Activation of Human Immunodeficiency Virus Type 1 in Monocytoid Cells by the Protozoan Parasite Leishmania donovani

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In this study, we demonstrated that the protozoan parasite Leishmania donovani and one of its major surface molecules, the lipophosphoglycan (LPG), can induce human immunodeficiency virus type 1 (HIV-1) expression in U1 and OM-10.1, two cell lines of monocytoid origin latently infected with HIV-1. Treatment of U1 cells with various concentrations of LPG (1, 5, and 10 μM) resulted in a dose-dependent secretion of tumor necrosis factor alpha (TNF-α). Suppression of LPG-induced HIV-1 expression by polyclonal anti-TNF-α antibodies further confirmed the involvement of this cytokine. Results from these studies indicate that the protozoan parasite L. donovani can induce the secretion of TNF-α that will function in an autocrine or paracrine manner to upregulate HIV-1 expression. Our data suggest for the first time that this protozoan parasite can be viewed as a potential cofactor in the pathogenesis of AIDS.

The development of AIDS is the culmination of a progressive infection with human immunodeficiency virus (HIV) whose course and outcome are determined by interactions between viral and host cellular factors; the course and outcome of HIV infection are possibly influenced by additional agents termed cofactors. It has been postulated that cofactors may be important in the progression of the enhancing cell-to-cell transmission or through up regulation of HIV type 1 (HIV-1) expression in infected cells (15). Little is known about the possible role of protozoan parasites in the progression of HIV disease. However, the fact that numerous intracellular protozoan parasites can also infect macrophages, which are the most frequently identified hosts of HIV-1 in tissues of infected individuals (13, 18), suggests that these microorganisms may be considered putative cofactors in the progression of HIV disease. In particular, Leishmania organisms will rapidly become more important as pathogens in HIV-infected individuals, since the presence of Leishmania organisms is overlapping with the presence of HIV in several countries. The fact that Leishmania organisms are opportunistic in immunosuppressed patients, such as those infected with HIV (2, 9), further reinforces this notion.

Parasites of the genus Leishmania are intracellular protozoa that infect macrophages of mammals, including humans. Transmission occurs during the blood meal of a phlebotomine sand fly. At this stage, the pathogen is in a flagellated promastigote form, but once engulfed by the phagocytic cells and surrounded by the phagolysosome, the parasite rapidly differentiates into an amastigote form. These microorganisms have a worldwide distribution and are found in tropical and subtropical areas, including major regions of endemicity in the Mediterranean basin, the Middle East, East Africa, South America, and the Indian subcontinent. More than 15 million people are estimated to be infected, with 500,000 new clinical cases per year (3). Furthermore, it has been estimated that one-third of the world population live in areas of endemicity and are at risk for contracting the infection (19). Recently, Leishmania donovani, which is endemic in some countries of southern Europe (e.g., France, Spain, and Italy), has emerged as an opportunistic pathogen mainly in HIV-infected patients (10, 14). Since both HIV and Leishmania organisms can invade and replicate within macrophages, it is possible that interactions between these pathogens can exacerbate the process of HIV infection.

To address the possibility that Leishmania parasites can positively modulate virus expression, we used the latently HIV-1-infected U1 cell line (12). These cells were first induced to differentiate with phorbol myristate acetate (PMA) since phagocytic activity of U1 cells has been found to be necessary to achieve a productive infection with the protozoan parasite L. donovani (unpublished observations). Differentiated U1 cells were used at 8 days after treatment with PMA, when reverse transcriptase activity had returned to basal levels of HIV-1 production (data not shown). The level of induction of HIV-1 expression was, at early time points, lower in cells treated with L. donovani than in U1 cells treated again with PMA (data not shown). However, at 9 days after incubation with L. donovani, a statistically significant increase in reverse transcriptase activity was seen in cells inoculated with L. donovani at 0:1:1 and 1:1 parasite/cell ratios (Fig. 1A). More importantly, the presence of L. donovani at a 1:1 parasite/cell ratio was shown to be a more potent inducer of HIV-1 activation than the phorbol ester PMA. No such enhancement of virus production was observed in differentiated U1 cells incubated with the highest parasite/cell ratio (10:1). This is most likely associated with the appearance of cell killing mediated by the parasite (data not shown). The promastigotes used for in vitro infection of the differentiated U1 cells were from day-8 cultures (stationary phase) of the L. donovani wild type (strain S2D).
We next investigated whether lipophosphoglycan (LPG), one of the major surface molecules of *Leishmania*, could be responsible for the observed induction of HIV-1 replication in differentiated U1 cells. LPG was chosen because it has been previously demonstrated to generate signal transduction in murine macrophages, leading to a rapid expression of the tumor necrosis factor (TNF) gene (6). This parasite surface molecule is recognized as playing an important role in various aspects of parasite-macrophage interaction, namely, at the level of the attachment and internalization of the promastigote and in the protection against the harsh environment and microbicidal functions of macrophages (26). Data in Fig. 1B indicate that a higher level of induction of HIV-1 replication was detected in differentiated U1 cells in response to the highest concentration of LPG (10 μM) than in PMA-treated cells. Furthermore, dose-dependent up regulation of HIV-1 expression was seen when differentiated U1 cells were treated with increasing concentrations of LPG. Experiments were carried out to evaluate whether different portions of LPG could result in activation of virus production. LPG from *L. donovani* promastigotes, phosphoglycan (PG) (the delipidated form of LPG), and the core phosphatidyl inositol (PI) were prepared as described previously (21, 27). The two distinct LPG fragments, PG and core PI, were able to up regulate virus production from latently HIV-1-infected U1 cells. Indeed, the addition of 1 μM PG or 10 μM core PI to the culture medium gave rise to higher levels of progeny virus than the use of PMA (Fig. 1C and D).

We next assessed whether treatment with LPG could result in a similar induction of HIV-1 expression in another cell line latently infected with HIV-1, namely, OM-10.1 cells (4). Such cells were not incubated with PMA because they rapidly undergo apoptosis following incubation with this phorbol ester (data not shown). Results from these experiments have indicated that, as expected, HIV-1 expression was up regulated in OM-10.1 cells after treatment with TNF alpha (TNF-α). Dose-dependent enhancement of HIV-1 expression was also observed when OM-10.1 cells were incubated with increasing concentrations of LPG (Fig. 2).

Endogenous TNF-α production was next monitored because this cytokine is known to be a very potent inducer of HIV-1 expression. Dose-dependent secretion of TNF-α was detected...
FIG. 2. Induction of HIV-1 expression in OM-10.1 cells by LPG. OM-10.1 cells were incubated with TNF-α (20 U/ml; Genzyme Diagnostics, Inc.) or different concentrations of purified LPG. HIV-1 expression was determined by measuring reverse transcriptase activity at 4 days following initiation of the experiment. Fold enhancement was calculated by dividing the level of reverse transcriptase activity after the addition of TNF-α or LPG by values measured for untreated cells. Results shown are the means (± standard error of the means) of three determinations. Asterisks indicate significant differences from untreated controls at P = 0.001.

TABLE 1. Decrease in the level of LPG-induced HIV-1 expression in differentiated U1 and undifferentiated OM-10.1 cells by anti-TNF-α antibodies

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>Fold enhancement of reverse transcriptase activity</th>
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<tbody>
<tr>
<td>U1 + PMA</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>U1 + LPG</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>U1 + LPG + 0.02 μg of anti-TNF-α per ml</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>U1 + LPG + 0.2 μg of anti-TNF-α per ml</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>U1 + LPG + 2 μg of anti-TNF-α per ml</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>OM-10.1 + TNF-α</td>
<td>1.4 ± 0</td>
</tr>
<tr>
<td>OM-10.1 + LPG</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>OM-10.1 + LPG + 0.02 μg of anti-TNF-α per ml</td>
<td>1.2 ± 0</td>
</tr>
<tr>
<td>OM-10.1 + LPG + 0.2 μg of anti-TNF-α per ml</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>OM-10.1 + LPG + 2 μg of anti-TNF-α per ml</td>
<td>0.1 ± 0</td>
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</table>

*a Differentiated U1 cells were treated with 10 nM PMA, and OM-10.1 cells were treated with TNF-α (20 U/ml) or a combination of LPG (10 μM) and increasing concentrations of goat anti-human TNF-α-neutralizing antibodies (R&D Systems, Minneapolis, Minn). The level of reverse transcriptase activity was determined after 6 days in culture.

*a Fold enhancement was calculated by dividing the level of reverse transcriptase activity in cells treated with TNF-α or the combination of LPG and anti-TNF-α antibodies by values measured for untreated cells. Results shown are the means (± standard error of the means) of three determinations.

when differentiated U1 cells were treated with increasing concentrations of LPG. In this experiment, 5 × 10⁵ cells per ml were plated in media containing 10 nM PMA or 1, 5, or 10 μM LPG. Culture supernatants were harvested after 6 days, when the level of reverse transcriptase activity was highest (data not shown), and TNF-α was quantitated by a commercial enzymatic assay (Genzyme Diagnostics, Inc., Cambridge, Mass.). U1 cells incubated in medium with no additions secreted 110 ± 0 (mean ± standard error of the mean, determined by one-way analysis of variance) pg of TNF-α per ml; U1 cells incubated with PMA secreted 450 ± 10 pg of TNF-α per ml; and U1 cells incubated with 1, 5, and 10 μM LPG secreted 130 ± 10, 160 ± 20, and 205 ± 15 pg of TNF-α per ml, respectively. It is of interest that a baseline level of secreted TNF-α was detected in unstimulated differentiated U1 cells; this level of TNF-α may account for the low level of HIV-1 production in such cells. The direct involvement of TNF-α was investigated by including in our cultures goat anti-human TNF-α-neutralizing antibodies to specifically block TNF-α bioactivity. Results in Table 1 clearly demonstrate that the presence of increasing concentrations of anti-TNF-α antibodies was associated with a dose-dependent reduction of HIV-1 expression in LPG-treated U1 and OM-10.1 cells.

These experiments were designed to determine whether protozoan parasites of the genus Leishmania, which are the causative agents of leishmaniasis, can modulate replication of HIV-1. This study is of great interest considering that more than 200 million people are estimated to be exposed to infection (3). More importantly, recent data demonstrate that 3 to 7% of individuals infected with HIV-1 in southern Europe develop visceral leishmaniasis, a chronic and often fatal disease (1). In this region, more than 50% of cases of visceral leishmaniasis among adults are related to infection with HIV, suggesting that Leishmania-HIV coinfections are frequent in areas where both microorganisms are endemic (5).

Results from our experiments showed that the promastigote form of L. donovani markedly activated virus production in two cell lines of monocytoid origin latently infected with HIV-1. We further determined that virus production was mediated by LPG, one of the major surface components of the protozoan parasite. Our data also suggest that PG and core PI, two distinct fragments of LPG, were both highly potent in inducing HIV-1 production. This observation is of prime importance, since the core PI fragment is present at the surface of the intracellular amastigote form throughout the progression of infection in the host. Thus, it is possible that the parasite in this form can induce virus replication in HIV-1-infected macrophages following infection of the parasite and host cell. The direct dose-dependent increase of secreted TNF-α, following treatment of latently HIV-1-infected cells with LPG, is a strong indication for the mechanism of action of LPG. Indeed, LPG-induced production of secreted TNF-α may function as a stimulator of HIV-1 expression in latently HIV-1-infected cells. Inhibition of LPG-mediated activation of HIV-1 production in cells incubated with increasing concentrations of anti-human TNF-α-neutralizing antibodies further confirmed the direct involvement of this cytokine in the observed phenomenon.

Over the past few years, several in vitro studies have convincingly demonstrated that TNF-α, a 17-kDa cytokine produced mainly by activated monocytes and macrophages, plays a pivotal role in the pathogenesis of HIV-1 (8). TNF-α has previously been reported to induce HIV-1 expression in cells of both T and monocytoid origins (11, 16, 17, 24, 28). The mechanism of activation of TNF-α is mediated via uncoupling of the cellular transcription factor NF-κB from its cytoplasmic inhibitor IκB. NF-κB can then translocate into the nucleus and bind to the two NF-κB-binding sequences located into the regulatory elements of HIV (7, 20, 22). Such an induction of HIV-1 expression by TNF-α has been demonstrated to function in a autocrine or paracrine manner in vitro both in cell lines and in primary cells (25, 28). The finding that L. donovani and LPG, one of its major surface molecules, can lead to TNF-α-mediated induction of HIV-1 expression supports the concept that this protozoan parasite can be envisaged as a cofactor in the pathogenesis of HIV-1 infection. The importance of the demonstration that L. donovani and LPG can lead to secretion of TNF-α is emphasized because of its clinical relevance for HIV-
infected patients. The fact that sera from 89% of patients with visceral leishmaniasis showed elevated levels of circulating TNF-α (23) is in agreement with our results. This paper presents the first demonstration that L. donovani can trigger TNF-α release from monocytoid cells latently infected with HIV-1. Our results confirm and extend the results of a previously reported study by Descoteaux et al. (6), who demonstrated that within 30 min following initial contact between LPG and murine macrophages, TNF mRNA was induced. The fact that purified LPG is as capable as LPG and murine macrophages, TNF mRNA was induced. The present study suggests that the protozoan parasite L. donovani is capable of inducing virus activation in monocytoid cells latently infected with HIV-1 and that the parasite may be seen as a cofactor for predisposition to progression of asymptomatic HIV-1 infection to symptomatic HIV-1 infection. Hence, in areas where both microorganisms are endemic, the medical community should be alerted to the predictable increase in the number of dual infections, which may result in exacerbation of HIV-related symptoms.

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