CD11c/CD18, A Transmembrane Signaling Receptor for Lipopolysaccharide

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Summary

CD11c/CD18 is a member of the leukocyte integrin family, heterodimeric adhesion molecules that interact with a diverse repertoire of ligands, including bacterial lipopolysaccharide (LPS). Their role as signal transducing receptors remains uncertain. We used a heterologous expression system to determine if CD11c/CD18 was capable of initiating signal transduction in response to LPS-binding, as assessed by the induced translocation of nuclear factor-κB. We have previously reported that Chinese hamster ovary (CHO)-K1 fibroblasts, normally unresponsive to LPS, acquire serum-dependent macrophage-like responses to LPS when transfected with CD14 (Golenbock, D. T., Y. Liu, F. Millham, M. Freeman, and R. Zoeller. 1993. *J. Biol. Chem.* 268:22055–22059), a known LPS receptor. In contrast, CHO cells acquired serum-independent responses to Gram-negative bacteria and LPS when transfected with CD11c/CD18 (CHO/CD11c). In comparison to CHO cells transfected with CD14 (CHO/CD14), responses in CHO/CD11c cells were slower, required higher endotoxin concentrations for maximal response, and were not inhibited by the presence of antibodies to CD14. CD11c/CD18 is, thus, the second phagocyte receptor, in addition to CD14, which has been shown to have the capacity to activate cells after binding to LPS. The function of this receptor in normal phagocytes may be limited to the recognition of LPS in infected tissues, where LPS-CD14 interactions are not favored because of the absence of serum proteins.

The leukocyte integrins, CD11a-c/CD18, are obligate α/β heterodimeric molecules consisting of a common 95-kD β subunit noncovalently attached to a unique α subunit: 180-kD αL (CD11a), 170-kD αM (CD11b), or 150-kD αX (CD11c). These transmembrane glycoproteins are expressed on the surface of leukocytes and participate in numerous cell–cell and cell–substrate interactions (1, 2). Deficiency of β-chain expression, as found in the autosomal recessive disease known as leukocyte adhesion deficiency, results in recurrent bacterial infections, presumably due to impaired chemotaxis and bacterial phagocytosis (3). This leads to impaired pus formation and defective wound healing (2).

A great deal is known about CD11a/CD18 and CD11b/CD18. CD11a/CD18 (LFA-1), present on all leukocytes, is involved in leukocyte–endothelial cell adhesion and helper and cytotoxic T lymphocyte interactions (4). CD11b/CD18 (CR3 or membrane attack complex [MAC-1][1]) is found primarily on mature neutrophils, monocytes, and NK cells. It functions as a complement receptor for iC3b and also participates in

monocyte and neutrophil adhesion to endothelial cells (2). The third leukocyte integrin, CD11c/CD18 (p150,95 or CR4), is found on a variety of cell types including monocytes, macrophages, granulocytes, and some T and B lymphocytes, and is a marker for hairy cell leukemia (3, 5, 6). It is also highly expressed on dendritic cells (7). In contrast to CD11a/CD18 and CD11b/CD18, the functional role of CD11c/CD18 has not been well defined. Like the other leukocyte integrins, it is involved in the adhesion of monocytes and granulocytes to endothelium (3, 8) and functions as a receptor for fibrinogen (9, 10) and iC3b opsonized particles (11, 12).

Although all three leukocyte integrins are capable of binding unopsonized bacteria and LPS to monocytes, macrophages, and granulocytes (13), it has never been clearly demonstrated that this interaction independently triggers a cellular response. CD18-deficient PBMC have been shown to respond normally to LPS, demonstrating that CD11/CD18 is not essential for cellular responses to LPS (14). Recent attention has focused on CD14, a 53-kD glycosyl phosphatidylinositol-linked protein present on monocytes, macrophages, and neutrophils, as an LPS-signaling molecule (15, 16). Binding of LPS to CD14 on mononuclear phagocytes results in the production of various cytokines, such as IL-1 and TNF-α, involved in the acute inflammatory response (16, 17). However, CD14 has no transmembrane domain, and several lines of recent evidence strongly suggest that the function of CD14 is to bind and amplify

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[1] *Abbreviations used in this paper:* CHO, Chinese hamster ovary; DHFR, dihydrofolate reductase; EMSA, electrophoretic mobility shift assay; ICAM, intercellular adhesion molecule; LBP, LPS-binding protein; MAC-1, membrane attack complex; NF-κB, nuclear factor-kappa B; ReLPS, LPS from *Salmonella minnesota* R595; sCD14, soluble CD14.

responses to LPS, but not to actually transduce a transmembrane signal. The best examples that CD14 does not transduce a signal come from studies of compounds which competitively antagonize the effects of endotoxin (18–22), such as LPS-induced prostaglandin and cytokine release. Careful ligand-binding studies demonstrated that LPS-receptor antagonists failed to inhibit the binding of LPS to CD14 at concentrations of inhibitor that completely blocked the ability of LPS to activate monocytes (22). A second line of evidence that CD14 does not transduce membrane signals comes from observations that some of the LPS antagonists actually activate macrophages from rodent species (18). For example, lipid IVa, a tetraacyl lipid A precursor of lipid A, inhibits LPS-induced TNF-α release from human monocytes, but stimulates TNF-α release from mouse macrophages. Studies of human and rodent cell lines transfected with murine or human CD14 demonstrated that the species-specific agonism or antagonism of the LPS antagonists does not correlate with the species of animal from which the cDNA for CD14 was derived, but correlates precisely with the species of cell in which CD14 is expressed (23). Thus, lipid IVa is an LPS antagonist in human fibroblasts transfected with mouse CD14.

We sought to explore what role, if any, CD18 played in LPS-induced cellular signaling. Because CD11c/CD18 activity cannot be easily separated from that of CD14 in a macrophage system, we used a Chinese hamster ovary (CHO)-K1 fibroblast line engineered to heterologously express the genes for CD11c/CD18 (CHO/CD11c) (24). CHO cells are normally unresponsive to LPS. However, when transfected with the gene for CD14 (CHO/CD14) (25), as little as 1 ng of LPS/ml will result in the release of arachidonic acid into the culture supernatant (25) and the translocation of the transcription factor, nuclear factor–kappa B (NF-κB), into the nucleus (26). We chose to examine NF-κB translocation in CHO/CD11c because it may be a sentinel event in the activation of proinflammatory cytokines (27–29). Furthermore, translocation of NF-κB is a convenient method for directly assessing LPS-induced signal transduction as it occurs in the absence of de novo RNA or protein synthesis (26). Our results demonstrate that CD11c/CD18, which is a known integral membrane protein, functions as a receptor capable of enabling responses to LPS.

Materials and Methods

Reagents. All solutions were guaranteed sterile and pyrogen-free by the manufacturer, unless otherwise stated. PBS, alpha-MEM, and Ham's F-12 were obtained from BioWhittaker, Inc. (Walkersville, MD). Ex-Cell 320 was obtained from JRH Biosciences (Lenexa, KS). FCS (LPS ≤10 pg/ml) was obtained from Hyclone Laboratories (Logan, UT). Human serum was derived from clotted whole blood from healthy volunteers and heat inactivated at 56° for 45 min. Ciprofloxacin was a gift from Miles, Inc. (West Haven, CT). Methotrexate, thymidine, and L-glutamine were obtained from Sigma Chemical Co. (St. Louis, MO). LPS from *Salmonella minnesota* R595 (ReLPS) was a gift from N. Qureshi and K. Takayama (University of Wisconsin, Madison, WI). Lipids were prepared as 1 mg/ml dispersed sonicates in pyrogen-free PBS and stored at –20°C. Before use, the suspensions were thawed and sonicated

for 3–5 min in a water bath sonicator (Lab. Supplies Co., Inc., Hicksville, NY).

Monoclonal Antibodies. Monoclonal antibody 60 bca (anti–human CD14) was from the American Type Culture Collection (ATCC) (Rockville, MD) and was provided as a serum-free hybridoma supernatant (Gibco Laboratories, Grand Island, NY) as a gift from Dr. Ronald R. Bach (Minneapolis Veterans Administration Hospital, Minneapolis, MN). TS1/18 mAb (anti–human CD18) (30) was also purchased from ATCC and used as ascites; mAbs L29 (anti–human CD11c) (31) and IB4 (13) were gifts from Dr. Samuel D. Wright (The Rockefeller University, New York). FITC-conjugated sheep anti–mouse IgG was obtained from Sigma Chemical Co.

Bacterial Cultures. *Salmonella minnesota* R595 was a gift of Dr. C. R. H. Raetz (Duke University, Durham, NC). A single colony from a fresh plate was inoculated into Luria broth medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.001 N NaOH) and grown to saturation. The number of bacteria was determined by serial dilutions and is expressed as CFU per milliliter. Bacteria were washed three times in PBS and stored in 200-μl aliquots at –80°C. Although whole bacteria were visibly present after freeze-thaw, freezing resulted in >99% cell death. Nevertheless, stimulation with whole bacteria was carried out in the presence of 10 μg of antibiotic ciprofloxacin/ml to inhibit potential bacterial growth during the stimulation assay.

Cell Lines. Cell lines were grown as adherent monolayers in tissue culture dishes at 37°C in 5% CO_2/95% air, and passaged twice a week to maintain logarithmic growth. The CHO-K1 cell line was obtained from the ATCC. CHO/CD14 was developed by stably cotransfecting the genes for human CD14 and neomycin resistance (CHO/CD14), while CHO/Neo was transfected with the gene for neomycin resistance alone, as described in Golenbock et al. (25). DG44, a CHO-K1 dihydrofolate reductase–deficient cell line, has been previously described (32) and represents the wild-type line for the CD11c/CD18 transfectants. Stable clonal cell lines of CHO/CD14, CHO/Neo, and DG44 were maintained in Ham's F-12 medium supplemented with 10% FCS and 10 μg of ciprofloxacin/ml.

Bulk CHO/CD11c and CHO intercellular adhesion molecule (ICAM) transfectants were gifts from Dr. T. Springer (Center for Blood Research, Boston, MA) and are described in detail elsewhere (33). Briefly, CHO/CD11c was engineered by cotransfection of DG44 with the genes for human CD11c/CD18 and a CHO–dihydrofolate reductase minigene, and selected in purine/pyrimidine-free medium. CHO/ICAM is DG44 cotransfected with the genes for a domain-deleted form of ICAM-1 and dihydrofolate reductase. Bulk CHO/CD11c transfectants were cloned by limiting dilution, and a single clonal line which expressed CD11c/CD18 was chosen at random to use for the experiments described. CHO/CD11c and CHO/ICAM were maintained in nucleotide-free alpha–minimum essential medium with 10% dialyzed FCS, 2 mM L-glutamine, 10 μg ciprofloxacin/ml, 16 μM thymidine, and 0.1 μM methotrexate. Cells were grown in the absence of methotrexate for several passages before experimentation.

Flow Cytometry Analysis of CHO Transfectants. CHO transfectants growing as monolayers in tissue culture dishes were detached from the surface using 1 mM EDTA in PBS and resuspended in medium with 10% FCS. 2.5 × 10^5 cells were incubated for 30 min on ice with unconjugated 60 bca mAb, TS1/18 mAb, or L29 mAb diluted in PBS/2% FCS for a final concentration of 10 μg/ml. Cells were washed with 2 ml PBS/2% FCS and counterstained with FITC sheep anti–mouse IgG (Sigma Chemical Co.), diluted 1:100 in PBS/2% FCS, on ice for 30 min. The cells were washed

again and resuspended in 0.4 ml PBS/2% FCS with 1 μg propidium iodide/ml. Stained cells were analyzed by flow microfluorimetry, using a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA), as described (25).

Cell Stimulation. Unless otherwise noted, cells were stimulated under strict serum-free conditions. Before the day of experimentation, cells growing as adherent monolayers in tissue culture dishes were washed three times with PBS, trypsinized, and resuspended in Ex-Cell 320 medium with 10 μg ciprofloxacin/ml (herein referred to as Ex-Cell). Cells were washed twice in Ex-Cell to remove FCS, and plated in six-well tissue culture dishes at a density of 1 × 10⁶. After overnight incubation at 37°C in 5% CO₂/95% air, adherent monolayers were washed twice with Ex-Cell and resuspended in 900 μl of Ex-Cell or Ex-Cell plus 2% human serum and the appropriate stimulant. For experiments with antibodies, cells were preincubated with the mAb diluted 1:30 for 15 min on ice before addition of the stimulus. Culture dishes were then returned to a 37°C, 5% CO₂/95% air environment. At the end of the incubation period, dishes were placed on ice to stop cellular activation during the preparation of nuclear extracts.

Preparation of Nuclear Extracts. The procedure used for the preparation of nuclear extracts is described in detail in Delude et al. (26). Briefly, adherent cells were washed in tissue culture plates with PBS containing 2% FCS, harvested using a rubber policeman, and pelleted in a microcentrifuge (Microfuge 11; Beckman Instruments, Palo Alto, CA). Cells were resuspended in 0.4 ml buffer (10 mM Tris HCl pH 7.8, 5 mM MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol, 0.3 mM EGTA, 1 mM phenylmethyl-sulfonyl fluoride, 10 mM β-glycerol-phosphate, 0.3 M sucrose, and 1.0 μg/ml each of the following protease inhibitors: aprotinin, antipain, leupeptin, chymostatin, and pepstatin), incubated on ice for 15 min, and lysed by adding Nonidet P-40 to 0.5%. Nuclei were collected by centrifugation and resuspended in 50 μl of buffer II (20 mM Tris pH 7.8, 5 mM MgCl₂, 320 mM KCl, 0.5 mM dithiothreitol, 0.2 mM EGTA, 0.5 mM PMSF, 10 mM β-glycerol-phosphate, 25% glycerol, and 1.0 μg/ml protease inhibitors, as above). After a 15-min incubation on ice, the nuclear extracts were cleared by centrifugation and transferred to a new tube. Protein concentration was determined using a protein assay dye reagent concentrate (Bio-Rad Laboratories, Hercules, CA).

Electrophoretic Mobility Shift Assay (EMSA). Two synthetic oligonucleotides containing the consensus sequence for NF-κB–binding from the murine Ig κ light chain gene enhancer (Oligos Etc., Guilford, CT) were annealed and labeled as described (26). 4 μg of crude nuclear extracts and 0.2 ng DNA probe (20,000 cpm) were used in a DNA-binding reaction containing 50 μg of polydeoxyinosinic-deoxycytidilic acid (the polymer poly[dI-dC]·poly[dI-dC]) per ml,

5% glycerol, and 1× band shift buffer (10 mM Tris HCl pH 7.8, 1 mM EDTA, 40 mM KCl, 1 mM dithiothreitol). The reaction was incubated at room temperature for 30 min and size fractionated by gel electrophoresis on a 4% native polyacrylamide gel (Protogel brand acrylamide:bisacrylamide [vol/vol 30:0.8]; National Diagnostics Inc., Atlanta, GA) run at 10 V/cm for 45 min at room temperature. Gels were transferred to 3-mm filter paper (Whatman Inc., Clifton, NJ), immediately dried under vacuum at 80°C, and exposed to x-ray film (XAR2; Eastman-Kodak Co., Rochester, NY) overnight at −80°C with an intensifying screen.

Results and Discussion

Bulk CHO/CD11c transfectants were incubated with whole *Salmonella minnesota* R595 under strict serum-free conditions over increasing periods of time, and nuclear extracts were analyzed for the induced translocation of NF-κB (Fig. 1). Nuclear localization of NF-κB in CHO/CD11c was evident within 30 min and peaked between 1 and 3 h. In contrast, CHO/CD14 responded within 15 min to the stimulus, peaked by 30 min, and returned to baseline at 3 h. CHO cells transfected with ICAM-1 (CHO/ICAM) (24), a member of the Ig super family, were unresponsive to treatment with whole bacteria. Because only 30–40% of the bulk-transfected population expressed CD11c/CD18 when analyzed by flow microfluorimetry (data not shown), a clonal population of CHO/CD11c was isolated by limiting dilution (Fig. 2). This clonal line responded in a similar manner to stimulation with whole *Salmonella minnesota* R595 and was used in all subsequent experiments.

We believed that the response of CHO/CD11c to whole Gram-negative bacteria was mediated through the interaction of LPS with the CD11c/CD18 receptor. To test this hypothesis, we incubated CHO/CD11c with graded amounts of ReLPS under serum-free conditions, and analyzed nuclear extracts for NF-κB. A comparison of the dose-response relationships shows that CHO/CD11c are modestly less responsive to LPS, compared to CHO/CD14 (Fig. 3). In CHO/CD14, maximum stimulation was seen between 0.1 and 1 μg of LPS/ml, while, in CHO/CD11c, peak activation occurred with 10 ng of LPS/ml. DG44, a DHFR-deficient CHO line (32) and the parent cell line to CHO/CD11c, was unresponsive to even 1 μg LPS/ml. In addition, CHO/ICAM and CHO-K1, transfected with the gene for neomycin resistance
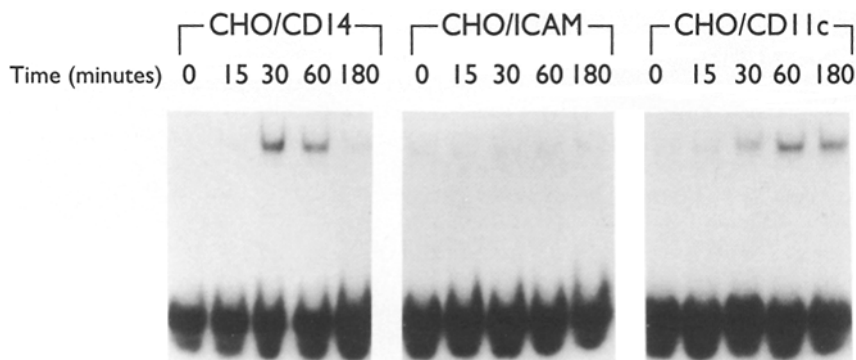

┌─CHO/CD14─┐ ┌─CHO/ICAM─┐ ┌─CHO/CD11c─┐
Time (minutes) 0 15 30 60 180   0 15 30 60 180   0 15 30 60 180

Figure 1. Time course of NF-κB translocation after exposure to whole *Salmonella minnesota* under serum-free conditions in CHO/CD14, CHO/ICAM, and CHO/CD11c. CHO/CD14, CHO/CD11c, and CHO/ICAM were prepared as cell monolayers under serum-free conditions, as described in Materials and Methods, and stimulated with 10⁶ CFU of whole *Salmonella minnesota* R595/ml for time periods ranging from 15 to 180 min. Nuclear extracts were prepared and localization of NF-κB was determined by EMSA. Nuclear NF-κB was evident in the CHO/CD11c line within 30 min and maintained throughout the 3-h time course, while, for CHO/CD14, the response is seen by 15 min and returns to baseline at 3 h. CHO/ICAM were unresponsive to this stimulus.
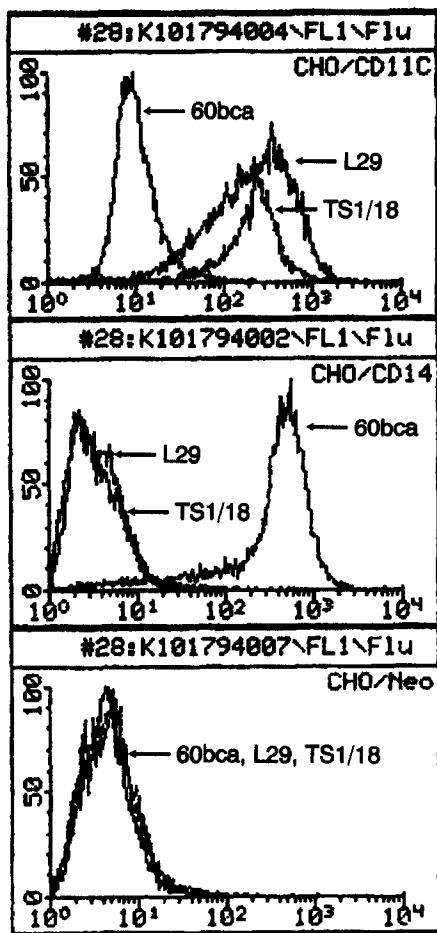
Figure 2. CHO cells transfected with CD11c/CD18 bind mAb to CD11c and CD18, but not CD14. A clonal isolate of the bulk CHO/CD11c transfection was generated by limiting dilution and subjected to FACS® analysis. Shown above is a FACS® histogram of CHO/CD14, CHO/CD11c, and CHO/Neo after staining with 60 bca (anti-CD14), TS1/18 (anti-CD18), or L29 (anti-CD11c).

(25), also failed to respond to LPS (data not shown). While the difference in sensitivity to LPS may be due to inherent differences between CD14 and CD11c/CD18, it is impossible to determine, at this time, if these observations simply reflect a lower copy number of receptors in the CHO/CD11c line.

CD14 is a glycosyl phosphatidylinositol–linked protein without a transmembrane domain. Efficient binding of LPS

to CD14 requires the presence of serum proteins, especially LPS-binding protein (LBP) (17, 34–36). A soluble form of CD14 (sCD14) is also present in serum, and several groups have observed that sCD14 is capable of enabling LPS responsiveness in certain cell types which do not express the glycosyl phosphatidylinositol–linked form of CD14 (34, 37–41). Despite the use of strict serum-free conditions, we could not eliminate initially the possibility that the signaling we observed in CHO/CD11c was being mediated through sCD14 contaminating our cell culture system. Several lines of evidence demonstrated that this was not the case. First, unlike endothelial cells and several other cell types which do not express CD14, neither soluble CD14, nor serum, enabled wild-type or mock-transfected CHO cells to respond to LPS (25, 26). Furthermore, anti-CD14 mAb inhibited LPS signaling in CHO/CD14 stimulated with 1–10 ng of LPS/ml, while the response to LPS by CHO/CD11c was not diminished by the presence of this antibody (Fig. 4). Although we have not performed extensive experiments with antibodies to the leukocyte integrins, experiments with the anti-CD18 mAb IB4 did not demonstrate specific inhibition of LPS signaling in CHO/CD11c (data not shown). This latter experimental result was not surprising, as soluble mAbs to CD18 have not been reported to block binding of LPS-coated erythrocytes to CD18 in the past (13), nor have any soluble α-chain antibodies been reported to block LPS-coated erythrocytes binding to CD11c, suggesting that the LPS-binding domain on CD11c/CD18 is not recognized by existing monoclonals.

CD14-mediated signaling is markedly enhanced by the addition of very small amounts of serum, due to the effects of LBP (16, 17, 42). CHO/CD14, for example, only minimally released arachidonate in response to LPS, even at concentrations as high as 1,000 ng of LPS/ml (25). Because LPS might competitively bind sCD14 present in serum, we predicted that signaling in our CHO/CD11c line might actually be diminished by the presence of serum. In contrast to CHO/CD14, NF-κB translocation in CHO/CD11c was significantly decreased in the presence of 2% human serum, compared to serum-free conditions (Fig. 5). Under parallel conditions, signaling in CHO/CD14 was enhanced by human serum. Thus, the responses observed in CHO/CD11c to LPS were not mediated via sCD14. CD11c/CD18 represents a transmembrane receptor for LPS that may activate macrophages through an entirely novel signaling pathway.

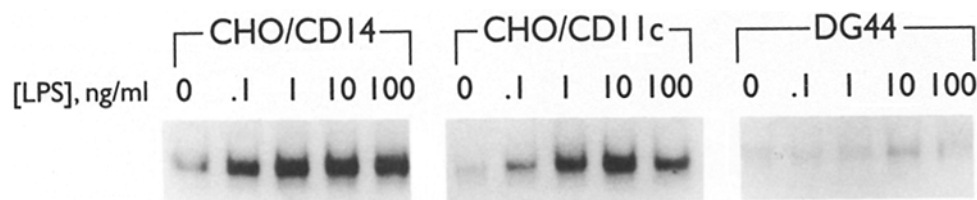The leukocyte integrins are clearly more than adhesion mol-

Figure 3. LPS dose-response in CHO/CD14, CHO/CD11c, and DG44 after exposure to LPS under serum-free conditions. CHO/CD14, CHO/CD11c, and DG44 were prepared and plated under serum-free conditions and stimulated with varying concentrations of ReLPS for 1 h. Nuclear extracts were prepared and analyzed by the EMSA. Both CHO/CD14 and CHO/CD11c responded to ReLPS, as reflected by the inducible translocation of NF-κB, in contrast to wild-type DG44.
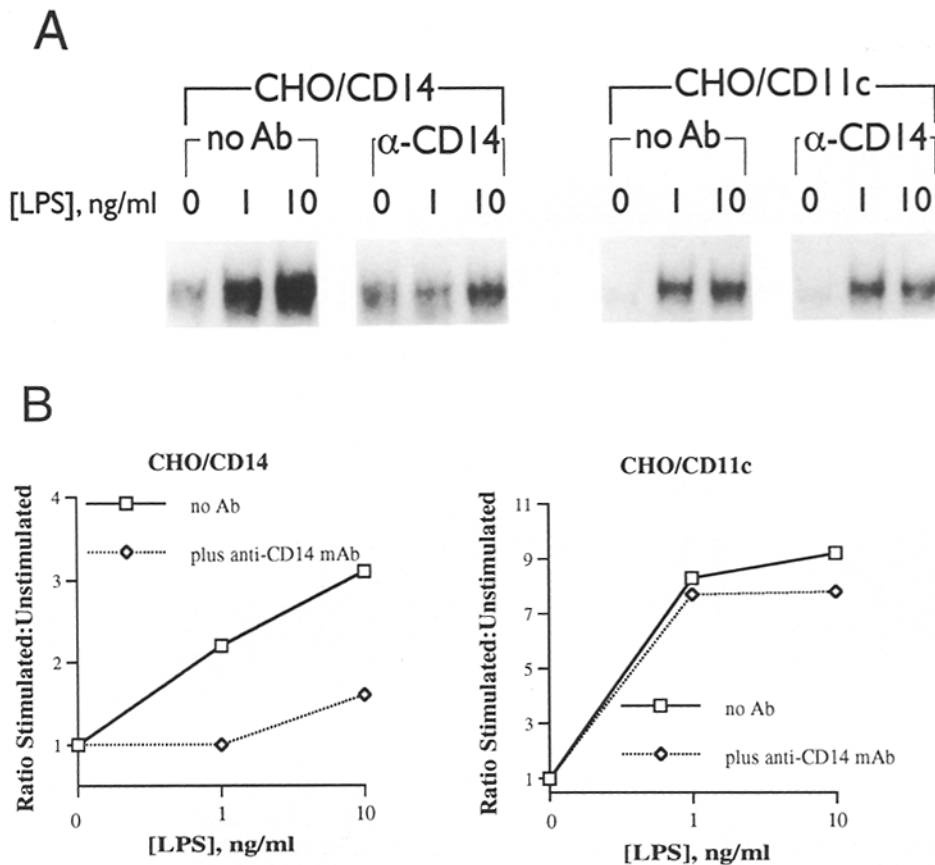
A

B

Figure 4. Effect of anti-CD14 mAb on NF-κB translocation in CHO/CD14 and CHO/CD11c exposed to LPS, under serum-free conditions. CHO/CD14 and CHO/CD11c were prepared and plated under serum-free conditions. Cells were preincubated with either PBS, or 60 bca (anti-CD14 mAb) diluted 1:30 for 15 min on ice. Stimulation was then carried out with varying concentrations of ReLPS for 1 h. Nuclear extracts were prepared and analyzed by the EMSA. Unlike CHO/CD14, stimulation in CHO/CD11c was not inhibited by the presence of anti-CD14 mAb (*A*). CHO/Neo were unresponsive to ReLPS in both the presence and absence of 60 bca (data not shown). Scanning densitometry of the autoradiograph was performed and illustrates graphically the ratio of nuclear NF-κB in stimulated/unstimulated cells (*B*).

ecules with a purely mechanical function. Several recent reports demonstrate their role in cell signaling. For example, spreading of macrophages, a CD11/CD18-dependent process, has been shown to induce the synthesis of IL-1α and IL-1β (43). Cell-associated IL-1β has also been observed in monocytes as a result of cross-linking CD11a, CD11b, or CD18 antigens with antibody (44). Similarly, binding of neutrophils to im-

Figure 5. Effect of human serum on NF-κB translocation in CHO/CD11c exposed to LPS. CHO/CD14, CHO/CD11c, and CHO/Neo were plated in either Ex-Cell, or Ex-Cell with 2% human serum heat-inactivated to 56°C for 45 min. Stimulation was carried out with varying concentrations of ReLPS for 1 h. Above is the EMSA of nuclear extracts prepared from CHO/CD11c, demonstrating that translocation of NF-κB was inhibited by the presence of serum. Simultaneous stimulation of CHO/CD14 was enhanced by the presence of serum (data not shown). Scanning densitometry of the autoradiographs showed that the ratio of nuclear NF-κB in stimulated/unstimulated CHO/CD14 cells increased by 2.8-fold in the presence of serum. In contrast, densitometry of the autoradiograph shown above revealed that serum reduced the effects of LPS by 1.4- and 2.6-fold (1 and 10 ng of LPS/ml, respectively).

mobilized anti-CD18, anti-CD11a, or anti-CD11c antibodies triggered the release of hydrogen peroxide (45), and concanavalin A binding to CD11c activated an oxidative burst in phagocytes (46). In view of these data, it seems likely that the leukocyte integrins are capable of directly transducing signals after binding to their respective ligands, including bacterial LPS.

The discovery that CD14 could function as a signaling receptor for LPS–LBP complexes was a significant contribution to the understanding of macrophage and neutrophil activation by LPS (16). Since then, several investigators have found evidence to suggest that alternative pathways coexist (22, 47, 48), but none of these CD14-independent pathways have been identified. CD11c/CD18 is, thus, the first alternative LPS-signaling pathway to be defined.

CD14 appears to be the predominant LPS receptor on peripheral monocytes. In blood stream infections, where serum and LBP are ample, CD14 is likely the major LPS-receptor involved in the acute cytokine response to invading Gram-negative bacteria. This would account for the ability of CD18-deficient monocytes, which express CD14, to respond normally to LPS (14). Although CD14-deficient knock-out mice were found to be extremely hyporesponsive to injected LPS (49), we propose that macrophages may encounter Gram-negative bacteria under a variety of situations which poorly resemble the interactions of injected LPS with blood monocytes. Tissue infections, especially infections at mucosal sur-
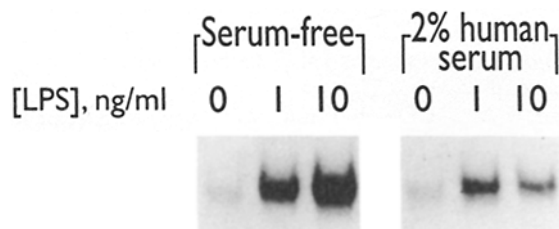
faces and in abscess cavities, may occur in the absence of serum-derived proteins which catalyze LPS interactions with CD14. In addition, tissue macrophages may differ from blood monocytes in the relative expression of CD14, in comparison to other LPS receptors. The importance of LPS is generally considered in the narrow context of sepsis and cytokine overproduction. Yet other effects of LPS, including the influence of LPS as a costimulatory molecule during the development of specific immunity to Gram-negative bacteria, may be just as profound. Further delineation of the mechanisms involved in activation of leukocytes by invading pathogenic Gram-negative bacteria will not only enhance our understanding of the host's response to infection, but may open up new avenues for therapeutic intervention.

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