells incubated with 0111B4 LPS still could migrate over a larger distance. Although less pronounced, similar results were seen when PMN were incubated with 16 to 125 μg of LPS per ml (data not shown).

Effects of LPS on neutrophil superoxide production upon stimulation. PMN were incubated with J5 and 0111B4 LPS. After 18 h, these PMN were washed and stimulated by *S. aureus*, and the amount of superoxide produced was measured. A dose-dependent effect of J5 LPS on the production of superoxide was observed (Fig. 2A). In contrast, superoxide production by 0111B4 LPS-incubated PMN upon stimulation with bacteria was enhanced.

Direct effects of LPS on PMN superoxide production. When PMN were incubated with LPS and the production of superoxide by these cells was measured directly, without stimulation by bacteria, superoxide production could be detected (Fig. 2B). The amount of superoxide produced varied directly from .7 to 17 nmol of superoxide per 10^7 PMN per 30 min when stimulated with 4 to 500 μg of J5 LPS per ml, as compared with 3 nmol of superoxide per 10^7 PMN per 30 min by control cells. 0111B4 LPS stimulated PMN to produce superoxide marginally. Incubation of PMN with J5 LPS (250 μg/ml) also resulted in oxygen consumption by the PMN (7.1 ± 0.4 nmol of O_2 per 10^6 PMN per 10 min). Leukocytes incubated with 0111B4 LPS consumed only 4.9 ± 0.3 nmol of O_2 per 10^6 PMN per 10 min.

Effects of LPS on neutrophil chemiluminescence response upon stimulation. When PMN had been incubated with J5 LPS (500 μg/ml) for 18 h, their ability to generate chemiluminescence upon stimulation with opsonized staphylococci (1% HPS) was clearly reduced when compared with control cells (Fig. 3A). The generation of chemiluminescence by PMN preincubated with 0111B4 LPS (500 μg/ml) was less affected. In the first 10 min, the amount of chemiluminescence generated was the same as the control PMN. After this time period, the generation of chemiluminescence declined 25% compared with con-

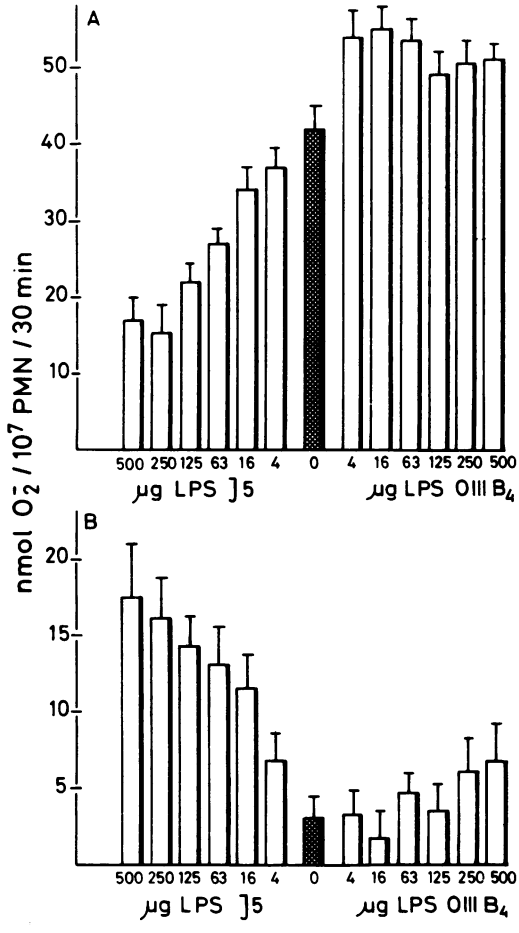

FIG. 2. (A) Production of superoxide by control PMN (▨) and J5 LPS (4 to 500 μg/ml) or 0111B4 LPS (4 to 500 μg/ml) incubated by PMN upon stimulation with opsonized *S. aureus*. (B) Production of superoxide by PMN directly stimulated with J5 LPS (4 to 500 μg/ml) and 0111B4 LPS (4 to 500 μg/ml). PMN (10^7) were mixed with 5×10^8 CFU of opsonized staphylococci (A) or 4 to 500 μg of LPS (B) and 0.18 mmol of ferricytochrome *c* in a total volume of 2.15 ml of Gel-HBSS. The incubation took place at 37°C for 30 min, and after removal of the PMN and bacteria by centrifugation, the amount of superoxide produced was determined by measuring the absorbance of the reaction mixture at 550 nm.

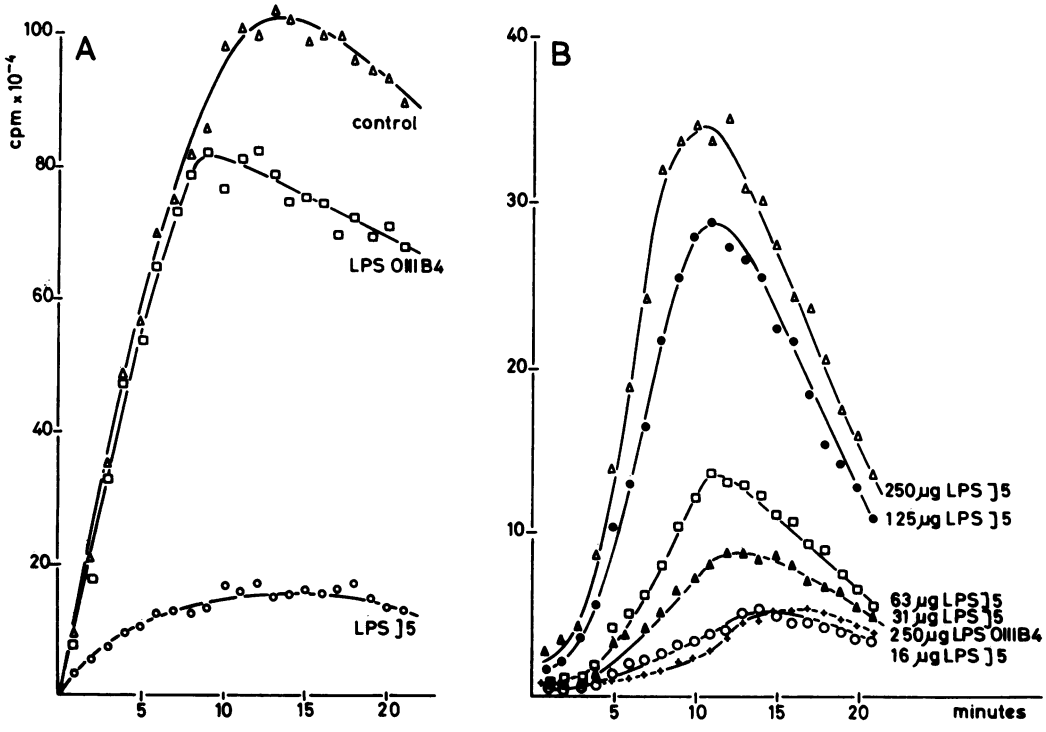

FIG. 3. (A) Generation of chemiluminescence by PMN incubated for 18 h with J5 LPS, 0111B4 LPS, or without LPS upon stimulation with opsonized staphylococci. (B) Generation of chemiluminescence by PMN directly stimulated with LPS. PMN (5×10^6) were incubated with 2.5×10^8 CFU of *S. aureus* (A) or 16 to 250 µg of LPS (B) in the presence of 0.03 µM luminol in a total volume of 1 ml of Gel-HBSS. The generation of chemiluminescence was measured for 0.1 min every 60 s over a 20-min period in a liquid scintillation counter in the out-of-coincidence mode.

trol PMN. Although, less pronounced, similar effects were observed when PMN were incubated with lower concentrations of LPS.

Direct effects of LPS on PMN chemiluminescence. J5 LPS was not only able to induce generation of superoxide by PMN directly, but also, when PMN were incubated with J5 LPS, generation of chemiluminescence could be detected (Fig. 3B). The amount of chemiluminescence varied from 5×10^4 to 35×10^4 cpm, depending on the amount of J5 LPS used. Again, 0111B4 LPS was able to generate chemiluminescence to a lesser extent.

Superoxide production by PMN incubated with lipid A and polysaccharide. The differences between results obtained with PMN incubated with J5 and 0111B4 LPS could be explained by the differences in molecular structure of the preparations. LPS J5 contains mainly lipid A, whereas 0111B4 LPS contains large polysaccharide side chains. Therefore, the effects of lipid A and the polysaccharide subfractions on PMN function were studied. PMN were incubated for 18 h with the separated fractions of 0111B4 LPS: the lipid A fraction and the polysaccharide fraction. PMN incubated with lipid A produced less superoxide after stimulation with bacteria compared with control PMN, and cells incubated with the polysaccharide portion of LPS produced more superoxide than did the control cells (Table 2). The dose-response effect on superoxide production by PMN which had been incubated with several amounts of lipid A was similar to the effects of J5 LPS on superoxide production by PMN. The hypothesis that the differences in the effects of J5 and 0111B4 LPS were caused by the differences in molecular structure was confirmed by the incubation of J5 LPS with polymyxin B, a cationic antibiotic that binds specifically to the lipid A region of LPS (5, 8, 18). By preincubating J5 LPS with an equivalent amount of polymyxin B, no decreased superoxide production by PMN could be caused by this LPS preparation (Table 2). Similar results were observed when the phagocytosis of bacteria was measured (data not shown).

When PMN were incubated with lipid A or the polysaccharide fraction and the production of superoxide was measured directly, it could be shown that lipid A induced more superoxide

TABLE 2. Effect of LPS components (250 μg/ml) and polymyxin B (250 μg/ml) on superoxide production by PMN

Component	nmol/10^7 PMN per 30 min[a]
Control	41.4 ± 2.0
Lipid A	19.7 ± 2.0[b]
Polysaccharide	45.9 ± 2.2[c]
J5 LPS	15.5 ± 3.5[b]
J5 LPS + polymyxin B	37.3 ± 2.2
Polymyxin B	41.2 ± 2.8

[a] PMN (10^7) were incubated with 5 × 10^8 CFU of opsonized *S. aureus* in the presence of 0.18 mmol of ferricytochrome *c* for 30 min at 37°C. After removal of PMN and bacteria by centrifugation, the amount of superoxide produced was determined from the increase in absorbance at 550 nm.

[b] $P < 0.001$.

[c] $P < 0.005$ compared with control value.

production than did the polysaccharide fraction (17.6 ± 5.2 versus 8.4 ± 2.9 nmol of superoxide per 10^7 PMN per 30 min; $P < 0.025$).

Effects of J5 LPS, in the presence of thiourea, on neutrophil superoxide production. It is possible that during incubation of PMN with J5 LPS, toxic oxygen species are produced (as measured by the enhanced superoxide production and chemiluminescence) through the interaction between PMN and lipid A. These oxygen species could be responsible for membrane lipid peroxidation of the PMN (4), thus affecting PMN function. If this assumption is true, oxygen-radical scavengers would prevent a J5 LPS-induced decrease in phagocytic cell function. Therefore, PMN were incubated with J5 LPS (500 μg/ml) together with the scavenger thiourea (0 to 100 mM). After 18 h of incubation, the rate of phagocytosis and the production of superoxide by the PMN upon stimulation by *S. aureus* were measured (Fig. 4). Cells incubated with J5 LPS produced only 48 ± 6% of the superoxide produced by control PMN ($P < 0.001$). When thiourea was present during the 18-h incubation with J5 LPS, the ability of the leukocytes to produce superoxide was similar to that of control PMN (89 ± 8% at 50 mM thiourea and 99 ± 5% at 100 mM thiourea). Also, phagocytosis of *S. aureus* by PMN incubated for 18 h with J5 LPS in the presence of thiourea was similar to that of control cells incubated with RPMI and thiourea only (data not shown). The amount of thiourea used had no effect on the production of superoxide and phagocytosis by the control PMN. Similar results were found when mannitol was used.

DISCUSSION

When PMN were incubated with LPS isolated from two different strains, *E. coli* 0111B4 and *E. coli* J5, a UDP-galactose-4-epimerase-deficient mutant of *E. coli* 0111B4, different effects could be measured. The phagocytic, chemotactic, and metabolic activities of PMN incubated with J5 LPS were diminished compared with control leukocytes. In contrast, incubation with 0111B4 LPS had no, or even an enhancing, effect on PMN function. The differences between the effects induced by J5 and 0111B4 LPS could be explained by their differences in molecular structure. J5 LPS contains mainly lipid A without polysaccharide side chains, whereas 0111B4 LPS is rich in polysaccharides. When lipid A was isolated from 0111B4 LPS and PMN were incubated with this fraction, similar effects on phagocytosis, production of superoxide, and chemiluminescence upon stimulation with *S. aureus*, as compared with J5 LPS, were observed. The effect of J5 LPS on PMN function could be neutralized by polymyxin B. Polymyxin B is a cationic antibiotic which binds to the lipid A fraction of LPS (5, 8, 18). Also, the polysaccharide fraction of 0111B4 LPS had no effect on PMN function. Therefore, we concluded that the effect of J5 LPS on PMN function was due to its large lipid A fraction. The de-

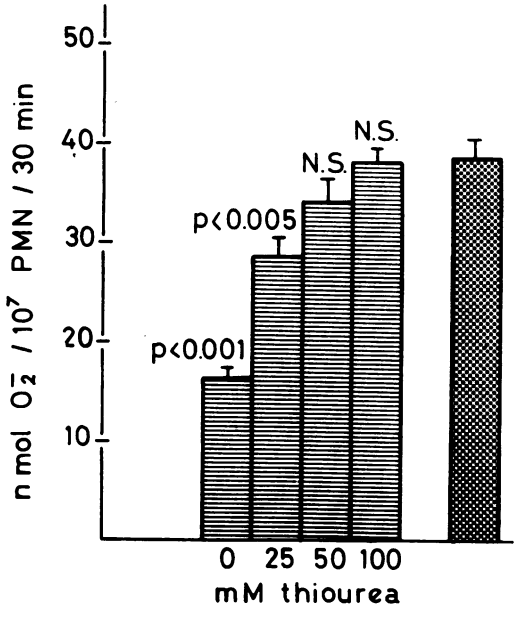

FIG. 4. Production of superoxide upon stimulation by opsonized staphylococci, by control PMN (▥), and by PMN incubated for 18 h with J5 LPS (▤) in the presence of 0, 25, 50, and 100 mM thiourea. PMN (10^7) were incubated with 5 × 10^8 CFU of opsonized *S. aureus* in the presence of 0.18 mmol of ferricytochrome *c* for 30 min at 37°C. PMN and bacteria were removed by centrifugation, and the amount of superoxide produced was determined from the increase in absorbance at 550 nm. N.S., Not significant.

crease of phagocytic cell function found by Proctor (23) and Davis et al. (9) was also mediated by the lipid A moiety of LPS. The lipid A portion of the LPS molecule consists of a 1-6 linked diglucosamine backbone containing both ester- and amide-linked long-chain fatty acids, as well as PP_i groups (16). This amphipatic character of the lipid A molecule could be responsible for an interaction between the LPS molecule and the cell surface of the PMN. The lipid A component is critical to most endotoxin activities (for reviews, see references 16, 20, and 32).

PMN were not only incubated with LPS and then stimulated with *S. aureus*, but also direct stimulation of PMN by LPS was measured. These experiments showed that PMN were able to produce superoxide, generate chemiluminescence, and consume oxygen when directly stimulated with LPS or LPS derivatives. J5 LPS and lipid A stimulated the leukocytes to a much greater extent than did 0111B4 LPS and the polysaccharide fraction. This led us to hypothesize that the oxygen species which are produced during direct stimulation of PMN by LPS are responsible for the observed defects in phagocytic cell function. It has been described that superoxide, hydroxyl radicals, and singlet oxygen are involved in the process of lipid peroxidation (4). The hydroxyl radical is capable of initiating lipid peroxidation by its ability to abstract hydrogen atoms from the allylic position of unsaturated lipids to yield hydroperoxides. Lipid peroxidation has been shown to result in severe damage to cellular membranes and organelles and their associated enzymes (4). This hypothesis was examined by experiments with thiourea that trapped toxic oxygen species (10) and largely prevented the effect of J5 LPS on PMN cell function.

The finding that 0111B4 LPS or the polysaccharide portion of the LPS was not deleterious to PMN function is in agreement with this hypothesis, because these preparations only induced production of oxygen species by PMN marginally, as measured by the production of superoxide and chemiluminescence. It is therefore possible that during incubation of PMN with 0111B4 LPS, no lipid peroxidation occurs. Since PMN could be stimulated in the presence of the polysaccharide portion of LPS by, e.g., phorbol myristate acetate or formylmethionyl-leucyl phenylalanine (data not shown), it seems unlikely that the polysaccharide portion of LPS just acts as a scavenger of oxygen radicals. However, it is possible that 0111B4 LPS is capable of capturing certain radicals. Further experiments, especially with cells from, e.g., patients with chronic granulomatous disease, are needed to prove unequivocally that the decrease in PMN function upon incubation with LPS is due to toxic oxygen species. PMN from patients with chronic granulomatous disease are unable to generate a burst in oxygen consumption and to produce oxygen metabolites (17, 25). Therefore, it would be of interest to study the effect of J5 LPS on these cells.

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