Identification and Characterization of Lipopolysaccharide-Binding Proteins on Human Peripheral Blood Cell Populations

JULIE L. HALLING, DANIELLE R. HAMILL, MEI-GUEY LEI, AND DAVID C. MORRISON*

Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, Kansas 66103

Received 2 July 1991/Accepted 16 December 1991

Previous research in this laboratory, using photoactivatable radioiodinated lipopolysaccharide derivatized with sulfosuccinimidyl-2-(*p*-azidosalicylamide)-1,3'-dithiopropionate (^{125}I -ASD-LPS), has resulted in the identification of a specific LPS receptor with a molecular mass of approximately 73 kDa on murine lymphocytes and splenic macrophages. The experiments presented in this report investigated whether a similar LPS-binding protein was also expressed on human peripheral blood populations, including monocytes, lymphocytes, neutrophils, platelets, and erythrocytes. Each cell population was incubated with ^{125}I -ASD-LPS, UV irradiated, washed, reduced, and solubilized, and the cell lysates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by autoradiography. On all of the cell populations, except erythrocytes, a similar 73-kDa LPS-binding protein was present. In addition, each population also expressed lower-molecular-weight secondary LPS-binding proteins, some of which were conserved among the populations. Binding of the photoactivatable LPS probe was found to be both time and temperature dependent. These data support the concept that the 73-kDa LPS-binding protein is conserved on multiple cell types from a variety of species.

In spite of the introduction of a large number of effective antibiotics and chemotherapeutic agents, the incidence of gram-negative septicemia has progressively risen since the early 1950s (38). The current mortality rate associated with gram-negative bacteremia has been estimated to be between 20 and 50% depending upon the age and health status of the patient and the appropriate use of antimicrobial drugs (15). The prognosis for the bacteremic patient is primarily dependent on whether shock ensues (13). Septic shock is a syndrome characterized by fever, hypotension, hypoxia, acidosis, and disseminated intravascular coagulation, often leading to multisystem organ failure and death (40).

The lipopolysaccharide (LPS) of the gram-negative bacterium has been implicated as a major contributing factor to the pathogenesis of septic shock (36). Of importance, the LPS itself is not toxic, rather it is the ability of the LPS macromolecule to interact with and stimulate a variety of host target cells which subsequently produces the potentially lethal mediators of septic shock (30). In this respect, experimental evidence accumulated during the last several decades has established that LPS can induce B-cell proliferation and differentiation into antibody-secreting plasma cells (29). Although endotoxin directly induces proliferation of only a minor subpopulation of T cells (25), evidence indicates that LPS profoundly affects T-cell responses in mediating its adjuvant properties (23, 24). Pretreatment of neutrophils with LPS causes a priming effect whereby subsequent stimulation with agents such as *N*-formyl-methionine-leucine-phenylalanine and phorbol myristate acetate results in a marked increase in the oxidative response and granule exocytosis (7). This priming effect can be augmented to an even greater degree in the presence of platelets, which have recently been reported to secrete a priming factor upon LPS stimulation (44). Platelet aggregation (35) and serotonin

release (32) have also been described following LPS stimulation *in vitro*.

Probably one of the most potentially important responses to LPS, however, occurs following LPS interactions with macrophages and monocytes. These mononuclear phagocytes synthesize and secrete a variety of immunologically relevant cytokines such as alpha/beta interferon (11), interleukin-1 (IL-1) (6), interleukin-6 (IL-6) (39), and tumor necrosis factor (3) in response to LPS stimulation. Strong evidence exists in support of the concept that IL-1, IL-6, and tumor necrosis factor play major roles in mediating the deleterious effects of septic shock (29); however, the roles of other host cells should not be understated.

In spite of the fact that LPS is well recognized as a potent stimulus for host inflammatory cells, the precise biochemical mechanisms involved have not, to date, been totally defined. Central to this understanding is the question of specific cellular receptors for LPS. Recent studies by Lei and Morrison (19, 20) using photoactivatable radioiodinated cross-linking derivatives of LPS have allowed the identification of a specific LPS-binding protein with a molecular mass of approximately 73 kDa expressed on mouse macrophages and B and T lymphocytes. Binding of LPS to this glycoprotein is specific for the lipid A moiety, since the addition of underivatized LPS or purified lipid A effectively inhibits the binding of the derivatized LPS (20). Inhibition was observed with a variety of LPS types but not with peptidoglycan (20), although recent studies by Dziarski (5) have not confirmed the latter result (22). Subsequent experiments by Bright et al. (2) and Chen et al. (4) have provided strong evidence that this protein may function as an LPS receptor. In those experiments, it was demonstrated that stimulation of mouse macrophages with a hamster monoclonal antibody (MAb 5D3) directed against the 73-kDa LPS-binding protein activated the macrophages to become cytotoxic for tumor cells (4).

Preliminary results reported by Roeder et al. (37) have provided evidence that similar LPS-binding proteins may be

* Corresponding author.

found on mononuclear cells of a variety of mammalian species, including humans. The data presented in this report extend these studies to a variety of populations of human peripheral blood lymphoreticular and inflammatory cells and demonstrate the existence of a similar 73-kDa LPS-binding protein on human monocytes, lymphocytes, neutrophils, and platelets. Specific LPS-binding proteins were not detected on human erythrocytes (RBC). Each cell population studied was found to express both unique and conserved lower-molecular-weight secondary LPS-binding proteins in addition to the 73-kDa LPS-binding protein. These results implicate the 73-kDa LPS-binding protein as a relatively conserved membrane constituent of human peripheral blood lymphoreticular and inflammatory cells.

(The research described in this report was carried out by Julie L. Halling in partial fulfillment of the requirements for the Ph.D. degree from the University of Kansas Medical Center, Kansas City.)

MATERIALS AND METHODS

LPS. LPS from *E. coli* O111:B4 was extracted and purified by a modification of the phenol-water procedure originally developed by Westphal and Luderitz (42) as previously described (28). The photoactivatable cross-linker, sulfo-succinimidyl-2-(*p*-azidosalicyl amide)-1,3'-dithiopropionate (SASD), was purchased from Pierce Chemical Co. (Rockford, Ill.). A photoactivatable, radioiodinated LPS from *E. coli* O111:B4 derivatized with SASD (125 I-ASD-LPS) was prepared exactly as described by Wollenweber and Morrison (43).

Cell separation. Normal human blood, anticoagulated with acid citrate-dextrose (stock concentration: 1.37% citric acid, 2.5% sodium citrate, and 2% dextrose, diluted 1:6 with blood), was obtained from healthy volunteers and was employed for the preparation of all peripheral blood cell populations.

For preparation of human platelets, anticoagulated blood was centrifuged at $350 \times g$ at room temperature for 15 min. The platelet-rich plasma was collected and centrifuged at $1,200 \times g$ for 15 min at 10°C to pellet the platelets. The platelets were washed in TBS (pH 6.5; 30 mM Tris, 120 mM NaCl, 5 mM glucose) by centrifugation and then resuspended in TBS (pH 7.4) at a concentration of 10^7 platelets per ml and placed on ice. Platelet numbers were determined by using an estimate of 3×10^7 platelets per ml of blood drawn.

Following the centrifugation step to obtain the platelet-rich plasma, RPMI 1640 (Hazelton Biologics Inc., Lenexa, Kans.) was added to restore the original volume of blood. One volume of 3% dextran (molecular mass, 400 kDa) in 0.9% NaCl was then added, and the tubes were allowed to stand at room temperature for 18 min to sediment RBC. The supernatant containing polymorphonuclear leukocytes (PMN) and mononuclear cells was collected, and the cells were pelleted by centrifugation at $700 \times g$ for 10 min at 4°C and then resuspended in RPMI 1640. Following the dextran sedimentation, RBC were obtained from the pellet. The RBC were washed in RPMI 1640 by centrifugation at $250 \times g$ for 10 min at 4°C . The cells were resuspended in RPMI 1640, counted, and placed on ice.

PMN and mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation. Briefly, the cell suspension was underlaid with Ficoll-Hypaque (density, 1.07) (Ficoll type 400 [Sigma Chemical Co., St. Louis, Mo.] and Hypaque-M, 75% [Wentrop-Breon Laboratories, New York,

N.Y.]) and centrifuged at $350 \times g$ for 40 min at room temperature.

PMN were obtained from the pellet of the Ficoll-Hypaque gradient. Residual RBC were hypotonically lysed, and the PMN were washed three times in RPMI 1640 by centrifugation at 4°C for 10 min at $250 \times g$. The cells were resuspended in RPMI 1640, counted on a hemacytometer, and placed on ice.

Mononuclear cells were obtained from the buffy coat of the gradient. Residual RBC were hypotonically lysed when necessary. The mononuclear cells were washed in RPMI 1640 by centrifugation at 4°C for 10 min at $250 \times g$. The mononuclear cells were then further fractionated to obtain monocytes and lymphocytes. Briefly, the cells were resuspended at a concentration of 10^7 cells per ml in RPMI 1640 containing 10% fetal calf serum (Hazelton Biologics) and 1% penicillin-streptomycin (Hazelton Biologics) (100 U/ml and 100 $\mu\text{g}/\text{ml}$, respectively). Five to eight milliliters of the cell suspension was plated on a tissue culture dish (100 by 20 mm) (Becton Dickinson, Lincoln Park, N.J.) overnight at 37°C to allow adherence of the monocytes. The following day, the nonadherent cell population, containing primarily lymphocytes, was collected, washed in RPMI 1640 by centrifugation at $250 \times g$ for 10 minutes, counted, and placed on ice. The lymphocyte preparation was determined to be approximately 82% CD2⁺, 6% CD19⁺, and 0.02% CD14⁺ by fluorescence-activated cell sorter (FACS) analysis. The adherent monocytes were gently washed with cold Hanks' balanced salt solution without Ca²⁺ or Mg²⁺ (Hazelton Biologics), and the plate was placed at 4°C for 30 min. The cells were then carefully removed from the plate by using a rubber policeman. The cells were washed with RPMI 1640 by centrifugation at $250 \times g$ for 10 min at 4°C , counted, and placed on ice. The monocyte preparation was determined to be approximately 81% CD14⁺ and 29% CD2⁺ by FACS analysis.

Photoaffinity labeling. For standard reactions, 2×10^6 cells resuspended in 20 μl of RPMI 1640 were incubated with 20 μl of 125 I-ASD-LPS (containing approximately 1 μg of LPS) at 37°C for 30 min exactly as described earlier (19). No serum was added to any of the reaction mixtures. When indicated, the cell populations were washed and resuspended in RPMI 1640 containing protease inhibitors, (1 mM phenylmethylsulfonyl fluoride, 2 μg of aprotinin from bovine lung per ml, 1 μg of leupeptin hemisulfate salt per ml, and 1 μg of alpha-1-antitrypsin from human plasma; all purchased from Sigma) and 5 mM EDTA prior to the addition of the 125 I-ASD-LPS. In some experiments, PMN were pretreated with 0.5 mM diisopropylfluorophosphate (DFP; Sigma) as described by Amrein and Stossel (1) prior to the addition of the 125 I-ASD-LPS. Following this incubation, the cell suspension was irradiated with short-wavelength UV light (4-W maximal emission at 254 nm) for 10 min to effect covalent association of the LPS with the target cells. The cells were washed with RPMI 1640 by centrifugation at $250 \times g$ for 10 min at 4°C following irradiation to remove excess unbound 125 I-ASD-LPS. The cells were solubilized in 50 μl of SDS sample buffer (5% 2-mercaptoethanol, 2.3% sodium dodecyl sulfate [SDS], 0.062 M Tris base, 1.09 M glycerol), and the cell lysates were electrophoresed on 11% SDS-polyacrylamide gels. SDS-polyacrylamide gel electrophoresis (PAGE) molecular weight standards were purchased from Bio-Rad Laboratories (Richmond, Calif.). Gels were stained with 0.2% Coomassie blue R250–50% methanol–12% acetic acid, destained with 20% ethanol–10% acetic acid, dried, and autoradiographed to detect LPS-binding proteins on target

FIG. 1. LPS-binding protein profiles of human peripheral blood monocytes. Monocytes were incubated for 30 min at 37°C with the 125 I-ASD-LPS derivative in the absence (lanes 1 and 5) or presence of a 10-fold (lanes 2 and 6) or 50-fold (lanes 3 and 7) excess of underivatized LPS. Cell lysates were analyzed by 11% SDS-PAGE under reducing conditions followed by autoradiography. The left panel shows the Coomassie blue-stained protein pattern, and the right panel shows the corresponding autoradiograph. The arrowhead indicates the position of the 73-kDa LPS-binding protein.

cells. Kodak X-Omat XK-1 film and a Dupont cassette with 2 Dupont Cronex Lightning-Plus intensifying screens were used for the autoradiography. The film was exposed to the gel for 1 to 3 days. In some cases, the intensity of the bands on the autoradiographs was determined with a Soft Laser Scanning Densitometer (Biomed Instruments, Inc., Fullerton, Calif.).

RESULTS

We initially assessed the binding of the radiolabeled, photoactivatable, derivatized LPS (125 I-ASD-LPS) to human peripheral blood monocytes and lymphocytes. Partially purified cell populations were incubated with 125 I-ASD-LPS for 30 min in the dark, UV irradiated, washed, reduced with 2-mercaptoethanol, and analyzed by 11% SDS-PAGE followed by autoradiography. Representative results of these studies are shown in Fig. 1 and 2. The left-hand panels of these figures show the Coomassie blue-stained protein profiles. The corresponding autoradiographs are shown in the right-hand panels. In these cell populations, an LPS-binding protein of approximately 73 kDa is readily detectable and is similar to that expressed on both of the analogous mouse cell populations and, as reported earlier, on unfractionated human peripheral blood mononuclear cells (37). Of interest, both populations express several additional lower-molecular-weight LPS-binding proteins, not all of which are equally reactive with LPS in the two cell populations. Particularly noteworthy in the lymphocyte preparation (Fig. 2) are three major proteins of approximately 50, 31, and 18 kDa and a less abundant protein of about 38 kDa. These secondary proteins are also present, although less prominent, in the adherent monocyte preparation.

Since it is possible that binding of the derivatized LPS to

FIG. 2. LPS-binding protein profiles of human peripheral blood lymphocytes. Lymphocytes were incubated for 30 min at 37°C with 125 I-ASD-LPS in the absence (lanes 1 and 5) or presence of a 10-fold (lanes 2 and 6) or 50-fold (lanes 3 and 7) excess of underivatized LPS. Cell lysates were analyzed by 11% SDS-PAGE under reducing conditions followed by autoradiography. The Coomassie blue-stained protein pattern is shown in the left panel, with the corresponding autoradiograph shown in the right panel.

the cell populations occurred by virtue of the ASD cross-linking moiety of the LPS probe rather than the LPS, it was important to demonstrate that the binding was specific for LPS. To this end, lymphocytes and monocytes were analyzed exactly as described above except that either a 10- or 50-fold excess of underivatized LPS was added simultaneously with the 125 I-ASD-LPS. The results, also shown in Fig. 1 and 2, clearly demonstrate that binding of the derivatized LPS to both the 73-kDa protein and the lower-molecular-weight proteins is specific for the LPS component of the LPS derivatized probe. Addition of increasing concentrations of underivatized LPS effectively competes with the 125 I-ASD-LPS for binding to all of the cell populations investigated.

Since it has been well documented that LPS can affect PMN and platelets, it was of interest to investigate whether these peripheral blood cell populations might also express LPS-binding proteins. These populations were purified from normal human blood as described in Materials and Methods and incubated with the 125 I-ASD-LPS. The cell lysates were then analyzed by 11% SDS-PAGE followed by autoradiography. The results of these studies are shown in Fig. 3 and 4. The left-hand panels of each figure again show the Coomassie blue-stained protein profiles, with the corresponding autoradiographs shown in the right-hand panels. As previously demonstrated for the human peripheral blood mononuclear cells, an LPS-binding protein similar to the 73-kDa mouse LPS receptor was expressed on both the platelet and the neutrophil populations. In addition, PMN (Fig. 3) express a number of lower-molecular-weight secondary LPS-binding proteins not detected on any of the other cell types. Platelets (Fig. 4), in contrast, express relatively few lower-molecular-weight secondary LPS-binding proteins. Interestingly, a difference in the LPS-binding protein pattern is seen when the concentration of platelets is increased from 2×10^6 to 2×10^7 . The secondary LPS-binding

FIG. 3. LPS-binding protein profiles of human peripheral blood PMN. PMN were incubated for 30 min at 37°C with 125 I-ASD-LPS in the absence (lanes 1 and 5) or presence of a 10-fold (lanes 2 and 6) or 50-fold (lanes 3 and 7) excess of underivatized LPS. Cell lysates were analyzed by 11% SDS-PAGE under reducing conditions followed by autoradiography. The Coomassie blue-stained protein pattern is shown in the left panel, and the corresponding autoradiograph is shown in the right panel. The arrowhead indicates the position of the 73-kDa LPS-binding protein.

proteins are not apparent when the higher concentration of platelets is used. However, note that the addition of increasing concentrations of underivatized LPS effectively competes with the 125 I-ASD-LPS for binding to both the 73-kDa protein and the lower-molecular-weight proteins on both of these cell populations (Fig. 3 and 4). This result indicates

FIG. 4. LPS-binding protein profiles of human peripheral blood platelets. Either 2×10^6 platelets (lanes 1 to 3 and 9 to 11) or 2×10^7 platelets (lanes 5 to 7 and 12 to 14) were incubated for 30 min at 37°C with 125 I-ASD-LPS in the absence (lanes 1, 5, 9, and 12) or presence of a 10-fold (lanes 2, 6, 10, and 13) or 50-fold (lanes 3, 7, 11, and 14) excess of underivatized LPS. Cell lysates were analyzed by 11% SDS-PAGE under reducing conditions followed by autoradiography. The Coomassie blue-stained protein pattern is shown in the left panel, and the corresponding autoradiograph is shown on the right. The arrowhead indicates the position of the 73-kDa LPS-binding protein.

FIG. 5. LPS-binding protein profiles of human peripheral blood RBC. RBC were incubated for 30 min at 4°C (lanes 2 and 6), 25°C (lanes 3 and 7), 37°C (lanes 4 and 8), or 42°C (lanes 5 and 9) with 125 I-ASD-LPS. Cell lysates were analyzed by 11% SDS-PAGE under reducing conditions followed by autoradiography. The Coomassie blue-stained protein pattern is shown in the left panel, with the corresponding autoradiograph shown in the right panel. The arrowhead indicates the position of the 73-kDa LPS-binding protein.

that binding of the radiolabeled LPS probe to these lower-molecular-weight proteins is also specific for the LPS portion of the 125 I-ASD-LPS. As shown in Fig. 5, no LPS-binding proteins were detected on human peripheral blood RBC.

Because a number of secondary lower-molecular-weight LPS-binding proteins were detected on neutrophils and, to a lesser extent, on mononuclear cells, it was possible that these lower-molecular-weight species arose from the proteolytic digestion of the 73-kDa LPS-binding protein. To address this possibility, purified PMN or mononuclear cells were resuspended in RPMI 1640 containing a cocktail of protease inhibitors (indicated in Materials and Methods) prior to the addition of the 125 I-ASD-LPS. The cells were treated as described in Materials and Methods, and the cell lysates were then analyzed by 11% SDS-PAGE followed by autoradiography. The results of one such experiment are shown in Fig. 6. Identical patterns of LPS-binding proteins were observed for both cell populations regardless of the presence of protease inhibitors. Additional experiments performed using PMN which had been pretreated with the irreversible serine protease inhibitor DFP yielded similar results (data not shown). In these experiments, detection of the vast majority of lower-molecular-weight LPS-binding proteins was independent of the presence of DFP. One exception was the disappearance of an LPS-binding protein of approximately 63 kDa in the PMN lysates from cells which had been pretreated with DFP. These results support the concept that proteolytic cleavage is most probably not a major contributing factor for the presence of most of the secondary LPS-binding proteins expressed on either human peripheral blood PMN or the mononuclear cell population.

To examine the dependence on temperature for optimal binding of the LPS derivatized probe to the cells, we incubated the cell populations with 125 I-ASD-LPS at either 4

FIG. 6. LPS-binding protein profiles of human peripheral blood PMN (lanes 4, 5, 8, and 9) and mononuclear cells (lanes 2, 3, 6, and 7) in the presence or absence of protease inhibitors. PMN or mononuclear cells were incubated with ^{125}I -ASD-LPS for 30 min at 37°C in the presence (lanes 2, 4, 6, and 8) or absence (lanes 3, 5, 7, and 9) of protease inhibitors. Cell lysates were analyzed by 11% SDS-PAGE under reducing conditions followed by autoradiography. The Coomassie blue-stained protein pattern is shown in the left panel, with the corresponding autoradiograph shown in the right panel. The arrowhead indicates the position of the 73-kDa LPS-binding protein.

or 37°C for 30 min. The cells were then UV irradiated for 10 min at 4 or 37°C, respectively, and solubilized in SDS sample buffer, and the lysates were analyzed by 11% SDS-PAGE and autoradiography. To obtain more quantitative results, the autoradiographs were analyzed by densitometric scanning. Figure 7 shows a representative densitometric tracing pattern obtained when mononuclear cells were studied. It is evident from these results that binding of the ^{125}I -ASD-LPS to the 73-kDa and lower-molecular-weight LPS-binding proteins is much more efficient at the higher temperature. Similar increases in binding of the LPS probe to the 73-kDa

FIG. 7. Temperature dependence for binding of ^{125}I -ASD-LPS to human peripheral blood mononuclear cells. Mononuclear cells were incubated at either 4 or 37°C for 30 min with ^{125}I -ASD-LPS. Cell lysates were analyzed by 11% SDS-PAGE under reducing conditions. The gel was autoradiographed, and the autoradiograph was densitometrically scanned. The arrowhead indicates the position of the 73-kDa LPS-binding protein.

FIG. 8. Time course for binding of ^{125}I -ASD-LPS to human peripheral blood mononuclear cells. Mononuclear cells were incubated with ^{125}I -ASD-LPS for 0, 1, 3, 10, or 30 min at 37°C. Cell lysates were analyzed by 11% SDS-PAGE under reducing conditions. The gel was autoradiographed, and the autoradiograph was densitometrically scanned. The arrowhead indicates the position of the 73-kDa LPS-binding protein.

LPS-binding protein were observed on all of the cell populations (data not shown).

It was also of interest to determine the optimal time required for binding of the ^{125}I -ASD-LPS to the cells. To accomplish this, cells were prepared as described in Materials and Methods and incubated with the ^{125}I -ASD-LPS for 0, 1, 3, 10, or 30 min at 37°C. Following this incubation, the cells were washed and solubilized in SDS sample buffer and analyzed by 11% SDS-PAGE followed by autoradiography. The resulting autoradiographs were then analyzed by densitometric scanning. The densitometric tracings for mononuclear cells and neutrophils are shown in Fig. 8 and 9, respectively. Binding of the derivatized LPS to the 73-kDa protein was optimal when the incubation was carried out for at least 10 min for all of the cell types. Optimal binding of the LPS probe to the mononuclear cell population (30 min) required additional time as compared with that to the PMN (10 min). In addition, incubation periods of less than 10 min were insufficient for efficient binding of the ^{125}I -ASD-LPS to not only the 73-kDa LPS-binding protein but also to secondary binding proteins present on PMN and mononuclear cells.

DISCUSSION

In this study we have examined the profiles of LPS-binding proteins on human peripheral blood cell populations. We have demonstrated that a 73-kDa LPS-binding protein

FIG. 9. Time course for binding of ^{125}I -ASD-LPS to human peripheral blood PMN. PMN were incubated with ^{125}I -ASD-LPS for 0, 1, 3, 10, or 30 min at 37°C . Cell lysates were analyzed by 11% SDS-PAGE under reducing conditions. The gel was autoradiographed, and the autoradiograph was densitometrically scanned. The arrowhead indicates the position of the 73-kDa LPS-binding protein.

similar to the LPS receptor previously identified on mouse splenic lymphocytes and macrophages (19) and peripheral blood mononuclear cells from a variety of mammalian species (37) can be readily detected on human monocytes, lymphocytes, PMN, and platelets. As previously demonstrated with sheep RBC (2), this 73-kDa LPS-binding protein is not detectable on human RBC. Binding of ^{125}I -ASD-LPS to the 73-kDa protein is dependent upon both time and temperature and is readily inhibited in a concentration-dependent manner by underivatized LPS. These data thus confirm and extend earlier results obtained in our laboratory in investigations of specific LPS receptors on immunologically relevant LPS-responsive mammalian cells (37).

Although we have confirmed the existence of this specific 73-kDa LPS-binding protein on human peripheral blood cell populations, and despite the fact that we have strong evidence which suggests that this protein has a functional role

in LPS-dependent activation of mouse bone marrow culture-derived macrophages (4), we do not currently have direct evidence that this protein serves as a functional receptor for cell activation in the human system. Since it has been established that the cell populations investigated in this study can respond to LPS *in vitro*, it is reasonable to speculate that this human 73-kDa protein may serve a role similar to that demonstrated earlier in the mouse. However, since lymphoreticular cells from endotoxin-low-responder C3H/HeJ mice also appear to express normal levels of the 73-kDa LPS receptor (19), the existence of this protein on a given cell population is not an *a priori* reason to assume a functional role for the protein in LPS-dependent cell activation.

The existence of secondary LPS-binding proteins is of potential interest. Both the lymphocyte and monocyte populations expressed major secondary binding proteins of approximately 50, 31, and 20 kDa. In addition, a minor secondary LPS-binding protein of approximately 38 kDa is found on both cell types. It is noteworthy that earlier studies of LPS-binding proteins have allowed identification of proteins of similar molecular mass. In this respect, the earlier studies of Roeder et al. showed a prominent protein of 51 kDa on human and rabbit peripheral blood mononuclear cells (37). More recent studies in our laboratory have revealed a protein of about 38 kDa which binds LPS on mouse splenocytes, a mouse pre-B cell line (70Z/3), and two mouse macrophagelike cell lines (J774 and P388D) (21). Of particular interest is the fact that the available evidence would suggest a binding specificity for the 2-keto-3-deoxyoctulosonate (KDO) residues (21). It is interesting that this 38-kDa protein may be involved in the recently reported human monocyte IL-1 response to KDO (17). Finally, Kirkland et al. have described a protein of approximately 18 kDa on the 70Z/3 pre-B cell line (14), and the 20-kDa protein identified in our own studies might be related to that LPS-binding protein.

The pattern of secondary LPS-binding proteins identified on neutrophils is far more complex than that observed for mononuclear cells. It is possible that these LPS-binding proteins result from proteolytic degradation, since PMN are a rich source of proteolytic enzymes. Several factors would argue against this possibility, however. First, we have observed identical patterns of LPS binding in the presence or absence of an extensive protease inhibitor cocktail. In addition, pretreatment of the PMN with the irreversible serine protease inhibitor DFP alters the detection of only one LPS-binding protein of approximately 63 kDa seen on this cell type. Second, examination of the time course of LPS binding to PMN indicates essentially equivalent qualitative profiles of LPS binding at all times up to 30 min. Were there significant degradation of higher-molecular-weight LPS-binding proteins, one might anticipate a greater accumulation of lower-molecular-weight LPS-binding proteins at longer incubation times. We have, however, occasionally noted in some PMN preparations an absence of high-molecular-weight proteins and an accumulation of low-molecular-weight species consistent with proteolytic degradation. In these circumstances, LPS-binding proteins are also of low molecular weight.

Our results, therefore, indicate the existence of multiple LPS-binding species on human PMN. Since binding can also be detected at 4°C , it is likely that many of these proteins are located on the cell membrane. It is of interest, however, that a number of neutrophil granule proteins with the potential to bind LPS have been identified, including lysozyme (33),

lactoferrin (26), bacterial permeability-inducing factor (41), cationic antimicrobial proteins (unpublished observations), and presumably acyloxyacylhydrolase (31). Although our studies have not, to date, investigated PMN uptake of LPS and binding to intracellular and/or granule constituents, we do not exclude the possibility that at least some of the LPS-binding proteins detected may represent granule constituents.

It is of interest that human platelets, in contrast to PMN, manifest two rather prominent LPS-binding proteins, including the 73-kDa protein. We have sometimes noted that the relative binding to these two proteins is dependent upon platelet concentration and that higher platelet concentrations favor binding to the 73-kDa protein. Currently, we do not understand the reason for this concentration dependence. It is, in any case, not totally without precedent that human platelets express LPS receptors, as has been previously postulated by Hawiger et al. (12). Although human platelet responses to LPS have been the subject of some controversy, there is solid evidence that these formed elements can respond to LPS. In this respect, as recently shown by Wright et al. (44), human PMN responses to LPS can be significantly enhanced by LPS-induced platelet-derived soluble factors. In addition, it has been shown that LPS will markedly enhance the human platelet secretory response to immune complexes and aggregated immunoglobulins (16).

The identification and characterization of LPS receptors on mammalian cells have been complicated by the fact that LPS can bind nonspecifically to cell membranes. In addition to interactions with membrane phospholipids, it is also possible that binding of LPS to membrane glycoproteins may occur through relatively nonspecific interactions. During the last several years, however, a number of groups have reported the results of experiments designed to identify specific LPS receptors (reviewed in references 8, 18, and 27). In addition to the results from our laboratory on the p73 LPS-binding protein, proteins of 96 kDa on the macrophage RAW263 cell line (9), 65 and 55 kDa on the macrophage J774 cell line (10), 47 kDa on hepatocytes (34), and 18 kDa on the mouse 70Z/3 pre-B cell line (14) have been identified. A study published by Dziarski has described a 70-kDa peptidoglycan-binding protein on mouse splenocytes which this investigator also reported binds LPS (5). The latter conclusion has not been confirmed in our own laboratory (22), and this question remains to be resolved. Further, Wright and Jong (45) and Wright et al. (47) have provided evidence that the CD11/CD18 membrane heterodimer adhesion proteins are responsible for the binding of LPS adsorbed to erythrocytes. Finally, Wright et al. (46) have reported that the CD14 membrane protein on monocytes serves as a binding site for complexes of LPS and the LPS-binding protein (LBP). Whether many or all of these interactions with LPS occur via specific binding has, to date, not been established, and this would apply to the binding interactions reported here as well. It would thus appear that there may be multiple mechanisms, both specific and nonspecific, by which macrophages and other host cells can recognize bacterial LPS.

In summary, we conclude that many of the human peripheral blood cell populations express an LPS-binding protein with physical and chemical properties similar to a 73-kDa receptor previously identified by our laboratory on mouse lymphocytes and macrophages. In addition, a number of secondary LPS-binding proteins have been identified, and the profiles of these binding proteins appear to be relatively unique for each of the cell populations investigated. The

precise role of these proteins in the various cellular responses to LPS remains to be established.

ACKNOWLEDGMENTS

This research was supported by research grant 2R37AI23447-06 from the National Institute of Allergy and Infectious Diseases, by a grant from Centocor Inc., Malvern, Pa., and by BRSG grant S07RR05373. Julie L. Halling is a scholar of the Wesley Foundation Cancer Research and Training Grant to the University of Kansas and Kansas State University.

We acknowledge Teresa Heitz, Department of Pathology, University of Kansas Medical Center, for expert technical assistance.

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