Gonococcal Lipooligosaccharide Suppresses HIV Infection in Human Primary Macrophages through Induction of Innate Immunity

Xinyan Liu, Arevik Mosoian, Theresa Li-Yun Chang, Bouchra Zerhouni-Layachi, Alexandra Snyder, Gary A. Jarvis, and Mary E. Klotman

1Division of Infectious Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, New York; 2Veterans Affairs Medical Center and Department of Laboratory Medicine, University of California, San Francisco.

Gonococcal Lipooligosaccharide (LOS) is a component of the gonococcal outer membrane that induces innate immunity through engagement of Toll-like receptor 4 (TLR4). We investigated the effects that LOS from 5 different strains of Neisseria gonorrhoeae have on HIV infection and on HIV provirus in primary human macrophages. LOS-treated human primary macrophages developed resistance to new HIV infection as well as to HIV provirus. Gonococcal LOS from the 5 strains and lipopolysaccharide (LPS) from Escherichia coli showed no significant difference in their anti-HIV activities. Suppression of HIV provirus resulted from the induction of interferon (IFN)-β and subsequent activation of signal transducer and activator of transcription 1. Neutralization of IFN-β, but not IFN-α, via antibody significantly reduced the anti-HIV activity induced by LOS and LPS. We conclude that LOS expressed by various strains of N. gonorrhoeae induce specific innate immune responses through TLR4 signaling, resulting in anti-HIV activity in human primary macrophages in vitro.

Epidemiologic and clinical studies provide strong evidence that gonococcal infection facilitates the transmission of HIV-1 infection [1, 2]. Yet despite these clinical observations, few studies have examined the underlying mechanisms.

Peripheral and resident macrophages are cellular targets of HIV infection and constitute a major reservoir for the persistence of HIV [3]. As a component of the first-line defense against microbes, macrophages recognize and engulf invading pathogens by phagocytosis, then bridge innate and adaptive immunity by processing antigens and presenting them to T cells. [4]. Because macrophages could be an important innate immune cell population in which coinfection with HIV and gonorrhea occurs, knowledge of the innate immune responses to coinfection with gonorrhea and HIV in macrophages is critical for understanding the pathogenesis of HIV.

Gonococcal lipooligosaccharide (LOS) is a critical virulence factor for Neisseria gonorrhoeae and is involved in many aspects of pathogenesis [5, 6]. LOS, together with pili, opacity-associated protein, and PorB porin protein, form the major adhesins for the gonococcal invasion of epithelial cells [7]. Locally, LOS causes toxic damage to the fallopian tube mucosa through induction of tumor necrosis factor (TNF)-α with resultant sloughing of ciliated epithelial cells [8, 9]. In addition, various bactericidal antibodies targeting LOS can be detected in the serum and secretions of patients with local infection, indicating that LOS at the mucosa induces a generalized as well as a local immune response [5]. Thus, the local and systemic impact that LOS has in gonococcal infection suggests that LOS plays an important role in HIV coinfections.
Gonococcal LOS has a basic structure that is similar to, but distinct from, that of lipopolysaccharides (LPSs) of enteric gram-negative bacilli. As an endotoxin, LOS consists of a hydrophobic lipid A that differs from the LPS of enteric bacilli in both the acylation and the chain length of the fatty acid residues as well as a hydrophilic oligosaccharide terminus that lacks the repeating O-carbohydrate antigenic side chain of enteric bacilli. LOSs show a high degree of heterogeneity both within and among strains [10, 11] in part because of the hydrophilic oligosaccharide core [12] and varied phosphorylation and fatty acid acylation of lipid A [13]. Even a single cell often simultaneously expresses ≥2 chemotypes of LOS in various proportions [14]. LOS also undergoes phase variation and distinct forms develop in different regions of the human body and at various time points during infection, a phenomenon that is considered important for the establishment of gonococcal infection [15, 16]. To address whether variations in LOS structure will lead to functional differences in the interaction between gonococcal LOS and host cells, thereby influencing HIV infectivity, in the present study, LOSs isolated from 5 strains of N. gonorrhoeae were investigated for the effect they have on HIV infection.

Bacterial LPS-induced activation of monocytes/macrophages mediated through Toll-like receptor 4 (TLR4) is well known [17]. It has been reported that LPS suppresses productive HIV-1 infection in primary macrophages in vitro. The mechanisms involved include LPS-induced, sustained down-regulation of HIV coreceptor CCR5 [18, 19]; secretion of type 1 interferon (IFN), which has nonspecific antiviral activity [20]; and inhibition of HIV provirus transcription [21]. TLR4 is one of the receptors of gonococcal LOS, but LOS evokes strain-specific differences in the induction of proinflammatory cytokines [22]. Using LPS as a reference, we addressed 2 questions: (1) whether gonococcal LOS induces innate immunity against HIV in human mature macrophages in a manner similar to LPS and (2) whether gonococcal LOS from different strains exhibits functional variation in the induction of innate immune responses and anti-HIV activities in macrophages in vitro.

**MATERIALS AND METHODS**

**Isolation and differentiation of human macrophages.**

Human peripheral-blood mononuclear cells were isolated from buffy coat samples from healthy donors by Ficoll density gradient centrifugation. Monocytes were selected by adherence to plastic flasks. Nonadherent cells were removed, and adherent cells were washed once with PBS. Macrophages were differentiated in Dulbecco’s MEM supplemented with 20% fetal bovine serum, 2 mmol/L glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin at 37°C in a humidified atmosphere of 5% CO₂ for 2 weeks. The entire procedure of macrophage isolation included 2 extensive washes at day 1 and day 7, and media was changed at least 4 times in the experiments that followed, with as many unattached cells as possible being removed. Purity of isolated macrophages was high, although small portions of T cells inevitably remained. Hornung et al. reported no TLR4 mRNA present in human T cells [23]. Therefore, the influence that the limited number of lymphocytes had on the effects of LOS was expected to be minimal.

**LOS.** LOS from 5 clinical isolates of N. gonorrhoeae (McK, F62, 1291, DOV, and PID2) was isolated by hot phenol extraction, using the method of Westphal and Jann [24]. Escherichia coli O55:B5 LOS was obtained from Sigma Chemical.

**Virus.** R5 HIV-1 Ba-L was obtained from Advanced Biotechnologies. Pseudotyped, replication-defective HIV VSV was generated in 293T cells by use of a 3-plasmid cotransfection system [25]. These 3 plasmids included a packaging construct pCMVΔR8.2; HIV-1 reporter construct pNL4-3-Luc.R-E with a firefly luciferase gene inserted into the pNL4-3 nef gene; and an envelope-expressing construct, pVSV, expressing vesicular stomatitis virus G glycoprotein. Transfection was performed in 75-cm² culture flasks, using Effectene (New England Biolabs).

**Effect that LOS has on HIV replication in macrophages.** To study the effects that LOS has on new HIV infection, macrophages were treated with LOS and LPS (100 ng/mL) for 24 h, followed by washing with PBS. Stimulated macrophages were infected with HIV-1 Ba-L at an MOI of 0.1 for 2 h. Unbound viruses were removed by washing with PBS, and macrophages were cultured postinfection for a total of 20 days. Virus replication was monitored in supernatants every 3–4 days by use of a HIV-1 p24 protein ELISA (National Cancer Institute at Frederick Cancer Research and Development Center).

To study the effects that LOS has on HIV provirus, pseudotyped, replication-defective HIV VSV was utilized to infect macrophages for 2 h, followed by washing with PBS and replacement with fresh media. HIV-1 integrase inhibitor L-731,988 (10 μmol/L in 0.5% dimethyl sulfoxide [DMSO] [Merck]) was added at 2 h or 72 h postinfection, and treatment continued for 96 h postinfection. Viral infection was determined by quantifying luciferase activity with Luciferase Assay Substrate (Promega).

**ELISA and IFN-β neutralization.** The IFN-β level in supernatant was determined by ELISA (Fujirebio) with a detection limit of 8.25 pg/mL. In the neutralization experiments, rabbit polyclonal antibodies (PAb) against human IFN-β and different isoforms of IFN-α (R&D) at a concentration of 5 μg/mL were added to HIV VSV-infected macrophages simultaneously with LOS and LPS treatment. As a control, 10 IU/mL of human IFN-α2 or IFN-β (R&D) in the presence or absence of the corresponding antibody was also used.

**Western blot for signal transducer and activator of transcription (STAT) 1 phosphorylation.** Whole macrophage cells were homogenized in buffer containing 20 mmol/L HEPES (pH 7.9), 140 mmol/L NaCl, 1 mmol/L MgCl₂, 1 mmol/L EDTA,
Gonococcal LOS Suppresses HIV Infection

25% glycerol, 1% NP-40, 0.5 mmol/L dithiothreitol, 1.5 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L NaVO₄, 1 mmol/L NaF, and protease inhibitor cocktail (Pierce). Protein concentration was assayed using a protein assay kit (Pierce). Cell lysates containing 50 μg of protein were separated by 4%–12% SDS-PAGE in MOPS buffer (Invitrogen) and transferred to polyvinylidene difluoride membranes (Millipore). Membranes were incubated overnight in Tris-buffered saline (TBST)–3% bovine serum albumin (BSA) containing phospho-STAT1 (Tyr701) PAb (1:1000 dilution). This antibody recognizes both the α and β forms of STAT1 (91 kDa and 84 kDa, respectively) (Cell Signaling). Blots were washed and incubated for 1 h with anti-rabbit horseradish peroxidase-conjugated antibody IgG (KPL) at a 1:20,000 dilution in TBST–3% BSA. Proteins were visualized by chemiluminescence (ECL). Blots were stripped in 0.1N glycine at room temperature for 30 min and were probed using total STAT1 PAb (Cell Signaling) as described above.

For the neutralization experiment, a 5 μg/mL concentration of rabbit PAb against human IFN-β (R&D) or 5 μg/mL of normal rabbit IgG was added to HIVVSV-infected macrophages simultaneously with LOS and LPS for 2 h. The activation of STAT1 under this setting was measured by Western blot as described above.

Statistics. Data are expressed as mean ± SD. Student’s t test was used to compare the means of different samples (treated vs. control). P < .05 was considered to be statistically significant.

RESULTS

Gonococcal LOS suppression of new infection with replication-competent HIV in mature human macrophages in vitro. Macrophages have been shown to be resistant to HIV infection when pretreated with LPS extracted from E. coli and Salmonella minnesota [21]. We compared the activities of E. coli LPS and gonococcal LOS by the use of the same concentration of LOS and LPS to stimulate human macrophages. Mature macrophages were treated with 100 ng/mL LOS or LPS for 24 h, washed, and then challenged with R5 HIVBa-L (MOI, 0.1) for 2 h. After 10 days of culture postinfection, all samples treated with LOS from 5 isolates, and 1 strain of LPS showed significant suppression of HIVBa-L production, compared with untreated cells (figure 1A). The peak inhibition occurred at day 17 with 80%–90% HIVBa-L suppression. No difference in HIVBa-L suppression was observed between LOS and LPS. There was no significant difference among LOSs from various gonococcal strains in their anti-HIV activities on macrophages; all 5 strains showed similar suppressive activity.

Infection of pseudotyped HIVVSV was also significantly suppressed in LOS-treated macrophages, as is shown in figure 1B. The suppression reached 90%–95%, with no strain difference between LOS and LPS at the dosage of 100 ng/mL.

We concluded that LOS-treated macrophages became resistant to both R5 HIV and pseudotyped HIVVSV. Because HIVVSV does not utilize traditional HIV coreceptors [27], the data suggested that the LOS-induced antiviral activity of macrophages...
Figure 2. Lipooligosaccharide (LOS) and lipopolysaccharide (LPS) suppression of HIV infection at the postintegration stage in human macrophages in vitro. Human macrophages were challenged with HIVVSV for 2 h and cultured for 72 h postinfection to complete proviral integration, followed by treatment with LOS, from 5 clinical isolates of *Neisseria gonorrhoeae* (MkC, F62, 1291, DOV, and PID2), or LPS, from *Escherichia coli*. A, Treatment of macrophages containing integrated HIV provirus for 24 h with LOS or LPS at concentrations of 0.01, 0.1, 1, 10, and 100 ng/mL. A luciferase assay was performed to quantify the expression of HIV provirus. B, Confirmation of HIV proviral integration, by addition of HIV-1 integrase inhibitor L-731,988 (10 μmol/L dissolved in 0.5% dimethyl sulfoxide [DMSO]), 2 h or 72 h postinfection, with treatment continuing for 96 h. In addition, the DMSO solvent was tested as a control. LOS or LPS at a concentration of 100 ng/mL was tested for HIV provirus suppression by measuring luciferase activity. For both panels, results are the mean luciferase values from triplicate wells and are representative of 3 individual experiments. RLU, relative light units.

in response to new HIV infection is due to mechanisms other than the down-regulation of CCR5 [18, 19].

**LOS suppression of the expression of HIV provirus at the postintegration stage in human macrophages.** Because HIV provirus persists in macrophages for long periods, it is possible that HIV-infected macrophages in the mucosa could be exposed to *N. gonorrhoeae* during gonococcal coinfection. Therefore, we used an in vitro HIV-integration model in macrophages to study whether gonococcal LOS can affect HIV infection. To this end, human macrophages were infected with replication-incompetent HIVVSV for 2 h, followed by 72 h of culture. To confirm HIV proviral integration, HIV-1 integrase inhibitor L-731,988 was added at 2 h or 72 h postinfection, and the treatment was continued for 96 h. The suppression of HIVVSV infection by the integrase inhibitor at 2 h but not at 72 h postinfection indicated that HIV proviral integration had been completed after 72 h of postinfection culture (figure 2B). As the solvent for L-731,988, DMSO alone did not affect the activity of integrated HIV in macrophages. LOS and LPS were applied for 24 h at doses of 0.01–100 ng/mL to macrophages containing integrated HIV provirus (figure 2A). Their anti-HIV activity increased in a dose-dependent manner, with no significant difference between LOS and LPS. The concentration of 100 ng/mL was used in all subsequent experimental conditions because previous work showed that this dose induced the highest level of TLR4 signaling [22]. As is shown in figure 2B, this dose of LOS and LPS resulted in significant suppression of HIV provirus in macrophages. No difference between LOS and LPS was observed in their anti-HIV activities (figure 2B). The data suggest that a common mechanism underlies the similar anti-HIV activity of LOS and LPS in macrophages, despite the known structural differences of the 2 endotoxin molecules.

**IFN-β as a critical mediator of suppression of HIV by LOS in macrophages.** When monocytes differentiate to macrophages in vitro, a variety of changes occur that influence HIV infection. Mature macrophages develop an increased susceptibility to HIV infection [28, 29]. At the same time, the response to type I and type II IFNs is also enhanced in macrophages because of the increased expression of IFN receptors on the plasma membrane [30]. Type I IFN has been shown to inhibit HIV-1 replication in human cells cultured in vitro [20], and IFN-β plays an important role in mediating the LPS-induced antiviral activity through TLR4 [31]. Therefore, we focused on comparing the anti-HIV function of LOS and LPS by studying the effect that IFN-β has on human macrophages.

Macrophages containing HIV provirus were stimulated with LOS and LPS for increasing periods. The secretion of IFN-β in the culture supernatants was analyzed by ELISA (figure 3). IFN-β was initially detectable (7–32 pg/mL) ∼1–2 h after treatment with LOS and reached peak concentrations of 680–990 pg/mL after 8 h, followed by a sharp decline in concentration at 24 h. IFN-β secretion from macrophages containing HIV provirus followed by stimulation of LOS showed a similar pattern across strains and with LPS, further supporting the notion of a similar mechanism for LOS and LPS.
Figure 3. Stimulation by lipooligosaccharide (LOS) or lipopolysaccharide (LPS) of interferon (IFN)-β secretion in the supernatants of macrophages containing HIV provirus. Macrophages were challenged with HIVVSV for 2 h and cultured for 72 h postinfection. A 100 ng/mL concentration of LOS, from 5 clinical isolates of Neisseria gonorrhoeae (MkC, F62, 1291, DOV, and PID2), or LPS, from Escherichia coli, was added and supernatants were collected after exposure of cells for 15 min, 30 min, 1 h, 2 h, 8 h, and 24 h. ELISA was performed to quantitate IFN-β levels in the culture supernatants. These results are representative of 3 independent experiments done in duplicate.

To confirm the role that IFN-β plays in LOS-induced suppression of HIV provirus, neutralizing antibodies were added simultaneously with LOS to macrophages containing HIV provirus. PAb against human IFN-β reduced the anti-HIV activities induced by LOS in macrophages, decreasing suppression from ~80% to 40% for each LOS and LPS (P < .05). However, neutralizing antibodies against human IFN-α had no effect on anti-HIV activity (figure 4A). Similar results were found with LPS. Purified human IFN-β and IFN-α (10 U/mL) showed anti-HIV activities in macrophages containing HIV provirus, and neutralizing antibodies blocked their anti-HIV functions (figure 4B). Therefore, IFN-β, but not IFN-α, plays an important role in LOS-induced HIV suppression in macrophages.

LOS-induced phosphorylation of STAT1 in macrophages infected with HIV provirus. STAT1 is a signal transducer of the IFN system. IFN binds to the extracellular domain of IFN cytokine receptors, leading to the phosphorylation of the cytoplasmic protein Janus kinase (JAK), which in turn phosphorylates STAT1 [32, 33]. Detection of STAT1 phosphorylation provides additional evidence that the IFN system is involved in LOS-induced anti-HIV activity in macrophages. As shown in figure 5A, treatment of macrophages containing HIV provirus with LOS or LPS from all strains led to the phosphorylation of STAT1, as assessed by Western immunoblots with phospho-STAT1 antibody. LOS or LPS at a dosage of 100 ng/mL induced STAT1 phosphorylation between 1 and 2 h after treatment, whereas STAT1 was not phosphorylated in the absence of LOS or LPS. When the neutralizing antibody to human IFN-β was added simultaneously with LOS, followed by incubation for 2 h, the activation of STAT1 induced by LOS was significantly inhibited (figure 5B). These results are consistent with the time of initiation of IFN-β secretion from macrophages after exposure to LOS and LPS (figure 3), indicating that IFN-β production by macrophages in response to LOS and LPS quickly induces STAT1 phosphorylation and subsequent expression of antiviral genes. In addition, as with IFN-β pro-
Figure 5. Signal transducer and activator of transcription (STAT) 1 phosphorylation, induced by lipooligosaccharide (LOS) or lipopolysaccharide (LPS) in macrophages with HIV provirus. Macrophages were challenged with HIV\textsubscript{VSV} for 2 h and cultured for 72 h postinfection, followed by treatment with LOS, from 5 clinical isolates of \textit{Neisseria gonorrhoeae} (MkC, F62, 1291, DOV, and PID2), or LPS, from \textit{Escherichia coli}. 

\textbf{A}, Addition of 100 ng/mL of LOS and LPS for 1 or 2 h. Phosphorylation of STAT1 in macrophages containing HIV provirus was analyzed by Western blot. 

\textbf{B}, Polyclonal antibody against human interferon-\textbeta (\alpha IFN-\textbeta) at a concentration of 5 \mu g/mL or normal rabbit IgG at 5 \mu g/mL was added simultaneously with LOS or LPS for 2 h. The inhibition of LOS-induced STAT1 activation was analyzed by Western blot. Total STAT1 was assessed as a loading control. Data are representative of at least 2 individual experiments. Cont, control macrophages; Inf, macrophages infected with HIV provirus; NT, no treatment; P-STAT1, phospho-STAT1.

<table>
<thead>
<tr>
<th></th>
<th>LPS</th>
<th>F62</th>
<th>DOV</th>
<th>MkC</th>
<th>PID2</th>
<th>1291</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont Inf 1 h 2 h</td>
<td>1 h 2 h</td>
<td>1 h 2 h</td>
<td>Cont Inf 1 h 2 h</td>
<td>1 h 2 h</td>
<td>1 h 2 h</td>
<td></td>
</tr>
<tr>
<td>P-STAT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>F62</td>
<td>DOV</td>
<td>MkC</td>
<td>PID2</td>
<td>1291</td>
</tr>
<tr>
<td>NT</td>
<td>\alpha IFN-\beta</td>
<td>IgG</td>
<td>\alpha IFN-\beta</td>
<td>NT</td>
<td>IgG</td>
<td>\alpha IFN-\beta</td>
</tr>
<tr>
<td>P-STAT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>F62</td>
<td>DOV</td>
<td>MkC</td>
<td>PID2</td>
<td>1291</td>
</tr>
<tr>
<td>NT</td>
<td>IgG</td>
<td>\alpha IFN-\beta</td>
<td>NT</td>
<td>IgG</td>
<td>\alpha IFN-\beta</td>
<td>NT</td>
</tr>
<tr>
<td>P-STAT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

production, the phosphorylation of STAT1 did not differ between strains of LOS.

**DISCUSSION**

In the present study, we investigated the innate immune response induced by LOS isolated from 5 clinical gonococcal strains and the effect it has on HIV infection in human macrophages and compared these results with similar treatment with \textit{E. coli} LPS. In contrast to clinical data suggesting synergy between HIV infection and bacterial or viral coinfection, we observed anti-HIV activity induced by gonococcal LOS in mature human macrophages in vitro. This effect was due to the production of IFN-\textbeta and the subsequent activation of the JAK-STAT1 pathway evoked by gonococcal LOS as well as LPS resulting in an innate anti-HIV immune response.

Of the broad range of mediators of the innate immune system, the TLR family constitutes the major innate recognition system for microbial invaders in vertebrates. Different TLRs recognize microbial molecules through pathogen-associated molecular patterns [34]. To date, 11 TLRs have been identified in humans. The limited number of TLRs indicates the large accessibility of each TLR to various molecules sharing some structural similarities. LOS from all 5 different strains used in these studies, as well as LPS, have been reported as TLR4 ligands [35, 36]. Although the lipid A structures of LOS and LPS differ, their similar anti-HIV activity provides some evidence of the relatively nonspecific nature of the innate immune response.

The involvement of TLR4 with meningococcal LOS in inducing macrophage activation and subsequent release of cytokines has also been reported [22, 37]. Pridmore et al. showed that different LOS from diverse strains of \textit{Neisseria} induced the IL-8 promoter variably and that TNF production was correlated...
with cell expression of TLR4 [22]. However, LOS from the 5 different strains in the present study did not show significant variation in their anti-HIV effects but instead showed similar patterns of IFN-β secretion and time points of STAT1 activation, which were also observed with induction by LPS. This discrepancy may be due to a differential induction of the 2 potential TLR4 signaling pathways related to MyD88.

On TLR4 engagement, the MyD88-dependent pathway is responsible for production of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α through NF-κB activation [38], whereas the MyD88-independent pathway accounts for production of cytokines such as IFN-β [39]. It has been reported that acylation and phosphorylation differences in the lipid A molecule account for different TNF-α production on stimulation of cells with LOS [22]. In the present study, LOS derived from different strains showed no differences in affecting the amount of IFN-β produced by macrophages. Given that we observed no variability in IFN-β induction by various strains, our data suggest that LOS lipid A variability does not affect the TLR4-MyD88–independent pathway, compared with its action on the TLR4-MyD88–dependent pathway, as is supported by the report of differential induction of TLR4 pathways by endotoxins [40]. Moreover, IFN-β, but not IFN-α, plays an important role in the antiviral activity derived from the LOS–induced TLR4-MyD88–independent innate immune response.

Activation of TLR3/4 can induce the MyD88–independent pathway and antiviral function through the adaptor TRIF (Toll–IL receptor-domain-containing adaptor protein inducing IFN-β [41]). Interferon regulatory factor (IRF) 3 and NF-κB are key transcription factors in the downstream signaling pathway. Translocation of both factors evokes early antiviral proteins, including IFN-β [31]. The autocrine and paracrine functions of IFN-β lead to the activation of the JAK-STAT pathway. Phosphorylated STAT1, STAT2, and active IRF7 form a complex that regulates a positive feedback loop of type I IFN, producing primarily IFN-α [42, 43]. In our neutralization experiments, antibody against IFN-β effectively blocked the induction of anti-HIV activities, whereas antibody against IFN-α did not, thus demonstrating the importance of IFN-β in early stages of the antiviral innate immune response induced by LOS and LPS.

IFN regulates hundreds of genes. Three families have been studied for their antiviral activity, including double-stranded RNA-activated protein kinases (PKRs), the 2′,5′-oligoadenylated synthetases (OASs), and the Mx proteins [44]. PKRs have multiple antiviral functions, such as inactivation of translation–initiation factor eIF-2α, induction of apoptosis, and regulation of IFN synthesis [45]. OASs catalyze the synthesis of 2′,5′-oligoadenylates, which activate RNase L to digest cytoplasmic mRNA and rRNA, preventing protein synthesis [46]. The antiviral activities of PKR and OAS are based on their destruction of viral RNA. Therefore, their contributions to LOS–induced antiviral activity could be limited. As GTPases, Mx proteins have been shown to interfere with the trafficking and/or the transcriptional activity of the viral ribonucleoprotein complexes [47]. Further investigation into the role that Mx proteins play in the mechanism of LOS–induced anti-HIV activity in macrophages is necessary. Other factors that may be involved in transcription regulation and IFN stimulation, such as the IRFs, may also contribute to this anti-HIV activity.

IRF is a family of transcriptional activators and repressors that bind to a consensus sequence, the IFN-stimulated response element (ISRE), existing within the promoter of IFN-β and most IFN-inducible genes [42]. Different HIV isolates have a conserved sequence at nt +200 and nt +217 that is homologous to ISRE [48]. Stimulation of IRF-1 results in activation of the HIV-1 LTR, whereas IRF-8 contributes to the latency of HIV by reducing HIV replication in vivo [49]. IRF is regulated by IFN; some members of the IRF families are also induced by the TLR pathway—for example, IRF3 is induced by TLR4 activation [31]. Therefore, LOS–induced transcriptional suppression of HIV in macrophages could be the net result of the induced IRF leading to coordinated enhancement and suppression of transcriptional activity.

The apparent contradiction between clinical data and the in vitro observations in the present study illustrates the complexity of local effects in the mucosa. Although local autocrine activity of the IFN system may produce a protective effect on macrophages themselves, secretion of proinflammatory cytokines by adjacent HIV-permissive cells within the mucosa may result in an enhanced HIV replication. This indicates that analysis of cell type–specific reactions and the manipulation of innate immunity should be done with both local and systemic effects taken into consideration. The ability of LOS to induce a structure-dependent proinflammatory response and a structure-independent protective response suggests that an optimal TLR4 ligand can be selected to evoke a protective innate immune response with minimized production of proinflammatory cytokines. Such candidates could be utilized for potential immune therapeutics.

References

46. Jacobs BL, Langland JO. When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. Virology 1996; 219:339–49.