independent of LBP and CD14 (*21*). LBP immunodepletion also inhibited TNF production induced with LPS isolated from S-form organisms (*Salmonella abortus equi* or *Salmonella typhimurium*).

The LBP binds to the lipid A moiety of LPS, and thus to a diverse array of Gram-negative organisms (*4*). The sequence identity to CETP suggests that LBP and CETP may be members of a family of proteins that bind to lipids and transport these ligands that are hydrophobic or amphipathic, or both, in aqueous environments. Our data show that LBP functions as a carrier protein for LPS in plasma and controls LPS-dependent monocytic responses. These data provide support for a model of LPS-induced monocytic stimulation under physiologic conditions, in which LPS first forms high-affinity complexes with LBP (*2, 4*), and these complexes then bind to cells through a specific receptor (*7*).

Recognition of the presence of LPS is important for an efficient response to infection with Gram-negative bacteria. Thus, a principal function of LBP may be to enhance the ability of the host to detect LPS early in infection; release of cellular products would then enhance natural resistance mechanisms that combat infection. As LBP concentrations rise during the acute phase response, its concentration in extravascular sites may also rise, which would provide an additional defense mechanism through its opsonic activity (*6*). The molecular details of LPS-LBP interactions will probably provide new approaches to managing the problems of Gram-negative sepsis and endotoxin shock.

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CD14, a Receptor for Complexes of Lipopolysaccharide (LPS) and LPS Binding Protein

SAMUEL D. WRIGHT,* ROBERT A. RAMOS, PETER S. TOBIAS, RICHARD J. ULEVITCH, JOHN C. MATHISON

Leukocytes respond to lipopolysaccharide (LPS) at nanogram per milliliter concentrations with secretion of cytokines such as tumor necrosis factor–α (TNF-α). Excess secretion of TNF-α causes endotoxic shock, an often fatal complication of infection. LPS in the bloodstream rapidly binds to the serum protein, lipopolysaccharide binding protein (LBP), and cellular responses to physiological levels of LPS are dependent on LBP. CD14, a differentiation antigen of monocytes, was found to bind complexes of LPS and LBP, and blockade of CD14 with monoclonal antibodies prevented synthesis of TNF-α by whole blood incubated with LPS. Thus, LPS may induce responses by interacting with a soluble binding protein in serum that then binds the cell surface protein CD14.

LIPOPOLYSACCHARIDE BINDING PROTEIN is a 60-kD serum glycoprotein that forms high-affinity stoichiometric complexes with bacterial endotoxin (LPS) (*1, 2*). LBP also functions as an opsonin (*3*). It binds to the surface of bacteria or to LPS-coated erythrocytes and mediates the adhesion of these coated particles to macrophages. Interaction of LPS-LBP complexes with macrophages subserves not only this adhesive function but also induces the synthesis of TNF by the macrophages (*4*). The molecule on the cell surface that mediates binding of LBP-coated particles is restricted to monocytes and macrophages, is mobile in the plane of the membrane, and is distinct from receptors for other known opsonins such as immunoglobulin and complement (FcγRI, FcγRII, FcγRIII, CR1, and CR3) (*3*). In order to determine the role of various surface structures in the binding of erythrocytes coated with LPS-LBP complexes (ELBPs), macrophages were allowed to spread on surfaces coated with monoclonal antibodies (MAbs) to macrophage surface proteins. Mobile membrane proteins diffuse to the substrate-attached portion of a spread macrophage and are trapped by interaction with the specific MAb, thus causing the apical surface to be specifically depleted of a single protein (*5, 6*). Depletion of proteins with MAbs to CD16 (FcγRIII), CD11b/CD18 (CR3), CD18, or HLA caused no diminution of the binding of ELBPs (Fig. 1). However, three separate MAbs to CD14 caused strong down-modulation of the binding of ELBP, suggesting that expression of CD14 on the apical surface of the macrophage is necessary for recognition of ELBPs.

For down-modulation to occur, antibody attached to the substrate must bind receptor, but it need not mask the ligand binding site of the receptor. Indeed, only two of the anti-CD14 MAbs tested appear to mask the binding site. Soluble MAb 3C10 and 60b both caused complete inhibition of binding of ELBPs to macrophages, with a half-maximal inhibition occuring at <1 μg/ml

S. D. Wright and R. A. Ramos, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.
P. S Tobias, R. J. Ulevitch, J. C. Mathison, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

*To whom correspondence should be addressed.

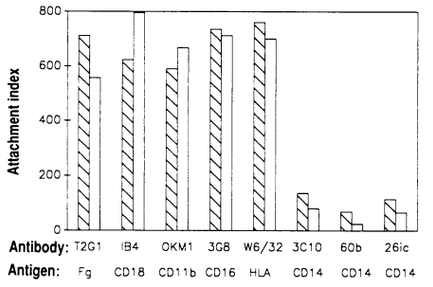

Fig. 1. Surface-bound MAbs to CD14 downmodulate binding of erythrocytes coated with LPS-LBP complexes (ELBPs). Monolayers of human macrophages were established on substrates coated with the indicated MAbs (25 μg/ml) by established methods (*18, 19*). ELBPs were prepared by coating sheep erythrocytes with LPS, then incubating the coated cells with LBP to form LPS-LBP complexes on the erythrocyte surface (*3*). The monolayers of macrophages were washed, ELBPs were added, and attachment of ELBPs to macrophages was measured after a 15-min incubation at 37°C, as described (*3*). Results are presented as attachment index (the number of erythrocytes bound per 100 phagocytes). Essentially all attachment was LBP-dependent, since omission of LBP lead to attachment indices of 3 and 5 in the two experiments shown. The MAbs were purified immunoglobulin Gs (IgGs). The MAb to human fibrinogen (Fg) was T2G1 (*20*). IB4 (*5*), OKM1 (*5*), 3G8 (*21*), W6/32 (*22*), 3C10 (*23*), 60b (*24*), and 26ic (*25*) were as described. Two experiments are shown (hatched bars and open bars) and were confirmed in two additional experiments.

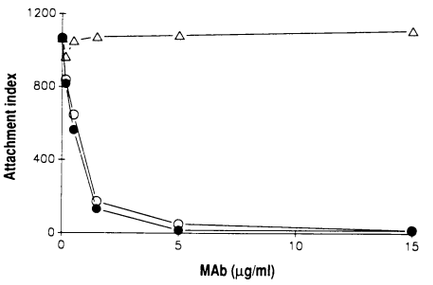

Fig. 2. Blockade of ELBP binding by MAbs to CD14. Monolayers of human macrophages were incubated for 15 min at 0°C with the indicated concentrations of the anti-CD14 MAbs 3C10 (○), 60b (●), or 26ic (△). ELBPs were added in the continued presence of antibody, and attachment was measured. Results are representative of three separate concentration dependence experiments and of ten experiments performed at a fixed concentration of 10 μg/ml. Parallel studies showed that incubation of macrophages with MAb to CD14 caused neither loss nor capping of the receptor. Saturating concentrations of a large panel of MAbs directed against other determinants on macrophages had no effect on the binding of ELBPs.

(Fig. 2). In contrast, the MAb 26ic (also anti-CD14) did not inhibit binding of ELBPs. This MAb binds to an epitope on CD14 that appears not to be involved in the interaction with LBP.

We exploited the ability of 26ic to bind CD14 without inhibiting function to determine if isolated CD14 recognizes LBP. Culture surfaces were coated with 26ic or other MAbs, then washed and incubated with a detergent lysate of macrophages. The surfaces were again washed and assayed for their ability to bind uncoated or LBP-coated erythrocytes. Surfaces coated with CD14 complexed to 26ic selectively retained ELBPs (Fig. 3). Uncoated erythrocytes did not bind to this surface, and surfaces coated either with other antigens or with CD14 complexed with blocking antibodies (3C10 or 60b) did not show appreciable affinity for ELBPs. Thus, CD14 that is purified from cell lysates by interaction with MAbs has the ligand-binding properties of a receptor for LPS-LBP complexes.

CD14 is a 55-kD glycoprotein that is attached to the membrane via a phosphatidylinositol glycan anchor (*7, 8*). Treatment of macrophages with phosphatidylinositol-specific phospholipase C (PI-PLC) results in the removal of 70% of cell surface CD14 (*7*) (Table 1), and we observed a concomitant 80% decrease in the capacity of the cells to bind ELBPs (Table 1), thus further establishing the role for CD14 in recognition of LBP.

Lipopolysaccharide stimulates mononuclear phagocytes to synthesize lymphokines such as TNF-α, interleukin-1, and interleukin-6 (IL-1 and IL-6). Excess secretion of these mediators, particularly TNF, is thought to be responsible for the phenomenon of endotoxic shock, an often fatal complication of infection (*9*). To determine if CD14 participates in the induction of TNF under conditions that approximate those in humans, we exposed whole blood to various concentrations of LPS in the presence or absence of MAbs to CD14. On the basis of the avidity of LBP for LPS (*2*), the LBP in human plasma (0.2 to 0.5 μg/ml) should complex virtually all LPS concentrations up to 5 ng/ml; experiments with normal rabbit plasma showed that both smooth and rough form LPS could be specifically precipitated with antibody to LBP (*10*). LPS at 1 ng/ml induced TNF-α synthesis in the blood, but addition of the blocking MAb to CD14, 3C10, nearly eliminated this response (Fig. 4). Similar results were obtained in experiments with both smooth and rough forms of LPS. Another blocking MAb to CD14, 60b, was also effective in inhibiting TNF-α production, but the nonblocking MAb to CD14, 26ic, was without effect (*11*). Similarly, no inhibition of TNF-α synthesis was observed with MAb to the equally prevalent antigen CD18 (Fig. 4), a protein that binds particulate LPS, but is unnecessary for responses of macrophages to LPS (*12*). Thus, the presence of CD14 is necessary for blood monocytes to respond to the concentrations of LPS (0.01 to 1 ng/ml) that are necessary to induce the symptoms of sepsis (*13*).

Responsiveness to Gram-negative LPS (endotoxin) is widely distributed in multicellular organisms and constitutes a defense against infections. Our data suggest the following model for the function of LBP and CD14 in the response of cells to endotoxin. Upon entry of LPS into the circulation, it is rapidly bound by LBP, and the resulting LPS-LBP complexes are recognized by the receptor CD14 on mononuclear cells. LBP is not bound by phagocytes in the absence of LPS (*3*). The ligated CD14 may then directly trigger the synthesis of TNF-α. Alternatively, CD14 may not trig-

Table 1. The receptor for LBP is removed by phosphatidylinositol-specific phospholipase C (PI-PLC). Monolayers of human macrophages were incubated for 60 min at 37°C in the presence or absence of PI-PLC (15 μg/ml). The monolayers were washed and the attachment of erythrocytes coated with C3bi [EC3bi (*18*)] or ELBPs was measured in a 15-min assay. Results are representative of four separate experiments. Suspensions of human macrophages were incubated for 60 min in the presence or absence of PI-PLC as described above. The expression of surface antigens was then measured by incubating the cells with MAb (10 μg/ml) to CD18 (IB4) or CD14 (3C10), then with fluoresceinated $F(ab')_2$ to murine IgG. Flow cytometry showed a single population of cells under all conditions with the indicated mean fluorescent intensity (linear scale).

Treatment	Attachment index		Mean fluorescent channel	
	EC3bi	ELBP	CD18	CD14
None	896	715	743	592
PI-PLC	1025	129	785	195

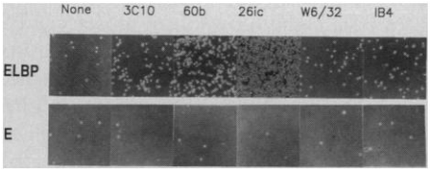

Fig. 3. Surfaces coated with CD14 bind ELBPs. Plastic tissue culture surfaces were coated with the indicated MAbs, washed, then incubated with a triton-extract of human macrophages [10^8 cells per milliliter, prepared as in (*5*)] for 60 min at 0°C. The surface-bound MAbs adsorb their target antigens from the lysate to yield a specific antigen-bearing surface. The surfaces were washed, and uncoated erythrocytes or ELBPs were added and incubated for 30 min at 20°C. After extensive washing, the wells were photographed. Many of the ELBPs bound to the 26ic + CD14–coated surface appear phase dark because of their close apposition with the substrate. Results are representative of four separate experiments.

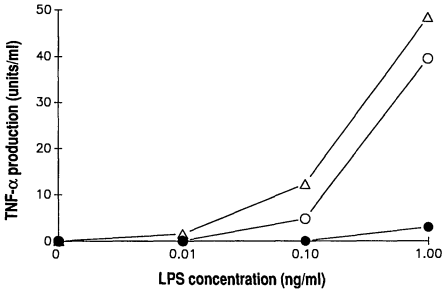

Fig. 4. Anti-CD14 MAb blocks the synthesis of TNF-α stimulated by LPS. Heparinized human blood was cultured with graded doses of LPS (type Re595) as described (○) (*26*). In parallel cultures, MAbs to CD14 (3C10, ●) and CD18 (IB4, △) were added to 10 μg/ml. After 16 hours at 37°C, TNF-α levels in the plasma were determined by the WEHI clone 13 assay (*27*). Parallel studies showed that all of the TNF-α activity in the supernatants could be neutralized by an antibody to TNF-α. Results are representative of four separate experiments, and similar results were also obtained after a shorter (4-hour) incubation of blood with LPS. The amount of TNF-α secreted could not be increased by raising LPS concentrations beyond the doses shown.

ger TNF-α release itself, but may serve to bring LBP-LPS complexes to the cell surface in such a way that other proteins (perhaps other subunits of a complex receptor) are stimulated. The latter possibility is supported by the observation that raising the concentration of LPS to 10 ng/ml causes TNF-α synthesis in the absence of either LBP (*4*) or CD14 (*11*). In either case, it is clear that the mechanism by which CD14 and LBP act represents an unanticipated departure from the mechanism by which eukaryotic cells respond to other agonists such as hormones or lymphokines. The binding of the agonist (LPS) is mediated by a soluble protein rather than a membrane-bound protein, and only after the formation of a soluble complex does the LPS interact with a membrane-bound "receptor."

CD14 is a well-known marker for monocytes and macrophages and is recognized by several monoclonal antibodies commonly used in flow cytometry studies (LeuM3, Mo2, and MY4). The genes for both human and murine CD14 have been cloned and sequenced (*14, 15*). The predicted protein sequence contains several copies of a leucine-rich 24-residue repeat also found in the serum protein, leucine-rich glycoprotein, in the *Drosophila* membrane proteins Chaoptin and Toll (*15*), and the lutropin-choriogonadotropin receptor (*16*). The gene for CD14 is located in a region of chromosome 5 known to encode several myeloid-specific growth factors or receptors, including IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), CSF-1, CSF-1 receptor, and the platelet-derived growth factor receptor (*17*). However, the function of CD14 has not been defined. Here we show that CD14 binds LBP-LPS complexes and thereby serves two important functions. LBP acts as an opsonin (*3*), and CD14 may thus function in the clearance of Gram-negative pathogens during infection. LBP also promotes the secretory responses of cells to LPS, and CD14 may thus function to heighten the sensitivity of the immune system to infection.

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Acceleration of Diabetes in Young NOD Mice with a CD4$^+$ Islet-Specific T Cell Clone

KATHRYN HASKINS AND MARCIA MCDUFFIE

Nonobese diabetic (NOD) mice develop an autoimmune form of diabetes, becoming hyperglycemic after 3 months of age. This process was accelerated by injecting young NOD mice with CD4$^+$ islet-specific T cell clones derived from NOD mice. Overt diabetes developed in 10 of 19 experimental animals by 7 weeks of age, with the remaining mice showing marked signs of the disease in progress. Control mice did not become diabetic and had no significant pancreatic infiltration. This work demonstrates that a CD4 T cell clone is sufficient to initiate the disease process in the diabetes-prone NOD mouse.

TYPE I DIABETES OR INSULIN-DEPENDENT diabetes mellitus (IDDM) is thought to be an autoimmune disease in humans. Two animal models for inherited autoimmune diabetes are the Biobreeding (BB) rat and the NOD mouse. A role for T cells in diabetes was implicated by studies with these diabetes-prone animals in which treatments that led to the depletion of T cells resulted in a decreased incidence of disease (*1*). More conclusive evidence was provided by transfer of disease into nondiabetic recipients with T cells from diabetic rats (*2*) or mice (*3*). Since depletion of either the CD4 or the CD8 T cell subsets from diabetic donor T cells can prevent transfer of disease, the consensus has been that both CD4 and CD8 T cells are needed for development of diabetes (*4*). To learn more about the T cells involved in the disease process, we isolated islet-specific T cell clones from NOD mice (*5, 6*) and used two of these clones to accelerate the diabetogenic process in unmanipulated, nondiabetic NOD recipients.

The islet-specific T cell clones in our panel (*6*) are all CD4$^+$, and they proliferate and produce interleukin-2 (IL-2) in response to whole islet cell antigen and irradiated NOD spleen cells as antigen-presenting cells (APC). The clones do not respond to islet antigen or APC alone, nor do they react in vitro with other cell types (thyroid, pituitary, or spleen) or in vivo with pituitary grafts. They are unlikely to be artifacts of in vitro immunization, as our attempts to pro-

Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262.