LIPOPOLYSACCHARIDE (LPS) BINDING PROTEIN OPSONIZES LPS-BEARING PARTICLES FOR RECOGNITION BY A NOVEL RECEPTOR ON MACROPHAGES

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The classical opsonins C3 and IgG bind to microorganisms and facilitate phagocytosis. Recent work suggests that additional proteins may also have opsonic activity. The acute-phase reactant, C-reactive protein (CRP), binds to phosphocholine residues on gram-positive bacteria and facilitates their interaction with phagocytes (1). Another acute-phase reactant, mannose binding protein (MBP),[1] binds to mannose residues on bacteria and fungi and facilitates recognition by phagocytes (2). We have investigated a third recently discovered acute-phase reactant, lipopolysaccharide binding protein (LBP) (3). LBP is a 60-kD glycoprotein present at concentrations <100 ng/ml in the serum of healthy rabbits. During the acute phase, LBP is synthesized by hepatocytes (Ramadori, G., K.-H. Meyer zum Buschenfelde, P. S. Tobias, J. C. Mathison, and R. J. Ulevitch, manuscript submitted for publication), and reaches concentrations of 30–50 μg/ml in serum. Here we show that LBP binds to LPS on the surface of bacteria and to LPS inserted into erythrocyte membranes. Binding of LBP to these particles dramatically enhances their interaction with macrophages. Particle-bound LBP is recognized by a receptor that is mobile in the plane of the membrane and that appears distinct from other opsonic receptors.

Materials and Methods

Reagents. LBP was purified from acute-phase rabbit serum (3), and appeared homogeneous on silver-stained gels. Anti–rabbit LBP was raised in goats. MBP was a gift of Dr. R. A. B. Ezekowitz (Harvard Medical School, Boston, MA). Bactericidal/permeability-increasing factor (BPI) was a generous gift of Dr. J. Gabay (Cornell Medical College, New York, NY). LPS from *Salmonella minnesota* (Re595 or wild type) was obtained from List Biological (Campbell, CA). mAbs IB4 against CD18 and 3G8 against FcγRIII (CD16) were as described (4); mAb 543 against CR1 was a gift of Dr. R. Schreiber (Washington University, St. Louis, MO); and mAbs 22 and IV.3, against FcγRI and FcγRII, were gifts from Dr. M. Fanger (Dartmouth Medical School, Hanover, NH). Pyrogen-free human serum albumin

[1] *Abbreviations used in this paper:* EBAV, avidin-coated biotinylated E; HSA, human serum albumin; LBP, LPS binding protein; MBP, mannose binding protein; MO, monocyte-derived macrophages.

(HSA) was from Armour Pharmaceutical Co. (Tarrytown, NY); and pyrogen-free PBS and DGVB^{2+} were from Whitaker MA Bioproducts (Walkersville, MD). NHS-biotin, Sulfo-NHS-biotin, and streptavidin were from Pierce Chemical Co. (Rockville, MD).

Surfaces. Tissue culture plastic surfaces were coated by incubation with 25 μg/ml protein (antibody, LBP, or HSA) or 1 μg/ml LPS for 1 h at 20°C. To form immune complexes, HSA-coated surfaces were incubated with anti-HSA antiserum (1:50) for an additional 30 min. In some cases, LPS-coated surfaces were subsequently treated with 10 μg/ml LBP for 30 min at 20°C. For assays of hydrogen peroxide production, all coated surfaces were exposed to 1 mg/ml HSA for 1 h before the addition of phagocytes. Coated surfaces were carefully washed with pyrogen-free PBS before the assays.

Cells. Monocyte-derived macrophages (MO) were obtained by culturing purified human monocytes in Teflon beakers for 3-10 d as described (5). Monolayers of fresh monocytes were obtained by allowing PBMC to adhere to protein-coated plastic for 45 min at 37°C. PBMC were purified from fresh blood by the method of English and Anderson (6). T cells, purified by rosetting with E, were a gift of J. Ming (The Rockefeller University). Human umbilical vein endothelial cell monolayers (7) were a gift of Dr. S. K. Lo (The Rockefeller University).

Sheep E were coated with IgG (EIgG) or IgM (EIgM) as described (5). C3bi was deposited on EIgM by incubating 2-10 × 10^8 EIgM in 1 ml of 10% C5-deficient human serum (Sigma Chemical Co., St. Louis, MO) for 30 min at 37°C. The erythrocytes were then washed and incubated for 10 min at 0°C in an EDTA-containing buffer. The resulting EC3bi bore no C3b as assayed by EDTA-resistant rosetting with MO. E were coated with LPS as described (8). The amount of LPS used in the preparation was varied to yield ELPS^{hi} (1-10 μg/4 × 10^7 E) or ELPS^{lo} (0.2-1 μg/4 × 10^7 E). ELPS^{lo} were coated with LBP by incubating equal volumes of ELPS^{lo} (10^8/ml) and LBP (10 μg/ml) for 20 min at 37°C. The resulting LBP-coated ELPS were washed and used immediately.

For certain experiments, E were also coated with LBP by an alternative method. E were first biotinylated by incubating 5 × 10^8 E with 250 μg Sulfo-NHS-biotin for 20 min at 5°C in 0.1 M sodium carbonate, pH 9.2, and LBP was biotinylated by incubating 50 μg LBP with 5 μg Sulfo-NHS-Biotin and dialyzing against PBS. The biotinylated protein was then linked to the biotinylated E through a streptavidin bridge. 10^8 washed, biotinylated E (EB) were incubated with 10 μg streptavidin for 30 min at 20°C to yield avidin-coated erythrocytes (EBAV). Preliminary experiments using fluoresceinated streptavidin showed that the EBAV were uniformly and intensely fluorescent, and no agglutination could be seen. 2.5 × 10^7 washed EBAV were incubated with 2.5 μg of biotinylated LBP for 30 min at 20°C to yield EBAV-LBP.

Salmonella typhimurium. LT2 *Gal* E was grown in the presence or absence of galactose to yield cells with a complete or truncated LPS, respectively (8). Exponentially growing cultures were washed, labeled with fluorescein, and adjusted to 2 × 10^8/ml in PBS as previously described (8).

Assays. Agglutination of LPS-coated erythrocytes was measured by shaking 10^6 ELPS^{hi} in 10 μl of diluted LBP for 30 min at 21°C in a round-bottomed microtest plate. Agglutination was read from the settling pattern.

Binding of ligand-coated E to MO was measured as described (5). Briefly, Terasaki tissue culture plates were coated with HSA or other proteins (see above), and monolayers of MO were established by incubating 5 μl of cells (0.5 × 10^6/ml in PBS containing 3 mM glucose, 0.5 mg/ml HSA, and 0.3 U/ml aprotinin [Sigma Chemical Co.]) for 45 min at 37°C. Ligand-coated E and the indicated proteins were added to the monolayers. E were allowed to settle for 10 min at 0°C, then the plate was warmed to 37°C for 15 min. Unattached E were removed by washing and attachment was scored by phase contrast microscopy. Binding of fluoresceinated *Salmonella* was assessed by a similar method using a 15-min incubation at 37°C as previously described (8). Results are reported as attachment index, the number of E or bacteria per 100 MO. Phagocytosis of ligand-coated E was measured by similar methods (5), with the exception that incubation of MO with the E was for 45 min at 37°C, and uningested E were lysed by brief exposure to hypotonic medium before scoring the wells. The attachment or phagocytic index obtained by a fixed procedure varied substantially from day to day. This variation prevented averaging of separate experiments, and representative experiments are thus displayed.

Release of hydrogen peroxide during spreading of MO on coated surfaces was measured as described (9). Briefly, 3-4 × 10⁴ MO (day 3 or 4) were added to protein-coated tissue culture wells containing horseradish peroxidase and 2.4 nmol of scopoletin. The plate was incubated at 37°C, and at intervals the consumption of scopoletin was measured using an automated fluorescence plate reader. Results are averaged from hexaplicate wells and are presented as nmol peroxide produced per well. Addition of the control stimulant, PMA (100 ng/ml), resulted in rapid evolution of peroxide that was identical in rate and extent for all coated surfaces tested.

Results

LBP Binds to LPS Inserted into Erythrocyte Membranes.   Addition of as little as 0.5 μg/ml of LBP to ELPS^{hi} caused agglutination. Since LPS partitions into the membrane of E by hydrophobic interactions with phospholipids, this observation suggests that LBP recognizes the exposed hydrophilic portion of the lipid A, and that LBP has the potential to form multimers. The ELPS were not strongly agglutinated and could be disrupted by gentle pipetting.

LBP Enhances Binding of ELPS and Salmonella to Macrophages.   Gram-negative bacteria and LPS-coated erythrocytes bind to MO through an interaction of LPS with members of the CD18 complex of receptors on leukocytes (8). We asked whether the addition of LBP could perturb that interaction. Initial experiments used E prepared with high levels of LPS. These ELPS^{hi} bound avidly to MO, and the addition of LBP slightly *enhanced* binding. To examine the nature of this enhancement, E were prepared with low levels of LPS. ELPS^{lo} were poorly bound by MO, but the addition of LBP caused a dramatic enhancement of binding (Figs. 1 and 2). Enhanced binding was dose dependent with a maximal effect at ∼1 μg/ml LBP. The specificity of this effect is indicated by the observation that another acute-phase reactant, mannose binding protein, did not affect binding of ELPS^{lo} to MO (Fig. 2) at concentrations as high as 100 μg/ml (data not shown); another LPS-binding protein, BPI, did not affect binding at concentrations as high as 10 μg/ml (not shown); and polyclonal anti-LBP antiserum (1:200) caused a 20-fold reduction in the rosetting of ELPS^{lo} caused by LBP.

The capacity of LBP to enhance interaction of ELPS with MO was also dependent on the amount of LPS in the erythrocyte membrane (Fig. 3). LBP could effectively mediate binding of E prepared with amounts of LPS 20-100-fold less than the amount needed to sustain a direct interaction between ELPS and MO.

Strains of gram-negative bacteria that express a truncated LPS (rough strains) are avidly bound by MO, but smooth strains, with a complete LPS, are bound poorly (8). Since LBP binds equally well to both smooth and rough LPS (10), we asked if LBP could opsonize smooth *Salmonella*. Addition of LBP caused a dramatic enhancement in the binding of smooth *Salmonella* to MO (Table I). Addition of LBP also enhanced the binding of rough *Salmonella*, but the effect appeared less dramatic due to the avid binding of unopsonized bacteria. Thus, LBP can enhance the interaction of a live bacterium with MO.

MO Recognize Complexes of LBP with LPS.   In the experiments above, LBP was added together with the MO and the ELPS. To determine if LBP binds to MO or ELPS, the cells were separately incubated with LBP, washed, and then combined. Pretreatment of ELPS^{lo} with LBP strongly enhanced binding to MO (Table II) with a dose-response curve identical to that observed in the coincubation experients (data
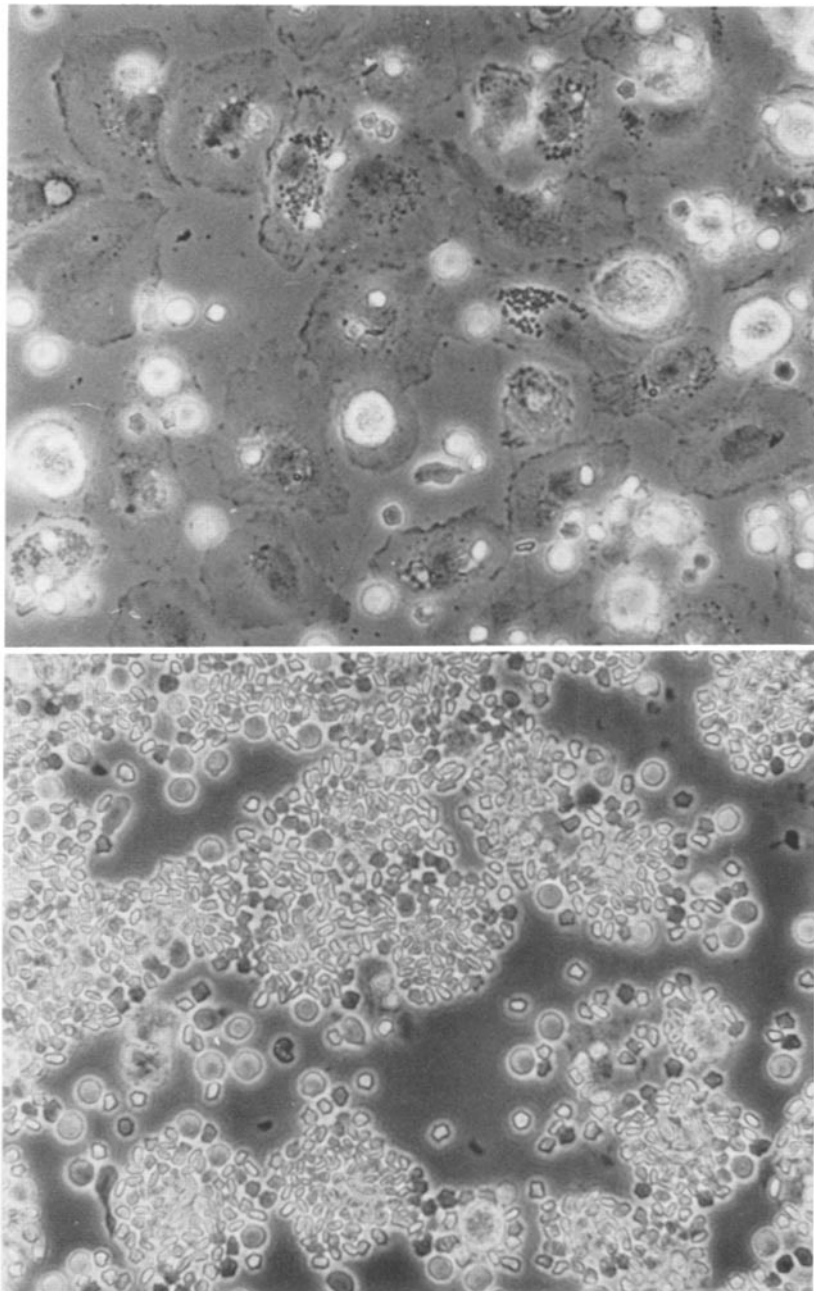
FIGURE 1.   LBP enhances the interaction of ELPS with MO. Monolayers of MO were incubated with ELPS[10] in the absence (*top*) or presence (*bottom*) of 5 μg/ml LBP. The monolayers were washed, fixed with glutaraldehyde, and photographed with phase contrast illumination.
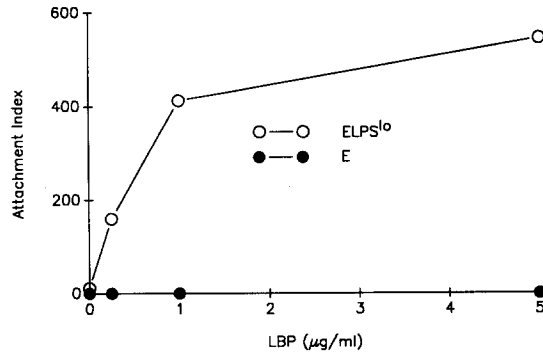
FIGURE 2. LBP enhances the interaction of ELPS with MO. Monolayers of MO were incubated with E or ELPS[lo] in the presence of varying doses of LBP, and attachment index was scored. A control acute-phase protein, MBP (5 μg/ml), caused no enhancement of binding of ELPS[lo] (attachment index = 4.9). Results are representative of four separate experiments.
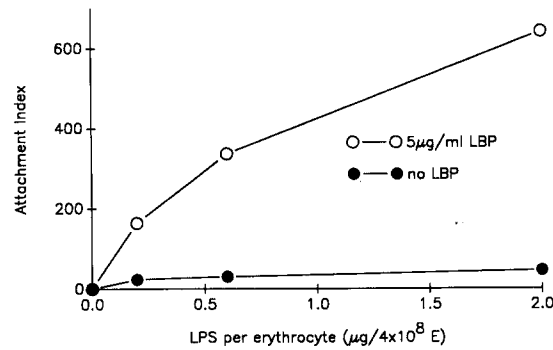

FIGURE 3. LBP-dependent binding of ELPS to MO depends on the density of LPS in the E membrane. ELPS were prepared with varying doses of LPS then incubated with monolayers of MO in the presence or absence of 5 μg/ml LBP. Results are representative of four separate experiments.

TABLE I

LBP Enhances Binding of Salmonella to MO

LBP	Attachment index	
	Smooth S. typhimurium	Rough S. typhimurium
−	273	1,096
+	1,661	2,109

Smooth and rough preparations of S. typhimurium LT2 were obtained by growing GalE mutants of this strain in the presence or absence of galactose as described (8). The binding of bacteria to monolayers of macrophages was then measured in the presence or absence of 2.5 μg/ml LBP. Addition of LBP caused a 5.9 ± 1.9 (n = 4)-fold enhancement in the binding of smooth bacteria to MO.

not shown). This result suggests that LBP associates stably with ELPS and that the surface-bound LBP is recognized by MO. Pretreatment of MO, on the other hand, did not affect the subsequent binding of ELPS (Table II).

LBP on the surface of ELPS is complexed with LPS. To determine if MO bind to LBP in the absence of LPS, LBP was biotinylated and attached to streptavidin-coated erythrocytes. The resulting EBAV-LBP were not bound by MO (Fig. 4), but addition of LPS caused strong attachment of the ELBP to MO. The LPS appeared to enhance adherence of EBAV-LBP by binding to LBP since the amount of LPS needed to cause attachment of ELBP was ~50-fold less than needed to cause attachment of E lacking LBP (Fig. 4). Further, the LPS-treated ELBP bound avidly

Table II

Pretreatment of ELPS But Not MO with LBP Enhances Their Interaction

	Attachment Index		
Condition	Exp. 1	Exp. 2	Exp. 3
No LBP	6	17	4
Pretreat ELPS^{lo}	820	715	942
Pretreat MO	5	21	16
Coincubate LBP, ELPS^{lo}, and MO	629	520	796

Binding of ELPS^{lo} (0.2 μg/4 × 10^8 E) to monolayers of MO was measured as described in Materials and Methods. ELPS^{lo} or MO were pretreated at 37°C with 5 μg/ml LPB for 20 min and washed before the assay. Alternatively, 5 μg/ml LBP was added during the assay of attachment.

to CD18-deficient MO, which do not bind ELPS (data not shown). Thus, LBP must be complexed with LPS in order to be recognized by MO.

LBP Is Recognized by a Mobile Receptor Restricted to Mononuclear Phagocytes. LBP-treated ELPS bound to virtually 100% of monocytes and MO, suggesting that binding activity is present on all members of these populations. To determine whether LBP interacts with other cell types, monolayers of PMN, T cells, and umbilical vein endothelial cells were incubated with LBP-treated ELPS^{lo}. No binding was observed (data not shown). Similarly, lymphocytes that occasionally contaminate MO preparations were never observed to bind LBP-coated E. Thus, the capacity to bind LBP-coated particles appears to be a property restricted to mononuclear phagocytes.

The existence of a specific receptor for LBP was demonstrated by allowing MO to spread on surfaces coated with complexes of LPS and LBP. Surface-bound LBP strongly downmodulated binding of LBP-treated ELPS^{lo} but had no effect on the binding of EIgG or EC3bi (Table III). These observations indicate that LBP is recognized by a molecule that is mobile in the plane of the membrane, and suggest that this receptor is different from CR3 and FcR.

LBP Does Not Interact with CR3, or FcR. Since LPS is known to be recognized by CR3 and other members of the CD18 complex (LFA-1 and p150,95) (8), it appeared possible that LBP enhanced binding of ELPS by facilitating the interaction of a low amount of LPS with these receptors. Several observations, however, rule out this possibility. LBP caused strong binding of ELPS to monocytes isolated from
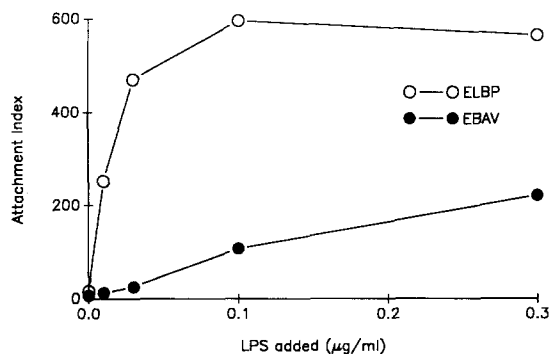

Figure 4.   MO do not recognize LBP in the absence of LPS. E-coated with biotin and streptavidin alone (EBAV) were incubated with biotinylated LBP to yield ELBP. Both ELBP and EBAV were incubated with graded doses of LPS for 20 min at 37°C, washed, and binding to monolayers of MO was measured.

TABLE III

Receptors for LBP Are Mobile in the Plane of the Membrane

Surface	ELPS^{lo} + LBP	ELPS^{hi}	EC3bi	EIgG
HSA	833	507	915	621
HSA–anti-HSA	795	455	1,051	45
IB4	846	149	200	253
LPS-LBP	147	628	1,161	762

Plastic surfaces were coated with HSA (500 μg/ml), mAb IB4 (25 μg/ml), or LPS (1 μg/ml) for 2 h at 21°C and washed thoroughly. Where indicated, anti-HSA (1:40 dilution of rabbit anti-HSA antiserum) or LBP (5 μg/ml) was added and incubated for 30 min at 20°C. MO were allowed to spread on the washed, coated surfaces for 45 min at 37°C, and after an additional wash, the ligand-coated E were added. ELPS^{hi} were prepared with 3 μg LPS/4 × 10^7 E. ELPS^{lo} were prepared with 0.3 μg LPS/4 × 10^7 E then treated with 5 μg/ml LBP as described in Materials and Methods. Data shown are representative of four separate experiments.

two patients with a congenital deficiency of CD18 (Table IV). As expected, the CD18-deficient cells exhibited negligible binding of ELPS^{hi} or EC3bi in parallel assays. Further evidence against the participation of CD18 molecules in recognition of LBP-treated ELPS^{lo} comes from experiments in which CD18 molecules were depleted from the apical surface of MO by allowing them to spread on surfaces coated with anti-CD18 mAbs. mAb IB4 downmodulated CD18 molecules as shown by the decreased binding of EC3bi and ELPS^{hi}, but LBP-treated ELPS^{lo} bound normally to these cells (Table III). Finally, depletion of Ca^{2+} and Mg^{2+} completely blocks binding of both C3bi and LPS to the CD18 complex (5, 8), but binding of LBP-treated ELPS^{lo} was equivalent in EDTA-containing buffers (data not shown).

The participation of Fc receptors in the recognition LBP was also ruled out. Spreading of cells on an immune-complex coated surface strongly downmodulated Fc receptors as assayed by the binding of EIgG. However, the binding of LBP-coated ELPS^{lo} was unaffected (Table III). Similar studies showed that surface-bound mannose binding protein and surface-bound mAbs against FcRI, FcRII, FcRIII, and

TABLE IV

LBP Mediates Binding of ELPS^{lo} to Monocytes from CD18-deficient Patients

Subject	Attachment index			
	ELPS^{hi}	ELPS^{lo}	ELPS^{lo} + LBP	EC3bi
Control 1	108	31	282	129
Control 2	185	27	437	162
Patient 1	17	15	394	4
Patient 2	5	14	529	16

Monolayers of monocytes from two CD18 deficient patients (Wright, S. D., P. A. Detmers, R. Adamowski, L. G. Kabbash, and M. Pabst, manuscript in preparation) and two normal adult controls were incubated with EC3bi, ELPS^{hi} (3μg/4 × 10^{8}E), ELPS^{lo} (1 μg/4 × 10^8 E), and attachment index was measured. Where indicated, 2.5 μg/ml LBP was added with the ELPS^{lo}.

CR1 had no effect on the binding of LBP to MO (not shown). These data suggest that LBP is not recognized by CR1, CR3, FcR or mannose binding protein receptors.

Receptors for LBP Enhance Fc-mediated Phagocytosis. Addition of anti-E IgG caused LBP-coated ELPS[lo] to be avidly phagocytosed by MO (Fig. 5). The dose of anti-E IgG needed for half-maximal phagocytosis was fivefold less than that needed to induce phagocytosis of uncoated E (Fig. 5). LBP thus appears to act synergistically with IgG to induce a phagocytic response. In keeping with previous reports (11), deposition of C3bi on E also enhanced phagocytosis mediated by IgG, and the extent of this enhancement was similar to that caused by LBP (Fig. 5).

We also measured phagocytosis mediated by LBP alone. Though LBP-coated ELPS formed florid rosettes with Mϕ, none of the bound E were phagocytosed by either resting (Fig. 5), fibronectin-, or PMA-stimulated MO (data not shown). Parallel studies showed strong fibronectin- and PMA-stimulated phagocytosis of EC3bi. A possible explanation for the absence of LBP-mediated phagocytosis is the high lateral mobility of LPS on the surface of an erythrocyte. The LPS could "cap" on the pole of the E attached to the MO, leaving insufficient ligand on the circumference of the E to guide an advancing pseudopod. To prevent such capping, biotinylated LBP was linked to biotinylated E proteins as described in Fig. 4 above. Again, none of the E bound in this way were phagocytosed by either resting or PMA-stimulated MO (phagocytic index = 0). Parallel studies showed that E coated with biotinylated F(ab)$_2$ of an anti-CD18 mAb (IB4) were readily phagocytosed (phagocytic index = 482). Thus, receptors for LBP cannot by themselves initiate phagocytosis of a coated erythrocyte.

Receptors for LBP Do Not Initiate an Oxidative Burst. To determine whether interaction of LBP with its receptor initiates a cytotoxic response from MO, we measured the production of hydrogen peroxide during the interaction of MO with coated surfaces (Fig. 6). Surfaces coated with IgG antibody-antigen complexes caused brisk release of peroxide from macrophages. Surfaces coated with LBP or LBP-LPS complexes, however, caused no release of peroxide above baseline. The failure to produce peroxide was not the result of inadequate interaction of LBP with its receptor. Binding to LPS-coated surfaces caused a small release of peroxide (12% of that stimulated by immune complexes or PMA), confirming that LPS was present on the surface. Addition to LBP to LPS-coated surfaces blocked the release caused by LPS, thus confirming that LBP effectively interacted with LPS in this experiment. Fi-
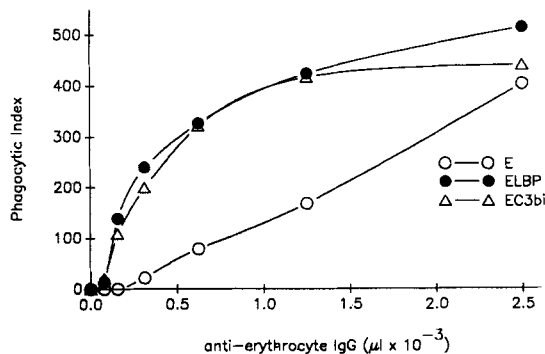

Figure 5. LBP enhances Fc-mediated phagocytosis. Monolayers of MO (day 5 culture) were incubated for 45 min with E, ELBP, or EC3bi in the presence of varying dilutions of anti-E IgG. Phagocytosis of the E was determined as described in Materials and Methods. ELBP were obtained by adding 1 μg/ml LBP to ELPS[lo] (0.3 μg LPS/4 $\times$ 10^{7}E) during incubation with MO. Attachment of these E in the absence of anti-E IgG was as follows: E, attachment index (AI) = 0; EC3bi, AI = 417; ELBP, AI = 404. Results are representative of six separate experiments.
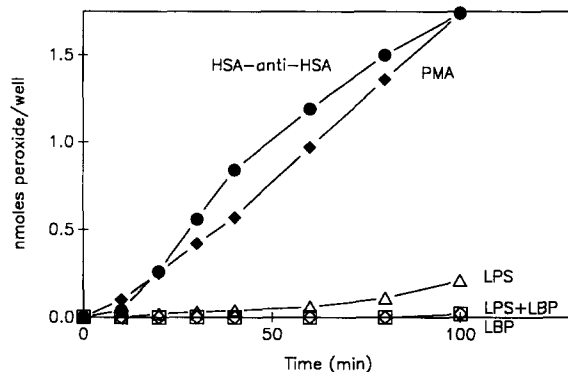
FIGURE 6. Secretion of hydrogen peroxide during spreading of MO on ligand-coated surfaces. 3×10^4 MO (day 3 of culture) were added to coated microtiter wells and the evolution of hydrogen peroxide was measured at intervals. Brisk production of peroxide occurred during spreading on immune complexes (HSA-anti-HSA, *filled circles*) or in response to the soluble agonist, PMA (*filled diamonds*). Low but reproducible peroxide release was observed during interaction with LPS-coated surfaces (*open triangles*). However, spreading on LBP-coated surfaces (*open square*) caused no release, and coating of LPS-coated surfaces with LBP (*open diamond*) prevented the LPS-induced generation of peroxide. Results are the average of hexuplicate determinations with standard error smaller than the symbols.

nally, parallel experiments showed that spreading of MO on LBP or LPS+LBP-coated surfaces caused down modulation of the binding of LBP-treated ELPS[lo] thus confirming that ligation of LBP receptors had occurred. LBP did not impair the production or measurement of peroxide since MO in LBP-coated wells exhibited normal peroxide evolution in response to PMA (data not shown). Thus LBP receptors appear incapable of triggering an oxidative burst.

Discussion

LBP recognizes the lipid A region of LPS and forms stoichiometric complexes with both rough and smooth forms (10). LBP bears NH_2-terminal sequence homology with another LPS-binding protein, BPI. BPI is stored in the specific granules of PMN (12) and kills gram-negative bacteria by binding LPS and disrupting the permeability barrier (13). In contrast to BPI, LBP is not directly cytotoxic (14) and its precise biological function has been obscure. Here we show that LBP functions as an opsonin since it binds bacteria and facilitates their binding and phagocytosis by macrophages. We presume that LBP binds LPS through a domain that is homologous with the LPS-binding domain of BPI, but we predict that the attachment of LBP to cells is mediated by a domain unique to LBP.

LBP on the surface of LPS-coated particles appears to be recognized by specific receptors on the MO which are mobile in the plane of the membrane. LBP-coated particles bind to MO but not other blood cells, and the binding activity on the apical surface of MO is depleted by spreading of cells on substrates coated with LBP-LPS complexes. The receptors for LBP appear distinct from other opsonic receptors since surface-bound antibodies to CR1, CR3, and FcR did not reduce the binding of LBP-coated particles. Experiments to identify the receptor for LBP are currently in progress.

The classical opsonin, IgG, facilitates the binding of IgG-coated particles, their phagocytic engulfment, and the release of toxic compounds such as hydrogen peroxide. The other classical opsonin, C3, facilitates principally the binding of C3-coated par-

ticles. Phagocytosis by unstimulated MO is observed only if the C3-coated particles also bear IgG (11), and evolution of hydrogen peroxide is not initiated (15). The opsonic activity LBP most closely resembles that of C3. LBP-coated particles are avidly bound by MO, but binding does not initiate phagocytosis (Fig. 5) or release of hydrogen peroxide (Fig. 6). LBP also acts like C3 in that it enhances phagocytosis of particles coated with low amounts of IgG (Fig. 5). The opsonic effect of LBP differs from that of C3 in only one respect. While complement proteins may initiate phagocytosis if MO are treated with an ancillary stimulus such as PMA (5) or fibronectin (16), LBP does not mediate phagocytosis even in such optimally stimulated cells.

By acting as an opsonin, LBP can be expected to limit the spread of gram negative bacteria in an animal. The appearance of LBP during the acute phase makes it well suited to combating infection, and we suggest that LBP represents a defense mechanism against gram negative bacteria.

Summary

Lipopolysaccharide binding protein (LBP) is an acute-phase reactant that binds bacterial LPS. We show that LBP binds to the surface of live *Salmonella* and to LPS-coated erythrocytes (ELPS), and strongly enhances the attachment of these particles to macrophages. LBP bridges LPS-coated particles to macrophages (MO) by first binding to the LPS, then binding to MO. Pretreatment of ELPS with LBP enabled binding to MO, but pretreatment of MO had no effect. Moreover, MO did not recognize erythrocytes coated with LBP unless LPS was also added, thus suggesting that interaction of LBP with LPS results in a conformational change in LBP that allows recognition by MO. Binding of LBP-coated particles appears to be mediated by a receptor found on blood monocytes and MO but not on other leukocytes or umbilical vein endothelium. The receptor is mobile in the plane of the membrane since binding activity on MO was downmodulated upon spreading of cells on surfaces coated with LBP-LPS complexes. The receptor appears to be distinct from other opsonic receptors since downmodulation of CR1, CR3, FcγRI, FcγRII, and FcγRIII with mAbs did not affect binding of LBP-coated particles, and leukocytes from CD18-deficient patients bound LBP-coated particles normally.

Coating of erythrocytes with LBP-LPS complexes strongly enhanced phagocytosis observed in the presence of suboptimal amounts of anti-erythrocyte IgG. However, binding mediated by LBP-LPS complexes alone caused neither phagocytosis of the LBP-coated erythrocytes nor initiation of an oxidative burst. The results of our studies define LBP as an opsonin. During the acute phase, LBP can be expected to bind gram-negative bacteria and bacterial fragments and promote the interaction of coated bacteria with phagocytes.

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