Inhibition of Lipopolysaccharide-Induced Inflammatory Responses by an Apolipoprotein AI Mimetic Peptide

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Abstract—Previous studies suggest that high-density lipoprotein and apoAI inhibit lipopolysaccharide (LPS)-induced inflammatory responses. The goal of the current study was to test the hypothesis that the apoAI mimetic peptide L-4F exerts antiinflammatory effects similar to apoAI. Pretreatment of human umbilical vein endothelial cells (HUVECs) with LPS induced the adhesion of THP-1 monocytes. Incubation of cells with LPS and L-4F (1 to 50 μg/mL) reduced THP-1 adhesion in a concentration-dependent manner. This response was associated with a significant reduction in the synthesis of cytokines, chemokines, and adhesion molecules. L-4F reduced vascular cell adhesion molecule-1 expression induced by LPS or lipid A, whereas a control peptide (Sc-4F) showed no effect. In contrast to LPS treatment, L-4F did not inhibit IL-1β or tumor necrosis factor-α–induced vascular cell adhesion molecule-1 expression. The inhibitory effect of L-4F on LPS induction of inflammatory markers was associated with reduced binding of LPS to its plasma carrier molecule, lipopolysaccharide binding protein, and decreased binding of LPS to HUVEC monolayers. LPS and L-4F in HUVEC culture medium were fractionated by fast protein liquid chromatography and were localized to the same fractions, suggesting a physical interaction between these molecules. Proinflammatory responses to LPS are associated with the binding of lipid A to cell surface receptors. The current studies demonstrate that L-4F reduces the expression of inflammatory markers induced by LPS and lipid A and suggest that apoAI peptide mimetics may be useful in the treatment of inflammation associated with endotoxemia. (Circ Res. 2005;97:236-243.)

Key Words: lipopolysaccharide ■ inflammation ■ vascular cell adhesion molecule-1 ■ apolipoprotein AI mimic peptide

Despite recent advances in antimicrobial and antiinflammatory therapy, sepsis continues to be a major cause of death in hospitalized patients. A recent observational cohort study provides estimates of 751 000 cases of severe sepsis in hospitals in the United States in 1995.1 Approximately 50% of patients in intensive care units develop severe sepsis, and the overall mortality rate of all affected patients is 29%.1 Mortality is attributable, in large part, to the cytotoxic actions of lipopolysaccharide (LPS) (endotoxin), a component of the outer membrane of Gram-negative bacteria. LPS is composed of a core oligosaccharide, a repeating polysaccharide side chain, and the glycolipid moiety lipid A.2 Proinflammatory and cytotoxic effects of LPS are mediated by lipid A.3 LPS is released from bacterial membranes into the circulation where it interacts with lipopolysaccharide binding protein (LBP), a member of the superfamily of phospholipid binding proteins. LBP binds to lipid A and mediates the disaggregation of LPS to form an LBP–LPS complex.4 LBP directs LPS to membrane-associated CD14 receptors (mCD14) on myeloid cells5 including monocytes and neutrophils. mCD14 is a cell surface–anchored protein that facilitates the binding of LPS and activation of Toll-like receptor (TLR) 4 which acts as the cellular transducer of LPS action.2,6 Plasma LPS–LBP may also interact with soluble CD14 to form a complex that activates TLRs on endothelial, epithelial, Kuppfer, and other cells.7 By activating nuclear factor κB–dependent signaling mechanisms, LPS stimulates the synthesis/release of inflammatory cytokines, which play an important role in the innate immune response.8,9 Dysregulation of this response leads to the development of endothelial dysfunction, intravascular coagulation, pulmonary injury, multiple organ failure, and death.

The acute-phase response to bacterial infection induces changes in plasma lipoprotein levels that are characterized by
a decrease in high-density lipoprotein (HDL). Increasing plasma HDL concentration, however, reduces complications associated with endotoxemia in mice. In this respect, it was shown that a 2-fold increase in plasma HDL enhances binding of intraperitoneally administered LPS to HDL, reduces plasma cytokine levels, and improves survival in transgenic mice. Intravenous infusion of reconstituted HDL (rhDL) or apoAI also confers protection against LPS in wild-type mice. Similar effects of rhDL in preventing the LPS-dependent induction of proinflammatory mediators have been demonstrated in vitro. Although these results suggest that HDL administration may be effective in treating sepsis, obtaining therapeutic quantities of the lipoprotein is impractical. Other pharmacological approaches to raise plasma HDL have yielded variable results.

Apolipoprotein mimetic peptides, previously developed in our laboratories, represent an emerging area in the field of HDL therapy. The apoAI mimetic peptide L-4F, the structure of which is based on the helical repeating domains of apoAI, dramatically reduces lesion formation in several dyslipidemic mouse models. In this article, we present the phase peptide synthesis method as previously described. The amino acid sequence of which is Ac-DWFKAAYDYF-KKAFVEEFAK-NH2, the L-4F binds to lipid A, thus preventing LPS from interacting with endothelial cells (HUVECs) and rat aorta. It is proposed that L-4F inhibits inflammatory responses induced by LPS and lipid A in human umbilical vein endothelial cells (HUVECs) and rat aorta. It is proposed that L-4F binds to lipid A, thus preventing LPS from interacting with LBP and endothelial cell surface receptors.

Materials and Methods

Materials

Cell adhesion was measured using a Vybrant Cell Adhesion Assay Kit (Molecular Probes Inc). Cell viability assays were performed using a CellTiter96 AQUos One Solution Cell Proliferation Assay kit (Promega Inc). LPS (Escherichia coli, serotype 026:B6) was obtained from Sigma. Purified lipid A was obtained from List Biological Labs. Antibodies to vascular cell adhesion molecule-1 (VCAM-1) (Santa Cruz Inc) and β-actin were obtained from Santa Cruz Inc. LPS/LBP binding assays were performed by ELISA (HyCult Biotechnology BV/Cell Sciences Inc). Bodipy-LPS was obtained from Molecular Probes. Superox 6 columns were from Amersham Biosciences.

ApoAI Mimetic Peptide Synthesis

L-4F, the amino acid sequence of which is Ac-DWFKAAYDYF-KKAFVEEFAK-NH2, was synthesized by the solid-phase peptide synthesis method as previously described. The scrambled peptide Sc-4F (Ac-DWFKAAYDYF-KKAFVEEFAK-NH2) was synthesized by rearranging the amino acid sequence of L-4F. Sc-4F is unable to form an amphipathic helix and was used as a control for L-4F. Peptide purity was ascertained by mass spectral analysis and analytical high-performance liquid chromatography. Peptide concentration was determined by monitoring the absorbance of tyrosine and tryptophan residues at 280 nm.

Cell Culture

Low passage (passage 3 to 6) HUVECs were cultured in complete M199 medium containing 10% FBS, heparin (100 μg/mL), and pituitary growth factor (50 μg/mL). THP-1 monocytes were propagated in RPMI medium 1640 containing 10% FBS and 0.05 mmol/L β-mercaptoethanol.

Cell Adhesion Assay

HUVECs were cultured (80% confluence) in 24-well plates and treated with LPS (1 μg/mL) in the absence and presence of L-4F (1 to 50 μg/mL) for 6 hours. After the treatment period, HUVECs were washed and media was removed. Concurrently, THP-1 cell suspensions were adjusted to 5×10⁶ cells/mL and loaded with the fluorescent dye calcein acetoxymethyl ester for 30 minutes at 37°C. THP-1 monocytes were washed to remove unincorporated calcein and then added to microwells containing HUVECs (5×10⁴ cells/mL) for 60 minutes. Nonadherent monocytes were then removed by gentle washing with PBS, and the fluorescence emission of HUVEC-bound THP-1 cells was monitored at 520 g/mL. The fluorescence signal of LPS-treated HUVECs in the absence of L-4F was designated 100% control. Nonspecific binding of calcein-loaded THP-1 cells was determined in saline-treated HUVECs and was subtracted from fluorescence signals obtained from LPS-treated cells.

Multiplex Protein Array

HUVECs were incubated with LPS (1 μg/mL) in the absence and presence of L-4F (50 μg/mL) for 6 hours. In control experiments, cells were treated with saline or L-4F alone. Conditioned media was then collected. Measurement of IL-6, IL-8, interferon-γ, tumor necrosis factor (TNF)-α, membrane cofactor protein-1, endothelial selectin (E-selectin), intercellular adhesion molecule-1 (ICAM)-1, and VCAM-1 was performed by Pierce/Endogen (Boston, Mass) using SearchLight protein array technology.

Measurement of VCAM-1 mRNA Expression

VCAM-1 mRNA levels were analyzed by reverse transcription followed by polymerase chain reaction (PCR), as described previously. A 354-bp segment of the human VCAM cDNA was amplified using a 24-mer upstream primer (5′-GGCTGTAATCCCTATTTTCC-3′) and a 29-mer downstream primer (5′-CGGTATTCCTCC AAAACTCTATATTCTCC-3′) of human VCAM-1 mRNA (GenBank No. NM 001078). A 505-bp segment of the cyclophilin cDNA was amplified using a 20-mer upstream primer (5′-AAGCTTCCCA AAGACAGCAGA-3′) and a 20-mer downstream primer (5′-GTCCTAAGTA GACGCAAAATGG-3′) of the human cyclophilin mRNA (GenBank No. Y00052). PCR conditions were 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 45 seconds for 28 cycles. PCR products were analyzed on a 1.2% agarose-ethidium bromide gel. The gels were photographed and the intensity of the VCAM-1 mRNA bands, and the cyclophilin mRNA bands were quantified by laser densitometric scanning. The ratio of VCAM-1 mRNA/cyclophilin mRNA band intensity was calculated. Effects of L-4F on VCAM-1 expression are presented as a percentage change compared with LPS treatment alone.

Analysis of VCAM-1 Protein Expression

Western blot analysis was performed to assess the effects of L-4F on VCAM-1 expression. LPS (1 μg/mL), lipid A (1 μg/mL), IL-1β (10 ng/mL), or TNF-α (5 ng/mL) were added to HUVECs bathed in complete media for a 6-hour time period. In some experiments, HUVECs were concurrently incubated with L-4F at concentrations between 1 to 50 μg/mL (~0.4 to 20 μmol/L). Sc-4F was used as a control peptide for L-4F. After treatment, cell lysates (50 μg) were subjected to 7.5% SDS-PAGE under denaturing conditions and transferred to nitrocellulose membranes (Hybond, Amersham). Blots were incubated with primary goat polyclonal IgG VCAM-1 antibody (Santa Cruz, Inc) overnight, followed by application of the secondary antibody (horseradish peroxidase–conjugated anti-goat IgG) for 30 minutes. Immunoreactive bands were visualized using ECL (Amerham). Membranes were probed with primary mouse β-actin antibody (Santa Cruz Inc) to control for protein loading. Concentration dependent effects of L-4F on VCAM-1 expression are presented as a percentage decrease compared with LPS treatment alone.

Measurement of LPS-LBP Binding

LPS–LBP binding and its modulation by L-4F were tested by ELISA (HyCult Biotechnology BV). Biotinylated LPS (1 μg/mL), lipid A (1 μg/mL), IL-1β (10 ng/mL), or TNF-α (5 ng/mL) were added to HUVECs coated with anti-LBP antibody, followed by addition of LBP (25 ng/mL) for 1 hour. Biotinylated LPS (1 μg/mL) was then added to each well in the absence and presence of L-4F (1 to 50 μg/mL) for 1 hour. Wells were then washed to remove unbound LPS and a secondary antibody coupled to streptavidin-peroxidase and directed
against biotinylated LPS was applied for 1 hour. The peroxidase substrate tetramethylbenzidine was then added, and the absorbance of the resulting colorimetric product was measured at A₄₅₀. The absorbance of the LPS–LBP binding complex in the absence of L-4F was designated 100% control.

**Derivatization and Radiolabeling of LPS**

Because commercial preparations of LPS are heterogeneous and often contaminated with proteins, purified *Escherichia coli* LPS (List Biochemicals Inc) was used in radioligand-binding studies. LPS was derivatized by covalent coupling to sulfosuccinimidyl 2-(p-azidosalicylamido)ethyl–1–3-dithiopropionate (SASD, Pierce, Inc) as previously described. The cross-linked reaction product LPS-ASD was separated from excess, free SASD by dialysis against PBS (18 hour, 4°C, 6 buffer changes). LPS-ASD was next iodinated using the iodogen method (Pierce, Inc). Free 125I was separated from 125I–LPS-ASD by dialysis and centrifugation (2000g, 1 hour, 5 times) using an Ultrafree-MC filter unit (Millipore, Inc). Seventy percent of the recovered counts were trichloroacetic acid precipitable.

**Radioligand-Binding Studies**

The endotoxin activity of commercial LPS (Sigma, Inc), purified LPS (List Biochemicals, Inc), and derivatized LPS-ASD were assessed using a Limulus amoebocyte lysate assay kit (BioWhittaker, Inc). The Limulus amoebocyte lysate activity of 125I–LPS-ASD, corresponding to 1 μg/mL commercial LPS, was added to HUVECs cultured in 6-well dishes. In preliminary experiments, competitive binding assays were performed in the presence of cold LPS-ASD (1 to 50 μg/mL). In subsequent experiments, we monitored the concentration-dependent effect of L-4F (1 to 50 μg/mL) on 125I–LPS-ASD binding over a 6-hour time period.

**Chromatographic Identification of an L-4F–LPS Complex**

The direct binding of L-4F to LPS was demonstrated by size exclusion chromatography using a Bio-Logic fast protein liquid chromatography system (Bio Rad). Two Superdex 6 columns in tandem were equilibrated with PBS (pH 7.4). In initial experiments, fluorescently labeled LPS (Bodipy-LPS: 10 μg) was suspended in complete M199 medium and incubated at 37°C for 30 minutes before injection on the columns and elution with PBS. 125I–L-4F (3.4 μg) was similarly incubated in M199 medium before chromatography. In some experiments, Bodipy-LPS (10 μg) and 125I–L-4F (3.4 μg or 10 μg) were coincubated before separation. Fractions (0.5 mL) were collected, and the presence of LPS was detected by measuring fluorescence emission at 530 nm (λex 485 nm) using a Bio-Tek plate reader (Bio-Tek Instruments, Vt). 125I–L-4F counts in each fraction were measured using a gamma counter.

**In Vivo Administration of L-4F**

Male Sprague-Dawley rats (250–300 g) were obtained from Harlan, Inc (Indianapolis, Ind). All animals received a standard laboratory diet and water ad libitum. Rats were maintained at constant humidity (60±5%), temperature (24±1°C), and light cycle (6 AM to 6 PM). To test the effect of L-4F on inflammatory events in vivo, male Sprague-Dawley rats (250 to 300 g) were injected with LPS (10 mg/kg) or vehicle. In some experiments, LPS-treated rats were also injected with L-4F (25 mg/kg). After 6 hours, animals were euthanized, and aortae excised. Tissue was homogenized and proteins extracted for Western blotting. VCAM-1 expression was assessed as described above. All protocols were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and were consistent with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

**Statistical Methods**

All results are reported as the mean±SEM. Statistical analysis was performed using SigmaStat version 3 software (Jandel Scientific Inc). Differences between the groups were assessed by 1-way ANOVA with post hoc testing (Student–Neuman–Keuls test). A value of P<0.05 was considered to be statistically significant.

**Results**

**L-4F Inhibits the Binding of THP-1 Monocytes to Endothelial Cell Monolayers and Expression of Inflammatory Mediators**

LPS is a potent inducer of inflammatory markers including cytokines and adhesion molecules. Upregulation of these mediators is associated with increased monocyte infiltration into the arterial wall. Figure 1 shows that LPS (1 μg/mL: 6-hour exposure) permits the firm adhesion of THP-1 monocytes to HUVEC monolayers. THP-1 monocyte binding in the absence of LPS was negligible (not shown). Concurrent incubation of HUVECs with LPS and L-4F resulted in a concentration-dependent reduction in THP-1 monocyte binding. At 50 μg/mL, L-4F inhibited LPS-induced THP-1 adhesion by 74±3% (Figure 1). Cellular effects of LPS and L-4F were not related to a cytotoxic action of either compound because HUVEC viability was not influenced by these incubation conditions (unpublished observation).

To test whether L-4F modulates the LPS-mediated induction of known markers of inflammation, the conditioned media of HUVECs exposed to LPS in the absence and presence of L-4F (50 μg/mL) was collected. These samples were analyzed for cytokine, chemokine, and adhesion molecule content using a multiplex protein array. All samples were tested in a blinded manner, and results are presented in the Table. IL-6, IL-8, interferon-γ, TNF-α, membrane cofactor protein-1, E-selectin, ICAM-1, and VCAM-1 were induced by LPS exposure and shed into the conditioned media of HUVECs. Induction of these metabolites was blocked when
L-4F (50 μg/mL) was added to incubation media containing LPS. L-4F treatment alone yielded similar results to saline vehicle controls.

**L-4F Specifically Inhibits LPS- and Lipid A–Induced VCAM-1 Expression**

Because VCAM-1 is thought to play a critical role in the firm adhesion of monocytes to the vascular endothelium, we tested the effect of L-4F on LPS-induced VCAM-1 expression in greater detail. L-4F reduced LPS-induced VCAM-1 mRNA and protein expression in a concentration-dependent manner (Figure 2A and 2B). Whereas VCAM-1 protein was undetectable in HUVECs treated with saline vehicle or L-4F alone, 50 μg/mL L-4F reduced VCAM-1 by 91±6%. The inhibition of LPS-induced VCAM-1 required the presence of L-4F in the incubation medium because pretreatment of HUVECs with peptide, followed by washing and addition of LPS, did not reduce VCAM-1 expression. Sc-4F (50 μg/mL) did not inhibit LPS-induced VCAM-1 (not shown), suggesting that the inhibitory effect of L-4F was related to its amphipathic helical nature. In related experiments, we tested the effect of L-4F on the induction of VCAM-1 by purified lipid A, the cytotoxic component of LPS (Figure 2C). The concentration dependence for inhibition of lipid A–induced VCAM-1 expression by L-4F was similar to that observed for LPS.

To test whether the inhibitory effect of L-4F on VCAM-1 expression was specific for LPS, we monitored the effect of the peptide on VCAM-1 expression induced by IL-1β and TNF-α. Treatment of HUVECs with 50 μg/mL L-4F, a concentration that inhibited LPS-induced VCAM-1 expression, did not reduce expression of the adhesion molecule in the presence of IL-1β or TNF-α (not shown).

**L-4F Limits Binding of LPS With LBP and Endothelial Cell Surface Receptors**

Because our data suggested that L-4F inhibits LPS action via an interaction with the lipid A moiety, we designed additional experiments to test the effect of L-4F on lipid A–dependent binding processes. LBP interacts with the lipid A group on LPS to form a plasma carrier complex that subsequently binds to either mCD14 or soluble CD14. Formation of the LPS–LBP complex is associated with an amplification of cellular responses to LPS stimulation. Using an ELISA-based binding assay, we found that L-4F reduced the association of LPS with LBP in a concentration-dependent manner (Figure 3A). On the basis of this finding, we predicted that L-4F would also reduce the association of LPS with endothelial cell surface receptors. To test this hypothesis, binding assays were performed using derivatized, radiolabeled LPS (125I–LPS–ASD). Cold LPS–ASD effectively competed with 125I–LPS–ASD for binding to endothelial cell surface receptors, thus

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**Effects of L-4F on the Release of Inflammatory Mediators From LPS-Treated HUVECs**

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Control</th>
<th>LPS</th>
<th>LPS + L-4F</th>
<th>L-4F</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>3.25 (+0.07)</td>
<td>11.33 (+1.13)*</td>
<td>4.63 (+0.46)</td>
<td>3.77 (+0.12)</td>
</tr>
<tr>
<td>IL-8</td>
<td>1391.88 (+57.33)</td>
<td>3451.46 (+78.77)*</td>
<td>2283.64 (+94.30)</td>
<td>1601.86 (+181.66)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>2.39 (+0.10)</td>
<td>3.60 (+0.11)*</td>
<td>2.81 (+0.40)</td>
<td>2.68 (+0.09)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>82.22 (+0.69)</td>
<td>120.35 (+11.89)*</td>
<td>88.25 (+6.56)</td>
<td>85.70 (+4.44)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>92.24 (+4.14)</td>
<td>4936.69 (+291.88)*</td>
<td>558.99 (+147.40)</td>
<td>171.42 (+11.82)</td>
</tr>
<tr>
<td>E-selectin</td>
<td>899.75 (+35.70)</td>
<td>3232.78 (+241.83)*</td>
<td>946.75 (+82.44)</td>
<td>973.18 (+49.69)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>15 792.99 (+798.52)</td>
<td>42 989.80 (+3884.89)*</td>
<td>21 560.15 (+2237.48)</td>
<td>14 461.22 (+1542.10)</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>738.80 (+1.25)</td>
<td>1743.12 (+118.91)*</td>
<td>752.06 (+71.01)</td>
<td>728.50 (+61.69)</td>
</tr>
</tbody>
</table>

Cells were treated with LPS (1 μg/mL) or L-4F (50 μg/mL) alone and in combination. The conditioned media were collected for measurement of cytokines, chemokines, and adhesion molecules. Control represents treatment of cells with an equivalent volume of saline. Units are pg/mg protein. Data are mean (+SEM). *P<0.05 vs all other treatments. N=3 for each condition.
demonstrating specificity of the radiolabeled complex for cell binding (not shown). Concurrent treatment of HUVECs with $^{125}$I–LPS-ASD and L-4F resulted in a decrease in cell-associated radioactive counts as the concentration of peptide was increased (Figure 3B).

### L-4F Directly Binds to LPS

To test whether inhibitory effects of L-4F on LPS-mediated responses were attributable to a direct binding interaction, chromatographic separation techniques were used. Bodipy-LPS or $^{125}$I–L-4F were dissolved in complete M199 medium and subjected to size exclusion chromatography on 2 Superdex 6 columns in tandem. Equilibration and elution was with PBS. Bodipy-LPS eluted in fractions 38 to 52 and radiolabeled L-4F eluted in fractions 57 to 73 (Figure 4A). In contrast, when Bodipy-LPS was incubated with $^{125}$I–L-4F, a single elution peak (fractions 47 to 63), distinct from the individual elution peaks of LPS and L-4F, was obtained. Both signals were retrieved in the same fractions, suggesting a direct physical interaction between L-4F and LPS (Figure 4B). A similar elution profile was observed when Bodipy-LPS (10 μg) was incubated with higher concentrations of $^{125}$I–L-4F (10 μg). The LPS-L-4F complex appears to be smaller in size than LPS alone suggesting that the LPS-L-4F interaction causes LPS to disaggregate.

### L-4F Inhibits the Action of LPS in Vivo

To determine whether L-4F is effective in reducing LPS-induced inflammatory responses in vivo, we tested the effect of IP injection of L-4F in LPS-treated Sprague-Dawley rats. VCAM-1 expression in rat aortae was assessed by Western blot. As shown in Figure 5, LPS injection was associated with a significant induction of VCAM-1 protein compared with vehicle-treated controls. IP injection of L-4F reduced LPS-stimulated VCAM-1 in rat aortae.

### Discussion

Antiinflammatory and antatherogenic effects of HDL have been ascribed to apoAI. Sepsis and septic shock, however, are associated with a reduction in total plasma cholesterol and
HDLP. Clinical data also suggest that reduced HDL is strongly associated with increased mortality in septic patients. Under these conditions, HDL is converted to an acute-phase lipoprotein that is enriched in serum amyloid A and depleted of apoAI. Acute-phase HDL also displays prooxidant and proinflammatory properties.[10,22]

Administration of rHDL to humans treated with low-dose LPS reduces TNF-α, IL-6, and IL-8 release and decreases expression of monocytic mCD14. HDL inhibits LPS action caused by the competitive binding of the LPS–LBP–CD14 complex to apoAI. Other data suggest that apoAI displaces and clears LPS bound to surface receptors on monocytes. The mechanism by which HDL neutralizes LPS is thought to occur by insertion and masking of the lipid A domain into the phospholipid leaflet that covers the surface of HDL.[11,25]

The apoAI mimetic peptide 4F has been shown to convert proinflammatory HDL to an anti-inflammatory form and improves HDL-mediated cholesterol efflux and reverse cholesterol transport in apoE-null mice.[27] We were, therefore, interested in studying whether L-4F modulates LPS-induced inflammatory markers under in vitro and in vivo conditions. We report here for the first time that the synthetic peptide L-4F reduces monocyte adhesion to endothelial cell monolayers. This response was associated with a significant reduction in the LPS-induced expression of cytokines, chemokines, and adhesion molecules. Specifically, L-4F prevented the upregulation of VCAM-1, a cell surface receptor known to participate in the firm adhesion of leukocytes to endothelial cells.[12] This response was specific for L-4F because coinubcation of HUVECS with LPS and the scrambled peptide Sc-4F, which does not form an amphipathic helix, failed to inhibit the expression of VCAM-1. L-4F, thus, mimics the effects of HDL and apoAI in inhibiting LPS action.

Synthetic apoAI mimetic peptides have been previously used to study changes in plasma cholesterol and fatty lesion formation in murine models of atherosclerosis. L-4F effectively reduces lesion size by decreasing the atherogenicity of low-density lipoprotein and very-low-density lipoprotein, monocyte chemotactic activity, and aortic macrophage infiltration.[14–16] These effects are mediated by the binding of peptide to phospholipid components of lipoproteins. In contrast, few studies have examined the actions of apoAI mimetic peptides in models of infection and inflammation. Of note is a recent report showing that L-4F inhibits IL-6 expression in influenza-infected mice.[20] In the context of sepsis, a single report shows that administration of the apoAI mimetic peptide 18A prolongs survival in LPS-injected mice.[21] Recent data suggest that oxidized phospholipids antagonize the proinflammatory response to LPS.[31] In this regard, it was shown that oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (OxPAPC) prevents the upregulation of LPS-dependent signaling intermediates, reduces endothelial cell expression of E-selectin, ICAM-1, and VCAM-1, and prolongs survival of mice treated with lethal doses of LPS.[30] OxPAPC, however, failed to inhibit adhesion molecule expression induced by TNF-α and IL-1β. These data suggest that OxPAPC specifically inhibits LPS action. The protective effect of OxPAPC was ascribed to inhibition of LPS binding with LBP and CD14, resulting in suppression of TLR-dependent signaling pathways.[31] In the current study, we examined the effects of L-4F on VCAM-1 expression induced by LPS, lipid A, TNF-α, and IL-1β. L-4F prevented induction by LPS and lipid A, but failed to suppress IL-1β- or TNF-α-stimulated VCAM-1 expression. These data suggested that a selective, physical interaction between the peptide and lipid A is responsible for the observed inhibition of LPS-induced VCAM-1 expression.

The binding of L-4F to model phospholipids has been described previously.[15] The lipid A moiety of LPS is composed of a diphosphorylated diglucosamine backbone linked to a variable number of fatty acyl chains via amide or ester bonds.[2,3] Because of its anionic and amphipathic structure, lipid A represents an ideal target for L-4F binding.[3] Although LPS may directly bind to receptors on the endothelial cell surface, the inflammatory response to LPS is significantly amplified when it is present as a complex with LBP and/or CD14.[4,5,25] Both molecules bind to LPS via the lipid A domain.[4,25] The role of LBP in inflammation has been disputed. Although studies in LBP knockout mice suggest that LPS administration induces a similar inflammatory response as that observed in wild-type mice, other data show that it plays an important role in the initiation of the inflammatory response. In this regard, it was demonstrated that immunodepletion of LBP significantly lowers the sensitivity of monocytes to LPS[6] and that LBP is essential for the rapid induction of an
inflammatory response to low doses of LPS. Similarly, anti-CD14 antibodies inhibit the expression of inflammatory mediators by LPS. In the current study, we found that L-4F prevented the binding of LPS to LBP in a concentration-dependent manner. Our data suggest that this could be attributable to an interaction between L-4F and LPS. Amphipathic molecules like LPS and lipid A form aggregates in aqueous solutions. It has been suggested that LPS is biologically active in the aggregated state and that the shape of the aggregate influences its binding to LBP/CD14. Our data suggest that L-4F binds LPS and alters its aggregation state. Because the lipid A domain of LPS is the binding site for LBP, we hypothesize that the binding of L-4F to LPS induces a conformational change in LPS by altering its aggregation state, which would render the lipid A region inaccessible to LBP, thus preventing assembly of an LPS–LBP complex and exposure to cell surface receptors.

In summary, our data show that L-4F prevents the LPS-induced expression of proinflammatory cytokines, chemokines, and adhesion molecules in vitro and reduces VCAM-1 expression in vivo. Clinical studies suggest that increased levels of IL-6 strongly correlate with increased mortality in septic patients. In this study, we found that LPS and LPS. Amphipathic molecules like LPS and lipid A form aggregates in aqueous solutions. It has been suggested that LPS is biologically active in the aggregated state and that the shape of the aggregate influences its binding to LBP/CD14. Our data suggest that L-4F binds LPS and alters its aggregation state. Because the lipid A domain of LPS is the binding site for LBP, we hypothesize that the binding of L-4F to LPS induces a conformational change in LPS by altering its aggregation state, which would render the lipid A region inaccessible to LBP, thus preventing assembly of an LPS–LBP complex and exposure to cell surface receptors.

In summary, our data show that L-4F prevents the LPS-induced expression of proinflammatory cytokines, chemokines, and adhesion molecules in vitro and reduces VCAM-1 expression in vivo. Clinical studies suggest that increased levels of IL-6 strongly correlate with increased mortality in septic patients. In this study, we found that L-4F prevented the upregulation of IL-6. This, coupled with our observation that L-4F administration prevents LPS-induction of VCAM-1 in vivo, suggests that apoAI mimetic peptides are useful in reducing inflammatory complications associated with sepsis. We propose that these responses are attributable to the association of peptide or a peptide–lipid complex with LPS that limits LPS action on cells. Different therapeutic approaches have been developed to treat endotoxemia including the use of inhibitors that target LPS, LBP, CD14, and TLRs and agents that elevate endogenous HDL-cholesterol. These therapies, however, have yielded mixed results. We recently observed that D-4F, the structure of which is identical to L-4F but composed of D-amino acids, Similarly inhibits LPS-induced VCAM-1 expression in vitro (unpublished observation). In contrast to L-4F, D-4F is orally active and is currently being tested in cholesterol reduction trials. ApoAI mimetic peptides thus represent a new class of therapeutic agents that shows promise in reducing vascular complications associated with inflammatory conditions including atherosclerosis and sepsis.

Acknowledgments

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Inhibition of Lipopolysaccharide-Induced Inflammatory Responses by an Apolipoprotein AI Mimetic Peptide

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