Lactoferrin Inhibits the Lipopolysaccharide-Induced Expression and Proteoglycan-Binding Ability of Interleukin-8 in Human Endothelial Cells

Elisabeth Elass,* Maryse Masson, Joël Mazurier, and Dominique Legrand

Laboratoire de Chimie Biologique et Unité Mixte de Recherche no. 8576 du Centre National de la Recherche Scientifique, Université des Sciences et Technologies de Lille, 59655 Villeneuve d’Ascq cedex, France

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Interleukin-8 (IL-8), a C-X-C chemokine bound to endothelium proteoglycans, initiates the activation and selective recruitment of leukocytes at inflammatory foci. We demonstrate that human lactoferrin, an antimicrobial lipopolysaccharide (LPS)-binding protein, decreases both IL-8 mRNA and protein expression induced by the complex Escherichia coli 055:B5 LPS/sCD14 in human umbilical vein endothelial cells. The use of recombinant lactoferrins mutated in the LPS-binding sites indicates that this inhibitory effect is mediated by an interaction of lactoferrin with LPS and CD14s that suppresses the endotoxin biological activity. Furthermore, since dimeric IL-8 and lactoferrin are both proteoglycan-binding molecules, the competition between these proteins for heparin binding was investigated. Lactoferrin strongly inhibited the interaction of radiola- beled IL-8 to immobilized heparin, whereas a lactoferrin variant lacking the amino acid residues essential for heparin binding was not inhibitory. Moreover, this process is specific, since serum transferrin, a glycoprotein whose structure is close to that of lactoferrin, did not prevent the interaction of IL-8 with heparin. These results suggest that the anti-inflammatory properties of lactoferrin during septicemia are related, at least in part, to the regulation of IL-8 production and also to the ability of lactoferrin to compete with chemokines for their binding to proteoglycans.

During the inflammatory process, the vascular endothelium expresses various chemoattractant and cell adhesion molecules that result in the selective recruitment of leukocytes to inflammatory foci (8, 34). In that process, the selectins and their ligands first initiate the rolling of leukocytes. Then chemokines are expressed that stimulate integrins responsible for the strong adherence of cells to endothelium (10). Interleukin-8 (IL-8), a potent C-X-C chemokine, activates LFA-1 integrins (LFA-1) on neutrophils (3, 10) and also binds, as a dimer and with a low affinity, to heparin and heparan sulfate molecules (16, 20, 40). Once firmly attached, the cells are directed by a chemotactic gradient to transmigrate into tissue affected by injury or infection (38). The cell surface proteoglycans increase the local concentration of IL-8 that, in turn, regulates the activation of neutrophils through specific interactions with a G protein coupled receptor (30). Thus, interactions between IL-8 and proteoglycans present on the endothelium or in the extracellular matrix drive the formation of haptotactic chemokine gradients to the inflammatory sites. This critical step in the innate immune response involves the concerted action of adhesion molecules and chemokines.

When highly expressed, IL-8 may have pathophysiological consequences for the organism. Various exogenous stimuli, such as lipopolysaccharides (LPS) and proinflammatory cytokines (tumor necrosis factor alpha, IL-1), induce the secretion of IL-8 from many cells, including endothelial cells (15, 36).

During septic shock, LPS strongly activate the endothelial cells and promote leukocyte infiltration and microvascular thrombosis. This contributes to the pathogenesis of disseminated intravascular inflammation, leading to severe damage of endothelium (9). Endotoxin stimulation of endothelial cells is mediated by soluble CD14 (sCD14), a specific LPS receptor present in serum (2, 14). sCD14 binds to LPS with a high affinity and interacts with a signaling molecule, the Toll-4-like receptor (7, 12). At low endotoxin levels, a serum acute protein called the LPS-binding protein (LBP) catalyzes the transfer of LPS monomers from aggregates to CD14 and enhances the sensitivity of cells to LPS (13).

Lactoferrin (Lf), an iron-binding glycoprotein found in exocrine secretions of mammals and released from granules of neutrophils during inflammation (28), modulates the endotoxin activation of cells in vivo and in vitro (5). Lf protects against sublethal doses of LPS in mice (26, 44) and in germfree piglets (21). Moreover, human Lf (hLf) binds specifically and with a high affinity to the lipid A regions of LPS (1, 11), sCD14, and the sCD14/LPS complex (4). Two N-terminal basic clusters of hLf, residues 1 to 5 and 28 to 34, are responsible for the binding to anionic molecules, such as LPS (11, 41), heparin, or cell-surface heparan sulfates (22, 27, 43). Recently interactions between Lf, LPS, and the LPS/CD14s complex were demonstrated that impede the expression of two adhesion molecules, E-selectin and the intercellular adhesion molecule 1 (ICAM-1), an integrin ligand, on human umbilical vascular endothelial cells (HUVEC) (4). These observations indicate that Lf may down-regulate the adherence of leukocytes to endothelial cells. We hypothesized that hLf may also inhibit the expression and the function of chemokines, thus limiting not only the binding...
of leukocytes to the endothelium but also their migration to inflamed tissues.

In this paper the effect of hLf on the expression of IL-8 induced by LPS in endothelial cells and its binding to proteoglycans was investigated. We first studied the inhibitory effect of hLf on the expression of IL-8 in LPS-activated HUVEC. Then we determined whether hLf could compete with IL-8 for binding to glycosaminoglycans. Finally, an insight in the role of domain N-1 of hLf in the inhibition of IL-8 production was gained with mutated recombinant hLfs or hLf variants.

MATERIALS AND METHODS

Materials. RPMI 1640 medium, obtained from Gibco-BRL (Egny, France), was supplemented with 10% fetal calf serum (FCS), which was purchased from Dominique Dutscher S.A. (Brumath, France), and with 2 mM L-glutamine from Gibco-BRL. Endothelial cell growth medium SMF, endothelial cell growth supplement (heparin, epidermal growth factor basic fibroblast growth factor (bFGF), hydrocortisone, gentamicin, and amphotericin B), and FCS (10%) were from PromoCell (Heidelberg, Germany). SP-Sepharose fast flow and PDI 0.5-25 columns were from Phth. the Limnology, Uppsalah, Sweden). The propylene glycol was from Cooper (Melun, France). Dulbecco’s phosphate-buffered saline (PBS), human serum transferrin, bovine serum albumin (BSA), collagenase, gelatin, heparin-BSA (H4043), heparin grade IA from porcine intestinal mucosal (H3149), and LPS 055:B5 from Escherichia coli were purchased from Sigma Chemical Co. (St. Louis, Mo.). Recombinant human IL-8 expressed in E. coli was from Sigma Chemical Co., and radiolabeled IL-8 was from Amersham (specific activity, 2000 Ci/mmol). Recombinant human sCD14 was purchased from BioMerieux (Grenfalld, Germany). sCD14 was obtained from serum-free culture supernatant of CHO cells transfected with human sCD14 cDNA cloned into pPOL-DHFR expression vector. Human IL-8/Nap-1 module set (BSTM204MST) was from Bender Medsystems Diagnostic (Vienna, Austria). All chemicals used were of the highest analytical grade, and LPS contamination was evaluated with Limulus amoeboocyte lysate assay kit (QCL1000; BioWhittaker, Walkersville, Md.).

Endothelial cell culture. Endothelial cells (HUVEC) were derived from human umbilical vein, according to a method previously described (17). Briefly, after treatment of the umbilical vein with 0.2% (wt/vol) collagenase in 37°C prewarmed RPMI for 30 min, HUVEC were collected by centrifugation (600 × g for 15 min). Cells were resuspended in endothelial cell growth medium SMF and were cultured in gelatin-coated 35-mm-diameter tissue culture wells at 37°C and 5% CO2. They were collected after trypsinization and then cultured in gelatin-coated 96- or 6-well flat-bottomed culture plates until confluency. Only cells of the third and fourth passages were used. Viability was over 96% as determined by trypan blue dye exclusion.

Preparation of hLf and hLf (−3N). Native hLf (nHLf) was purified from fresh human milk (provided by the milk bank of “Jeanne de Flandres” Hospital, Lille, France) by cation exchange chromatography and was iron saturated, as previously reported (29, 39). Homogeneity of the protein was checked by sodium dodecyl sulfate–7.5% polyacrylamide gel electrophoresis (SDS-PAGE).

Expression and purification of recombinant and mutated hLfs. The expression and purification of various hLf mutants were performed as previously described (23, 35). A full-length 2.3-kbp cDNA coding for hLf was obtained from a human mammary gland cDNA library (Clontech, Palo Alto, Calif.). Three human recombinant hLf (rHLF) variants were obtained by site-directed mutagenesis of the cDNA-coding hLf sequence by using the Sculptor in vitro mutagenesis system kit (Amersham International, Buckinghamshire, United Kingdom). Nonmodified rHLf; G4R-rHLf, a mutated rHLf in which sequence 1GRRRR5 was deleted; EGS-rHLf, an hLf whose sequence 1RKVRGPP19 was replaced by EGS (the 365 to 367 C-terminal counterpart of sequence 28 to 34); and 4G4R-EGS-rHLf, a rHLf with both G4R and EGS modifications, were produced in a baculovirus expression system and were purified as previously reported (23). The purity of the rHLf mutants was assessed by SDS–7.5% PAGE. The N-terminal amino acid sequence was checked by the Edman degradation procedure by using an Applied Biosystem 477 protein sequencer.

Activation of endothelial cells by LPS in the presence of hLf. HUVEC were seeded into 6-well plates for studying IL-8 mRNA expression or into 96-well plates for assaying the IL-8 production. HUVEC grown to confluence were washed twice and incubated in RPMI-FCS in the presence of 10 to 1,000 ng of E. coli 055:B5 LPS/ml. The effect of hLf on IL-8 mRNA expression by HUVEC was investigated in the presence of 50 µg of nHLf/ml. To study the effect of hLf on IL-8 production, 5- to 150-µg/ml concentrations of either nHLf, rHLf, hLf (−3N), or mutated rHLfs (EGS-rHLf, G4R-rHLf, G4R-EGS-rHLf) were incubated with the cells. These hLf concentrations are those encountered at the inflammatory sites (6, 25). Before incubation with cells, nHLf and its variants were preincubated for 30 min at room temperature with LPS. Controls were performed without LPS and hLf or with hLf alone. After 6 h of incubation at 37°C and 5% CO2, the cells were washed immediately prior to RNA extraction. Prior to IL-8 protein assays, confluent cells were incubated at 37°C with RPMI-FCS for 1 h. Viability was over 96% as determined by trypan blue dye exclusion. The supernatants were collected and centrifuged prior to quantitation of IL-8 by enzyme-linked immunosorbent assay (ELISA).

RNA extraction from LPS-induced endothelial cells and RT-PCR. Extraction of total RNA from HUVEC was performed with the Total Quick RNA Cells and Tissues kit (Talent srl, Trieste, Italy) according to the manufacturer’s instructions. Five micrograms of each total RNA preparation was reverse transcribed (RT) into first-strand cDNA by using oligo(dT) primers (Stratagene) and 20 U of Moloney murine leukemia virus reverse transcriptase (Promega). One twelfth of the mixture was then amplified by PCR with a primer pair (Cybergénique, Saint-Malo, France) designed for the specific detection of human IL-8. 5′-CCA AAXTTTGCACGAGAAT-3′ (sense primer) and 5′-AAXTTTACCGGAA TCTGTG-3′ (antisense primer). ICAM-2 was used as a reference, since it was found to be constitutively expressed in HUVEC cells (31). First-strand sequence amplification was performed with Tt polymerase (Promega) and the following steps: an initial denaturation at 94°C for 5 min, multiple cycles (25 and 35 cycles for IL-8 and ICAM-2, respectively) consisting of denaturation at 94°C for 5 min, annealing at the optimal temperature (55 and 60°C for IL-8 and ICAM-2, respectively) for 1 min, and a primer extension at 74°C for 1 min, followed by a final extension step at 74°C for 1 min. PCR assays were performed in triplicate. Twenty out of 25 µl of each PCR was loaded on a 1% agarose gel stained with 0.5 µg of ethidium bromide/ml. The gel was then analyzed by computerized densitometric imaging with the Bio-Rad GelDoc analysis system and Quantity One software, version 4.1.0 (Bio-Rad, Milano, Italy). Amplification products were subjected to (Taq) cloning (Invitrogen, Zoetermeer, Netherlands) and were sequenced to confirm the specificity of the PCR products. The results were expressed as the ratio of the fluoresence intensities of IL-8 and ICAM-2 PCR products.

Quantitation of IL-8 release by ELISA. HUVEC plated into gelatin-coated 96-well plates and grown to confluence were stimulated by LPS as described above. Cell culture supernatants were diluted fourfold, and 100 µl was processed for IL-8 quantification by sandwich ELISA according to the manufacturer’s instructions (Bender Medisystems Diagnostic). Briefly, microtiter plates were coated overnight at 4°C with 100 µl of (5 µg/ml) anti-IL-8 monoclonal antibody diluted in PBS, washed with PBS–0.05% (vol/vol) Tween 20, and blocked for 2 h with 200 µl of PBS–0.05% Tween containing 0.5% (wt/vol) BSA. Supernatants diluted in PBS–0.05% Tween–0.1% Triton X-100 were then transferred to the wells. After 2 h of incubation at room temperature with a second peroxidase conjugated anti-IL-8 polyclonal antibody (100 µl of a 1:6,500 dilution), the wells were washed and detection was performed with 0-phenylenediamine-dihydrochloride (150 µl) for 20 min at room temperature. The reaction was stopped with 50 µl of 2 M H2SO4 per well, and the absorbance at 490 nm was measured on a microplate reader. IL-8 concentrations in the cell culture supernatant were quantitated in comparison with a standard curve generated with recombinant human IL-8.

The number of unstimulated cells present in each well was estimated by assaying the protein contents according to Lowry’s method (24).

Competitive inhibition of the IL-8 binding to immobilized heparin. Microtiter plates (Immobilon) were coated for 24 h at 4°C with 100 µl of (10 µg/ml)
heparin-BSA in PBS. After being washed with PBS, wells were blocked with 200 µl of 1% BSA in PBS for 2 h at room temperature. The solution was discarded and plates were washed three times with PBS. The binding of IL-8 to immobilized heparin-BSA was assessed in the presence of 0.5 nM 125I-IL-8 and 5 nM unlabelled chemokine by using binding buffer (PBS pH 7.4) containing 5 mM MgCl2, 1 mM CaCl2, and 0.5% BSA) for 4 h at 20°C. As previously reported (16, 20), the addition of unlabelled IL-8 in the nanomolar range increases the binding of radiolabelled IL-8 to heparin by forming chemokine multimers. Under similar conditions, 125I-IL-8 binding to heparin-free tubes coated with BSA was estimated and the amounts of IL-8 bound to heparin, in the presence or absence of competitor, respectively, and [IL] is the concentration of the competing ligand.

Statistical analysis. Data are presented as the means ± standard errors for the indicated number of independent experiments. Statistical significance was analyzed with a Student’s t test for unpaired data. Values of P < 0.05 were considered to be significant.

RESULTS

Effect of hLf on the expression of IL-8 by LPS-stimulated endothelial cells. In order to determine if hLf may modulate the expression of IL-8 by LPS-stimulated endothelial cells, we studied the IL-8 mRNA expression and protein production by HUVEC cells in the presence of various amounts of LPS and nhLf.

First, the expression of IL-8 mRNA was assessed by RT-PCR (Fig. 1). The IL-8 mRNA expression by HUVEC was clearly dependent on the LPS concentration (0.1 to 1,000 ng/ml) and was maximal when cells were incubated with 100 ng of LPS/ml. The preincubation of LPS with 50 µg of nhLf/ml induced a twofold decrease of IL-8 mRNA expression (Fig. 1A). This phenomenon was observed at any LPS concentration used for the activation of cells. The inhibition was nhLf dose-dependent and reached 53% with 100 ng of LPS/ml in the presence of 100 µg of nhLf/ml (Fig. 1B).

Second, the production of IL-8 by LPS-stimulated HUVEC in the presence of nhLf was assessed by ELISA. As shown in Fig. 2A, the exposure of cells to LPS in the presence of 10% FCS resulted in a dose-dependent increase in IL-8 secretion, whereas nhLf alone had no effect. Similar to findings of a previous study (15), we detected about three- and fourfold higher production of IL-8 with 10 and 100 ng of LPS/ml, respectively, compared to that of unstimulated cells. The IL-8 secretion induced by endotoxin was significantly decreased in the presence of nhLf; 32% ± 3% and 51% ± 5% inhibitions were measured when cells were incubated in the presence of 100 µg of nhLf/ml and induced with 10 and 100 ng of LPS/ml, respectively. Furthermore, hLf blocked the secretion of IL-8 in a concentration-dependent manner (Fig. 2B). Up to 54% ± 3% inhibition was measured with 100 ng of LPS/ml in the presence of 150 µg of nhLf/ml. These findings demonstrate that Lf interferes with both endotoxin-induced IL-8 mRNA expression and production by endothelial cells.

Importance of the N-terminal domain of hLf in the inhibition of IL-8 production by LPS-stimulated endothelial cells. The N-terminal domain I of hLf and, more precisely, the sequences involving amino acid residues 1GRRRR5 and 28RKVRGPP34, have been identified as LPS-binding sites; thus, we investigated by ELISA whether the hLf mutated in these two N-terminal basic clusters could modify the LPS-induced IL-8 production. As illustrated in Fig. 3, IL-8 secretion was decreased by 43% ± 3% in the presence of 100 µg of rhLf/ml. In contrast, neither rhLf changed between residues 28 to 34 (EGS-rhLf) nor G4R-EGS-rhLf lacking residues 1 to 5 and changed at residues 28 to 34 were able to prevent the IL-8 production in HUVEC. No more than 6% ± 6% inhibition was detected in the presence of 100-µg/ml concentrations of these mutants. Moreover, no clear inhibitory effect was measured with 50 µg of G4R-rhLf/ml, and the addition of 100 µg of the protein/ml resulted in a twofold less inhibition (18% ± 4%) than that of rhLf. The deletion of only the first three residues from hLf [hLf(−3N)] partially modified the IL-8 production of cells. About 15% ± 3% inhibition was obtained with 50 µg of hLf(−3N)/ml and up to 30% ± 5% with 100 µg of the protein. Nonmutated rhLf exhibited inhibitory effects similar to those of nhLf. These experiments...
indicate that the two basic N-terminal clusters involving both residues 1 to 5 and 28 to 34 of hLf are essential for the inhibitory effect of hLf on the LPS-stimulated IL-8 production.

Inhibition by hLf of the IL-8 production induced by the sCD14-LPS complex in endothelial cells. Serum sCD14 mediates the binding of LPS to endothelial cells (14). Elsewhere, interactions between Lf and either sCD14 or the sCD14-LPS complex were recently described (4). These interactions may be relevant to the inhibiting properties of hLf on IL-8 production by LPS-stimulated HUVEC. Thus, we studied the production of IL-8 induced by the sCD14-LPS complex in the presence of various concentrations of nhLf. As shown in Fig. 4, the production of IL-8 was threefold increased in the presence of complexed sCD14 and LPS compared to that of the controls with medium, sCD14, sCD14 with nhLf, or LPS alone. Inhibition of IL-8 production was dependent on the nhLf concentration (data not shown). About 46% ± 3% inhibition was detected at 200 μg of nhLf/ml (Fig. 4). These results show that the inhibition effect of hLf is related to its interaction with the sCD14-LPS complex.

Inhibition of IL-8 binding to immobilized heparin by hLf. Cell surface proteoglycans of endothelial cells are required for presenting highly diffusible inflammatory chemokines to leukocytes (16, 20, 40). IL-8 homodimers bind to heparan sulfate and heparin. Since hLf interacts with proteoglycans (22, 27, 43), we hypothesized that hLf may interfere with the presentation of IL-8 on glycosaminoglycans. To check this hypothesis, the binding of radiolabeled IL-8 to immobilized heparin-BSA was analyzed in the presence of increasing concentrations of nhLf (Fig. 5A). Human serum transferrin and soluble heparin were used as controls (Fig. 5A). Both nhLf and soluble heparin were able to strongly compete with 125I-IL-8 for the binding to immobilized heparin.
immobilized heparin-BSA. As little as 10 μg of nhLf/ml yielded 58% ± 3% inhibition. Lf was a more effective competitor than the soluble heparin molecule. In contrast, serum transferrin, whose structure is highly homologous to that of hLf, did not prevent the 125I-IL-8 binding to heparin-BSA.

**Importance of the N-terminal domain of hLf for the inhibition of the IL-8 binding to immobilized heparin.** We investigated the role of hLf basic residues 1 to 4 and 28 to 34 in the inhibition of radiolabeled IL-8 binding to proteoglycans (Fig. 5B). Both sequences were reported to act as cationic cradles for heparin binding (27, 43). Increasing concentrations of N-terminally modified hLf variants were used in competitive binding experiments to immobilized heparin. About 10 μg of rHLF/ml and 35 μg of EGS-rHLF/ml reduced the amount of 125I-IL-8 bound to heparin by 50%. Maximal inhibition (around 70%) was gained from 100-μg/ml concentrations of any of the two proteins. Competition between 125I-IL-8 and G4R-rHLF or Lf(-3N) for heparin-BSA binding was less effective than that obtained with rHLF. The IC₅₀ values calculated for G4R-rHLF and Lf(-3N) were 72 and 88 μg/ml, respectively. Very low inhibition (9% ± 4%) was detected with G4R-EGS-rHLF. These results demonstrate the ability of Lf, through its glycosaminoglycan-binding site, to compete with IL-8 for binding to heparin.

**DISCUSSION**

Endothelial cells express chemokines that initiate the activation and recruitment of circulating leukocytes at inflammatory tissue sites. Nevertheless, during septicemia chemokines participate in a series of cellular events that severely damage the endothelium and surrounding tissues (9, 30). Anti-inflammatory therapies aiming to regulate the chemokine responses may thus prevent those septic shock-associated events. Lactoferrin, a glycoprotein released from neutrophils during inflammation and present in most secretions, exhibits anti-inflammatory properties (5). In particular, the protective effect of exogenous Lf against endotoxin lethal shock in various animals was previously reported (19, 21, 26, 44). Recently, we brought evidence that Lf strongly interacts with LPS, sCD14, or the sCD14-LPS complex, thus lowering the expression of adhesion molecules, E-selectin, and ICAM-1 on endothelial cells (4). However, its effect on the expression and function of chemokines in the leukocyte recruitment process was not investigated.

We first investigated the effect of hLF on the IL-8 expression induced in LPS-activated endothelial cells. In the present report, we provide evidence that hLF decreases the IL-8 expression by LPS-stimulated HUVEC at both transcriptional and translational levels. Our results obtained by activating cells with LPS in the presence of recombinant sCD14 suggest that interactions between Lf and LPS or the sCD14-LPS complex are responsible for that inhibitory effect. The experiments with hLF variants modified at residues ²RRRR⁵ and ²RKVRGPP³⁴, which participate in the recognition of LPS (11, 41), strongly support this assertion. Mutations in both sequences totally abrogated the inhibitory effect of Lf. Furthermore, removal of the first three N-terminal amino acid residues (Lf-3N), which are not essential for the binding to LPS (11), had a lesser influence on the inhibition properties of Lf. From these results we determined that hLF released from neutrophils during infection or used as a therapeutic agent may down-regulate the expression of endothelial IL-8 and the recruitment of immune cells during inflammation. Since IL-8 is a key molecule which triggers neutrophils degranulation (18) and subsequent release of Lf, a reciprocal negative feedback regulation of both molecules is likely under physiological conditions. In contrast to the results of Shinoda et al. (37), who reported that Lf stimulates the release of IL-8 from neutrophils, hLF alone has no effect on IL-8 production by HUVEC. This difference could be explained either by the presence of LPS contaminants in the Lf fractions used in their experiments or by different Lf binding sites on HUVEC and neutrophilic cells. Furthermore, these authors have not investigated the activity of Lf on LPS-activated cells. A previous study by Wang et al. (42) demonstrates that iron-free hLF decreases the oxidative burst induced by LPS in neutrophils. This result may be supported by the hypothesis that Lf could also down-regulate the IL-8 production induced by LPS in neutrophils.
In a second step we investigated whether Lf, a glycosaminoglycan-binding molecule (27, 32, 43), could compete with IL-8 for its binding to proteoglycans. Endothelial glycosaminoglycans may act as storage sites for chemokines. Hence, their presentation to leukocyte-expressed chemokine receptors may be facilitated (30). IL-8 binds to heparin with a moderate affinity ($K_a$ of 0.39 to 2.63 $\mu$M) (20) while the affinity between Lf and heparin molecules is significantly higher (15 to 124 nM) (27, 32).

Our results show that Lf, at concentrations encountered at the inflammatory sites (6, 25), strongly inhibits the binding of IL-8 to heparin. This inhibition was specific, since serum transferrin, another heparin-binding member of the transferrin family (33), did not compete with IL-8 for heparin binding. Further evidence of such a competition was brought by some experiments with mutated Lf. We show that alterations of only one out of the two N-terminal basic stretches, 5-GRRR and 28-KKVGP, do not significantly alter the competitive binding properties of Lf. In contrast, alterations at both sites abrogate these properties. These results are supported by the requirement of residues 1-GRRRR-5 and 28-KKKVR-31 to form a cationic cradle for heparin binding (27, 43). Moreover, we previously demonstrated that R2 to R4 but not R3 contribute to the binding of hLf to the heparan sulfate molecule present at the surface of Jurkat lymphocytic cells (22). These observations in total strongly suggest that the heparin-binding features of Lf are responsible for its ability to compete with IL-8. The heparin structural motifs recognized by Lf and those interacting with IL-8 have been characterized previously (16, 20, 40).

Furthermore, the fact that both Lf and IL-8 may interact with the surface of Jurkat lymphocytic cells (22). These observations in total strongly suggest that the heparin-binding features of Lf are responsible for its ability to compete with IL-8. The heparin structural motifs recognized by Lf and those interacting with IL-8 have been characterized previously (16, 20, 40). About five N- and O-sulfated saccharide units are required for the optimal binding of one IL-8 monomer (20, 40). Furthermore, the 2-O-and 6-O-sulfated disaccharides structure, and more precisely the di-O-sulfated disaccharides unit –Idca(2-OSO$_3$)-GlcNSO$_3$(6-OSO$_3$), promotes the binding of IL-8 (40). Concerning Lf, octosaccharides were the smallest heparin oligosaccharides showing significant binding, and the 2-O-, 6-O-, and N-sulfate groups were of equal importance for Lf binding. Consequently, the fact that both Lf and IL-8 may interact with common sulfate groups on heparin and the fact that Lf binds to heparin with a higher affinity than IL-8 probably accounts for the displacement of chemokine from heparin in the presence of Lf.

In conclusion, our results demonstrate that the anti-inflammatory activity of Lf is relevant, at least in part, to its ability to inhibit both expression and presentation of IL-8 on endothelial cells, therefore regulating the recruitment of leukocytes at inflammatory sites. Recently, Lf was shown to prevent gut mucosa damages in mice during LPS endotoxemia (19). Our findings shed light on one of the mechanisms that explain the protective effect of Lf against the septic shock and subsequent tissue damages. An optimal effect is seen when exogenous Lf is administered to animals prior to provoked septicaemia (44) and may be explained by the coating of endothelium proteoglycans with Lf, thus impeding further IL-8 presentation. The demonstration that Lf inhibits IL-8 expression and presentation by cells opens the way to investigating the role of Lf in septic shock and in other pathologies.


