INFECTION AND IMMUNITY, Nov. 1996, p. 4638–4642 Vol. 64, No. 11
0019-9567/96/$04.00+0

Lipopolysaccharide Binding Proteins on Polymorphonuclear Leukocytes: Comparison of Adult and Neonatal Cells

GEFEI QING,[1,2] SUSAN HOWLETT,[3] AND ROBERT BORTOLUSSI[1,2]*

Departments of Pediatrics,[1] Pharmacology,[3] and Microbiology and Immunology,[2] Dalhousie University, Halifax, Nova Scotia, Canada

Received 15 April 1996/Returned for modification 6 June 1996/Accepted 12 August 1996

We have previously shown that polymorphonuclear leukocytes (PMN) from cord blood of normal full-term infants have a decreased priming response to lipopolysaccharide (LPS) compared with PMN of adults. Because the reason for this difference is poorly understood, we compared LPS binding on PMN from adults and newborns by using a photoactivatable iodinated LPS (from *Escherichia coli* O111:B4), coupled to 2-(*p*-azidosalicylamido)-1,3′-dithopropionate (LPS-ASD) to covalently link LPS to the PMN membrane. We incubated 2×10^4 adult or neonatal PMN with ^{125}I-ASD-LPS (100 ng/ml) together with unlabelled LPS (0 to 100,000 ng/ml) for 20 min at 4°C. The maximum total ^{125}I-ASD-LPS binding to newborn PMN (1,004 ± 103 cpm) was lower than that binding to adult PMN (3,583 ± 444 cpm; $P < 0.01$ with respect to newborn PMN). However, the concentration of unlabelled LPS that displaced 50% of the maximum specifically bound ^{125}I-ASD-LPS was similar for PMN from adult and newborn infants (-4.85 ± 0.04 and -5.13 ± 0.14 log g of LPS per ml, respectively; $P > 0.05$). We further assessed the membrane binding of ^{125}I-ASD-LPS to PMN by using membrane extracts analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. LPS binding proteins were found at approximately 73, 55 to 57, and 25 kDa in both adult and neonatal PMN. However, PMN from newborn infants had markedly lower membrane-associated ^{125}I-ASD-LPS at the 55- to 57- and 25-kDa protein bands as indicated by the intensity of the autoradiograph. Binding of LPS at these bands was specific for the lipid A portion of LPS, since purified unlabelled lipid A displaced ^{125}I-ASD-LPS in a dose-dependent manner. Thus, PMN from newborn infants bind less LPS than do PMN from adults, even though the sites for LPS membrane binding appear to be the same.

Phagocytic cells such as polymorphonuclear leukocytes (PMN) and monocytes play a key role in host defense against bacterial infection. Phagocytic function can be modulated by cytokines as well as by bacterial products produced during infection. For example, following exposure to lipopolysaccharide (LPS) in vitro, PMN develop an increased ability to produce oxidative radicals which can damage and kill bacteria (1). This process has been called priming (10). PMN obtained from adults with bacterial infection produce increased amounts of oxidative radicals when stimulated with bacterial formyl-methionyl-leucyl-phenylalanine compared with PMN from noninfected adults (2, 18). In contrast to adult cells, PMN obtained from newborn infants with bacterial infection have decreased bactericidal activity and oxidative radical production compared with noninfected newborns (23, 33). We have shown that PMN from neonates are primed less effectively in vitro with LPS than are PMN from adults, a factor that we believe may contribute to the increased susceptibility of neonates to bacterial infection (4).

Ulevitch and Tobias have identified a plasma protein, LPS binding protein, that interacts with the cell receptor CD14 when bound to LPS to enhance phagocytic cell activity (24, 25, 27). However, LPS can interact with cells via at least two pathways, one dependent and the other independent of plasma or serum proteins (3, 7, 8, 13, 17, 26, 28, 30). The two pathways of LPS activation may serve different purposes for the PMN. Plasma-dependent LPS activation may be the predominant pathway for PMN in the bloodstream, where plasma proteins are in high concentration and LPS is in low concentration during sepsis. In contrast, plasma-independent pathways may be important at the local site of infection, where plasma protein concentrations are low and the LPS concentration is high. Understanding both pathways of cellular activation by LPS will be important to understand the host response to gram-negative infection.

The underlying mechanism for the lower response of neonatal PMN to LPS and the role of plasma in mediating this response are unknown. In this study, we examined the possibility that LPS receptors on adult and neonatal PMN differ qualitatively or quantitatively. Halling et al. have identified several cell membrane LPS binding proteins on adult human lymphocytes, monocytes, and PMN by using a photoactivatable iodinated LPS coupled to 2-(*p*-azidosalicylamido)-1,3′-dithiopropionate (^{125}I-ASD-LPS) (11). We used this technique to compare LPS binding to adult and neonatal PMN in the presence and absence of plasma. We found marked differences in the amount of LPS binding to adult and neonatal PMN membranes, with neonatal PMN membranes having decreased total and specific LPS binding sites. By using membrane extracts analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography, the predominant LPS-binding proteins were seen at approximately 73, 55 to 57, and 25 kDa for both adult and neonatal PMN. The 55- to 57- and 25-kDa LPS receptors were prominently expressed on adult but not on neonatal PMN.

MATERIALS AND METHODS

Reagents. *Escherichia coli* LPS (serotype O111:B4) was purchased from List Biological Labs (Campbell, Calif.). Sulfosuccinimidyl-2-(*p*-azidosalicylamido)-1,3′-dithiopropionate (SASD) and Iodo-Beads were bought from Pierce Chemical Co. (Rockford, Ill.). SDS-PAGE reagents, molecular weight standards, and the Affi-Gel Blue column (Econo-Pac blue cartridge) were purchased from

* Corresponding author. Mailing address: Izaak Walton Killam and Grace Health Center, 5850 University Ave., Halifax, Nova Scotia, Canada B3J 3G9. Phone: (902) 428-8498. Fax: (902) 428-3217. Electronic mail address: BBORTOLUSSI@IWKGRACE.NS.CA.

Bio-Rad Laboratories (Mississauga, Ontario, Canada). Sterile water (no preservative, nonpyrogenic) was obtained from Abbott Laboratories (Montreal, Quebec, Canada). Hanks' balanced salt solution was obtained from GIBCO Laboratories (Burlington, Ontario, Canada). Percoll was purchased from Pharmacia Chemical Co. (Montreal, Quebec, Canada). Iodine-125 was purchased from Amersham (Oakville, Ontario, Canada). Dialysis bags, borax (sodium tetraborate), boric acid, bovine serum albumin (BSA), E-Toxate kits, and other chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). Buffers and plasma were checked for endotoxin by a *Limulus* amebocyte lysate assay to ensure that LPS was not detectable (<12 pg/ml).

Radiolabeling LPS. We conjugated SASD to LPS from *E. coli* O111:B4 by the method described by Wollenweber and Morrison (32). Briefly, LPS was reconstituted with sterile pyrogen-free water at a concentration of 2 mg/ml, using sonication to ensure an even distribution of LPS in water. Of this solution, 500 μl of LPS (1 mg) was added to SASD (0.4 mg), followed immediately by 500 μl of 0.1 M borate buffer (pH 8.5). The reaction mixture was kept at room temperature for 30 min, sonicated for 30 s every 10 min, and subjected to an additional incubation with fresh SASD (0.4 mg) under the conditions described above. LPS-ASD was freed from excess reagent by centrifugation at 2,000 × *g* for 2 min. The clear supernatant (LPS-ASD) was dialyzed against phosphate-buffered saline (PBS; pH 7.2) overnight at 4°C in the dark (minimum of six changes). LPS-ASD was aliquoted and stored at −70°C.

Two Iodo-Beads were washed, dried with filter paper, added to 10 μl (1 mCi) of ^{125}I solution, diluted with 200 μl of 50 mM PBS (pH 6.5), and left at room temperature for 5 min. LPS-ASD (100 μl) was added to preloaded beads and incubated at room temperature for 15 min. Radioactive samples were dialyzed against 50 mM PBS (pH 7.4) in the dark until less than 0.5% of the total radioactivity could be measured in the outer dialysate. Radiolabeled LPS-ASD was aliquoted and stored at −70°C. The specific activity of ^{125}I-ASD-LPS was assessed by the *Limulus* amoebocyte lysate test with purified LPS as the standard. The ^{125}I-ASD-LPS we used had an approximate specific activity of 5.2 μCi/μg. All reactions with photosensitive compounds were carried out under reduced light conditions with a 25-W red light source.

PMN preparations. Fresh peripheral venous blood from healthy adult volunteers and umbilical cord blood from normal full-term neonates were collected under sterile conditions and anticoagulated with heparin (10 U/ml). PMN were isolated from blood by a Percoll gradient technique described previously (19). Cells were routinely purified to >90% purity and >98% viability in approximately 2 h and were assayed immediately afterward. All experiments were completed within 6 h of obtaining the blood. Contaminating erythrocytes were removed by brief hypotonic lysis with 0.15 M NH_4Cl. The cells were then washed and resuspended in Hanks balanced salt solution.

Preparation of albumin-depleted plasma. Albumin-depleted plasma was made from pooled adult plasma with Affi-Gel Blue cartridges as specified by the manufacturer. The albumin concentration in plasma was decreased by more than 99.9% by this process, from 39 to 0.01 g/liter. After passage, albumin was the only protein whose concentration was substantially decreased by this method as indicated by SDS-PAGE (data not shown).

LPS binding assay with PMN from adults or neonates. Adult or neonatal PMN (2×10^4 cells suspended in 100 μl of Hanks balanced salt solution) were incubated in duplicate samples with ^{125}I-ASD-LPS (100 ng/ml) in an ice-water bath for 20 min in the presence of unlabelled LPS (0 to 100,000 ng/ml). Plasma or serum was not present in the mixture. After incubation with ^{125}I-ASD-LPS, cells were exposed to UV light (UV cross-linker; wavelength, 254 nm; FB-UVXL-1000; Fisher Scientific) for 10 min. The cells were then washed with PBS, and radioactivity in the cell pellets was assessed. Background counts (those in tubes without cells) were subtracted from all test samples.

Cross-linking of ^{125}I-ASD-LPS to PMN membrane proteins. Covalent linking of LPS to PMN membrane proteins was done as described by Halling et al. (11) by incubating PMN (2×10^6 cells suspended in 40 μl) with 1 μg of ^{125}I-ASD-LPS at 37°C for 30 min. We found, as they had, that LPS bound less intensely to the PMN membrane in experiments conducted at 4°C than in identical experiments at 37°C. Unless otherwise stated, plasma or serum was not added to the reaction mixtures. Cells were exposed to UV light for 10 min and then washed with Hanks balanced salt solution and solubilized in 0.5% Triton X-100 with 0.5 M mannitol, 5 mM $CaCl_2$, 0.02% sodium azide, and 10 μM phenylmethylsulfonyl fluoride. After 30 min on ice, the mixture was sedimented by microcentrifugation and the supernatant with solubilized cell membranes was collected and stored at −70°C until analyzed. In some experiments, a 10- or 50-fold excess of unlabelled LPS, O111:B4, J5, or lipid A was added to PMN at the same time as ^{125}I-ASD-LPS to determine whether these compounds could displace ^{125}I-ASD-LPS.

To determine the effect of plasma and albumin on ^{125}I-ASD-LPS binding to PMN, some experiments were done as described above except for the addition of whole plasma, BSA, or albumin-depleted plasma during the incubation of PMN with ^{125}I-ASD-LPS.

SDS-PAGE and autoradiography. The solubilized cell samples were subjected to SDS-PAGE (11% polyacrylamide). The gels were stained with Coomassie blue G-250 in 40% methanol–10% acetic acid and destained with 40% methanol–10% acetic acid. Autoradiographs were prepared with X-Omat AR film and cassettes with X-Omatic intensifying screens (Eastman-Kodak, Rochester, N.Y.). The radiographs were exposed for 2 days to 3 weeks at −70°C. Autoradiography density analysis was done with a scanning densitometer.

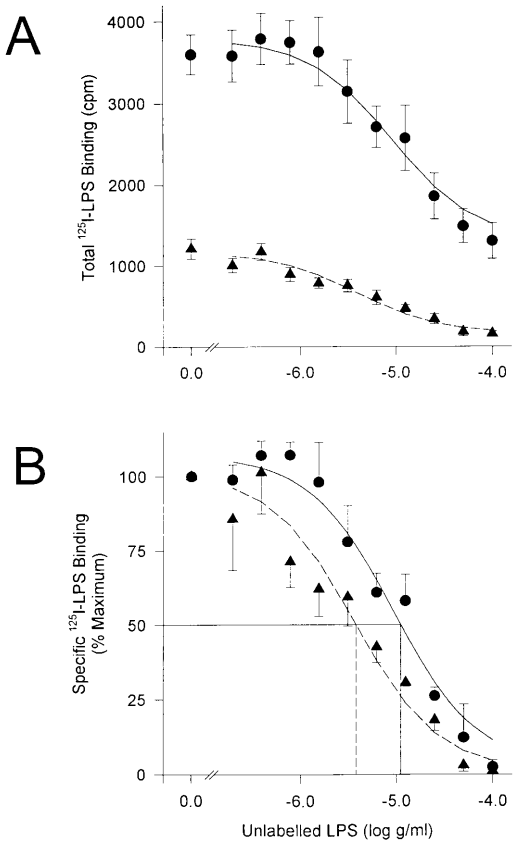

FIG. 1. ^{125}I-ASD-LPS binding to PMN in the presence of increasing concentrations of unlabelled LPS. Adult (circles) or newborn (triangles) PMN (2×10^4 PMN per well) were incubated for 20 min with ^{125}I-ASD-LPS (100 ng/ml) in the dark, exposed to UV light, washed, and counted. The results represent the mean ± standard error of the mean for four experiments in each group. (A) Total ^{125}I-ASD-LPS bound to PMN in the presence of various concentrations of unlabelled LPS. The maximum amounts of ^{125}I-ASD-LPS bound to PMN of neonates and adults were 1,004 ± 103 cpm and 3,584 ± 444 cpm, respectively ($P < 0.01$). (B) Specific ^{125}I-ASD-LPS (total minus nonspecific) plotted as a function of increasing concentrations of unlabelled LPS. The data in panel B were normalized as a percentage of maximum for neonatal and adult PMN. Positions on the abscissa shown by the vertical lines indicate the IC_{50}.

Statistical analysis. All data are presented as the mean ± standard error of the mean. For comparison between samples from adults and newborns, a nonpaired *t* test was used; $P < 0.05$ was considered significant. Statistical determinations were done with statistics software (Statistix; Analytical Software, Minneapolis, Minn.). Nonlinear curve fitting was done with Sigmaplot for Windows (Jandel Scientific, Corte, Madera, Calif.).

RESULTS

^{125}I-ASD-LPS binding assays. Optimal incubation time and cell number were determined in preliminary experiments for total ^{125}I-ASD-LPS binding to PMN (data not shown). On the basis of these results, we used 2×10^4 cells per well and incubated samples for 20 min. We then compared ^{125}I-ASD-LPS binding to PMN from adults and neonates in the absence of serum. Figure 1A illustrates the effect of increasing concentrations of unlabelled LPS on total ^{125}I-ASD-LPS binding to adult and neonatal PMN. While increasing concentrations of unlabelled LPS displaced ^{125}I-ASD-LPS binding to both adult and neonatal PMN, there was significantly less ^{125}I-ASD-LPS binding to neonatal PMN than to adult PMN (Fig. 1A; 1,004 ± 103 cpm/2×10^4 PMN for neonatal PMN [$n = 4$] versus 3,583 ± 444 cpm/2×10^4 PMN for adult PMN [$n = 4$]; $P <$

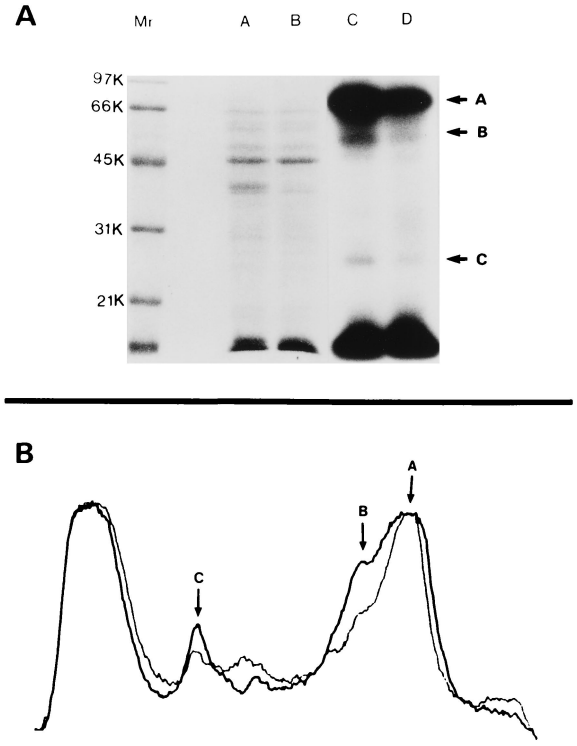

FIG. 2. LPS binding proteins on PMN from neonates and adults. (A) Adult or neonatal PMN were incubated with ^{125}I-ASD-LPS, and cell lysates were analyzed by SDS-PAGE (11% polyacrylamide) under reducing conditions followed by autoradiography. Lanes A (adult) and B (newborn) show the Coomassie blue-stained protein patterns, and lane C (adult) and D (newborn) show the corresponding autoradiographs. Arrows A, B, and C indicate the positions of the 73-, 55- to 57-, and 25-kDa LPS binding proteins (read from top to bottom). The figure is representative of three experiments. (B) Adult (thick line) and newborn (thin line) PMN membrane autoradiographs were prepared as described above. The autoradiograph was scanned and analyzed by densitometric image analysis. The height of the curve is proportional to the intensity of the autoradiograph bands. Arrows indicate the position of the 25-kDa (C), 55- to 57-kDa (B), and 73-kDa (A) LPS binding proteins (read from left to right).

0.01). Similar differences between adult and newborn PMN were found when specific LPS binding was compared (876 ± 86 cpm/2 × 10^4 PMN for neonatal PMN [$n = 4$] versus 2,481 ± 336 cpm/2 × 10^4 PMN for adult PMN [$n = 4$]; $P < 0.05$). Specific ^{125}I-ASD-LPS binding was determined by subtracting ^{125}I-ASD-LPS binding which remained in the presence of a 1,000-fold-greater amount of unlabelled LPS (the nonspecific binding) from the total ^{125}I-ASD-LPS bound. Next, we determined whether the concentrations of unlabelled LPS that displaced 50% of the maximal specific binding (IC_{50}) differed between adult and neonatal PMN. Data were then normalized to the ^{125}I-ASD-LPS bound in the absence of unlabelled LPS, as shown in Fig. 1B. Normalized ^{125}I-ASD-LPS specific binding curves were similar in shape in adult and neonatal PMN, although the curve for neonatal PMN appeared to be shifted slightly to the left (Fig. 1B). However, mean IC_{50} for ^{125}I-ASD-LPS binding were not significantly different between the two groups (−4.85 ± 0.04 and −5.13 ± 0.14 log g of LPS per ml for adult and neonatal PMN, respectively [$P > 0.05$]).

^{125}I-ASD-LPS binding on membrane proteins of adult and neonatal PMN. We assessed the membrane binding of ^{125}I-ASD-LPS to adult and neonatal PMN by using membrane extracts analyzed by SDS-PAGE and autoradiography. A representative autoradiograph is shown in Fig. 2A. Both adult and neonatal PMN had similar LPS binding profiles on corresponding autoradiographs, with a predominant LPS-binding protein of approximately 73 kDa. In addition to the 73-kDa protein, both adult and neonatal PMN showed LPS-specific binding at 55 to 57 and 25 kDa (Fig. 2A). Two other bands at approximately 45 and 32 kDa were present in some subjects but not all. The density of ^{125}I-ASD-LPS at the 73-kDa site was as intense with neonatal as with adult PMN; however, the band was wider with adult cells, suggesting the presence of overlapping proteins (Fig. 2). The LPS binding proteins at 55 to 57 and 25 kDa were less intense with neonatal than with to adult cells.

To demonstrate that the binding was specific for LPS, PMN autoradiographs were done with a 10- or 50-fold excess of unlabelled LPS: lipid A (Fig. 3), O111:B4, or J5 (data not shown). Unlabelled LPS was added at the same time as the ^{125}I-ASD-LPS. Unlabelled LPS and lipid A blocked ^{125}I-ASD-LPS binding to the PMN membrane in a dose-dependent manner, indicating that binding is specific for the lipid A portion of ^{125}I-ASD-LPS. In Fig. 3, LPS-binding bands were evident at 32 and 45 kDa in addition to 73, 55 to 57, and 25 kDa. These bands (32 and 45 kDa) were not seen in PMN preparations of all subjects.

In these studies, LPS binding to PMN was assessed in the absence of plasma. We also evaluated the interaction of LPS with PMN in the presence of plasma to simulate what may occur in blood during infection. In these experiments, we found that LPS binding to PMN was not seen when either whole plasma or BSA was present. Because this study suggested that albumin interfered with LPS binding to PMN in this technique, we further assessed the specific effect of albumin on ^{125}I-ASD-LPS binding to PMN by using albumin-depleted plasma. As shown in Fig. 4, the binding of ^{125}I-ASD-LPS to PMN occurred when albumin-depleted plasma was used, while LPS binding was not detected when control plasma was used. LPS-binding profiles on autoradiographs obtained by using ^{125}I-ASD-LPS with albumin-depleted plasma were similar to those obtained in the absence of plasma; specific LPS binding was identified at approximately 73, 55 to 57, and 25 kDa.

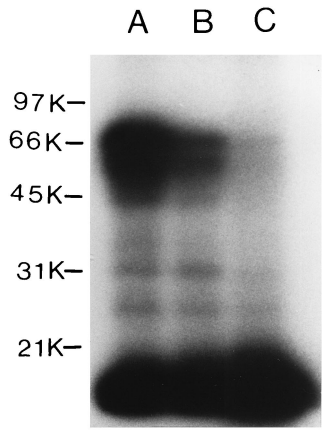

FIG. 3. LPS binding protein profiles on adult PMN inhibited by unlabelled LPS. Adult PMN were incubated with ^{125}I-ASD-LPS in the absence (lane A) or presence of lipid A in 10-fold (lane B) and 50-fold (lane C) excess. Cell lysates were analyzed by SDS-PAGE (11% polyacrylamide) under reducing conditions followed by autoradiography. Similar results were obtained with LPS O111:B4 and J5. In the autoradiograph shown here, bands were seen at 73, 55 to 57, 45, 32, and 25 kDa. However, the 45- and 32-kDa bands were not seen for all subjects (see the text).

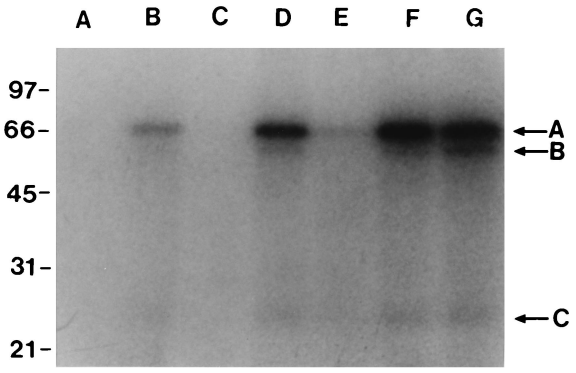

FIG. 4. Effect of albumin on LPS binding to PMN. The albumin-depleted plasma (lanes B, D, and F) and whole plasma (lanes A, C, and E) were normalized to the same concentration of nonalbumin protein. For lanes A and B, 1.4 mg of nonalbumin plasma protein per ml was present during PMN, LPS, and protein incubation. Protein concentrations for LPS and PMN used in lanes C and D and lanes E and F were 0.35 and 0.09 mg/ml, respectively. The PMN preparation for lane G was incubated with LPS and HBSS. Arrows A, B, and C indicate the positions of the 73-, 55- to 57-, and 25-kDa LPS binding proteins respectively.

DISCUSSION

In this report, we compared the LPS binding to neonatal and adult PMN by using ^{125}I-ASD-LPS. LPS binding proteins on adult PMN membrane in our studies were similar to those described by Halling et al. (11) and Rabin et al. (22) with human adult PMN and monocytes. PMN had a predominant 73-kDa LPS binding protein as well as others at approximately 55 to 57 and 25 kDa.

Because the functional activity of primed PMN from neonates differs markedly from that of primed PMN from adults, we further assessed LPS binding proteins by using neonatal PMN. This is the first report to describe LPS membrane binding proteins when PMN from neonates were used. Our studies show that PMN from newborn infants have lower specific and total LPS binding than do PMN from adults under the conditions tested. In spite of the marked difference in total LPS binding, adult and neonatal PMN had similar IC_{50} and similar membrane protein sites for LPS binding, at 73, 55 to 57, and 25 kDa as shown by autoradiography. We also assessed the specificity of these binding sites for LPS with adult PMN. Unlabelled LPS (O111:B4, J5, and lipid A) competed with ^{125}I-ASD-LPS for binding to PMN, suggesting that binding of ^{125}I-ASD-LPS to these membrane sites is specific for lipid A component of LPS.

It is possible that one or more of the LPS binding proteins contributes to the marked difference in LPS priming between neonatal and adult PMN. However, the functions of these membrane LPS binding proteins are not fully known. A 73-kDa membrane-binding protein is the predominant binding site on murine lymphocytes and splenic macrophages (14, 15) and on human monocytes, lymphocytes, PMN, and platelets (11). The physiological importance of the 73-kDa binding protein is supported by the observation that a hamster monoclonal antibody, MAb5D3, against the murine 73-kDa LPS binding protein, inhibits the binding of LPS to murine splenocytes (5, 20) and protects against the lethal effects of endotoxin in a murine model (21). Finally, MAb5D3 activates the macrophage (6). However, on the basis of studies with mouse and human cell lines, Dziarski recently proposed that a cellular 70-kDa peptidoglycan/LPS binding protein is actually cell-bound albumin which originates from serum (9). This protein may be identical to the 73-kDa LPS binding protein that we found in our study and as described by Halling et al. (11) and Rabin et al. (22). The functional activity of cell-bound albumin is not known.

Two molecules of special interest, at approximately 55 to 57 and 25 kDa, have been described previously and were identified in our preparations of PMN. Both are specific for the core region on LPS, since interaction of ^{125}I-ASD-LPS at each site is inhibited by purified lipid A. In addition, both are found on PMN from adults in considerably greater amounts than on PMN from neonates. An LPS binding protein of approximately 55 kDa has been described by several researchers, who have suggested that it is CD14 (20, 31). However, Weersink et al. have reported the presence of a 55-kDa LPS binding protein on PMN that they believe to be bactericidal/permeability-increasing protein (29, 30). The second LPS binding site (25 kDa) that we identified has been seen by others (11, 12). However, its function has not been determined.

We also wished to assess the effect of plasma on LPS-PMN membrane binding. Previous studies with ^{125}I-ASD-LPS to identify cell membrane receptors have been done in the absence of serum or plasma. Studies to identify LPS binding proteins in plasma or on cell membranes in the presence of serum have been done with [^{3}H]LPS or fluorescein isothiocyanate-conjugated LPS preparations (16, 24, 29). Our initial attempts to assess ^{125}I-ASD-LPS interactions with PMN in the presence of plasma were unsuccessful. When either whole plasma or BSA was present, ^{125}I-ASD-LPS binding to membrane proteins of PMN was undetectable. This finding prompted us to speculate that albumin might interfere with ^{125}I-ASD-LPS binding to PMN. When albumin-depleted plasma was used, ^{125}I-ASD-LPS binding to PMN membrane became detectable. In the presence of albumin-depleted plasma, ^{125}I-ASD-LPS membrane recognition proteins were evident at 25, 55 to 57, and 73 kDa. The physiologic significance of the interference of LPS binding by albumin is unclear. We speculate that ^{125}I-ASD-LPS may have a weak affinity for albumin. In spite of this, a large portion of ^{125}I-ASD-LPS may combine with albumin when photoactivation initiates the formation of covalent binding to proteins, since albumin is the predominant protein in plasma. The concentration of the remaining "free" ^{125}I-ASD-LPS may be insufficient to be detected on cell membranes by autoradiography, in which microgram-per-milliliter concentrations are required, yet sufficient to prime the cell, in which nanogram-per-milliliter amounts are sufficient.

Thus, we found that PMN from newborn infants have lower total and specific LPS cell binding than do PMN from adults. Although neonatal PMN had a similar IC_{50} and the same LPS membrane-binding sites as adult cells, they had smaller amounts of LPS membrane-binding proteins at 55 to 57 and 25 kDa than did adult PMN. PMN from newborn infants have also diminished LPS-stimulated priming activity. Further studies are need to understand the role of LPS binding proteins in PMN priming.

ACKNOWLEDGMENTS

We acknowledge Peter Nicholl for his technical assistance, K. Barrett for typing the manuscript, and the Case Room nurses of the Grace Maternity Hospital for their assistance in collecting umbilical cord blood.

Support for this research was provided by the Medical Research Council of Canada (MT7610).

REFERENCES

1. **Aida, Y., and M. J. Pabst.** 1990. Priming of neutrophils by lipopolysaccharide for enhanced release of superoxide. J. Immunol. **145:**3017–3025.
2. **Barbour, A. G., C. D. Allred, C. O. Solberg, and H. R. Hill.** 1980. Chemiluminescence by polymorphonuclear leukocytes from patients with active bac-

terial infection. J. Infect. Dis. **141:**14–25.
3. **Bohmer, R. H., L. S. Trinkle, and J. L. Staneck.** 1992. Dose effects of LPS on neutrophils in a whole blood flow cytometric assay of phagocytosis and oxidative burst. Cytometry **13:**525–531.
4. **Bortolussi, R., S. Howlett, K. Rajaraman, and S. Halperin.** 1993. Deficient priming activity of newborn cord blood-derived polymorphonuclear neutrophilic granulocytes with lipopolysaccharide and tumor necrosis factor-α triggered with formyl-methionyl-leucyl-phenylalanine. Pediatr. Res. **34:**243–248.
5. **Bright, S. W., T.-Y. Chen, L. M. Flebbe, M.-G. Lei, and D. C. Morrison.** 1990. Generation and characterization of hamster-mouse hybridomas secreting monoclonal antibodies with specificity for lipopolysaccharide receptor. J. Immunol. **145:**1–7.
6. **Chen, T., E. Scott, and D. C. Morrison.** 1994. Differential effects of serum on lipopolysaccharide receptor-directed macrophage activation for nitric oxide production. Immunol. Lett. **40:**179–187.
7. **Cohen, L., A. Haziot, D. R. Shen, X.-Y. Lin, C. Sia, R. Harper, J. Silver, and S. M. Goyert.** 1995. CD14-independent responses to LPS require a serum factor that is absent from neonates. J. Immunol. **155:**5337–5342.
8. **Corrales, I., A. J. L. Weersink, J. Verhoef, and K. P. M. van Kessel.** 1993. Serum-independent binding of lipopolysaccharide to human monocytes is trypsin sensitive and does not involve CD14. Immunology **80:**84–89.
9. **Dziarski, R.** 1994. Cell-bound albumin is the 70-kDa peptidoglycan-, lipopolysaccharide-, and lipoteichoic acid-binding protein on lymphocytes and macrophages. J. Biol. Chem. **269:**20431–20436.
10. **Hallett, M. B., and D. Lloyds.** 1995. Neutrophil priming: the cellular signals that say 'amber' but not 'green'. Immunol. Today **16:**264–268.
11. **Halling, J. L., D. R. Hamill, M.-G. Lei, and D. C. Morrison.** 1992. Identification and characterization of lipopolysaccharide-binding proteins on human peripheral blood cell populations. Infect. Immun. **60:**845–852.
12. **Kielian, T. L., and F. Blecha.** 1995. CD14 and other recognition molecules for lipopolysaccharide: a review. Immunopharmacology **29:**187–205.
13. **Kitchens, R. L., and R. S. Munford.** 1995. Enzymatically deacylated lipopolysaccharide (LPS) can antagonize LPS at multiple sites in the LPS recognition pathway. J. Biol. Chem. **270:**9904–9910.
14. **Lei, M.-G., and D. C. Morrison.** 1988. Specific endotoxic lipopolysaccharide-binding proteins on murine splenocytes. II. Membrane localization and binding characteristics. J. Immunol. **141:**1006–1011.
15. **Lei, M. G., and D. C. Morrison.** 1988. Specific endotoxic lipopolysaccharide-binding proteins on murine splenocytes. I. Detection of lipopolysaccharide-binding sites on splenocytes and splenocyte subpopulations. J. Immunol. **141:**996–1005.
16. **Luchi, M., and R. S. Munford.** 1993. Binding, internalization, and deacylation of bacterial lipopolysaccharide by human neutrophils. J. Immunol. **151:** 959–969.
17. **Lynn, W. A., Y. Liu, and D. T. Golenbock.** 1993. Neither CD14 nor serum is absolutely necessary for activation of mononuclear phagocytes by bacterial lipopolysaccharide. Infect. Immun. **61:**4452–4461.
18. **McCarthy, J. P., R. S. Bodroghy, P. B. Jahrling, and P. Z. Sobocinski.** 1980. Differential alterations in host peripheral polymorphonuclear leukocyte chemiluminescence during the course of bacterial and viral infections. Infect. Immun. **30:**821–831.
19. **Metcalf, J. A., W. M. Nauseef, J. I. Gallin, and R. K. Root.** 1986. Neutrophil purification, p. 2–6. *In* Laboratory manual of neutrophil function. Raven Press, New York.
20. **Morrison, D. C., T. Kirikae, F. Kirikae, M.-G. Lei, T. Chen, and S. W. Vukajlovich.** 1994. The receptor(s) for endotoxin on mammalian cells. Prog. Clin. Biol. Res. **388:**3–15.
21. **Morrison, D. C., R. Silverstein, S. W. Bright, T.-Y. Chen, L. M. Flebbe, and M.-G. Lei.** 1990. Monoclonal antibody to mouse lipopolysaccharide receptor protects mice against the lethal effects of endotoxin. J. Infect. Dis. **162:**1063–1068.
22. **Rabin, R. L., M. M. Bieber, and N. N. H. Teng.** 1993. Lipopolysaccharide and peptidoglycan share binding sites on human peripheral blood monocytes. J. Infect. Dis. **168:**135–142.
23. **Shigeoka, A. O., J. I. Santos, and H. R. Hill.** 1979. Functional analysis of neutrophil granulocyte from healthy infected and stressed neonates. J. Pediatr. **95:**454–460.
24. **Tobias, P. S., K. Soldau, J. A. Gegner, D. Mintz, and R. J. Ulevitch.** 1995. Lipopolysaccharide binding protein-mediated complexation of lipopolysaccharide with soluble CD14. J. Biol. Chem. **270:**10482–10488.
25. **Tobias, P. S., K. Soldau, L. Kline, J.-D. Lee, K. Kato, T. P. Martin, and R. J. Ulevitch.** 1993. Cross-linking of lipopolysaccharide (LPS) to CD14 on THP-1 cells mediated by LPS-binding protein. J. Immunol. **150:**3011–3021.
26. **Ulevitch, R. J., and P. S. Tobias.** 1994. Recognition of endotoxin by cells leading to transmembrane signaling. Curr. Opin. Immunol. **6:**125–130.
27. **Ulevitch, R. J., and P. S. Tobias.** 1995. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu. Rev. Immunol. **13:**437–457.
28. **Wagg, I., M. Nakamura, A. Honda, I. Ferby, S. Toyoshima, S. Ishiguro, and T. Shimizu.** 1993. Two distinct signal transduction pathways for the activation of guinea-pig macrophages and neutrophils by endotoxin. Biochem. Biophys. Res. Commun. **197:**465–472.
29. **Weersink, A. J. L., K. P. M. van Kessel, M. E. van den Tol, J. A. G. van Strijp, R. Torensma, J. Verhoef, P. Elsbach, and J. Weiss.** 1993. Human granulocytes express a 55-kDa lipopolysaccharide-binding protein on the cell surface that is identical to the bactericidal/permeability-increasing protein. J. Immunol. **150:**253–262.
30. **Weersink, A. J. L., K. P. M. van Kessel, M. E. van der Tol, and J. Verhoef.** 1994. Modulation of lipopolysaccharide binding to human granulocytes. Immunology **83:**617–623.
31. **Weingarten, R., L. A. Sklar, J. C. Mathison, S. Omidi, T. Ainsworth, S. Simon, R. J. Ulevitch, and P. S. Tobias.** 1993. Interactions of lipopolysaccharide with neutrophils in blood via CD14. J. Leukocyte Biol. **53:**518–524.
32. **Wollenweber, H.-W., and D. C. Morrison.** 1985. Synthesis and biochemical characterization of a photoactivatable, iodinatable, cleavable bacterial lipopolysaccharide derivative. J. Biol. Chem. **260:**15068–15074.
33. **Wright, W. C., B. J. Ank, J. Herbert, and E. R. Steihm.** 1975. Decreased bactericidal activity of leukocytes of stressed newborn infants. Pediatrics **56:**579–584.

Editor: R. E. McCallum