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Bacterial Lipopolysaccharide Activates HIV Long Terminal Repeat Through Toll-Like Receptor 4

Ozlem Equils,* Emmanuelle Faure,* Lisa Thomas,* Yonca Bulut,† Sergey Trushin,‡ and Moshe Arditi*

In HIV-infected patients, concurrent infections with bacteria and viruses are known to induce HIV replication as assessed by increases in plasma HIV RNA levels. In the present study, we determined the cell surface receptor and molecular mechanisms of enterobacterial LPS-induced HIV transcription. Human dermal microvessel endothelial cells (HMEC) were transfected with an HIV-long terminal repeat (LTR)-luciferase construct and subsequently stimulated with purified bacterial LPS. Our studies demonstrate that human Toll-like receptor 4 (TLR4) mediates LPS-induced NF-κB and HIV-LTR activation in HMEC through IL-1 signaling molecules, namely myeloid differentiation protein, IL-1R-associated kinase, TNFR-associated factor, and NF-κB-inducing kinase. Cotransfection of HMEC with HIV-LTR-luciferase and TLR4 cDNA from LPS-hyporesponsive C3H/HeJ mice abrogates LPS-induced HIV transcription as does the use of dominant-negative mutants of the IL-1 signaling molecules. Transfection of HMEC with an HIV-LTR-mutant that lacks the NF-κB binding site or pretreatment of cells with chemical inhibitors of the NF-κB pathway also blocked LPS-induced HIV-LTR transactivation. These data support the conclusion that TLR4 mediates enterobacterial LPS-induced HIV transcription via IL-1 signaling molecules and NF-κB activation plays an important role in HIV-LTR transactivation. The Journal of Immunology, 2001, 166: 2342–2347.

Gram-negative bacterial LPS or endotoxin is generally recognized as one of the most potent stimulants of host immune and inflammatory cells, including monocytes, macrophages, and endothelial cells (1, 2). In all of these cells, LPS is known to induce the production of pro-inflammatory cytokines, adhesion molecules, and chemokines through activation of transcriptional factors such as NF-κB (3–7). The initiation of the cellular responses to LPS has been shown recently to involve a human homologue of the Drosophila Toll receptor, termed Toll-like receptor 4 (TLR4) (reviewed in Ref. 8). Studies conducted over the past several years have demonstrated that the Toll family of signaling receptors play a crucial role in both Drosophila and mammalian innate immune responses to microbial Ags (8–10). Genetic studies using the mutant LPS-hyporesponsive C3H/HeJ and LPS nonresponsive C57BL/10ScCr mouse strains have revealed that TLR4 functions as the long sought signaling receptor that mediates enteric LPS-induced cellular activation (reviewed in Ref. 10).

We have recently shown that endothelial cells and macrophages express the TLR4 LPS receptor and respond to LPS through this membrane receptor by induction of NF-κB activation via IL-1R-associated signaling molecules, including myeloid differentiation protein (MyD88), IL-1R-activated kinase (IRAK), TNFR-associated factor-6 (TRAF6), and NF-κB-inducing kinase. Cotransfection of HMEC with HIV-LTR-luciferase and TLR4 cDNA from LPS-hyporesponsive C3H/HeJ mice abrogates LPS-induced HIV transcription as does the use of dominant-negative mutants of the IL-1 signaling molecules. Transfection of HMEC with an HIV-LTR-mutant that lacks the NF-κB binding site or pretreatment of cells with chemical inhibitors of the NF-κB pathway also blocked LPS-induced HIV-LTR transactivation. These data support the conclusion that TLR4 mediates enterobacterial LPS-induced HIV transcription via IL-1 signaling molecules and NF-κB activation plays an important role in HIV-LTR transactivation.

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1 Abbreviations used in this paper: TLR, Toll-like receptor; HMEC, human dermal microvessel endothelial cells; IRAK, IL-1R-associated kinase; MAPK, mitogen-activated protein kinase; MyD88, myeloid differentiation protein; NIK, NF-κB-inducing kinase; TRAF, TNFR-associated factor; LTR, long terminal repeat; cpg, cyclopentenone PG; PT3, phosphatidylserine 3; PPAR, peroxisome proliferator-activated receptor; IκB, inhibitory κB; IKK, IκB kinase; 15d-PGJ2, 15-deoxy-Δ12,14-PGJ2.

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NIK. Furthermore, our findings indicate that LPS-induced transactivation of HIV-LTR-luciferase is dependent on NF-κB activation in this experimental system and is modulated by both the p38 mitogen-activated protein kinase (MAPK) and phosphatidylinositols (PI3) kinase pathways. A more complete understanding of the molecular mechanism(s) of LPS-induced HIV replication is expected to have potential therapeutic implications, as concurrent and opportunistic infections such as sexually transmitted diseases in HIV-infected patients are known to be associated with an increase in plasma HIV RNA (17).

Materials and Methods

Cells and reagents
The immortalized HMEC (a generous gift of Dr. F. J. Candal, Centers for Disease Control and Prevention, Atlanta, GA) (21) were cultured in MCDB-131 medium supplemented with 10% heat-inactivated FBS, 2 mM glutamine, and 100 μg/ml penicillin and streptomycin in 24-well plates. The cells were routinely used between passages 10 and 14 as described earlier (11). Tissue culture reagents were purchased from Life Technologies (Rockville, MD). The NF-κB proteasome inhibitor I was purchased from Calbiochem (San Diego, CA), 15-Deoxy-Δ12,14-PGJ2 (15d-PGJ2) was purchased from Cayman Chemical (Ann Arbor, MI), and PI3 kinase inhibitor LY294002 was obtained from Biomol (Plymouth Meeting, PA). Highly purified, phenol-water-extracted Escherichia coli K235 LPS (≤0.008% protein), which was prepared according to the method of McIntyre et al. (22), was generously provided by Dr. Stefanie Vogel (Uniformed Services University of Health Sciences, Bethesda, MD). All reagents were verified to be LPS-free by the Limulus amoebocyte lysate assay (≤0.03 endotoxin U/ml; Pyrotell; Associates of Cape Cod, Woods Hole, MA).

Expression vectors
Dominant-negative cDNA constructs of MyD88, IRAK, TRAF6, and NIK have been characterized and described earlier (11, 23–25); C3H/HeJ TLR4 cDNA (0.5 μg) was a generous gift from Dr. Bruce Beutler (University of Texas Southwestern Medical Center, Dallas, TX); dominant-negative IKKβ was a kind gift from Dr. Michael Karin (University of California, San Diego, CA). The HIV-LTRwt-Luc and a mutant lacking the NF-κB binding site (HIV-LTRΔβ-Luc) expression vectors have been described previously (26). Briefly, they carry U2 + R regions of the HIV-LTR (laboratory adapted xytropic HIV-1 isolate (LAI) strain) from nucleoside −644 (XhoI) to +78 (HindIII), except that the tandem κB-responsive elements were deleted in HIV-LTRΔκB-Luc and replaced by the consensus sequence for Bell (27). Endothelial leukocyte adhesion molecule (ELAM)-NF-κB-luciferase and pCMV-κB-galactosidase vectors were used as described previously (11).

Transfection of HMEC
HMEC were plated at a concentration of 50,000 cells/well in 24-well plates and cultured overnight in MCDB-131 with 10% serum as described above. Cells were cotransfected the following day with FuGene 6 transfection reagent (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer’s instructions. The reporter genes pCMV-κB-galactosidase (0.1 μg), HIV-LTRwt-Luc (0.5 μg), or HIV-LTRΔκB-Luc expression vectors (0.5 μg), as well as dominant-negative mutants of MyD88, IRAK, TRAF6, or NIK (0.5 μg each), were transfected into HMEC as reported previously (28). Reporter genes pCMV-κB-galactosidase (0.1 μg) and C3H/HeJ TLR4 (0.5 μg) were cotransfected with either HIV-LTR wild-type (0.5 μg) or HIV-LTRΔκB luciferase construct (0.5 μg). pCMV-κB-galactosidase was transfected to normalize the results for transfection efficiency as described earlier (11). Cells were transfected for 24 h and then stimulated for 6 h with 50 ng/ml LPS suspended in growth medium or with recombinant human TNF-α (20 ng/ml; Genzyme, Cambridge, MA). Cells then were lysed and luciferase activity was measured with a Promega kit (Promega, Madison, WI) and a luminometer. β-galactosidase activity was determined by colorimetric method as described earlier (11). For experiments using chemical inhibitors, the overnight-transfected HMEC were pretreated for 1 h with chemical inhibitors of various signaling molecules, including 15d-PGJ2 (10 μM), which has been found to block NF-κB activation via specific inhibition of inhibitor κB (IκB) kinase (IKK) β (29), PI3 kinase inhibitor LY294002 (20 μM), proteasome inhibitor (100 μM), or p38 MAPK inhibitor SB203580 (20 μM). The cells were then stimulated with either LPS (50 ng/ml) or human TNF-α (20 ng/ml) for 6 h. These inhibitors did not affect the viability of HMEC at the concentrations used, as assessed by trypan blue staining (data not shown).

Results

LPS induces HIV-LTR-driven luciferase gene expression
In HIV-infected patients, opportunistic infections, including Gram-negative bacterial infections, lead to an increase in plasma HIV RNA levels, suggesting an induction of virus replication (16–19). To assess possible mechanisms that might be responsible for these observations, we evaluated the role of the Gram-negative bacterial cell wall component LPS on HIV replication by investigating LPS-stimulated HIV-LTR transactivation in vitro. Because we have shown previously that LPS can activate human endothelial cells through a mechanism involving activation of the transcription factor NF-κB via TLR4 (28), we used HMEC for the HIV-LTR-luciferase transfection experiments. In HMEC previously transfected with HIV-LTR-luciferase construct, the addition of LPS efficiently transactivates HIV-LTR in a dose-dependent manner (Fig. 1). However, under otherwise identical experimental conditions, LPS was unable to induce transactivation of HIV-LTRΔκB, which lacks the NF-κB binding site (Fig. 1). These results indicate that LPS stimulates HMEC activation of intracellular signaling pathways, which leads to enhancement of HIV-1 LTR transactivation, and that NF-κB activation is important in this event.

LPS induces HIV-LTR activation in endothelial cells via TLR4
Because enterobacterial LPS activates HMEC through TLR4 (28), we undertook experiments to investigate the functional role of...
TLR4 in LPS-induced HIV-LTR transactivation in HMEC. For these studies, HMEC were transiently cotransfected with C3H/HeJ TLR4 cDNA. In C3H/HeJ mice, a missense mutation in the cytoplasmic domain of TLR4 allows this mutant TLR4 to act as a dominant-negative molecule, most likely by sequestering the downstream adapter and signaling molecules (e.g., MyD88 and IRAK1), thus preventing LPS-induced signaling and NF-κB activation (30–32). Cells were also transfected with the HIV-LTR-dependent luciferase gene and then tested for their responses to LPS by measuring the luciferase activity. In the absence of C3H/HeJ TLR4 cDNA, LPS induces a high level of luciferase activity. However, transient transfection of the C3H/HeJ TLR4 cDNA construct blocked the LPS-induced HIV-LTR activation in a dose-dependent manner (Fig. 2). As anticipated, the C3H/HeJ TLR4 cDNA was without significant effect on TNF-α-induced HIV-LTR transactivation (data not shown). These findings suggest that the functional TLR4 expressed on HMEC and the associated TLR4-mediated signaling pathway play important roles in LPS-mediated HIV-1-LTR-dependent transcription.

**LPS induces HIV-LTR transactivation through IL-1 signaling molecules**

The IL-1 signaling pathway in mammals is strikingly similar to the Toll-signaling pathway in *Drosophila* (33–36). LPS activates NF-κB through IL-1R-associated signaling molecules in human endothelial cells, namely adapter protein MyD88, IRAK, TRAF6, and the protein kinase NIK (11). Therefore, we examined whether similar or identical signaling mechanisms were involved in LPS-induced HIV-LTR transactivation. For these studies, HMEC were transiently cotransfected with the HIV-LTR-luciferase construct and dominant-negative mutants of the IL-1R-associated signaling elements. Dominant-negative constructs for MyD88 (ΔMyD88), IRAK (ΔIRAK), TRAF-6 (ΔTRAF6), and NIK (ΔNIK) all significantly inhibited LPS-mediated HIV-LTR-dependent reporter gene expression (Fig. 3). Overexpression of the empty vector had no detectable effect on LPS-induced HIV-LTR transactivation.

**FIGURE 2.** LPS induces HIV-LTR activation in HMEC via TLR4. HMEC were cotransfected with various concentrations (0.1, 0.2, 0.5, 1 μg) of C3H/HeJ TLR4 cDNA, HIV-LTR-luciferase (0.5 μg), and β-galactosidase (0.1 μg) reporter vectors, and LPS induction of HIV-LTR was assessed. HMEC transfected with empty vector were used as control. Results are shown as mean and SD of three or more independent experiments and reported as a percentage of LPS-stimulated HIV-LTR promoter activity cotransfected with a vector control.

**LPS activation of NF-κB is important for induction of HIV-LTR transactivation**

Potent activators of NF-κB, such as IL-1, TNF-α, or LPS, induce rapid phosphorylation of the IKK complex (e.g., IKKα, IKKβ, IKKδ) (37–39). Phosphorylated IκBα is then polyubiquitinated and targeted for rapid degradation by the 26S proteasome, thus allowing the liberation and translocation of NF-κB into the nucleus. Inhibition of the activity of 26S proteasome by proteasome inhibitor I has been shown to block the degradation of IκBα and block the activation, the nuclear translocation, and DNA binding of NF-κB (40). To investigate further the participation of NF-κB in LPS-induced HIV-LTR transactivation, HMEC were transfected with a dominant-negative construct of IKKβ (ΔIKKβ) along with HIV-LTR-dependent reporter gene, and luciferase activity was measured after 5 h of LPS stimulation. In separate experiments, HMEC were pretreated with the proteasome inhibitor I (100 μM) for 1 h, and the HIV-LTR-luciferase activity was then assessed after LPS stimulation. The inhibition of NF-κB activation by transient transfection of ΔIKKβ (Fig. 3) or by preincubating the cells with proteasome inhibitor I (Fig. 4) prevented LPS-induced HIV-LTR transactivation. These observations, together with our findings that LPS is unable to induce transactivation of HIV-LTRΔxB, support the conclusion that LPS-TLR4-induced NF-κB

**FIGURE 3.** LPS induces HIV-LTR transactivation through IL-1 signaling molecules. HMEC were cotransfected with HIV-LTR-luciferase cDNA (0.5 μg), 0.5 μg of dominant-negative mutants of IL-1 signaling molecules (MyD88, IRAK, TRAF6, and NIK), or dominant-negative IKKβ and β-galactosidase reporter vectors (0.1 μg). HMEC transfected with empty vector were used as control. Results are shown as mean and SD of three or more independent experiments and reported as a percentage of LPS-stimulated HIV-LTR promoter activity cotransfected with a vector control.

**FIGURE 4.** Role of NF-κB, p38 MAPK, and PI3 kinase in LPS-induced HIV-LTR transactivation. HMEC were cotransfected with HIV-LTR-luciferase and β-galactosidase reporter vectors and treated with various NF-κB inhibitors such as 26S-proteasome inhibitor (100 μM), 15d-PGJ2 (10 μM), PI3 kinase inhibitor LY294002 (20 μM), or the p38 MAPKinase inhibitor SB203580 (20 μM), 1 h before stimulation with LPS (50 ng/ml). HIV-LTR-luciferase activity was measured with luciferase assay and normalized with β-galactosidase activity. Data shown are mean and SD obtained from three experiments and reported as a percentage of LPS-stimulated HIV-LTR promoter activity cotransfected with a vector control.
Activation is required for HIV replication in the experimental system that we used.

**Effect of 15d-PGJ₂ on LPS-induced HIV-LTR transactivation**

Cyclopentenone PGs (cyPGs) have been reported to exert anti-inflammatory activity through the activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) (41–45). The bioactive cyPG, 15d-PGJ₂, which is physiologically formed by dehydration and isomerization of cyclooxygenase metabolite PGG₂, can activate PPAR-γ, a nuclear receptor that interferes with NF-κB transcriptional activity (41, 43). A recent study has demonstrated a novel mechanism of the anti-inflammatory activity of cyPGs that appears to be PPAR-γ independent and is based upon a direct inhibition and modification of the IKKβ subunit of IKKs (29), resulting in blocking of NF-κB DNA binding. To investigate the potential role of 15d-PGJ₁ on LPS-induced HIV-LTR transcription, we pretreated HIV-LTRwt-luciferase transfected-HMEC with 15d-PGJ₂ (10 μM) for 1 h before LPS stimulation. 15d-PGJ₂ also completely blocked the LPS-induced HIV-LTR-luciferase activity (Fig. 4), as well as NF-κB activation (data not shown). These findings further support the important role of NF-κB activation in LPS-induced HIV-LTR transactivation. Our observations also suggest that bioactive cyPGs, such as 15d-PGJ₂, and possibly more potent derivatives, may potentially be useful for down-modulating LPS-induced HIV replication.

**Role of PI3 kinase on LPS-induced HIV-LTR transactivation**

PI3 kinase has been shown previously to be activated rapidly after LPS stimulation (46). PI3 kinase regulates the activation of p65/RelA by phosphorylation and transactivation of p65/RelA (47). PI3 kinase is also involved in TNF-α-mediated activation of NF-κB-dependent genes; the inhibition of PI3 kinase activity by wortmannin or LY294002 has been reported to greatly potentiate TNF-α-induced apoptosis (48). However, the interaction between the PI3 kinase pathway and the LPS-TLR4-mediated NF-κB signaling pathway is not well understood. To study the role of the PI3 kinase pathway in LPS-induced HIV-LTR transcription, we pretreated HMEC with the PI3 kinase inhibitor LY294002 (20 μM) for 1 h before LPS stimulation. Pretreatment of HMEC with LY294002 significantly blocked LPS-induced HIV-LTR-dependent reporter gene activation (Fig. 4). These findings suggest that PI3 kinase plays a role in LPS-TLR4-mediated NF-κB activation and HIV-LTR transcription. PI3 kinase inhibitors strongly block NF-κB p65 phosphorylation and inhibit both PI3 kinase and NF-κB transactivation and NF-κB-dependent gene expression but have no effect on degradation of IκBα, nuclear translocation, or the ability of NF-κB to bind DNA (47).

**Role of p38 MAPK activation on LPS-induced HIV-LTR transactivation**

The cytoplasmic enzyme p38 MAPK present in monocytes and endothelial cells is activated by a variety of environmental stresses, as well as UV light, LPS, and inflammatory cytokines (49, 50). In monocytes, inhibition of the p38 MAPK pathway blocks the LPS-induced production of IL-1 and TNF-α (51, 52). In T lymphocytes, p38 MAPK contributes to HIV-LTR activation by IL-1, TNF-α, UV light, and osmotic stress, which can be blocked by the p38 MAPK inhibitor SB203580 (53). The NF-κB sites in HIV-1-LTR are required for p38 MAPK-mediated responses to cytokines (53), and a recent study showed that p38 MAPK pathway is required for NF-κB activation (54). We next investigated the role of p38 MAPK pathway in LPS-TLR4-induced HIV-1-LTR transactivation. HMEC transfected with HIV-LTRwt-luciferase construct were preincubated with the specific p38 MAPK inhibitor (SB203580) for 60 min and stimulated with LPS for 5 h. Pretreatment of cells with SB203580 inhibited the LPS-induced HIV-LTR-dependent luciferase activity (Fig. 4), as well as NF-κB-luciferase activity (data not shown). The inhibition was ~50%, which is consistent with a previous report showing a similar degree of inhibition of TNF-α-induced HIV-LTR activation by the p38 MAPK inhibitor SB203580 (55). These observations support the concept that, in addition to PI3 kinase, the p38 MAPK pathway also contributes to LPS-induced NF-κB activation and HIV-LTR transactivation.

**Discussion**

In this study, we show that LPS promotes HIV-LTR activation in HMEC by activating NF-κB through TLR4 and IL-1 signaling molecules, namely MyD88, IRAK, TRAF6, and NIK. The activation of NF-κB is important and required for LPS-induced HIV-1 transcription in endothelial cell system that we used because an HIV-LTRΔκB-luciferase construct lacking the NF-κB binding site could not be activated by LPS. However, our data were obtained in an endothelial cell system, and HIV-LTR expression can be differentially regulated on different cell types. Indeed, in HIV-susceptible monocytic cell lines, LPS-induced HIV-LTR transactivation was shown to be mediated by transcription factor P.1, which is specifically expressed in monocytes in addition to NF-κB (56). Furthermore, we have shown that p38 MAPK and PI3 kinase are also involved in LPS induction of HIV-1 LTR. Thus, we conclude that the innate immune receptor TLR4 mediates enterobacterial LPS-induced HIV-LTR transactivation via IL-1R-associated signaling molecules and that LPS-mediated HIV transcription is dependent on the activation of NF-κB and modulated by the p38 MAPK and the PI3 kinase pathways.

The expression of integrated HIV-1 is controlled at the level of transcription by cellular factors and the viral transactivator Tat, acting through the HIV-1-LTR (57–59). The HIV-1-LTR contains cis acting elements required for transcription initiation as well as binding sites for several transcription factors, including NF-κB. The activation of HIV-1 gene expression by many extracellular stimuli is critically dependent on activation of NF-κB, which is known to bind to two κB sites within the HIV-1-LTR enhancer region (27, 60, 61). The predominant complex binding to the LTR κB sites in activated cells is NF-κB p50-p65 heterodimer. In unstimulated cells, NF-κB is restricted to the cytoplasm through its interaction with inhibitor proteins belonging to IκB family. Activation of NF-κB occurs through phosphorylation and proteolysis of the IκB inhibitor, with subsequent translocation of the active NF-κB into the nucleus, where it can bind to its cognate binding sites (62). Multiple factors can induce translocation of NF-κB into the nucleus, including Gram-negative bacterial LPS. Although LPS is known to be a very potent stimulator of HIV-1 replication in latently HIV-1-infected monocyctic cell line (U1 cells) through the activation of NF-κB (63), the cellular signaling mechanisms and the receptor(s) involved in LPS induction of HIV replication have not been fully defined. While CD14 was shown to play a Role in LPS-induced HIV replication (64), this GPI-linked receptor lacks an intracytoplasmic tail and therefore is not capable of providing intracellular signals. Recent studies have established that TLR4 is the primary signaling receptor for enterobacterial LPS. TLR4 contains a cytoplasmic domain that is homologous to the receptor(s) involved in LPS induction of HIV replication. Thus, we conclude that TLR4 is responsible for LPS induction of HIV-LTR transactivation. Because TLR4-induced NF-κB activation pathway shares IL-1R signaling molecules (11), it is of interest to note that IL-1 has also been shown to...
induce HIV-LTR transactivation and HIV replication (65). Furthermore, IL-1 and TNF-α concentrations were shown to be consistently elevated in cervical secretions during bacterial vaginosis, which was recently identified as a cofactor that promotes sexual transmission of HIV (66).

In HIV-1-infected patients, concurrent infections with Gram-negative bacteria are associated with increased plasma HIV RNA levels, suggesting LPS-enhanced HIV replication in vivo. LPS also has been shown to enhance HIV replication in monocyte cell lines in vitro (63); however, several other in vitro studies reported that LPS induces resistance of macrophages to HIV infection (67–69). This apparent discrepancy was suggested to be the result of several potential factors, including the protective effect of LPS-induced cytokines/chemokines, the activation state of the cells, and induction of a negative regulatory factor that may have an inhibitory effect on HIV-LTR and viral expression (67–70). One recent study reported a differential expression pattern of Toll receptors on human leukocytes and found that TLR4 is undetectable in resting or activated human T lymphocytes, although it is readily detectable in myelomonocytic cells (71). The exposure of human monocytes to bacterial products such as LPS or to proinflammatory cytokines increased TLR4 expression. Of potential importance, TLR4 expression by LPS was blocked after IL-10 treatment (71). These novel observations on differential expression patterns of Toll receptors on human leukocytes may also help explain the contradictory published findings regarding LPS effects on HIV replication in various cells.

Macrophages, which are thought to be the initial target cells of HIV infection and one of the major HIV reservoirs, play a crucial role in the overall host immune responses to HIV, especially in the polarization of Th cell responses to Th1 and Th2 cells through the production of IL-12 and IL-10 (72–74). We and others have recently shown a preferential replication pattern of chemokine receptors CCR5 and CXCR4 trophic primary HIV-1 isolates in Th1 and Th2 cells, respectively (75, 76). One well-characterized property of LPS is its ability to induce a low responsive state termed “LPS tolerance” in macrophages exposed to low levels of LPS priming (77). Furthermore, LPS “reprogramming” of macrophages has been shown to lead to up-regulation of IL-12 production and down-regulation of IL-10 production, which subsequently influences the Th1 and Th2 cell balance (78, 79). HIV-infected patients may be continuously exposed to low levels of LPS in vivo, during intercurrent infections, sexual intercourse, and i.v. drug use, which may lead to “reprogramming” of their macrophages and, as a consequence, significantly influence the cytokine environment. Reprogramming of macrophages may subsequently effect the susceptibility to HIV infection and efficiency of HIV replication. Therefore, understanding the molecular mechanisms and receptors involved in LPS induction of HIV replication may have important implications for the understanding of HIV immunopathogenesis.

In summary, our data indicate that TLR4 mediates the LPS induction of NF-κB activation and HIV-LTR transactivation through the IL-1R-associated signaling molecules MyD88, IRAK, TRAF-6, as well as p38 MAPK and PI3 kinase. To our knowledge, this is the first time that LPS-TLR4 signaling and NF-κB activation are being linked to increased HIV replication via HIV-LTR transactivation and that the LPS-TLR4-NF-κB activation pathway is being tied to the PPAR-γ and PI3 kinase pathways. Our results also suggest that the cyPG, 15d-PGJ2, and possibly more potent derivatives may have potential therapeutic value in preventing LPS-mediated enhancement of HIV replication during opportunistic bacterial infections where suppression of NF-κB activity may be desirable.

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