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LACTOFERRIN-LIPOPOLYSACCHARIDE INTERACTIONS

Effect on Lactoferrin Binding to Monocyte/Macrophage-Differentiated HL-60 Cells[1]

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Lactoferrin (LF) has been implicated in a number of functions including the negative regulation of myelopoiesis in vitro and in vivo, an effect mediated by suppression of cytokine release from monocytes/macrophages. This suppression is abrogated by bacterial LPS. In the present study, HL-60 cells were induced to differentiate to monocytes/macrophages by 12-O-tetradecanoyl phorbol-13-acetate, and LF-binding assays were performed. After differentiation, HL-60 cells showed a twofold increase of LF-binding sites with no difference in the specificity or affinity of LF between pre- and post-differentiated cells. CD11a, CD11b, and CD11c Ag, which have been associated with specific binding sites for LPS on monocytes/macrophages, were also increased three- to fourfold after differentiation. With the use of this system, the effect of LPS on LF binding was studied. At 37°C, LPS enhanced LF binding on HL-60 cells, especially after differentiation. Conversely, at 4°C, LPS inhibited LF binding. There was little effect of temperature on LF binding in the absence of LPS. In the presence of polymyxin B sulfate, the enhanced LF binding by LPS was abrogated. Also, pretreatment with mAbCD11 and/or mAb5D3, which are associated with or directed against candidate LPS receptors, reduced LF binding. Cross-linking studies using an iodinated, photoactivatable LPS derivative ([^{125}I]ASD-LPS) demonstrated directly the specific binding of LPS to LF. These data indicate a dichotomous nature of LF binding on monocyte/macrophage-differentiated HL-60 cells—one being mediated by specific LF receptors whereas the other is apparently mainly via LPS receptors after formation of an LF-LPS complex. These interactions, for which a model is proposed, help to explain the mechanism behind LPS abrogation of the myelopoietic suppressive effects of LF, and a situation that probably occurs during bacterial infection.

LF,[4] an iron-binding glycoprotein found in biologic secretions such as milk, tears, semen and plasma, and a major component of the specific (secondary) granules of neutrophilic granulocytes, has been implicated in a number of functions (reviewed in Reference 1). These include bacteriostatic and bactericidal effects (2, 3) believed to involve removal of iron from the microorganism's environment (4) and the negative regulation of myelopoiesis in vitro (1, 5–9) and in vivo (10, 11). This latter function is mediated through suppression from monocytes and macrophages of cytokines such as CSF (5, 7) and IL-1 (12). IL-1 can trigger other cells to release CSF (13, 14). Monocytes and macrophages have specific receptors for LF (1, 15, 16), and initiation of LF suppression of cytokine release is considered to involve an interaction of LF with these receptors (1, 5). In this context, it is of potential relevance that bacterial LPS can abrogate the myelosuppressive effects of LF in vitro (5, 6) and in vivo (11). Because fucoidin, a polyanionic algal polysaccharide, blocks LF binding to mouse macrophages, an effect resulting from the formation of a stable LF-fucoidin complex (17) that apparently cannot bind to the LF-receptor, it was possible that LPS modulation of LF action involved a direct LPS-LF interaction. To evaluate this possibility, undifferentiated and TPA-induced monocyte/macrophage differentiated HL-60 cells were used as target populations to evaluate LF-receptor binding. The results, using mAb known to be associated with, or to directly recognize, LPS-receptors, suggested a direct interaction between LPS and LF, a finding confirmed by using a photoactivatable, cleavable cross-linking derivative of LPS. A model for this interaction is proposed.

MATERIALS AND METHODS

Cell culture and induction of monocytic differentiation. HL-60 cells, obtained from the American Type Culture Collection (Rockville, MD), were maintained in continuous culture in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% FCS (Hyclone, Logan, UT), 2 mM L-glutamine, penicillin (50 U/ml) and streptomycin (100 μg/ml). Induction of monocytic differentiation was performed

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[4] Abbreviations used in this manuscript: LF, lactoferrin; TPA, 12-O-tetradecanoyl phorbol-13-acetate; FeLF, iron-saturated lactoferrin; DPBS, Dulbecco's PBS; ASD-LPS, LPS derivatized with sulfosuccinimidyl 2-(*p*-azidosalicylamido) ethyl-1,3′-dithiopropionate; PMX, polymyxin B sulfate.

as described by others (18). Briefly, TPA (Sigma Chemical Co., St. Louis, MO) solubilized in acetone at 0.16 mM was added to the 2.5×10^5 cells/ml in suspension to achieve a final concentration of 16 nM and cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. Control cultures contained equivalent amounts of acetone. After culture with TPA, adherent cells were harvested, washed twice in DPBS supplemented with 2% FCS before use in LF-binding studies. Viability was over 98% as determined by trypan blue dye exclusion. Cellular morphology was assessed after staining with May-Grunwald-Giemsa and nonspecific esterase (19).

Surface Ag analysis. TPA treated and control HL-60 cells were stained with FITC-labeled mAb and immunofluorescence was analyzed by a FACS-440 flow cytometer (Becton Dickinson, Sunnyvale, CA). Before staining, cells were preincubated with heat-inactivated human AB serum to block antibody binding to FcR and then incubated with one of the following mAb for 20 min at 4°C: CD11a mAb (Dako, Carpinteria, CA, directed against the α-chain of LAF-1 (20)); CD11b mAb (Mo-1, Ortho, Raritan, NJ, directed against the α-chain of C3bi receptors (21)); or CD11c mAb (Dako, directed against the α-chain of p150,95 (22)). These three surface Ag have been reported to consist of adhesion-promoting receptors and have been associated with binding sites for LPS (23). Cells were then washed twice in DPBS with 2% FCS and incubated with the appropriate FITC-conjugated goat anti-mouse secondary antibody for 20 min at 4°C. Background fluorescence was determined by using an isotype-matched mouse mAb that does not react with human cells. The cells were washed three times and maintained at 4°C until they were analyzed.

Molecules. Purified human milk LF (Sigma Chemical Co., St. Louis, MO), found to migrate as a single band on 12% nonreducing SDS-PAGE gels after Coomassie blue staining or after autoradiography of ^{125}I-labeled LF (data not shown), was iron saturated with ferric ammonium sulfate and L-ascorbic acid as previously described (6). Iron saturation was assessed spectrophotometrically by an increase in the ratio of OD at 460 nm wavelength vs 280 nm. This ratio was consistently 0.040 and was considered to reflect a fully iron saturated state. Chromatography on Superose-12 HR10/30 (Pharmacia, Piscataway, NJ) gel-filtration column revealed that over 95% of Fe LF was in a monomeric form.[5] LF was iodinated with IODO-BEADS (Pierce, Rockford, IL) according to the manufacture's instructions. Free ^{125}I was removed by using an Econo-Pac 10GD column (BioRad, Richmond, CA). The specific activity of iodinated LF was about 65,000-80,000 mCi/mM. [^{125}I]LF was stored at −20°C and used within 2 wk to avoid radiolysis during storage. Any contaminating endotoxin was removed using Detoxi-Gel (Pierce) immediately before each binding assay. Endotoxin-depleted LF and binding buffer had <10 pg/ml endotoxin as measured by the limulus amebocyte lysate assay (Sigma). Protein concentration was measured by the Bio-Rad protein assay kit using BSA (Sigma) as a standard. Other reagents used were: *Escherichia coli* LPS (serotypes 0111:B4, 026:B6, and J-5), polymyxin B sulfate, human transferrin, human liver ferritin, human γ-globulin and fucoidin were purchased from Sigma. mAb to the 80-kDa LPS receptor was as previously described (24).

[^{125}I]Lactoferrin binding assay. Binding assays were performed using a phthalate oil procedure for separating cell-bound and -free radioactivity. Cells (4×10^6) were suspended in 100 μl of binding medium, RPMI 1640 and 20 mM Hepes, pH 7.4, in microcentrifuge tubes. This medium also contained a metabolic inhibitor (0.2% sodium azide) to minimize receptor internalization as well as 0.2% human transferrin to minimize nonspecific binding. Preliminary data indicated that the use of transferrin gave more reproducible results than when BSA was used (data not shown). Cells were incubated with various concentrations of [^{125}I]LF for 90 min at 4°C (maximum binding was reached after 45 min of incubation, and no significant difference was noted when binding was done at 37°C; data not shown). Nonspecific [^{125}I]LF binding was determined in the presence of a 100-fold molar excess of unlabeled LF. Three 30-μl aliquots from each tube were removed and layered on top of 100 μl of a mixture of dibutyl phthalate (Sigma) and bis(2-ethylhexyl)phthalate (Aldrich, Milwaukee, WI) (1.5/1; v/v), respectively. Samples were centrifuged at 13,000 × *g* for 2 min. Cellbound and cellfree [^{125}I]LF was quantitated with a 5500B Beckman Gamma Counter. Binding data were analyzed by the method of Scatchard (25).

Affinity cross-linking of LF and LPS. Sulfosuccinimidyl-2-(*p*-azidosalicylamido)ethyl-1,3′-dithiopropionate (Pierce), a photosensitive, cleavable cross-linking reagent, was covalently coupled with *Escherichia coli* 0111:B4 LPS and iodinated as described elsewhere (26). This [^{125}I]ASD-LPS probe was incubated with LF or other proteins for 30 min at 37°C in borosilicate glass tubes, and irradiated with short wavelength UV light for 10 min at 20°C. Samples were analyzed by SDS-PAGE under reducing or nonreducing conditions according to the method of Laemmli (26). Proteins were stained with Coomassie brilliant blue R250 (BioRad). Gels were then destained and dried. Autoradiography was performed on Kodak X-OMAT x-ray film with intensification screen (DuPont, Wilmington, DE) at −80°C.

[5] **Mantel, C. R., K. Miyazawa, and H. E. Broxmeyer.** 1990. Lactoferrin polymerization during iron saturation. *Submitted for publication.*

RESULTS

Lactoferrin binding to HL-60 cells. Cells cultured with TPA for 72 h were >95% adherent, had the morphology of monocytes/macrophages, and were greater than 95% nonspecific esterase positive. Cells cultured without TPA were nonadherent and nonspecific esterase negative. Induction of monocytic differentiation was accompanied by a three- to fourfold increase, respectively, in the mean channel number of fluorescence intensity of cells stained by CD11a, CD11b, and CD11c mAb, as assessed by flow cytometric analysis (Fig. 1). TPA treatment of cells was also associated with an approximately twofold increase in specific LF binding; enhanced binding reached a plateau between 72 h and 120 h of cell culture (Fig. 2). Because culture for 96 h or more with TPA resulted in increasing numbers of nonviable cells, a 72-h treatment was chosen for further studies. LF binding became saturated at 4°C after 90 min for both differentiated and

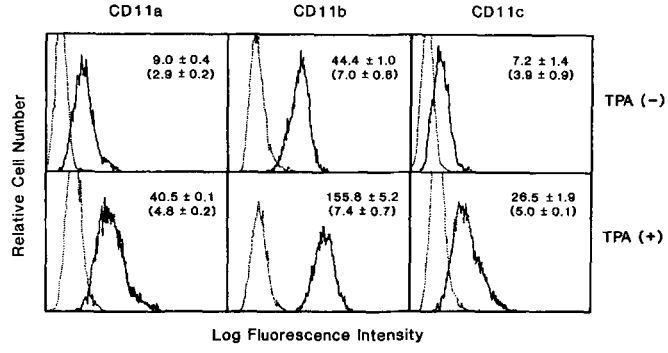

Figure 1. CD 11 Ag expression on HL-60 cells after TPA-induced differentiation. Cells were cultured for 72 h with or without 16 nM TPA and analyzed by flow cytometry with the use of CD11a, CD11b, and CD11c mAb (——) and a nonreactive isotype identical mAb (· · ·). The first number of each panel represents the fluorescence intensity of the mean channel number. The number in parentheses represents the mean channel number of background. (Mean ±SD for two separate experiments.)

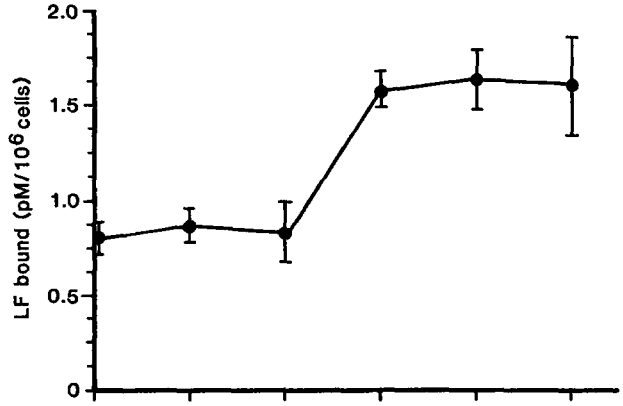

Figure 2. Kinetics of specific LF binding to HL-60 cells during culture with TPA. TPA-treated cells were incubated with 7.1×10^{-8} M [^{125}I]FeLF for 90 min at 4°C. Nonspecific binding was measured in the presence of 100-fold unlabeled LF and was subtracted from total binding to obtain specific binding.

undifferentiated cells (Fig. 3). There was an increase in the total number of binding sites on differentiated cells as compared with control cells. Scatchard analysis of the binding data indicated two classes of binding to both differentiated and nondifferentiated cells: a higher affinity binding class ($Kd_1 = 1.3 \times 10^{-7}$ M) and a lower binding affinity class ($Kd_2 = 1.2 \times 10^{-6}$ M). The number of high affinity binding sites per cell was estimated to be 1.9×10^6 whereas the number of low affinity binding sites was 6.3×10^6 on differentiated cells. Control cells had 9.6×10^5 sites/cell for high affinity and 3.1×10^6 sites/cell for low affinity-binding sites. Thus, the increased binding after differentiation was probably caused by an equal increase in the number of both classes of binding sites, and not by a change in affinity. However, the twofold increase of total binding may in part have been related to an increase in cell-surface area.

As shown in Table I, among a number of related molecules only FeLF or native LF (8% iron saturated) could compete for [^{125}I]FeLF binding. No differences were noted in competition of specific binding between differentiated and undifferentiated HL-60 cells. Also, fucoidin, an algal polysaccharide that binds LF directly by formation of a stable complex, was found to be a potent competitor of LF binding, as reported by others (17).

Effect of LPS on LF binding to differentiated HL-60 cells. Because fucoidin, a polyanionic polysaccharide, effectively inhibited binding of LF to differentiated HL-60 cells, we evaluated the effect of several forms of bacterial LPS, also polyanionic polysaccharides, in this system. As seen in Figure 4, *E. coli* 026:B6 LPS (40 μg/ml) had no effect on LF-binding at 4°C, however, at 37°C, it significantly enhanced LF-binding. Also differentiation of HL-60 cells to monocytes/macrophages dramatically increased LF binding in the presence of LPS at 37°C. When the concentration of [^{125}I]LF was increased from 7.1×10^{-8} M (Fig. 4*A*), to 6.7×10^{-7} M (Fig. 4*B*), differ-

TABLE I

Competitive Inhibition of LF Binding to HL-60 Cells[a]

Competitor	% of LF Bound (Mean ± SD)	
	Untreated cells	TPA-treated cells
None (control)	100	100
FeLF (unlabeled)	18 ± 1	20 ± 1
Native LF (unlabeled)	22 ± 8	24 ± 1
Fe transferrin[b]	96 ± 1	97 ± 0
Apo transferrin[b]	101 ± 1	100 ± 1
Liver ferritin	98 ± 3	103 ± 3
γ-Globulin	106 ± 6	99 ± 1
BSA	106 ± 4	102 ± 6
Fucoidin	11 ± 0	9 ± 1

[a] Differentiated and undifferentiated HL-60 cells were incubated with 7.1×10^{-8} M [^{125}I]FeLF in the presence of 100-fold molar excess unlabeled competitor as shown above for 90 min at 4°C and LF binding was quantified. Each number represents the percentage of LF binding compared with control cells (without competitor). All values shown are the mean ± SD of triplicate experiments.

[b] For transferrin-competition experiments, 2.0% BSA was included in binding buffer instead of the usual 0.2% transferrin.

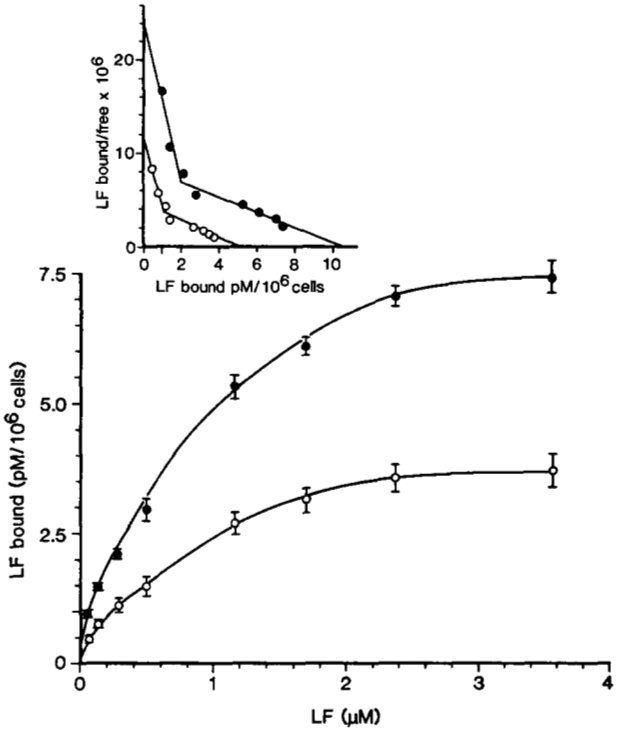

Figure 3. Specific dose-response binding of [^{125}I]FeLF to differentiated or control HL-60 cells. Nonspecific binding was measured as before and was subtracted from total binding to obtain specific binding. The mean ±SD of three experiments is shown. ○, Undifferentiated cells; ●, differentiated cells. Scatchard plot analysis (*insert*) indicates two types of LF-binding sites—high affinity and low affinity. Linear regression analysis was used to estimate the x-intercepts for calculating the number of binding sites per cell.

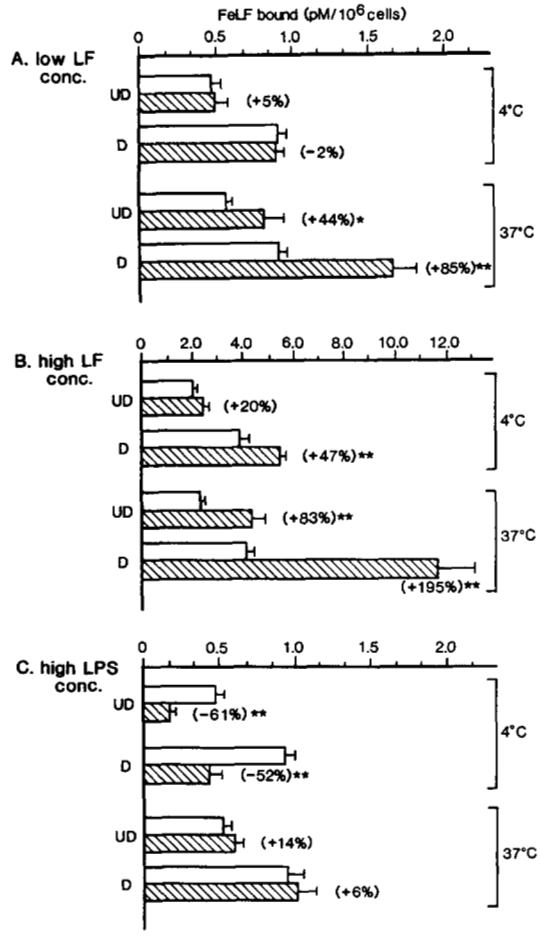

Figure 4. Effect of LPS on LF binding to HL-60 cells. Differentiated (*D*) or undifferentiated (*UD*) HL-60 cells were incubated with [^{125}I]FeLF in the presence (▨) or absence (▭) of *E. coli* 026:B6 LPS, and the total LF binding to the cells at 4°C or 37°C was assessed. Binding conditions were (*A*) 7.1×10^{-8} M [^{125}I]FeLF and 40 μg/ml LPS; (*B*) 6.7×10^{-7} M [^{125}I]FeLF and 40 μg/ml LPS; (*C*) 7.1×10^{-8} M [^{125}I]FeLF and 400 μg/ml LPS. The numbers in parentheses show the percent change of LF binding to the cells with LPS compared with that without LPS. (* $p < 0.05$; ** $p < 0.001$).

entiated cells became much more responsive to LPS-enhanced binding as compared with control cells at 37°C (Fig. 4*B*). However, when the LPS concentration was raised from 40 to 400 μg, LF binding was inhibited 39% for control cells and 48% for differentiated cells at 4°C (Fig. 4C). This suppressive effect was abrogated by incubation at 37°C instead of 4°C. In the absence of LPS, temperature had no significant effect on LF binding.

This "ambivalent" behavior of LPS on LF binding, i.e., enhanced binding at 37°C and inhibited binding at 4°C, was also observed for other strains of *E. coli* LPS (Fig. 5). The data using J-5, which lacks the O-Ag polysaccharide and contains only the core polysaccharide and lipid A portion of the LPS molecule, suggests that the O-Ag portion of the molecule probably does not participate in the interaction. However, when the LF-binding experiments were done in the presence of LPS pretreated with PMX, which binds to the lipid A portion of the LPS molecule and consequently blocks LPS receptor binding to macrophages (28, 29), the enhanced LF binding caused by LPS was abolished, even at 37°C, and resulted in 66% inhibition of binding. PMX itself did not have any effect on LF binding in the absence of LPS.

We also compared FeLF and native LF (8% iron saturated) binding to HL-60 cells in the presence of LPS (Fig. 5*A,B*). Because no difference was observed between FeLF and native LF, this interaction appeared not to depend on the iron content of LF, in the context of LF containing iron.

When differentiated HL-60 cells were preincubated with mAb CD11 and/or mAb 5D3 (24), both of which are associated with or directed against putative LPS receptors, LPS-enhanced LF binding was reduced or abolished (Fig. 6). Mouse IgG_1, IgM, mAbCD11, and mAb5D3 had no significant effect on LF binding in the absence of LPS.

Cross-linking of LF and [^{125}I]ASD-LPS. The above results suggested that modified LF binding in the presence of LPS was caused by binding to the LPS receptor. To evaluate whether this was caused by a direct binding of LPS to LF, the complex of which was then bound to LPS receptors, cross-linking experiments were performed by using a photoactivatable, iodinated 0111:B4 LPS derivative ([^{125}I]ASD-LPS). In this cross-linking reagent, the iodinated group and the photoactivatable azido group are distal to a reducible disulfide bond. Therefore, cleavage of this bond by reduction after cross-linking effectively transfers the label from the ASD-LPS to LPS-binding proteins (26).

As shown in Figure 7, radioiodine could be detected after reduction at the gel positions of FeLF and native LF (*lanes 3* and 4). Also visible is the "step-ladder" binding pattern characteristic of this LPS derivative (26). *Lane 2* shows the cross-linking of [^{125}I]ASD-LPS to BioRad marker proteins. Only BSA could be cross-linked with this reagent as previously reported (26). When FeLF and native LF are compared, native LF showed a denser radioactive band, presumably caused by a stronger binding of [^{125}I]ASD-LPS. Because identical amounts of native and FeLF were used, this could be interpreted as being caused by a higher affinity of native LF for LPS. In the presence of excess unlabeled LPS, no radioactivity was detected comigrating with LPS. These data suggest a direct and specific interaction of LF and LPS.

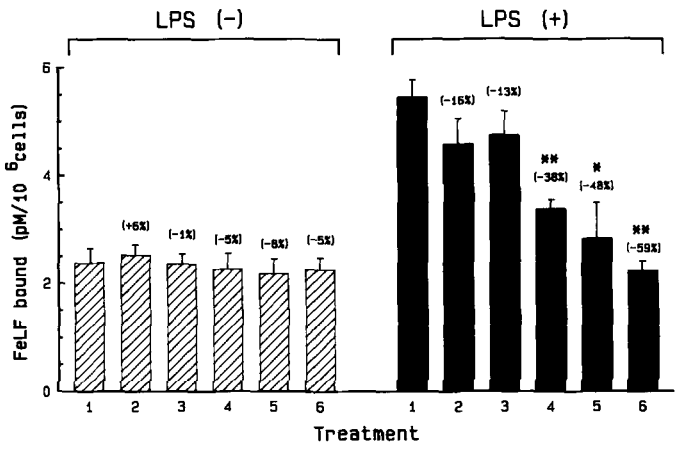

Figure 6. Effect of mAb directed against LPS receptors on enhanced LF binding to differentiated HL-60 cells in the presence of LPS. Differentiated HL-60 cells (5.0×10^6 in 500 μl DPBS with 2% FCS) were preincubated with 10 μg of mAb CD11a, CD11b, CD11c, and/or mAb 5D3 for 30 min at 4°C. The cells were washed twice with DPBS, incubated with 1.75×10^{-7} M FeLF for 90 min at 37°C in the presence (■) or in the absence (▨) of 40 μg/ml LPS (*E. coli* 0.111:B4), and LF binding to the HL-60 cells was measured. Columns: 1) no treatment, 2) mouse IgG_1 pretreatment, 3) mouse IgM pretreatment, 4) mAb CD11 pretreatment, 5) mAb 5D3 pretreatment, 6) mAb CD11 plus mAb 5D3 pretreatment. The number in parentheses is the percent change of LF binding to cells pretreated with antibodies compared with those without pretreatment. The data are the results of two separate experiments (* $p < 0.01$ vs column 1; ** $p < 0.001$ vs column 1.).

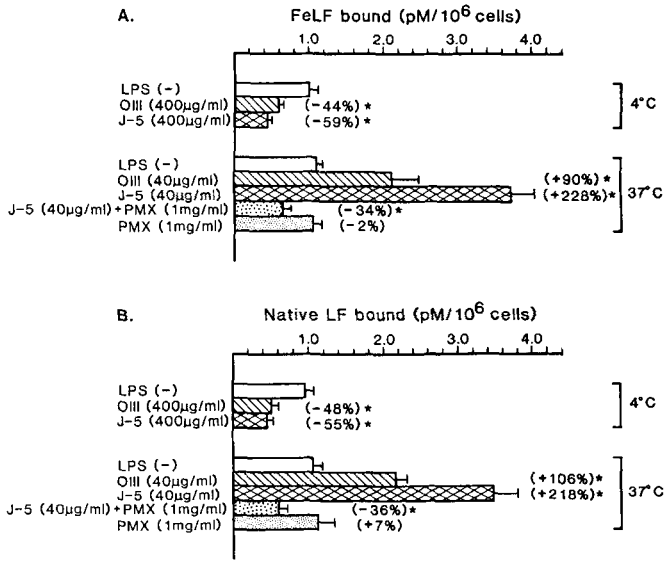

Figure 5. The influence of PMX and LF iron content on the interaction between LF and LPS. Differentiated HL-60 cells were incubated with [^{125}I] FeLF (7.1×10^{-8} M, sp. act. 79,400 mCi/mM); [^{125}I]native LF (6.9×10^{-8} M, sp. act. 75,000 mCi/mM) in the presence of *E. coli* 0111:B4; or J-5 LPS and/or PMX at 4°C or 37°C. Optimal enhancing (37°C) or inhibiting (4°C) concentration of each LPS was chosen based on data from experiments described in Figure 4. The number in parentheses represents the percentage of LF binding to the cells with LPS or PMX as compared with controls. (* $p < 0.001$ vs controls).

DISCUSSION

Our report has demonstrated that bacterial LPS can modulate the binding of LF on HL-60 cells in vitro, especially after the cells are induced to terminal monocyte/macrophage differentiation. This modulation is temperature dependent with enhancement seen at 37°C and inhibition seen at 4°C. It is proposed that the nature of these effects of LPS on LF binding are caused by a direct interaction of the two molecules, a conclusion supported

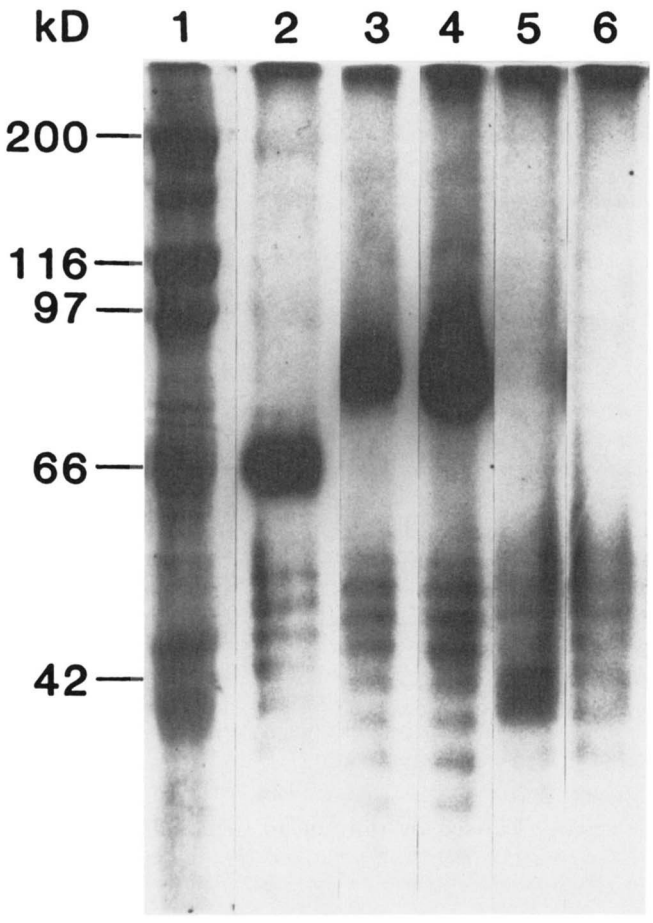

Figure 7. Cross-linking of [^{125}I]ASD-LPS to LF and other kinds of proteins. Proteins were incubated with [^{125}I]ASD-LPS (1 μg in 20 μl PBS; 1.4 μCi) and irradiated with short wavelength UV light. Samples were reduced with 5% 2-ME and applied to 11% SDS-PAGE gels. *Lanes: 1*) marker proteins (myosin, β-galactosidase, phosphorylase b, BSA, and OVA) stained with Coomassie blue; *2*) autoradiography of *lane 1*; *lanes 3* to *6* are also autoradiographs; *3*) FeLF (5 μg), *4*) native LF (5 μg), *5*) FeLF (5 μg) with excess unlabeled LPS (100 μg), and *6*) native LF (5 μg) with excess unlabeled LPS (100 μg).

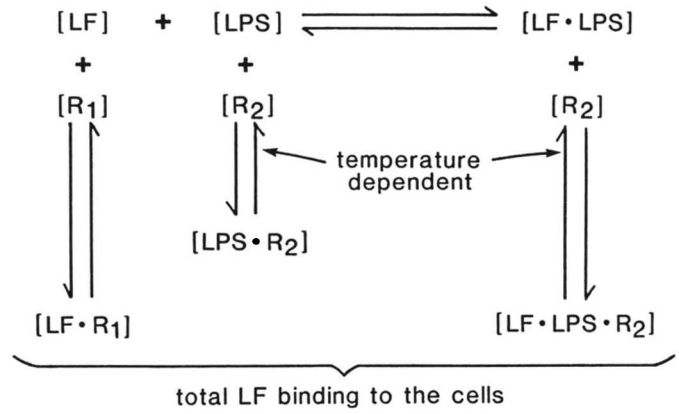

Figure 8. Proposed model for the interaction between LF and LPS on monocyte/macrophage. R_1: LF receptor; R_2: LPS receptor.

by several lines of evidence: 1) LPS binding to monocytes/macrophages is temperature dependent (23), similar to its effects on LF binding to HL-60 cells; 2) the LPS effect on LF binding is heightened after terminal differentiation of HL-60 cells, a process that is shown to increase the density of CD11 Ag, a group of surface molecules that are associated with LPS binding (23, 30); 3) polymyxin B sulfate, which binds to the lipid A portion of the LPS molecule and which blocks LPS receptor binding on macrophages (23, 28, 29), abrogates LPS-enhanced LF binding; 4) anti-LPS receptor mAb essentially abolish the enhancing effects of LPS treatment on LF binding; 5) LPS is directly and specifically cross-linked to LF by using a photosensitive derivatized LPS cross-linking reagent.

It is suggested that the phenomena noted can be explained by four equilibrium states as shown in Figure 8. When the concentration of LF is relatively low, a large excess of LPS shifts the equilibrium to the right to form the LF-LPS complex, which reduces the net-free LF concentration and suppresses LF binding to the cells at 4°C (because LPS binds to its receptor only weakly at 4°C). These conditions are similar to those in Figure 4*C* and also the fucoidin experiment (Table I). However, when these conditions are applied at 37°C, this suppression is masked by the binding of the LPS-LF complex to the LPS receptor instead of the LF receptor. When there is excess LF, the equilibrium is shifted to the left showing a small effect of LPS at 4°C, while showing the highest binding at 37°C. This is similar to the conditions in Figure 4*B*. It is possible that LPS stimulates LF receptor expression on HL-60 cells during the 90 min of incubation for the binding assay by "activation" of the differentiated HL-60 cells. However, we suggest that an influence of LPS activation would be very little, if any, because when the LPS concentration was increased from 40 to 400 μg/ml, enhanced LF binding was decreased, even at 37°C (Fig. 4, *A* and *C*), as a result of competitive binding of uncomplexed LPS to its receptor, leaving LF uninvolved.

A pivotal conclusion suggested by these data is that LF loses the ability to bind to the LF receptor after forming an LF-LPS complex. This notion is supported by experiments with PMX and anti-LPS-receptor antibodies. PMX is reported to inhibit binding of LPS by blocking the lipid A portion of the LPS molecule (23, 28) and thereby would inhibit binding of the LF-LPS complex as well. It is possible that PMX might inhibit the binding of LF to LPS, which would also result in reduced binding of LF to cells via LPS receptors. This is unlikely however, because LF binding in the presence of LPS and PMX is still reduced relative to LF alone (Fig. 5). Furthermore, earlier studies have shown that the binding site for PMX on LPS is distal from that for another cationic protein, lysozyme (31). Finally, when LPS receptors were blocked by pretreating cells with antibodies to LPS receptors, the enhancing effects of LPS were reduced. Recent data have established that the LF molecule consists of two lobes (N- and C-lobe) (32). Domain I of the N-lobe appears to be the critical region for LF receptor interaction (33). As the N-lobe of LF is cationic, it is reasonable to think that this important region might be involved in LPS (polyanion) binding and may change the conformation of this region and alter the ability of LF to bind to its receptor.

Several proteins have been reported to bind LPS. These include bacterial permeability increasing protein (34), lipopolysaccharide-binding protein (35), cationic antimicrobial protein (36), lysozyme (37), and serum albumin, which is usually added to buffers for receptor-binding assay for the purpose of reducing nonspecific binding (26). Thus, LF and LPS may interact under conditions in vivo that may be more complicated than that suggested in Figure 8. However, the data presented here may help

explain a number of biologic effects of LF.

As described above, LF acts as a negative myelopoietic regulator, an effect mediated by suppression of release of cytokines such as IL-1 from monocytes/macrophages (5–7, 12), and these effects are neutralized by LPS in a dose-dependent manner in vitro (5, 6). This can now be explained by a direct LF-LPS interaction. After formation of the LF-LPS complex, transmission of signal transduction for hematopoietic regulation may be impaired. However, it may still participate in IL-1 induction by the interaction between LPS and LPS receptors (38). Pertinent to this point are recent results showing that molecular complexes of LPS and lysozyme maintain this capacity to stimulate the production of IL-1 in murine macrophages (39).

LF released from neutrophils during the acute phase of infection in vivo (40) may be prevented from acting as a myelopoietic suppressor molecule by binding LPS at sites of infection. It has been shown by others that LF can protect mice injected with a lethal dose of *E. coli* (41) and that it has a prophylactic effect during infections in neutropenic patients (42) suggesting LF participation in the regulation of these important host-defense mechanisms and potential clinical application.

LF has been reported to have direct bacteriostatic and bacteriocidal activities (2, 3). The specific binding of LF to LPS on bacterial cells would be an appropriate recognition site to initiate these direct biologic activities. LF also has other indirect functions related to host defense mechanisms against infection in vivo. These include the promotion of polymorphonuclear leukocyte adhesiveness (43), reduction of lysozyme regeneration (44), and increased hydroxyl radical production by neutrophils (45).

Some of these biologic activities are influenced by iron saturation, i.e., LF needs to be in an iron-unsaturated state to function as a bacteriostatic agent (2, 3), but in an iron-saturated form to act as a myelopoietic suppressor factor (5, 6). Iron binding has been shown to alter the three dimensional conformation of the LF molecule (32). For this reason the effect of iron saturation on LPS modification of LF binding was investigated. No significant difference was detected. However, the data from the cross-linking study suggests that native LF may have a higher affinity for LPS than FeLF. The meaning of this is at present not clear.

Terminal monocyte/macrophage differentiation of HL-60 cells causes loss of ability of cells to proliferate (18); this is coincident with a twofold increase in total LF receptor number per cell (Fig. 3). The same phenomenon was observed by us with U-973 cells (46) induced to differentiate with granulocyte-CSF and granulocyte macrophage-CSF (data not shown). Scatchard analysis of HL-60 data revealed two classes of LF-binding sites—high and low affinity. These data are in agreement with previous reports by others (47) with a LF-dependent cell line HT29-D4. The individual role of each class of LF binding site is unknown.

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