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Bacterial Lipopolysaccharide and IFN-γ Induce Toll-Like Receptor 2 and Toll-Like Receptor 4 Expression in Human Endothelial Cells: Role of NF-κB Activation

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Toll-like receptor (TLR) 4 has been identified as the primary receptor for enteric LPS, whereas TLR2 has been implicated as the receptor for Gram-positive and fungal cell wall components and for bacterial, mycobacterial, and spirochetal lipoproteins. Vascular endothelial cell (EC) activation or injury by microbial cell wall components such as LPS is of critical importance in the development of sepsis and septic shock. We have previously shown that EC express predominantly TLR4, and have very little TLR2. These cells respond vigorously to LPS via TLR4, but are unresponsive to lipoproteins and other TLR2 ligands. Here we show that LPS, TNF-α, or IFN-γ induce TLR2 expression in both human dermal microvessel EC and HUVEC. Furthermore, LPS and IFN-γ act synergistically to induce TLR2 expression in EC, and LPS-induced TLR2 expression is NF-κB dependent. LPS and IFN-γ also up-regulate TLR4 mRNA expression in EC. These data indicate that TLR2 and TLR4 expression in ECs is regulated by inflammatory molecules such as LPS, TNF-α, or IFN-γ. TLR2 and TLR4 molecules may render EC responsive to LPS and may help to explain the synergy between LPS and lipoproteins, and between LPS and IFN-γ, in inducing shock associated with Gram-negative sepsis. The Journal of Immunology, 2001, 166: 2018–2024.

Lipopolysaccharide, or endotoxin, a major component of the outer surface of Gram-negative bacteria, is a potent activator of cells of the immune and inflammatory systems, including macrophages, monocytes, and endothelial cells (EC), and contributes to the systemic changes seen in septic shock (1–4). The endotoxic shock syndrome is characterized by systemic inflammation, multiple organ damage, circulatory collapse, and death (1, 2, 4). Sepsis and septic shock cause ~200,000 deaths annually in the United States (3).

The endothelium, although initially envisioned as a passive, inert vascular lining, is now considered important in the regulation of vascular tone, coagulation and fibrinolysis, cellular growth, differentiation, and immune and inflammatory responses (5–7). Furthermore, the vascular EC, like macrophages, are critical targets for LPS and many cytokines (5, 7–10). Activation of vascular endothelium by LPS results in EC production of various proinflammatory molecules, including leukocyte adhesion molecules, as well as soluble cytokines and chemokines (5–7).

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3 Abbreviations used in this paper: EC, endothelial cells; HMEC, human dermal microvessel EC; TLR, Toll-like receptor; sCD14, soluble CD14; cyPG, cyclopentenone PG; 15d-PGJ2, 15-deoxy-d12,14-PGJ2; PPAR-γ, peroxisome proliferator-activated receptor-γ; IKK, IκB kinase.

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for Gram-positive cell wall components; bacterial, mycobacterial, and spirochetal lipoproteins; and fungi (19, 21–25, 36–38). Therefore, a concept of “division of labor” between these two receptors in recognizing diverse microbial pathogens and altering the immune system has emerged (39). Bacterial lipoprotein, a TLR2 ligand, is one of the most abundant proteins in the outer membrane of Gram-negative and Gram-positive bacteria (40) and acts synergistically with LPS to induce proinflammatory cytokine production and lethal shock (41).

We have reported recently that human dermal microvessel EC (HMEC) and HUVEC express TLR4 predominantly (42). HMEC and HUVEC express little TLR2 and respond vigorously to Escherichia coli LPS via TLR4, but not to Mycobacterium tuberculosis 19-kDa lipoprotein, a TLR2 ligand (42). Transfection of TLR2 confers to HMEC responsiveness to 19-kDa lipoprotein (42). Because several microbial Ags, e.g., Gram-positive cell wall fragments; bacterial, spirochetal, and mycobacterial lipoproteins; and fungal Ags, require TLR2 to activate cells, the regulation of TLR2 mRNA expression in vascular EC could determine EC responses to these TLR2 ligands and influence the overall immune responses. We now describe the regulation of TLR2 expression in EC in response to inflammatory stimuli such as LPS, TNF-α, and IFN-γ. To investigate whether the transcriptional events that lead to LPS-mediated TLR2 mRNA up-regulation in EC are mediated by NF-κB, we studied the effects of various NF-κB inhibitors and NF-κB p65 antisense oligonucleotide. Our results indicate 1) that LPS, TNF-α, and IFN-γ up-regulate TLR2 expression in vascular EC, 2) that LPS-induced TLR2 expression and up-regulation in EC is NF-κB dependent, and 3) that LPS and IFN-γ synergize to induce expression of TLR2 in EC. LPS and IFN-γ also induced TLR4 up-regulation in EC. Induction and up-regulation of TLR2 in response to inflammatory stimuli such as LPS, TNF-α, or IFN-γ in vascular EC may have important implications in host defense against bacterial, mycobacterial, and spirochetal infections and may help explain the well-known synergy between LPS and lipoproteins in the induction of septic shock. The up-regulation of TLR4 by IFN-γ may represent a novel mechanism for the well-described synergy between IFN-γ and LPS.

Materials and Methods

Cells and reagents

The immortalized HMEC (43), a generous gift of Dr. Candal (Center for Disease Control and Prevention, Atlanta, GA), were cultured in MCDM-131 medium supplemented with 10% heat-inactivated FBS, 2 mM glutamine, and 100 μg/ml penicillin and streptomycin in 24-well plates and used between passages 10 and 14. Tissue culture reagents were obtained from Life Technologies (Gaithersburg, MD). HUVEC were isolated and cultured as previously described (44) and were cultured in Medium 199 supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, 0.3% sodium bicarbonate, and 10% FBS. Highly purified, phenol-water-extracted E. coli K235 LPS (<0.008% protein), which was prepared according to the method of McIntire et al. (45), was obtained from Stefanie N. Vogel (Uniformed Services University of the Health Sciences, Bethesda, MD). The purity of this LPS preparation has been previously demonstrated (46, 47), and this preparation of E. coli LPS is active on TLR4-transfected HEK 293 cells and not on TLR2 transfecants (Stefanie N. Vogel, unpublished observation). Human TLR2-α and IFN-γ were purchased from R&D Systems (Minneapolis, MN), the 15-deoxy-Delta(2,14)-PGF2α (15d-PGF2α) and proteasome inhibitor I from Cayman Chemicals (Ann Arbor, MI), and cycloheximide from Sigma. The anti-human TLR2 mAb (TL2.1), which recognizes a TLR2-associated epitope and was generated by immunization of BALB/c mice with Chinese hamster ovary-TLR2 cells (46), was kindly provided by Terje Espevik (The Norwegian University of Science and Technology, Trondheim, Norway).

The specificity of this Ab for TLR2 has been reported elsewhere (48). Phosphorothioate-modified oligodeoxynucleotides for NF-κB p65 sense (5’-GCCATGGGACAACTGTTCCCC-3’) and antisense (5’-GGAAC CAGTTGTCCTAGTTGCGC-3’) were obtained from Isao Kitajima (Kagoshima, Japan) (49).

RT-PCR analysis

Total RNA was isolated from resting and LPS-, TNF-α-, or IFN-γ-stimulated HMEC and HUVEC using an RNA Stat60 isolation reagent (TelTest, Friendswood, TX) following the manufacturer’s instructions and treated with RNase-free DNase I. For reverse transcription reaction, the Moloney murine leukemia virus preamplification system (Life Technologies, Gaithersburg, MD) was employed. PCR amplification was performed with Taq polymerase (Perkin-Elmer, Foster City, CA) for 32 cycles at 95°C for 45 s, 54°C for 45 s, and 72°C for 1 min (for TLR2 and TLR4), as described earlier (42). The TLR2 and TLR4 oligonucleotide primers used for RT-PCR have been described elsewhere (42). GAPDH primers were obtained from Clontech (Palo Alto, CA). The TLR2 and TLR4 RT-PCR fragments were purified and sequenced to confirm the identity of the fragments. Real-time quantitative PCR was done on the iCycler (Bio-Rad, Hercules, CA) using PE Applied Biosystems (Foster City, CA) SYBR Green PCR kit and the TLR primers described above. The semiquantitative RT-PCR experiments were repeated with cells pretreated for 1 h with 15d-PGJ2 (20 μM), proteasome inhibitor I (100 μM), or cycloheximide (10 μg/ml). EC were pretreated with NF-κB p65 antisense and sense oligonucleotide (30 μM) for 24–48 h before LPS stimulation (50 ng/ml) as described earlier (49, 50). For densitometry analysis, the intensity of the bands was measured by the Kodak Digital Science 1D Program with Kodak (Rochester, NY) camera DS40-D2120 and normalized with GAPDH intensity.

Western blot

Conditioned EC or PBMC were lysed for 30 min on ice in a lysis buffer containing 10 mM Tris-HCl (pH 7.8), 50 mM NaF, 1% Triton X-100, 2 mM EDTA, 100 mM NaCl, 1 mM sodium orthovanadate, 0.1 mM aprotinin, and 1 μg/ml of each of the protease inhibitors pepstatin, leupeptin, aprotonin, antipain, and chymostatin, 30 μg/ml TPCK, and 1 mM PMSF. Following lysis, cell debris was removed by centrifugation (14,000 × g, 4°C, 15 min), and supernatants were collected and stored at −80°C until use. Protein concentrations were determined using the Bio-Rad assay kit. One-hundred-thirty micromolars of total protein was added in Laemmli buffer for 5 min, followed by SDS 10% PAGE in Tris-glycine/SDS buffer (25 mM Tris, 250 mM glycine, 0.1% SDS), and blotted onto Immobilon P transfer membranes (Amersham Pharmacia Biotech) (100 V, 1.5 h, 4°C). After blocking for 2 h in TBST (20 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20) containing 5% nonfat milk, membranes were washed three times in TBST and probed for 18 h at 4°C with anti-TLR2 mAb TL2.1 (final concentration, 1 μg/ml) in TBST. After washing three times in TBST, membranes were incubated with secondary HRP-conjugated goat anti-mouse IgG (1:2500 dilution; Bio-Rad) and washed five times in TBST, and bands were detected using ECL reagents (Amersham Pharmacia Biotech) according to the manufacturer’s description.

Immunohistochemistry staining

HMEC were cultured on eight-chamber microscope slides. Cells were fixed in 4% paraformaldehyde and without drying were incubated with anti-TLR2 mAb TL2.1 (10 μg/ml) or with mouse IgG and irrelevant IgG2a controls for 1 h. Primary Ab was detected from FITC-conjugated goat anti-mouse antisemir following the instructions from the manufacturer (ABC vector kit) (Vector Laboratories, Burlingame, CA). The cells were counterstained with hematoxylin. Samples were viewed and photographed using a Zeiss (Oberkochen, Germany) Axioskop microscope.

Transfection and NF-κB-luciferase assay

HMEC were plated at a concentration of 50,000 cells/well in 24-well plates and cotransfected the following day with reporter genes pCMV-β-galactosidase (0.1 μg) and ELAM-NF-κB-luciferase (0.5 μg) using the FuGene 6 Transfection Reagent (Boehringer Mannheim, Indianapolis, IN) as described earlier (31). After overnight transfection, HMEC were stimulated for 5 h with 50 ng/ml LPS with and without pretreatment (for 60 min) with the proteasome inhibitor I or 15d-PGJ2. Cells were also pretreated with NF-κB p65 antisense oligonucleotide (30 μM) for 24 h times before LPS stimulation. Cells were lysed in 60 μl of reporter lysis buffer (Promega, Madison, WI), and luciferase activity was measured with a Promega kit and a luminometer (Promega), and β-galactosidase activity was determined by the colorimetric method to normalize transfection efficiency as described earlier (28).

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Results and Discussion
To investigate whether TLR2 expression in EC is regulated by inflammatory stimuli, we first analyzed the effects of LPS, TNF-α, and IFN-γ on TLR2 mRNA and protein expression in HMEC and HUVEC by RT-PCR, Western blot analysis, and immunohistochemical staining. HMEC were stimulated with *E. coli* LPS (50 ng/ml), TNF-α (20 ng/ml), or IFN-γ (20 ng/ml or 200 IU/ml) for 1, 2, 4, and 5 h, and semiquantitative and real-time RT-PCR were performed by using human TLR2 primers as described earlier (42). TLR2 mRNA expression was significantly up-regulated by LPS, TNF-α, and IFN-γ in HMEC (Fig. 1A). LPS-induced TLR2 mRNA up-regulation was apparent by 30 min (data not shown) and maximal at 2 h. Similar observations were made from primary HUVEC stimulated with LPS (Fig. 1B) or IFN-γ (data not shown). Real-time quantitative PCR confirmed the results obtained by semiquantitative RT-PCR (data not shown). To determine whether LPS-mediated TLR2 mRNA up-regulation in EC was dependent on new protein synthesis, we preincubated EC with cycloheximide (10 μg/ml) for 60 min before LPS stimulation. Cycloheximide pretreatment inhibited LPS-induced TLR2 up-regulation, suggesting the need for new protein synthesis, i.e., that TLR2 is not an immediate early gene or primary response gene for LPS activation (Fig. 1C). To test whether LPS stimulation of TLR2 synthesis also requires de novo transcription, HMEC were preincubated with the transcription inhibitor actinomycin D before LPS stimulation. Actinomycin D also inhibited LPS-induced up-regulation of TLR2 mRNA (data not shown), suggesting that LPS-TLR4 activation triggers both transcriptional and translational events in EC that lead to TLR2 induction. Although our data suggest that the regulation of TLR2 at the transcriptional level may be important, these preliminary experiments about the mechanism of TLR2 mRNA up-regulation do not rule out additional possible mechanisms, including mRNA stabilization.

LPS-induced TLR2 protein expression and up-regulation in HMEC was also observed by Western blot analysis (Fig. 2A) and by immunohistochemistry (Fig. 2B) using an anti-human TLR2 mAb (TL2.1). Unstimulated HMEC exhibited a low expression of TLR2 protein that was markedly up-regulated in response to LPS stimulation for 4 h (Fig. 2A). Likewise, following 4 h of LPS (50 ng/ml) stimulation, cellular expression of TLR2 protein was significantly enhanced on fixed HMEC, whereas unstimulated HMEC showed very weak TLR2 expression (Fig. 2B). An isotype-matched control Ab (data not shown) and IgG control stainings were negative. Vascular EC can be activated by LPS in one of two ways: a direct pathway where LPS-sCD14 (soluble CD14) complexes directly activate ECs, and an indirect pathway through various inflammatory mediators induced by LPS-activated immune cells (i.e., IL-1β, TNF-α, IFN-γ) (reviewed in Ref. 51). Induction and up-regulation of TLR2 in response to inflammatory stimuli such as LPS, TNF-α, and IFN-γ in vascular endothelium may have important implications in host defenses against bacterial, mycobacterial, and spirochetal infections by rendering these cells responsive and more sensitive to microbial ligands that specifically use TLR2, and may help explain the well-known synergy between LPS and lipoproteins in the induction of shock (41). This study provides the basis for further investigating the functional correlation of TLR2 up-regulation in EC by various inflammatory mediators. Our observations also suggest that the induction of TLR2 expression is not limited to TLR4 activation by LPS. TNF-α and IFN-γ stimulation also result in a similar level of TLR2 up-regulation, suggesting that multiple signaling pathways induced by various inflammatory mediators converge to induce TLR2 expression.

We observed that, in addition to TLR2, LPS also induced the up-regulation of TLR4 expression in HMEC (Fig. 3) and in primary HUVEC (data not shown). To investigate whether IFN-γ also induces TLR4, we incubated HMEC with IFN-γ. We show...

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**FIGURE 1.** Up-regulation of human TLR2 mRNA in EC in response to LPS, TNF-α, and IFN-γ by RT-PCR. A, Semiquantitative RT-PCR for human TLR2 (347 bp) in HMEC was performed. HMEC were stimulated with *E. coli* LPS (50 ng/ml), TNF-α (20 ng/ml), or IFN-γ (20 ng/ml) for 1, 2, 4, and 5 h. GAPDH expression was used as control. Data are from an experiment representative of six independent experiments yielding similar results. Graphs indicate relative TLR2 mRNA expression (densitometric quantification of TLR2 mRNA transcript over the GAPDH mRNA transcript) for each time point, and constitutive expression is expressed as 100%. B, HUVEC were stimulated with LPS (50 ng/ml) for 1, 2, and 5 h. Semiquantitative RT-PCR was performed. GAPDH expression was used as control. C, HMEC were pretreated with medium or 10 μg/ml of the protein synthesis inhibitor, cycloheximide (CHX), for 1 h and then stimulated with LPS for 4 h. Total RNA was extracted, and RT-PCR was performed. GAPDH expression was used as control.
that, in addition to TLR2, IFN-γ also induces the up-regulation of TLR4 mRNA in EC (Fig. 3). It is well known that IFN-γ enhances both sensitivity and the magnitude of cellular responses to LPS and causes synergistic activation of cells (52–54). In the presence of IFN-γ, otherwise nontoxic doses of LPS become highly lethal for mice (55). The mechanisms of this synergistic toxicity are not well understood. In addition, the combination of the direct and indirect pathways of LPS-induced EC activation would provide a much more realistic representation of the LPS-triggered inflammatory stimuli that the endothelium is exposed to in the course of response to infection and might reveal changes that are much more potent.

To investigate whether LPS and IFN-γ can act synergistically to induce TLR2 expression in EC, we incubated HMEC with suboptimal concentrations of LPS (1 or 5 ng/ml), IFN-γ (1 or 5 ng/ml), or various combinations of LPS plus IFN-γ and assessed TLR2 mRNA expression by RT-PCR. When used together, LPS and IFN-γ acted synergistically to induce significant up-regulation of TLR2 expression in HMEC at concentrations that were ineffective when each was used alone (Fig. 4). We postulate that IFN-γ-induced up-regulation of TLR4 and TLR2 may transform a small amount of LPS or lipoprotein into a lethal stimulus capable of causing shock. This mechanism may also enable the host to respond quickly to relatively low doses of LPS and lipoproteins, thereby enhancing the innate immune response and antibacterial defenses.

We have previously shown that human microvascular ECs, which are one of the first lines of defense against invading microbial agents including Gram-negative bacteria, actively participate in innate immune responses and that enterobacterial LPS-induced responses in EC are specifically mediated via TLR4 (31, 42). TLR4 in human vascular EC participates in sensing LPS by a mechanism that is sCD14 dependent (42). After detecting the LPS

FIGURE 2. LPS up-regulates TLR2 protein expression in HMEC. A. Western blotting. Confluent HMEC monolayers were treated for 4 h with either medium or 50 ng/ml LPS, rinsed with ice-cold PBS, detached with trypsin/EDTA, and centrifuged, and total cellular extracts were prepared as described (31). As a positive control for human TLR2 expression, cellular extracts from $25 \times 10^6$ human PBMC were used. Protein samples were subjected to SDS-PAGE (130 μg/ml of total protein per lane) followed by blotting with anti-human TLR2 mAb TL2.1. The signal was detected with a secondary HRP-labeled Ab by application of standard ECL techniques. The positions of the m.w. standards are shown on the left. B. Immunostaining. HMEC were incubated for 4 h with LPS (50 ng/ml), fixed in 4% paraformaldehyde/PBS, and stained with anti-human TLR2 mAb or control mouse IgG. Panel A, LPS-stimulated HMEC stained with control mouse IgG; panel B, resting HMEC stained with anti-human TLR2 mAb TL2.1 (10 μg/ml); panel C, LPS-stimulated HMEC with anti-human TLR2 mAb TL2.1 (10 μg/ml).

FIGURE 3. LPS and IFN-γ up-regulate TLR4 mRNA in HMEC. HMEC were stimulated either with LPS (50 ng/ml) for 1 and 2 h or with IFN-γ (20 ng/ml) for 1 and 4 h. Expression of human TLR4 (548 bp) in HMEC was analyzed by semiquantitative PCR following reverse transcription. RT-PCR analysis of GAPDH expression was used as control (lower panel, 983 bp). Data are from an experiment representative of three independent experiments performed with similar results.

FIGURE 4. LPS and IFN-γ act synergistically to up-regulate TLR2 expression in HMEC. HMEC were stimulated for 4 h with suboptimal concentrations of LPS (1 or 5 ng/ml) or IFN-γ (1 or 5 ng/ml) and with different combinations of LPS/IFN-γ concentrations. Expression of human TLR2 (347 bp) was analyzed by semiquantitative PCR following reverse transcription. GAPDH expression was used as control. Data shown are from an experiment representative of three independent experiments performed with similar results.
molecules, TLR4 transmits the information across the cell membrane via the IL-1R intracellular signaling molecules, leading to the activation of NF-κB and regulation of several responsive genes (42). To investigate whether LPS-induced TLR2 up-regulation in EC is dependent on NF-κB activation, we pretreated HMEC with various chemical inhibitors of the transcription factor NF-κB and with sense and antisense p65 NF-κB oligonucleotides and analyzed their effects on LPS-mediated TLR2 mRNA up-regulation by RT-PCR. We observed that pretreatment of HMEC with proteasome inhibitor I, an NF-κB inhibitor (56), or with antisense phosphorothioate oligodeoxynucleotide against p65 NF-κB inhibited LPS-induced TLR2 up-regulation (Fig. 5A) and NF-κB-luciferase activity (Fig. 5B), whereas NF-κB p65 sense oligonucleotide had no significant effect on the mRNA TLR2. The housekeeping enzyme, GAPDH, was unaffected in these cells (Fig. 5A). These inhibitors did not result in EC death as measured by trypan blue uptake. Collectively, these results suggest that LPS-induced TLR2 mRNA expression in EC is NF-κB dependent. Our findings are consistent with a recent report that mouse TLR2 promoter contains NF-κB binding sites (57). Another study, investigating the putative 5′-proximal promoter of the human TLR4 gene, did not find any NF-κB binding site, at least in the proximal portion of this promoter (58). Because LPS-mediated NF-κB activation in EC is sCD14 dependent (42), as expected, the LPS-induced TLR2 up-regulation in EC was sCD14 dependent as well (data not shown).

Of interest, 15d-PGJ2, a cyclopentenone PG (cyPG), also inhibited LPS-induced TLR2 up-regulation (Fig. 5A) and NF-κB-luciferase activity (Fig. 5B). It has been suggested that cyPGs exert anti-inflammatory activity through the activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) (59, 60). The bioactive cyPG, 15d-PGJ2, which is physiologically formed by dehydration and isomerization of the cyclooxygenase metabolite PGD2, can activate PPAR-γ, a nuclear receptor that interferes with NF-κB transcriptional activity (59, 60). A recent study demonstrated a novel mechanism of anti-inflammatory activity of cyPGs that is PPAR-γ independent and based on the direct inhibition and modification of the IkB kinase (IKK) β subunit of IKKs (61). Elevated cyPG synthesis has been detected in late phases of inflammation (59) and is associated with resolution of inflammation (62). Concentrations of cyPG that are sufficient to inhibit IKK may occur locally during the late phase of inflammation, and down-regulation of TLR2 in response to LPS or cytokines may be part of the resolution of inflammation. Our results would also suggest that cyPG, and possibly more potent derivatives, may have therapeutic value in the treatment of sepsis and inflammatory diseases in which inhibition of NF-κB activity may be desirable, and where LPS-induced TLR2 up-regulation may synergistically augment the inflammatory responses.

Our data indicate that TLR2 and TLR4 expression in EC can be regulated at sites of infection and inflammation, either directly by LPS or indirectly by inflammatory cytokines such as TNF-α and IFN-γ. Our observations are consistent with and extend the findings of two recent reports showing that LPS induces TLR2 in mouse adipose cells (39) and TLR4 mRNA expression in rat coronary EC (63). In the latter study, the investigators showed that NF-κB is involved in LPS induction of TLR4 mRNA, but found no accompanying increase in TLR4 protein (63). Although LPS was found to inhibit the expression of TLR4 mRNA in two earlier studies using RAW 264 mouse macrophage cell lines (26, 64), a more recent study reported that LPS and proinflammatory cytokines increase TLR4 mRNA expression in human monocytes and polymorphonuclear cells (65). This divergence most likely reflects differences in cell types and differentiation stages. The observation that EC express the receptor for LPS and induce the receptor for lipoproteins and Gram-positive, sprochetal, and fungal cell wall components in response to LPS suggests that the eventual responsiveness of EC to various TLR2 ligands may be modulated by external stimuli. Furthermore, a recent study showed that TLR2

**FIGURE 5.** Up-regulation of TLR2 is NF-κB dependent. A. RT-PCR. HMEC were pretreated for 1 h with either proteasome inhibitor I (100 μM) or 15d-PGJ2 (20 μM) and stimulated with LPS (50 ng/ml) for 4 h. NF-κB antisense and sense oligonucleotides (30 μM) were added to the medium three times during 48 h before stimulation with 50 ng/ml LPS. Expression of human TLR2 (347 bp) was analyzed by semiquantitative PCR following reverse transcription. GAPDH expression was used as control. Data shown are from an experiment representative of three independent experiments performed for each inhibitor with similar results. B. NF-κB-luciferase activity. HMEC were transiently transfected with NF-κB-luciferase and β-galactosidase reporter vectors overnight. Cells were then stimulated with LPS (50 ng/ml) for 5 h with and without pretreatment with proteasome inhibitor I, p65 antisense oligos, and 15d-PGJ2, and luciferase and β-galactosidase assays were performed as described under Materials and Methods. Data shown are mean ± SD of three or more independent experiments and are reported as a percentage of LPS-stimulated NF-κB promoter activity.
can also function as a ‘death receptor’ that mediates apoptosis in addition to NF-κB activation in response to bacterial lipopolysaccharides (66). Therefore, TLR2 expression and up-regulation in EC cells by LPS and various inflammatory cytokines may also augment and contribute to endothelial cell apoptosis in bacterial sepsis and septic shock leading to vascular collapse. Indeed LPS has been shown to induce disseminated endothelial apoptosis resulting in endotoxic shock (67).

Our data also indicate that EC are involved at the earliest stages of the immune response and are capable of directly sensing the presence of bacterial cell wall components. These observations underline the importance and direct role of the vascular endothelium as an integral component of the innate immune response.

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