Influence of Coinfecting Pathogens on HIV Expression: Evidence for a Role of Toll-Like Receptors

André Bárica, Charles A. Scanga, Marco Schito, Damien Chaussabel and Alan Sher


http://www.jimmunol.org/content/172/12/7229

---

**References**

This article cites 66 articles, 37 of which you can access for free at:

http://www.jimmunol.org/content/172/12/7229.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Influence of Coinfecting Pathogens on HIV Expression: Evidence for a Role of Toll-Like Receptors

André Báfica,‡ Charles A. Scanga,* Marco Schito,‡ Damien Chaussabel,* and Alan Sher‡

Immune activation of HIV gene expression as a consequence of the host response to coinfecting pathogens has been implicated as an important factor in AIDS progression. Immune responsiveness to many of the infectious agents associated with HIV has been demonstrated to depend on a family of innate recognition molecules, known as Toll-like receptors (TLR). Therefore, TLR-pathogen interactions could play an indirect role in regulating HIV-associated disease. In this review, we summarize emerging evidence for the influence of TLR recognition on HIV gene activation and AIDS progression.

The number of people living with HIV/AIDS reached 40 million in 2003 (1). Coinfection with non-HIV pathogens has been postulated to be an important exogenous factor that influences the severity and rate of disease progression in HIV+ individuals, reducing survival and increasing the risk of HIV transmission (2–6). The heterologous infectious agents involved include viruses such as human herpesvirus-6, HSV-1, several protozoan parasites (e.g., Leishmania donovani, Toxoplasma gondii, Plasmodium falciparum), and bacteria (e.g., Mycobacterium sp. and Neisseria gonorrhoea) (6–10). In certain regions of the world and, in particular, sub-Saharan Africa, the high prevalence of chronic and recurrent acute infections with such pathogens and the limited availability of treatment and control measures are factors that potentially compound this problem (11).

Enhanced HIV induction as a consequence of microbial-induced immune activation has been implicated as the mechanism responsible for the elevated viral expression observed in coinfected individuals (3, 12). The term immune activation has been used to refer to stimulation of the HIV long terminal repeat (LTR)3 by means of signals triggered by the immune response to non-HIV Ags or infectious agents (13). These signals can operate in trans through proinflammatory cytokines (e.g., TNF, IL-1) induced in a responding immune cell and/or in cis—by transcription factors activated in HIV-infected cells upon coinfection with a secondary pathogen. Both mechanisms depend on triggering events initiated when pathogens are recognized by receptors belonging to the innate immune system.

The major innate recognition system for microbial invaders in vertebrates is now thought to be the Toll-like receptor (TLR) family (14, 15). TLR are descended from similar receptors (Toll) originally discovered in Drosophila (16) and share a TOLL/IL-1R (TIR) domain required for intracellular signaling as well as an extracellular region containing leucine-rich repeats. At present, 10 TLR members are known to exist in humans and at least 11 in mice. In addition to the TLR themselves, IL-1 and IL-18 receptors share similar intracellular domains and therefore are included as members of this family. Importantly, the different TLR are triggered by conserved molecular structures (pathogen-associated molecular patterns (PAMP)) expressed by microbial agents. These PAMP include lipid or lipoprotein moieties (recognized by TLR1, TLR2, TLR4, and TLR6), protein motifs (recognized by TLR5), as well as nucleotide sequences (TLR3 and TLR9) (14, 15). Although originally thought to be restricted to individual TLR, it is becoming evident that recognition of a given pathogen can involve multiple TLR (17).

Ligation of TLR results in the triggering of a cascade of events that leads to the transcription of many of the genes involved in immune activation (18). These signals involve the recruitment of the adaptor molecule myeloid differentiation factor 88 (MyD88) and in certain cases TIR domain-containing adapter inducing IFN-β (TRIF), TRIF-related adapter molecule (TRAM), and TIR domain-containing adapter protein (TIRAP), followed by the binding of IL-1R-associated kinase (IRAK) family members and the activation of TRAF-6, ultimately leading to the activation of NF-κB and mitogen-activated protein (MAP) kinases (Fig. 1). The latter events are important transcriptional regulators for the genes encoding the proinflammatory cytokines TNF and IL-1, which as discussed above can enhance HIV LTR activity. In addition, TLR signaling can lead to direct NF-κB-dependent LTR triggering and/or the activation of transcriptional factors such as NF-κB, STAT3, and AP-1, which are known to up-regulate HIV transcription.

Received for publication March 2, 2004. Accepted for publication April 14, 2004.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by the National Institutes of Health Intramural AIDS Targeted Antiviral Program.

2 Address correspondence and reprint requests to Dr. André Báfica or Dr. Alan Sher, Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 50, Room 6146, 50 South Drive, Bethesda, MD 20892. E-mail addresses: abafica@niaid.nih.gov and asher@niaid.nih.gov

3 Abbreviations used in this paper: LTR, long terminal repeat; TLR, Toll-like receptor; TIR, Toll/IL-1R; PAMP, pathogen-associated molecular pattern; MyD88, myeloid differentiation factor 88; TRIF, TIR domain-containing adapter inducing IFN-β; MAP, mitogen-activated protein; Tg, transgenic.

Copyright © 2004 by The American Association of Immunologists, Inc.
of other transcription factors such as AP-1 that have been implicated in HIV induction (19, 20).

In the following review, we will summarize the existing evidence linking microbial-stimulated TLR signaling with HIV LTR induction and viral expression. This information is of importance for understanding the interaction of coinfecting pathogens with HIV as well as for designing approaches for counteracting the effects of immune activation on AIDS progression.

**In vitro evidence for a role of TLR ligands in HIV induction in human cells**

The first evidence for an association of TLR signaling and HIV induction came from studies in which LPS was shown to stimulate viral LTR activity in chloramphenicol acetyl transferase reporter-transfected monocyte/macrophage-like cell lines via a mechanism associated with NF-κB activation (21). In the same report, the authors showed that LPS stimulation also induces HIV replication in chronically infected U1 cells (a monocyte/macrophage cell line), an effect that had been noted previously in a study of cytokine production by HIV-infected THP-1 cells (22). Importantly, no virus induction was observed in a transfected T cell line stimulated in the same fashion with LPS (21). Although TLR4 had not yet been identified as the receptor for NF-κB activation by LPS, the latter findings were consistent with TLR4 involvement, because this TLR family member is not normally expressed by T cells, LPS was also shown in subsequent studies to stimulate HIV replication in infected primary human monocyte/macrophage cultures (22). Additional publications documented the ability of lipoarabinomannan (24), lipophosphoglycan (25), and bacterial DNA (26) to promote HIV LTR transactivation in stably transfected cell lines, although the authors were not aware that they were dealing with TLR ligands.

The above studies involved the effects of immune activation on integrated LTR or viral expression. Somewhat opposing findings were obtained when HIV infection of monocyte/macrophages was initiated immediately before or simultaneously with microbial stimulation. In these experiments, LPS or mycobacterial exposure was found to inhibit HIV replication either as a result of chemokine induction and coreceptor blockade or suppression of other preintegration steps in the viral life cycle (27–29).

With the discovery of mammalian TLR signaling in 1997 (30), it was logical to conclude that TLR ligation is a key event in the induction of HIV expression by microbial ligands. Formal proof was not obtained until 2001 when it was shown by Equils et al. (31) that LPS stimulates LTR transactivation in transfected human dermal microvessel endothelial cells secondarily transfected with wild-type, but not mutated TLR4. In a follow-up study, these authors (32) used a similar approach to establish a role for TLR2 in LTR transactivation by bacterial TLR2 ligands. More recently, Sundstrom et al. (33) have shown that HIV-infected human mast cells display increased HIV replication when stimulated with known TLR2, -4, and -9 ligands.

In a parallel line of investigation, it had been shown that live microorganisms or crude microbial extracts also promote HIV expression in monocytic cells in vitro (25, 26). For example, both live *Mycobacterium tuberculosis* (24) and *L. donovani* (25) were found to stimulate HIV LTR promoter activity in transfected cells as well as virus replication in chronically infected monocyte/macrophage cell lines. Importantly, in these two studies, pathogen extracts as well as purified components (e.g., mycobacterial lipoarabinomannan and phosphatidylinositol mannoside, and leishmanial lipophosphoglycan) were also shown to stimulate the same viral responses, and all of these stimuli were later shown to be or contain TLR2 agonists (34, 35). Taken together with the later findings of Equils et al. (31, 32), these reports suggested that TLR signaling is the major pathway for microbial-induced HIV expression, and to date, three of the known TLR—TLR2, TLR4, and TLR9—have
been shown to possess this activity (Table I). Nevertheless, the involvement of additional microbial recognition receptors and signaling pathways in the induction of HIV has not been formally ruled out, although as discussed below, there is strong evidence in a murine transgenic system arguing that TLR signaling is in itself sufficient.

Mechanisms implicated in TLR-induced HIV expression

The pathways by which microbial TLR ligands trigger HIV expression have not been fully elucidated, although there are a number of obvious candidates (Fig. 1). Cytokine-mediated induction of the HIV LTR through NF-κB activation is thought to be a major mechanism by which concurrent infectious agents enhance proviral transcription (11, 36) and may contribute to TLR-dependent triggering. Thus, serum TNF levels have been correlated with increased viral loads in patients dually infected with HIV and M. tuberculosis (11, 36). Furthermore, in vitro neutralization of TNF has been shown to partially inhibit HIV replication induced by purified protein derivative from mycobacteria in monocytes (36) as well as the enhancement in viral expression stimulated by live M. tuberculosis in a chronically infected macrophage cell line (24). In contrast, anti-TNF treatment had no effect on HIV replication in U1 cultures stimulated with LPS (21), and in a different study not involving M. tuberculosis infection, no correlation was observed between TNF and HIV gene expression in lymph nodes of HIV+ individuals (37). Thus, these findings suggest that, although influencing HIV expression, TNF is not an obligatory signal for HIV LTR induction.

In addition to indirect cytokine-mediated HIV induction, the interaction of PAMPs with TLR may result in the MyD88-dependent activation of HIV transcription through NF-κB itself or MAP kinase-dependent AP-1 (19, 20) (Fig. 1). Other transcription factors implicated in HIV LTR activation such as C/EBP, CREB, and Sp1 could also be induced as a consequence of MAP kinase signaling and promote HIV expression (19, 20, 38–42). LTR induction may also involve mechanisms dependent on other adaptor molecules (e.g., TRIF and TRAM) distinct from those requiring MyD88 or by means of phosphatidylinositol 3-kinase-dependent NF-κB activation (43) (not shown in Fig. 1). So far, however, none of the above mechanisms have been formally demonstrated to explain TLR-mediated immune activation in HIV-infected human cells. This issue is important because targeting of cytokine-independent inductive pathways could represent a useful strategy for decreasing HIV loads in coinfected individuals.

Evidence for TLR involvement in HIV induction in experimental models

In addition to in vitro studies using HIV+ human cells, the role of immune activation in regulating viral expression has been examined in a number of experimental animal models involving either SIV infection of rhesus monkeys (44) or transgenic mice incorporating proviral DNA clones or LTR reporters constructs (45–49). Although not involving live virus infection, the transgenic mouse models offer the best opportunity to directly assess the function of TLR signaling in regulating HIV expression in vivo. Moreover, these models allow a direct analysis of the effects of immune activation on the postintegration phase of the viral life cycle. In general, the studies on HIV transgenic mice have confirmed the ability of TLR stimuli such as LPS and mycobacteria to trigger the HIV LTR and, in the case of the two systems involving full-length provirus, the induction of viral proteins and even infectious virions (46, 47, 50).

Our group has directly investigated the role of TLR signaling in HIV induction using a transgenic mouse model (HM 166) that incorporates full-length copies of the NL4-3 HIV clone (51). In vitro studies indicated that TLR2, TLR4, and TLR9 agonists are each potent inducers of HIV p24 expression in transgenic splenocytes, and that, when combined, these microbial stimuli have an additive effect on the viral response (32). To directly confirm the role of TLR signaling in immune activation in vitro and in vivo, we created TLR2-deficient HIV-transgenic mice (Tg/TLR2−/−) by crossing HM 166 Tg mice with TLR2−/− animals (51). Splenocytes from these mice, in addition to failing to produce p24 in response to TLR2 ligands (e.g., Pam3Cys, phosphatidylinositol mannoside (PIM)1,2), displayed severely impaired in vitro p24 production when stimulated with live as well as heat-killed M. tuberculosis or Mycobacterium avium. Interestingly, the same mycobacteria-exposed Tg/TLR2−/− spleen cell cultures showed only a partial reduction in TNF production, and the addition of neutralizing anti-TNF mAb failed to inhibit the bacterial-induced p24 response. The latter observations support the existence of a TLR-independent pathway for immune activation consistent with the findings on HIV-infected human cells discussed above (21, 36, 37).

A major advantage afforded by HIV animal models is the ability to analyze the regulation of HIV expression in vivo. When Tg/TLR2−/− mice were infected with live M. avium, these animals, in contrast to TLR2-sufficient Tg mice, failed to mount a significant HIV p24 response as detected in either plasma or ex vivo spleen cell cultures and showed dramatically inhibited viral replication (Kleeberger, unpublished observations).

Table 1. Major evidence for TLR involvement in immune activation of HIV in microbial systems

<table>
<thead>
<tr>
<th>TLR</th>
<th>Critical Observations</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>TLR2 dependence of HIV-LTR induction by soluble tuberculosis factor from mycobacteria.</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Requirement for TLR2 in induction of HIV by M. avium and M. tuberculosis in a HIV-transgenic mouse model.</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Enhancement of HIV replication in human mast cells by peptidoglycan.</td>
<td>33</td>
</tr>
<tr>
<td>TLR4</td>
<td>Activation of HIV-LTR by LPS in TLR4 transfected cells and murine HIV transgenic cells.</td>
<td>31, 32</td>
</tr>
<tr>
<td></td>
<td>LPS induced HIV replication in virus-infected human mast cells.</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Partial protection against AIDS onset in HIV+ individuals heterozygous or homozygous for a TLR4 allele.</td>
<td>S. Kleeberger, unpublished observations</td>
</tr>
<tr>
<td>TLR9</td>
<td>Activation of HIV by CpG oligonucleotides in human cell lines and infected mast cells as well as murine HIV-transgenic cells.</td>
<td>32, 33</td>
</tr>
<tr>
<td></td>
<td>Increased HIV viral loads in patients given the CpG containing gag antisense GEM91.</td>
<td>57</td>
</tr>
</tbody>
</table>
reduced levels of gag and env transcripts in the same tissue. The above findings point to TLR2 agonists as the major stimulus of HIV gene expression in this animal model of mycobacterial coinfection. Interestingly, TLR2 has been shown to play only a limited role in in vivo control of mycobacterial infection in murine infection models (52–55). If a similar situation occurs in humans, it may be possible to target TLR2-dependent HIV induction without enhancing susceptibility to mycobacterial pathogens.

In vivo evidence for a role of TLR signaling in regulating HIV expression in humans

Although the in vitro and experimental animal models data strongly support a role for TLR in regulating HIV expression, the evidence for a relationship with human HIV infection and disease has so far been indirectly investigated. Indeed, the in vivo situation in humans is complex, because TLR signaling by triggering chemokine production and interfering with reintegration steps in the viral life cycle could actually result in inhibited HIV replication as evidenced in certain studies (27, 28, 56). Nevertheless, findings from a previous clinical trial with an antisense construct complementary to gag (GEM91) are consistent with a positive effect of TLR ligation with enhanced viral load in HIV+ patients (57). In this work, GEM91, which had been shown to potently inhibit HIV replication in vitro studies (58), was found to actually increase viral burdens when administered by continuous i.v. infusion for 8 days in a blinded dose escalation study. On later examination, it was found that GEM91 contains CpG-like motifs and elimination of these in a later construct, GEM92, resulted in a loss of the viral enhancement seen with GEM91 (59). The authors of the study speculated that the increased viral loads stimulated by GEM91 may have been the result of TLR9 stimulation by the CpG-like motifs present in the construct. These observations also suggest that DNA vaccine constructs containing CpG motifs run the possible risk of elevating virus production when administered to HIV+ individuals.

A second approach to assessing the role of TLR signaling in the regulation of HIV expression would be to quantitate viral loads and disease progression in coinflcted individuals with defined TLR polymorphisms. Such studies correlating association of human TLR polymorphisms with disease are in their infancy (60, 61), and we know of no published study examining this issue in HIV infection. Nevertheless, preliminary data have suggested that individuals heterozygous or homozygous for a TLR4 allele are protected against AIDS onset (S. Kleeberger, unpublished data). This interesting study opens up a possible system in which to dissect the function of TLR signaling in immune activation-induced AIDS progression.

TLR signaling as a candidate intervention target in HIV disease

If indeed TLR-dependent immune activation is a contributing factor to AIDS progression, then targeting of this process may be of therapeutic benefit. Antagonists as well as Ab and antisense inhibitors have been developed that suppress signaling by several TLR family members, and such interventions could potentially be used for this purpose (62, 63). An alternative strategy would be to attempt to inhibit the process of TLR signaling in a selected manner. One well-known procedure of this type is TLR reprogramming (64, 65). In this technique based on the phenomenon of LPS tolerance, prior exposure to one TLR stimulus results in down-modulation of responsiveness to secondary challenge with the same or related TLR agonist (65–67). To assess the potential usefulness of this procedure in inhibiting TLR-dependent HIV induction, we stimulated HM 166 Tg macrophages or spleen cells with TLR2, TLR4, or TLR9 ligands, and then assessed HIV p24 production following homologous or heterologous challenge with the same ligands 48 h later (68). Although prior reprogramming resulted in a dramatic decrease in TNF, IL-1, and IL-6 production following secondary challenge, p24 production was found to be unexpectedly increased in the same cell cultures. Because, as predicted from previous studies (65, 66), NF-κB activity was markedly reduced in the reprogrammed cells, these observations support the existence of non-NF-κB-dependent pathways for TLR-dependent immune activation of HIV and suggest that, at least in the case of this experimental model, reprogramming cannot be used to suppress TLR-mediated HIV induction. More recently, experiments using in vitro HIV-infected primary human macrophages indicate that reprogramming may lead to inhibition of HIV replication despite increased and more sustained TNF release (O. Equils, unpublished observations). Although the explanation for the discrepant results of tolerization on HIV expression in the murine and human cell culture experiments is presently unclear, it is of interest that, in each system, reprogramming had opposing effects on HIV vs TNF induction following secondary TLR stimulation.

Other strategies that target TLR signaling may offer alternative therapeutic approaches. For example, TLR-induced HIV p24 production can be abrogated by pharmacological inhibition of p38 or phosphatidylinositol 3 kinases in HM 166 transgenic spleen cells (M. Schito, unpublished results) or transfected human dermal microvessel endothelial cells (31). These observations suggest the existence of defined pathways for TLR-mediated immune activation that could be blocked with specific biochemical inhibitors as described by others in different systems (41–43).

Concluding remarks and questions for future research

It should be clear from this brief review of the literature that much remains to be done both to confirm the role of TLR signaling in AIDS progression as well as to establish this pathway as a realistic intervention target for reducing viral burdens in HIV+ individuals coinfected with other pathogens. First and foremost will be to determine whether HIV expression in humans is indeed subject to TLR regulation. Although already supported by published data using transfected cell lines stimulated with microbial extracts (31, 32), this work needs to be confirmed by means of TLR antagonism or suppression in primary human cells exposed to intact pathogens. More importantly, studies cross-correlating TLR polymorphisms with HIV loads in patients with defined coinfections are needed to establish a more formal in vivo link between these two parameters and for providing an epidemiological setting for clinical trials aimed at intervention based on TLR targeting. On a more basic front, further information is needed concerning the signaling pathways by which TLR stimulation regulates HIV expression. In particular, it will be important to determine whether, as suggested by the animal model studies (51), mechanisms independent of either cytokine induction or NF-κB activation significantly contribute to TLR-dependent triggering of the HIV LTR. If indeed TLR signaling can be established as a potent
driving force for HIV disease progression, these data will, at the very least, add further weight to the argument for aggressive treatment of coinfections in HIV+ persons (8). Whether TLR-directed antagonism will in certain groups of patients provide a superior strategy for treating with immune activation of HIV remains to be determined.

Acknowledgments

We are grateful to Drs. Claire Chougnet, Moshe Arditi, Leonid Margolis, and Johan Van Weyenberg for their helpful comments and suggestions, and we thank Drs. Ozlem Equils and Steven Kleeberger for allowing us to cite their unpublished data.

References


