ADHESION-PROMOTING RECEPTORS ON HUMAN MACROPHAGES RECOGNIZE *ESCHERICHIA COLI* BY BINDING TO LIPOPOLYSACCHARIDE

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Bacterial LPS (endotoxin) causes profound physiologic effects in humans and animals. These include fever, shock, and induction of the acute-phase response (1). The cell type primarily responsible for these effects is the macrophage which synthesizes large amounts of IL-1 and cachectin/tumor necrosis factor (TNF) in response to LPS (2, 3). It is assumed that the high sensitivity to LPS derives from receptors for LPS on the macrophages. Because LPS is located on the outer leaflet of the bacterial outer membrane, we hypothesized that receptors for LPS might mediate the binding of bacteria to macrophages previously observed by several investigators (4–6). We report here that macrophages bind *Escherichia coli* by recognizing LPS.

We have also characterized the receptors responsible for the recognition of *E. coli*. It is known that human macrophages express three structurally homologous receptors, CR3, lymphocyte function–associated antigen (LFA-1),[1] and p150,95. Each of these surface glycoproteins consists of an $\alpha_1\beta_1$ dimer composed of a 150–190-kD α chain and a 95-kD β chain. The β chain is identical in each of the three proteins, but the α chains of CR3, LFA-1, and p150,95 are structurally and antigenically distinct (7, 8). Here we show that each of these dimers can promote the binding of *E. coli* to human macrophages.

Materials and Methods

Reagents. Polymyxin B sulfate, chlorpromazine, aprotinin, and yeast mannan were obtained from Sigma Chemical Co. (St. Louis, MO), 2,4-dinitrophenol from Eastman Kodak Co. (Rochester, NY), phosphatidylcholine from Supelco, Inc. (Bellefonte, PA), [^{3}H]phosphatidylcholine (40 mCi/mmol) from New England Nuclear (Boston, MA), and LPS (*Salmonella minnesota* R595 [Re]) from List Biological Laboratories (Campbell, CA). LPS was iodinated with Bolton-Hunter reagent as recommended by the manufacturer (New England Nuclear), and unbound label was removed by gel filtration. The specific activity of the product was 1.1×10^4 cpm/μg. Lipid IVa was a generous gift of Dr. C. R. H. Raetz (Univ. of Wisconsin, Madison, WI), and β-glucan was a gift of Dr. J. Czop (Harvard Medical School, Boston, MA).

Antibodies. Monoclonal antibody (mAb) OKM1, directed against the α chain of CR3 (8), was a gift of Dr. G. Goldstein (Ortho Pharmaceuticals, Raritan, NJ); mAb TS1/22,

This work was supported by grants AI-22003 from the U.S. Public Health Service and JFRA-103 from the American Cancer Society.

[1] *Abbreviations used in this paper:* HSA, human serum albumin; LFA-1, lymphocyte function-associated antigen; MO, monocyte-derived macrophage.

 J. EXP. MED. © The Rockefeller University Press · 0022-1007/86/12/1876/13 $1.00
Volume 164 December 1986 1876–1888

directed against the α chain of LFA-1 (9), was a gift of Dr. T. Springer (Dana-Farber Cancer Institute, Boston, MA); mAb Leu-M5, directed against the α chain of p150,95 (10) was a gift of Dr. Louis Lanier (Becton, Dickinson & Co., Mountain View, CA); mAb IB4, directed against the β chain of CR3, LFA-1, and P150,95 (8), and mAb 3G8, directed against the low-avidity Fc receptor of neutrophils (11) were as previously described.

Cells. Human monocytes were purified from buffy coats on Percoll gradients and were cultured in 12.5% human serum in Teflon beakers as previously described (12). Upon cultivation for 4–10 d, the cells matured into macrophages (MO). Neutrophils were purified from fresh human blood on Ficoll-Hypaque gradients (13).

Sheep erythrocytes (E) were coated with C3b (EC3b), C3bi (EC3bi), or IgG (EIgG) as described (12). E were coated with LPS by a modification of the method of Pangburn et al. (14). Freshly sonicated dilutions of LPS were incubated for 60 min at 37°C with E at 10^8 E/ml in an EDTA-containing buffer (5 mM veronal buffer, pH 7.5, 140 mM NaCl, 0.1% gelatin, 10 mM EDTA). Cells were then washed three times in buffer containing divalent cations (2.5 mM veronal buffer, pH 7.5, 75 mM NaCl, 2.5% dextrose, 0.06% gelatin, 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$). In some experiments, radioiodinated LPS was used and the amount of incorporation into E was directly measured. Uptake varied from 0.5 to 1.5% of the amount of LPS offered.

Bacteria. *E. coli* K12 (strain MC1061) was obtained from Dr. D. Portnoy (The Rockefeller University). Exponentially growing cultures were obtained by innoculating L broth (1% tryptone, 0.5% yeast extract, 0.15 M NaCl) with a freshly prepared overnight culture as described (15). Under these growth conditions, our isolate exhibited neither motility nor the capacity to agglutinate sheep or guinea pig E, thus suggesting that functional flagella and type I pili were not expressed. Cells were washed three times in PBS (16) and labeled with FITC by a modification of the method of Hed (17). Bacteria were suspended to a density of 10^9 cells/ml in 0.05 M sodium carbonate, pH 9.2, 0.1 M NaCl containing 1 mg/ml FITC (Calbiochem-Behring Corp., San Diego, CA). After 15 min incubation at 20°C, the cells were washed three times in PBS. Control experiments indicated that fluoresceination did not alter the viability of the cultures.

E. coli 0111B4 *Gal*E and *S. typhimurium* LT2 *Gal*E were obtained from Dr. R. C. Goldman (National Institutes of Health, Bethesda, MD). *Gal*E strains lack UDP-glucose epimerase and thus cannot synthesize galactose (18). In the absence of exogenous galactose, these cells synthesize a truncated, "rough" LPS molecule (see *arrow*, Fig. 1). Addition of galactose to cultures of these cells allows the synthesis of wild-type "smooth" LPS (18, 19). Exponentially growing cultures of these cells were prepared in L broth to yield rough bacteria, and parallel cultures were incubated with 100 μM galactose to yield smooth bacteria. The cells were then washed and labeled with FITC as described above.

Attachment of Bacteria and Ligand-coated Erythrocytes to MO. Monolayers of phagocytes were prepared as follows. Cells were suspended at 0.5×10^6 cells/ml (MO) or 2×10^6 cells/ml (neutrophils) in PBS containing 3 mM glucose, 0.5 mg/ml human serum albumin (HSA; Worthington Biochemical Corp., Freehold, NJ), and 0.3 U/ml aprotinin. Terasaki tissue culture plates (Miles Laboratories Inc., Naperville, IL) were coated with HSA (1 mg/ml), or with purified mAb (30–50 μg/ml) by a 60-min incubation at 20°C. The culture surfaces were washed, 5 μl of phagocyte suspension was added per well, and the cells were allowed to spread at 37°C for 45 min.

Attachment of ligand-coated E to monolayers of phagocytes was determined by adding 5×10^5 E/well and incubating for 45 min at 37°C. The attachment of E to phagocytes was scored by phase-contrast microscopy as previously described (12). Results are reported as attachment index, the number of E bound per 100 phagocytes. The attachment of bacteria was measured by adding 2.5×10^5 FITC-labeled bacteria per well. To bring the bacteria into proximity with the monolayer, the plates were centrifuged at 600 *g* for 5 min. After an incubation of 30–45 min, unattached bacteria were washed away, and attachment was scored with the aid of a fluorescence microscope. The fluoresceination rendered the bacteria readily distinguishable from similarly sized organelles in the MO. Control experiments in which the binding of bacteria was measured by transmitted-light

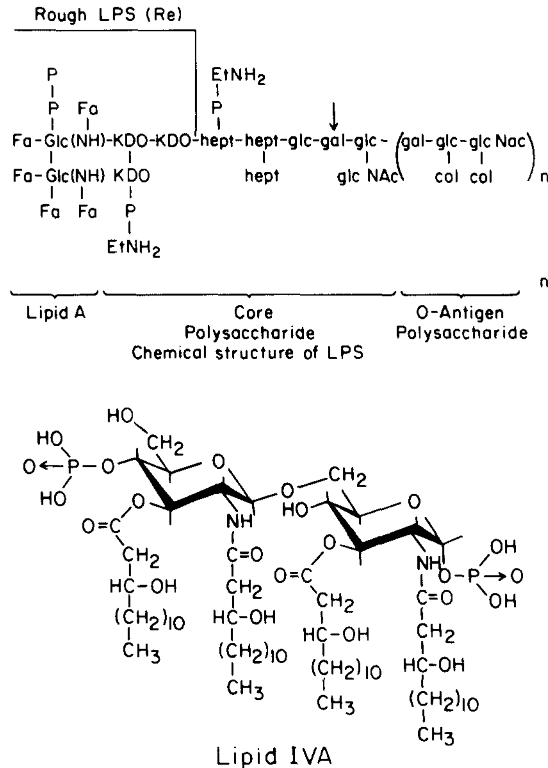

FIGURE 1. Structure of bacterial lipopolysaccharide

microscopy after staining the cells with Wright-Giemsa indicated that the FITC did not alter the extent or the specificity of the binding.

Results

MO Bind E. coli in the Absence of Serum Opsonins. Exponentially growing *E. coli* K12 were added to monolayers of human MO at a ratio of ~50 bacteria per MO. After 45 min at 37°C, 3–12 bacteria were found adherent to each MO. The attachment of *E. coli* to MO was unlikely to be mediated by serum-derived opsonins because the assay was performed with cells washed into PBS. To rule out the possibility that anti-*E. coli* antibodies were carried over into the assay through interaction with receptors for IgG on the phagocytes (Fc receptors), MO were cultured for 6 d in the presence of serum that had been adsorbed with *E. coli*. Removal of anti-*E. coli* antibodies had no effect on the attachment of *E. coli* (data not shown).

A contribution of Fc receptors to the binding of *E. coli* was further eliminated by observing the attachment of bacteria to MO spread on surfaces coated with IgG in antigen–antibody complexes. After phagocytes spread on such ligand-coated surfaces, Fc receptors diffuse in the plane of the membrane and are trapped by interaction with substrate-bound ligand (20, 21). The apical portion of the MO membrane is thus depleted of receptors. Table I shows that substrate-bound immune complexes caused significant downmodulation of Fc receptors,

TABLE I

Binding of E. coli by MO Does Not Require Fc Receptors, Secreted Complement Components, Mannosyl/Fucosyl Receptors, or Mannose-sensitive Adhesins

Inhibitor	Attachment index				
	E. coli	EIgG	EC3b	EC3bi	ELPS
None	475	1,014	1,026	1,140	661
Immune complexes*	526	105	1,179	1,135	690
50 mM mannose	453	871	1,350	1,205	693
1 mg/ml mannan	331	1,008	1,013	1,152	698
Fab anti-C3	453	930	49	36	756

Monocytes were cultured for 4 d in Teflon beakers, and monolayer cultures were then established. The binding of *E. coli* or the indicated ligand-coated E was then measured in the presence of 50 mM α-D-mannose, 1 mg/ml mannan, or 50 μg/ml Fab anti-C3 antibody. Data are presented as attachment index, the number of particles attached per 100 phagocytes.

* To remove Fc receptors from the apical surface of the MO, monolayers were formed on substrates coated with DNP groups and anti-DNP IgG (20). The resulting immune complex–coated surface withdraws Fc receptors as indicated by the inhibition of the binding of EIgG.

in that IgG-coated erythrocytes (EIgG) could no longer bind to the apical portion of the MO. The binding of *E. coli*, however, was not affected.

MO synthesize and secrete proteins of the complement cascade, and it has been suggested that phagocytes may opsonize particles with this endogenous store (22). To eliminate the possible contribution of MO-derived C3 to the recognition of *E. coli*, binding assays were performed in the presence of saturating concentrations of Fab anti-C3 antibodies. These antibodies completely block the binding of erythrocytes coated with ~80,000 C3b or C3bi (Table I). However, Fab anti-C3 has no effect on the binding of *E. coli*. It is thus unlikely that autoopsonization of *E. coli* with MO-derived C3 accounts for the observed recognition.

Several naturally occurring isolates of *E. coli* possess pili that recognize mannose residues on the surfaces of eukaryotic cells, and these adhesins can promote binding of bacteria to phagocytes (23, 24). Such mannose-recognizing structures do not play a role in the recognition described here because concentrations of mannose that completely block the mannose-sensitive adhesins (50 mM, reference 24) have no effect on the binding of *E. coli* (Table I). MO also express a receptor for mannose and fucose that participates in the binding of zymosan (25) and *Leishmania* (26). This receptor appears not to participate in the recognition of *E. coli* inasmuch as concentrations of yeast mannan that block binding of zymosan and *Leishmania* (1 mg/ml mannan) do not inhibit binding of *E. coli* (Table I). Finally, MO recognize several particles (rabbit E and neuraminidase-treated mouse E) that activate the alternative pathway of complement, and this recognition can be blocked with soluble β-glucan (27). The binding of *E. coli* to MO, however, is not blocked by 100 μg/ml β-glucan, a concentration that is 100-fold higher than required to block the binding of alternative pathway activators (data not shown). These data suggest that MO recognize *E. coli* by a novel mechanism that does not require IgG, complement proteins, adhesins on the bacterial surface, mannose receptors, or β-glucan receptors.

The characteristics of the binding of *E. coli* to MO were studied. Binding

TABLE II

Binding of E. coli to MO Requires Divalent Cations

Divalent cation	Attachment index				
	E. coli	EC3b	EIgG	EC3bi	ELPS
Ca^{2+} and Mg^{2+}	765	803	870	1,217	853
Ca^{2+}	186	796	850	286	300
Mg^{2+}	145	719	850	228	120
None	85	803	781	68	121

Monocytes were cultured for 4 d in Teflon beakers. Monolayers of phagocytes were then prepared and the cells were washed into PBS made without divalent cations (PD). The indicated cations were added (0.5 mM), and the binding of *E. coli* or the indicated ligand-coated E was determined as described in Materials and Methods. Data are presented as attachment index, the number of particles attached per 100 phagocytes. Results shown are representative of three separate experiments.

TABLE III

Temperature Dependence of the Binding of E. coli to MO

Temperature	Attachment index				
	E. coli	EC3bi	EIgG	EC3b	ELPS
37°C	273	1,479	881	1,843	1,800
18°C	54	596	1,009	1,521	80
0°C	17	153	1,045	1,456	21

Monolayers of MO (7-d cultures) were incubated for 30 min with *E. coli* or the indicated ligand-coated E at the temperatures shown, and the attachment of particles was then determined. Results shown are representative of four separate experiments.

required both Ca^{2+} and Mg^{2+} (Table II) and occurred only at warm temperatures. Binding was lowered three- to fivefold when the temperature was reduced to 18°C and was absent at 0°C (Table III). The requirements for divalent cations and warm temperatures are unlike those for the binding of IgG or C3b-coated particles (Table III). However, these characteristics closely resemble the requirements for binding of C3bi-coated particles to MO (12, and Tables I and II).

Receptors on MO Recognize LPS. In order to bind live *E. coli*, receptors on the phagocyte must recognize an external constituent of the bacterium. Because the strain of *E. coli* used in these experiments has neither pili nor flagella, we examined the most prevalent molecule on the outer membrane, LPS. We first observed the binding of strains of bacteria with modified LPS. *E. coli* K12 is considered a rough strain because its LPS is truncated at the ketodeoxyoctonate-containing core region and does not bear polymers of sugars that compose the O antigens (Fig. 1). To observe the role of the O antigen, we compared the binding of isogenic smooth strains (with O antigen) and rough strains (without O antigen) of *E. coli* and *S. typhimurium*. The presence of O antigen strongly depressed the nonopsonic recognition of both *E. coli* and *Salmonella* to macrophages (Table IV). These observations suggest that modification of LPS structure affects the interaction of *E. coli* with receptors on the MO.

To further examine the role of LPS in the binding of *E. coli*, MO were allowed

TABLE IV
Binding of Gram-negative Bacteria to Human Phagocytes

Bacterium	Attachment index		
	Macrophages	Monocytes	Neutrophils
E. coli K12 (rough)	559	615	668
E. coli 0111B4, rough	409	460	407
E. coli 0111B4, smooth	25	130	171
S. typhimurium LT2, rough	399	602	418
S. typhimurium LT2, smooth	31	101	36

Smooth and rough preparations of *E. coli* and *S. typhimurium* were obtained by growing *Gal*E mutants of these strains in the presence or absence of galactose as described in Materials and Methods. The binding of bacteria to monolayers of macrophages, monocytes, or neutrophils was then measured. Results are representative of three experiments.

TABLE V
Inhibition of the Binding of E. coli by Surface-bound LPS

Coating of surface	Attachment index		
	E. coli	EC3bi	EIgG
None	1,191	782	412
Rough LPS	318	281	457
Rough LPS + polymyxin	1,044	676	478
Lipid IVa	230	288	389
Lipid IVa + polymyxin	1,209	652	438
Phosphatidylcholine	1,200	696	450

Tissue culture plastic surfaces were coated for 60 min at 37°C with 50 μg/ml of the lipids shown. Surfaces were then incubated for 60 min at 20°C with 1 mg/ml HSA in the presence or absence of 10 μM polymyxin B sulfate. Surfaces were then washed and MO (5-d cultures) were allowed to spread for 45 min. The attachment of *E. coli* or ligand-coated E was then measured using a 30-min assay. Control experiments showed that the tissue culture wells bound 3×10^5 molecules of [^{3}H]phosphatidylcholine per square micrometer and 6×10^5 molecules of ^{125}I-LPS per square micrometer. This density of LPS is approximately equal to that in the outer membrane of *E. coli*. Addition of polymyxin did not cause the loss of lipids from the culture surface.

to spread on surfaces coated with purified rough LPS. These cells exhibited a marked reduction in the capacity to bind *E. coli*, whereas the capacity to bind IgG-coated particles remained unchanged (Table V). By analogy with experiments described above with surface-bound IgG, we believe that specific receptors for LPS diffuse to the substrate-attached portion of the phagocyte, where they are trapped by interaction with surface-bound LPS. Removal of receptors for LPS thus appears to block the binding of *E. coli*.

The lipid A portion of LPS appears critical for its interaction with receptors on MO. Pretreatment of LPS-coated surfaces with polymyxin B sulfate, a drug that binds the lipid A region, completely abolished their capacity to inhibit the binding of *E. coli* (Table V). Further, downmodulation of the binding of *E. coli* occurred with a chemically defined biosynthetic precursor of LPS termed lipid IVa (Table V). Lipid IVa comprises a portion of lipid A (Fig. 1, and reference

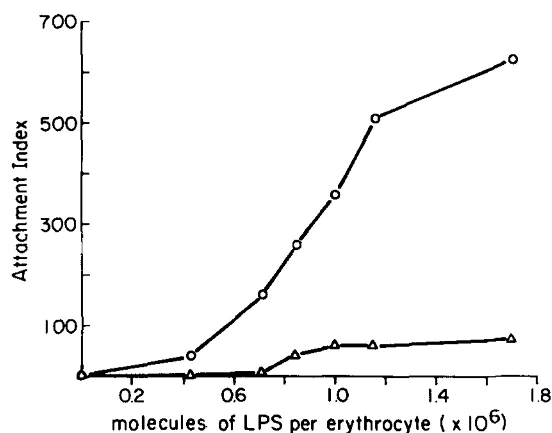

FIGURE 2. Attachment of ELPS to MO. E were coated with various amounts of ^{125}I-LPS. Monolayers of MO (7-d cultures) were then established on surfaces coated with HSA (O) or on surfaces coated with the anti-β mAb, IB4 (Δ). The binding of the ELPS was then measured. The results shown are representative of three separate experiments.

28). Surfaces coated with a phospholipid unrelated to LPS, phosphatidylcholine, caused no depression in *E. coli* binding (Table V).

To characterize further the ligand specificity of the receptors for LPS, we observed the binding of purified LPS preparations to MO. LPS was allowed to partition into the membranes of erythrocytes. We believe the LPS becomes inserted into the bilayer because the addition of polymyxin B causes prompt lysis of LPS-coated erythrocytes (ELPS) but not control E (data not shown). In this inserted configuration, the hydrophilic portion of LPS is exposed to the medium as it is in a bacterial membrane. ELPS bound avidly to MO, and the binding was dependent on the number of LPS per E (Fig. 2). The binding of ELPS to MO was not a consequence of deformation of the red cell membrane caused by inserted lipid. The membrane-active agents, chlorpromazine and 2,4-dinitrophenol, which intercalate into the inner and outer leaflets of the membrane, respectively (29), caused marked shape changes in the erythrocytes, but did not enable the E to bind to MO (data not shown).

MO recognize ELPS by the same mechanism used for *E. coli.* The binding of ELPS required divalent cations (Table II), and was inhibited at temperatures lower than 37°C (Table III). Further experiments indicated that ELPS were recognized by the same MO receptors as *E. coli* (see below).

E. coli Are Recognized by CR3, LFA-1, and p150,95. MO express a family of heterodimeric cell surface glycoproteins that are involved in adhesion reactions (for review, see reference 30). CR3 (the C3bi receptor) mediates the binding of C3bi-coated particles (8), LFA-1 mediates the binding of tumor cells (31), and the function of the third protein, p150,95, has not been determined. MO express approximately equal numbers of these three surface proteins (S. D. Wright, unpublished observations). The adhesion events mediated by CR3 and LFA-1 are dependent on divalent cations and warm temperatures (12, 32, and Table III), thus suggesting that these or similar receptors may function in the recognition of LPS. Our first indication that CR3 binds to LPS was that LPS-coated

TABLE VI
Attachment of E. coli to MO Is Inhibited by Substrate-bound Monoclonal Antireceptor Antibodies

Antibodies on surface			Attachment index		
Ab:OKM1 Ag:αCR3	TS1/22 αLFA-1	LeuM5 αp150,95	*E. coli*	ELPS	EC3bi
−	−	−	444	441	700
+	−	−	380	420	20
−	+	−	469	412	570
−	−	+	452	466	682
+	+	−	389	550	32
−	+	+	521	323	672
+	−	+	509	553	42
+	+	+	132	52	34
		IB4 (anti-β)	129	71	56
		3G8 (anti-FcR)*	417	512	622

Monocytes were cultured for 7 d in Teflon beakers. The phagocytes were then allowed to spread on surfaces coated with the indicated mAb for 45 min at 37°C. The binding of *E. coli* or ligand-coated E was then measured using a 45-min assay. Results are representative of four separate experiments.

* The mAb 3G8 is directed against the low avidity Fc receptor of neutrophils and reacts weakly with MO (11).

surfaces reduced the capacity of MO to bind EC3bi (Table V). This observation suggests that the C3bi receptor (CR3) also binds LPS.

To assess directly the role of CR3, LFA-1, and p150,95 in the binding of unopsonized *E. coli,* MO were plated on surfaces coated with monoclonal anti-receptor antibodies. We have previously demonstrated that such antibody-coated surfaces cause specific downmodulation of their target antigen from the apical portion of the MO (8, 33), and experiments with C3bi-coated particles verified that CR3 was removed by anti-CR3 but not by anti-LFA-1 or anti-p150,95 (Table VI) under the conditions used here. Removal of all three members of the CR3/LFA-1/p150,95 family with an antibody against their common β chain (IB4) caused profound inhibition of the binding of *E. coli* (Table VI), suggesting that at least one of these receptors binds bacteria. Removal of individual receptors with antibodies against the α polypeptides, however, caused negligible depression of binding, and removal of any pair of these receptors caused only modest inhibition. Profound inhibition of binding was only observed when all three family members were removed, either using an anti–β-chain antibody or a combination of three anti–α-chain antibodies. These results indicate that each of the members of the CR3/LFA-1/p150,95 family is independently capable of binding *E. coli.*

All three members of the CR3/LFA-1/p150,95 family appear to recognize LPS. The binding of ELPS was inhibited when all three of these proteins were removed from the cell surface, but the binding of ELPS was still observed when any one of the family members remained on the cell surface (Table VI). We conclude that the receptors, CR3, LFA-1, and p150,95 can each recognize LPS, and this binding reaction can result in the attachment of bacteria to MO.

CR3 is found not only on MO but also on monocytes and neutrophils. We

tested whether the expression of these receptors coincides with the ability to bind *E. coli*. Each of these cell types bind *E. coli* (Table IV), and the binding of each of these cell types is strongly reduced after interaction of the cell with an IB4-coated surface (data not shown).

Discussion

We show here that a family of three structurally related receptors on MO, CR3, LFA-1, and p150,95, are each capable of recognizing *E. coli* (Table VI). Removal of all three of these receptors from the MO membrane halts the binding of *E. coli*, but interestingly, removal of any pair of the receptors has negligible effect on binding. For example, removal of CR3 and LFA-1 from MO does not reduce the binding of *E. coli*. Thus the remaining member of the family, p150,95 in this case, is capable of mediating binding on its own. The capacity to recognize *E. coli*, therefore, appears to be a property common to all the members of the CR3/LFA-1/p150,95 family of receptors.

Our observations strongly suggest that the ligand on *E. coli* that is recognized by CR3, LFA-1, and p150,95 is LPS. Red cells coated with purified LPS were recognized by MO with the identical temperature (Table III), divalent cation (Table II), and receptor (Table VI) requirements as *E. coli*. Further, LPS-coated surfaces downmodulated the capacity of MO to bind *E. coli* (Table V).

The site on LPS that is recognized by CR3, LFA-1, and p150,95 appears to be in the lipid A region of the molecule, in that polymyxin B sulfate blocks the recognition of LPS (Table V). We have also observed that MO recognize the biosynthetic precursor of lipid A, termed lipid IVa (Table V, and S. D. Wright, and C. R. H. Raetz, preliminary observations).

We presume that because CR3, LFA-1, and p150,95 bind to LPS that is inserted into *E. coli* membranes or into red cell membranes, the receptors must bind the hydrophilic portion of the LPS molecule. Inasmuch as the hydrophilic portion of lipid IVa is composed of diglucosamine bisphosphate, we suspect that a disaccharide or sugar phosphate provides the structure that is recognized. It is possible that the three proteins, CR3, LFA-1, and p150,95, each recognize subtly different residues in order to provide a greater spectrum of target molecules.

CR3, LFA-1, and p150,95 have previously been recognized as receptors that function in adhesion events. CR3 mediates the binding of C3bi-coated E to MO (8), LFA-1 mediates adhesion between killer T cells and their targets (9), and neutrophils from patients deficient in this family of receptors show defects in chemotaxis and attachment to glass surfaces (34). Recent work further suggests that all three of these receptors function in the recognition of the fungal pathogen, *Histoplasma capsulatum* by MO (35). The experiments described here show that CR3, LFA-1, and p150,95 also recognize LPS. The recognition of LPS requires divalent cations (Table II) and warm temperatures (Table III) as have all the previously described actions of these receptors. Thus, MO employ a common mechanism and a common set of receptors to recognize a diverse spectrum of ligands. The explanation for this broad specificity is not clear. It is possible that LPS, C3bi, and *H. capsulatum* display a common structure, such as a sugar phosphate, that can be recognized by the receptor CR3. Alternatively, CR3 may contain multiple binding sites with different specificities, as recently

suggested by Ross et al. (36). Whether a single binding site on CR3 serves both for the binding of LPS and C3bi or if multiple binding sites exist is currently under study.

MO can recognize *E. coli* in the absence of IgG, complement proteins, or bacterial adhesins. This novel mechanism of recognition may thus serve in the initial response of cells to bacteria, before the generation of specific immunity. Consistent with this view are the observations on several patients whose cells lack the common β chain of CR3, LFA-1, and p150,95 and express none of the mature $\alpha_1\beta_1$ dimers on their cell surface (37). These patients suffer recurrent bacterial infections (30). The precise role of this recognition system in host defense, however, remains to be determined. Unopsonized *E. coli* appears to be phagocytosed by MO (S. D. Wright, unpublished observations), but the efficiency with which the bacteria are subsequently killed has not yet been determined. The full spectrum of bacterial species that can be recognized nonopsonically is also not known. We have observed that MO recognize rough strains of *E. coli, S. typhimurium* (Table IV), and *Pseudomonas aeruginosa* (D. P. Speert and S. D. Wright, unpublished observations), but smooth strains of *E. coli* and *Salmonella* are recognized poorly (Table IV). Since many pathogenic gram-negative bacterial strains are smooth (18), clearance of these pathogens may require the action of IgG and complement.

It is of interest that all cell types that express CR3, LFA-1, or p150,95 have been reported to exhibit a striking physiological response to low levels of LPS: MO secrete IL-1 and TNF, PMN show enhanced capacity to release toxic oxygen intermediates (38), and B lymphocytes undergo blast transformation (39). We show here that CR3, LFA-1, and p150,95 can act as receptors for LPS. Whether these receptors directly mediate the biological effects of LPS such as release of IL-1 is currently under investigation.

Summary

We report here that human macrophages bind *Escherichia coli* by recognizing bacterial lipopolysaccharide (LPS). Purified LPS was inserted into erythrocyte membranes, and the resulting LPS-coated red cells were bound by macrophages with the same temperature and cation dependence as observed for *E. coli.* When receptors for LPS were withdrawn from the plasma membrane by spreading the macrophages on LPS-coated surfaces, the binding of *E. coli* was blocked. We have also identified the receptors on macrophages that recognize LPS. Macrophages express three structurally homologous cell surface proteins, CR3, lymphocyte function–associated antigen (LFA-1), and p150,95. We used surface-bound monoclonal antireceptor antibodies to selectively remove these proteins from the apical surface of macrophages. We found that each of these proteins mediated the binding of *E. coli* to macrophages.

We thank Drs. P. A. Detmers, D. Portnoy, R. S. Steinman, and Z. A. Cohn for their critical reading of the manuscript.

Received for publication 25 July 1986.

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