Inhibition of HIV-1 Replication in Monocyte-Derived Macrophages by *Mycobacterium tuberculosis*

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Controversial results have been obtained in studies of the effect of *Mycobacterium tuberculosis* on human immunodeficiency virus type 1 (HIV-1) replication in cells of the macrophage lineage. In the present study, monocyte-derived macrophages (MDMs), previously incubated for 2 days with heat-inactivated *M. tuberculosis*, were infected with HIV-1. *M. tuberculosis* consistently inhibited viral replication, and a similar result also was observed in the presence of supernatants from *M. tuberculosis*-stimulated MDMs, which indicates that this effect was mediated by soluble factors. Although CCR5-binding chemokines were induced by *M. tuberculosis* stimulation, the results of neutralization experiments indicated that it is unlikely that they were responsible for viral suppression. Inhibition occurred mainly after viral entry (demonstrated by use of a vesicular stomatitis virus G–pseudotyped HIV-1 and by analysis of HIV-1 early and late reverse-transcription products). Therefore, *M. tuberculosis*-induced factors may inhibit in vitro HIV-1 replication in macrophages by affecting an early postentry step in the HIV-1 cycle.

Worldwide, 1.86 billion persons are infected with *Mycobacterium tuberculosis*, and, notably, 8% of all tuberculosis infections occur in individuals who are coinfected with HIV-1. HIV-1 can productively or latently infect mononuclear phagocytes, mostly tissue macrophages [1], which serve as a major viral reservoir in nonlymphoid districts [2, 3]. Human macrophages also represent the first line of defense for the containment of *M. tuberculosis* infection.

Different effects of *M. tuberculosis* on HIV-1 replication have been described, both in vivo and in vitro. In HIV-1–infected individuals, *M. tuberculosis* enhances both systemic [4] and local (in infected lungs) [5] viral replication. In vitro, discordant effects of *M. tuberculosis* on HIV-1 replication have been reported elsewhere [6–13]. *M. tuberculosis* has been shown to induce viral transcription and production in cells of the monocytic lineage and in chronically infected monocytic cell lines [6–8], as well as to induce viral replication in acutely infected primary monocyte-derived macrophages (MDMs) [9]. Similar enhancing effects by *M. tuberculosis* were observed in vitro in primary peripheral blood mononuclear cells (PBMCs) that were acutely infected with HIV-1 [4] and in CD8+ T cell–depleted PBMCs isolated from HIV-1–infected individuals and cultivated ex vivo [4, 10]. In contrast, *M. tuberculosis* has been reported to exert antiviral effects via modulation of type I interferon (IFN) response and via stimulation of CCAAT/enhancer bind-
ing protein β expression [11, 12] in MDMs acutely infected in vitro with HIV-1. Others have reported lack of cross-regulatory effects by concurrent HIV-1 and M. tuberculosis infection of MDMs [13].

M. tuberculosis induces synthesis and secretion of numerous cytokines and chemokines [14–16] that are potentially capable of modulating HIV-1 infection and/or replication. In particular, proinflammatory cytokines, such as tumor necrosis factor (TNF–α) and interleukin (IL)–1β, increase HIV-1 replication through transcriptional activators via binding of NF-κB to a consensus sequence present in the 5′ long terminal repeat (LTR) of HIV-1 [17], as well as via NF-κB-independent mechanisms [18]. In addition, other cytokines, including IL-6 and IFN-γ, have been shown to modulate HIV-1 expression, mostly by acting at a posttranscriptional level [19]. On the other hand, chemokines CCL3, CCL4, and CCL5 inhibit viral replication [20] by the engagement of CCR5, the main HIV-1 coreceptor expressed on MDMs [21]. More recently, α-defensins released by activated CD8+ T cells have been indicated as potential anti-HIV determinants [22].

The epidemiological evidence with regard to the effect of tuberculosis on the natural history of HIV infection is controversial [23]. Tuberculosis has been found to be associated with adverse outcomes, including an increased risk of death and shorter median survival, in HIV-coinfected patients, compared with HIV-infected individuals who did not develop tuberculosis [24, 25]. However, the difference in outcomes was not statistically significant [24, 25]. Others have reported that tuberculosis is associated with a more favorable prognosis, including a statistically significant increase in the median survival time, compared with other HIV-associated opportunistic infections [26–29]. Therefore, although certain studies have shown that HIV-infected patients with active tuberculosis have a high mortality rate, it is unclear whether this is the result of tuberculosis itself or rather is the consequence of the HIV-related immunosuppression. Thus, the controversial results obtained in vitro with regard to the effect of M. tuberculosis on HIV-1 replication also are present in epidemiological studies of the effect of active tuberculosis on HIV-1 disease progression.

The aim of the present study is to evaluate M. tuberculosis–mediated effects on viral replication in acutely HIV-1–infected MDMs. Our findings support the concept that unknown soluble factors regulate HIV-1 replication in macrophages that are either stimulated by or infected with M. tuberculosis, mainly at an early postentry step.

MATERIALS AND METHODS

**Antibodies and MDMs**  Monoclonal antibodies (MAbs) specific for CD14, CD3, and IgG2a subclasses (Pharmingen) were used, either unconjugated or conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Peripheral venous blood samples (Blood Bank of University “La Sapienza,” Rome) were obtained from healthy donors who had provided written informed consent. PBMCs from freshly collected buffy coats were isolated by density gradient centrifugation, using Lympholyte-H (Cederlane). Monocytes were purified by positive sorting, using anti-CD14–conjugated magnetic microbeads (Miltenyi); purification was followed by T cell depletion with anti-CD3 beads (Dynal). More than 99% of selected cells were CD14+, and <1% were CD3+, as determined by flow cytometry. MDMs were obtained by cultivation of adherent monocytes for 5 days in complete medium consisting of RPMI 1640 medium (BioWhittaker Europe), 2 mmol of l-glutamine, 15% lipopolysaccharide (LPS)–free fetal calf serum (FCS; BioWhittaker Europe), 100 IU/mL penicillin, and 100 μg/mL streptomycin (BioWhittaker Europe), with the addition of 0.1 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF; Schering-Plough). Cells were seeded either in flat-bottom 6-well tissue culture plates (2 × 106 cells in 2 mL of complete medium; Costar) to harvest supernatants or in 96-well plates (0.2 × 106 cells in 200 μL of complete medium) for in vitro HIV-1 infection experiments (see below). The MDM cultures were maintained at 37°C in 5% CO2. Antibiotics were never added to the medium in cultures of MDMs infected with live M. tuberculosis.

**M. tuberculosis and HIV-1 infection of MDMs.** M. tuberculosis H37Rv (ATCC 27294) was cultivated as described elsewhere [18]. All mycobacteria preparations were analyzed for LPS contamination by the Limulus amoebocyte lysate assay (BioWhittaker Europe) and contained <10 pg/mL of LPS. M. tuberculosis was used either live or after heat inactivation (HI) at 100°C for 1 h. Addition of HI M. tuberculosis was described as “stimulation with M. tuberculosis,” whereas addition of live M. tuberculosis was called “infection.” HI M. tuberculosis or live M. tuberculosis were added to MDMs at an MOI of 10 bacteria/cell. After 48 h of incubation, the cells were infected with a CCR5-dependent (R5) laboratory-adapted HIV-1 strain (ADA) and were pretreated for 30 min with DNase (220 U/μg of virus; Boehringer Mannheim) at an MOI of 0.05 for 1 h. Fifty percent of the culture supernatant was removed, and the cells were washed 3 times with complete medium maintained at room temperature.

To determine whether the observed effect was caused by the action of soluble factors, rather than the presence of whole bacteria, 0.22 μm–filtered culture supernatants (Millipore) were used in parallel experiments. In particular, supernatants were harvested from unstimulated (control), M. tuberculosis–stimulated, or M. tuberculosis–infected MDMs after 2 days of culture. The supernatants derived from unstimulated MDMs are referred to as “control supernatants,” whereas those derived from MDM cultures treated with HI M. tuberculosis or infected with live M. tuberculosis are called “HI M. tuberculosis supernatants”
or “live M. tuberculosis supernatants,” respectively. The following is the protocol used for most experiments, unless otherwise specified: 110 μL of these supernatants was added to MDM cultures, in a total volume of 200 μL/well in 96-well culture plates 2 h before HIV-1 infection. Supernatants were added to autologous (supernatants and MDMs from the same donor) or heterologous (supernatants and MDMs from different donors) MDM cultures. In vitro HIV-1 infection and washes were performed as described above. After the last wash, 100 μL of control supernatants and M. tuberculosis supernatants was added again to the HIV-infected MDM cultures. Supernatants from HIV-1–infected cultures were harvested every 2–3 days and were replaced with fresh complete medium. Supernatants were stored at −80°C until testing for HIV-1 p24 Gag antigen (Ag) by ELISA (Vironostika HIV-1 Antigen MicroELISA System; Organon Teknika). In selected experiments, in vitro HIV-1 infection was performed in the presence of a pool of recombinant human (rh) CCR5–binding chemokines (CCL3, CCL4, and CCL5; R&D Systems) added at 100 ng/mL each or in the presence of rhIL-10 (R&D Systems) at 10 ng/mL.

Cytokine and chemokine determination. Control supernatants and M. tuberculosis supernatants from MDM cultures that had been established from samples from independent donors were harvested at different times after M. tuberculosis infection and were stored at −80°C. ELISA kits for IL-1β, IL-6, TNF-α, IL-10, IL-12 p70, IL-16, leukemia inhibitory factor (LIF), α-defensin, transforming growth factor (TGF)–β, macrophage-defined chemokine (MDC), and the CC chemokines CCL2, CCL3, CCL4, CCL5, and CCL22 were purchased from R&D Systems. The ELISA kit for IFN-α was obtained from PBL Biomedical Laboratories. All ELISAs were carried out according to the manufacturers’ instructions.

Vesicular stomatitis virus G (VSV-G)–pseudotyped HIV-1 preparation, MDM infection, and detection. VSV-G–pseudotyped HIV-1 preparations were obtained as supernatants of the 293T cell line 2 days after cotransfection with the HIV-1 pNL4-3 molecular clone and as VSV-G–expressing plasmids (molar ratio, 5:1) performed by the calcium phosphate method [30]. The supernatants were clarified and concentrated by ultracentrifugation, as described elsewhere [31]. Virus preparations were titrated by measuring HIV-1 p24 Gag Ag contents by ELISA. Pseudotyped HIV-1 (10 ng/10⁶ cells) was used to infect 7-day-old MDMs in the presence or absence of control supernatants or HI M. tuberculosis supernatants. Cells expressing intracytoplasmatic HIV-1 Gag Ag were evaluated by fluorescence-activated cell sorter analysis. In brief, MDMs were incubated with FITC-activated anti-CD14 MAb for 1 h at 4°C. Cells were washed and treated with Permeafix (Ortho Diag-

![Figure 1](image-url)  
**Figure 1.** *Mycobacterium tuberculosis* inhibits viral replication in acutely HIV-1–infected monocyte-derived macrophages (MDMs), an effect that is mediated by soluble factors. In vitro HIV-1 infection was performed in MDMs treated with heat-inactivated *M. tuberculosis* (HI Mtb), in untreated MDMs (control [CTR]), and in MDMs treated with 2-day-old supernatants (SNs) (HI Mtb SNs, CTR SNs, and live *M. tuberculosis* SNs [live Mtb SNs]). *M. tuberculosis* significantly inhibits HIV-1 replication in acutely HIV-1–infected MDMs tested by measurement of HIV-1 p24 Gag Ag contents by ELISA. *P* values were calculated for untreated HIV-1–infected MDMs and HI *M. tuberculosis*–treated cultures. Data are the mean of 8 independent experiments for the data reported at day 12 after infection and of 6 independent experiments for the data reported at days 16 and 20 after infection.
nostic) for 30 min at room temperature and were labeled, after 2 additional washes, with KC57-RD1 PE-conjugated anti–HIV-1 Gag MAb (Coulter) for 1 h at room temperature.

**Immunodepletion.** Supernatants were incubated overnight at 4°C with a mixture containing goat neutralizing anti–CCL3, -CCL4, and -CCL5 MAbs (10 μg/mL each) and anti–IL-10 (1 μg/mL; R&D Systems) linked to protein A–G agarose beads (Pierce). This procedure was performed to deplete endogenous CCR5-binding chemokines present in control supernatants and *M. tuberculosis* supernatants. As a control, supernatants also were incubated with equal concentrations of goat IgG isotype MAbs. Afterward, immunocomplexes bound to protein A-G agarose were discarded by centrifugation; supernatants were passed through 0.22-μm filters (Millipore) and added to MDM cultures.

**DNA purification from HIV-1–infected MDMs.** HIV-1–infected and HIV-1–uninfected MDMs were collected by washing with FCS-free warm medium, centrifuged at 14,000 g for 15 s, and frozen at −80°C. Commercial kits for DNA purification were used according to the manufacturer’s instructions (QIAamp DNA Mini Kit; Qiagen).

**Polymerase chain reaction (PCR) amplification of HIV-1.** PCR amplification was performed as follows: each reaction contained (0.25 mmol each of 4 dNTPs, 50 mmol of NaCl, 25 mmol of Tris-HCl (pH 8.0), 2 mmol of MgCl2, and 2.5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer). The reaction program was 94°C for 15 min, 60°C for 2 min, and 72°C for 2 min for 1 cycle and then 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min for the following 49 cycles. The amplified products resulting from the PCR were analyzed by use of electrophoresis on 1.8% agarose. The sequences of the oligonucleotide primers used for HIV-1 DNA detection were the following: for M667, sense, 5′-CTG TGG ACA TCA GAA CCA CTG-3′; and for M661, antisense, 5′-CCT GCG TCG AGA GAG CTC CTC TGG-3′; the amplified product resulting from the PCR was cloned in a 200-bp fragment. An oligonucleotide primer pair specific for the U3 region of the HIV-1 LTR consisted of M665 (sense, 5′-GCT AGG ATG CTC CAT GGG ATG GAG GAC GCG GAG-3′) and M666 (antisense, 5′-GAG GGA CGC ACC CCA GTC-3′); this pair formed an amplified product of 207 bp. The primer AA55 was designed as an antisense primer (5′-CTG CTA GAG ATT TTC CAC ACT GAC-3′), and a 140-bp fragment was amplified when this primer was used in conjunction with M667 [32]. As an internal control, the region of the β-actin gene was amplified in a separate PCR by use of the following primers: 5′-GGC GGC ACC ACC ATG TAC CTT-3′ and 5′-AGG GCC CGG ACT GTG CAT ACT-3′ [33]. Serial dilutions of MDMs were performed for the quantification of HIV-1 DNA, with cell numbers ranging from 2 × 10^4 to 2 × 10^5 cells.

**Chronically HIV-1–infected U1 cells.** The U1 cell line is a promonocytic cell line chronically infected with HIV-1 LAI/IIIB that contains 2 integrated proviruses/cell [34, 35]. U1 cells were seeded at 10^5 cells/well in 96-well flat-bottom plates in the presence of control supernatants and HI-M. tuberculosis MAbs. Afterward, supernatants were passed through 0.22-μm filters (Millipore) and added to MDM cultures. 32

**Results**

**Table 1.** Chemokine and cytokine production after 2 days of culture in *Mycobacterium tuberculosis*–stimulated (heat-inactivated [HI]) *M. tuberculosis* or *M. tuberculosis*–infected (live *M. tuberculosis*) monocyte-derived macrophages (MDMs).

<table>
<thead>
<tr>
<th>MDM culture</th>
<th>CCL2</th>
<th>CCL3</th>
<th>CCL4</th>
<th>CCL5</th>
<th>CCL22</th>
<th>TNF-α</th>
<th>IL-6</th>
<th>IL-1β</th>
<th>IL-10</th>
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<tbody>
<tr>
<td>Control</td>
<td>336 ± 76</td>
<td>47 ± 13</td>
<td>36 ± 13</td>
<td>50 ± 10</td>
<td>17,600 ± 5100</td>
<td>44 ± 30</td>
<td>294 ± 76</td>
<td>28 ± 20</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>HI <em>M. tuberculosis</em></td>
<td>21,700 ± 2600</td>
<td>8200 ± 2900</td>
<td>5046 ± 1200</td>
<td>158 ± 56</td>
<td>15,328 ± 4900</td>
<td>670 ± 268</td>
<td>1961 ± 650</td>
<td>60 ± 20</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>Live <em>M. tuberculosis</em></td>
<td>23,960 ± 1400</td>
<td>12,000 ± 1400</td>
<td>9332 ± 1500</td>
<td>1622 ± 406</td>
<td>10,490 ± 4100</td>
<td>3972 ± 487</td>
<td>4750 ± 291</td>
<td>1739 ± 330</td>
<td>1166 ± 14</td>
</tr>
</tbody>
</table>

**Note.** IFN, interferon; IL, interleukin; LIF, leukemia inhibitory factor; TGF, transforming growth factor; TNF, tumor necrosis factor.

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Mycobacterium tuberculosis–mediated inhibition of HIV-1 replication is independent of the release of CCR5-binding chemokines (CCs) and only partially dependent on the endogenous production of interleukin (IL)–10.

A, Immunodepletion of the endogenous CCs was performed in supernatants (SNs) that were subsequently added to monocyte-derived macrophages (MDMs) before HIV-1 infection, as described in Materials and Methods. In parallel cell cultures, a pool of recombinant human (rh) CCs (100 ng/mL each) was exogenously added to MDMs. HIV-1 replication was tested by measuring HIV-1 p24 Gag antigen contents by ELISA.

B, Immunodepletion of endogenous IL-10 was performed in SNs that were subsequently added to MDMs before HIV-1 infection, as described in Materials and Methods. In parallel cell cultures, exogenous rhIL-10 (10 ng/mL) was added. Data are representative of 3 independent experiments. Error bars indicate the mean ± SE of triplicate experiments (bars do not appear when smaller than symbols). CTR, control; HI Mtb, heat-inactivated M. tuberculosis.

Effect was completely reproduced, and the difference was still statistically significant (P < .01 to P < .0005, depending on the day analyzed). The antiviral effect was concentration dependent and was obtained with supernatants from autologous (MDMs and supernatants obtained from the same donor) or heterologous (MDMs and supernatants obtained from different donors) MDM cultures (data not shown). Therefore, for practical reasons, the following experiments were performed in a heterologous cell system. In addition, after we observed a substantial homology between the effects induced by live M. tuberculosis and HI M. tuberculosis, the use of HI M. tuberculosis was preferred for biological safety reasons also. Thus, all results were generated using supernatants derived from MDMs stimulated for 48 h with HI M. tuberculosis.

A 2-h period of pretreatment with supernatants before HIV-1 infection was adopted, instead of a cotreatment (simultaneous addition of supernatants and virus) or a posttreatment (addition of supernatants 2 h after virus infection) approach, although no significant difference was observed among the 3 different protocols (data not shown) in terms of antiviral effect. The M. tuberculosis–mediated decrease of viral replication was associated with a reduction of the cytopathic effects typically induced by HIV-1 infection, with a 40%–70% decrease in cell mortality, coupled with prolonged cell survival (data not shown). Thus, M. tuberculosis inhibited HIV-1 replication and cytopathicity in MDMs acutely infected in vitro, and this effect was mediated by soluble factors.

**Effects of M. tuberculosis on secretion of chemokines, cytokines, and α-defensins by MDMs.** We expanded the assessment of cytokine induction by live M. tuberculosis described elsewhere [15] by also evaluating the presence of a broad range of HIV-1–inhibiting or –enhancing soluble factors [19, 36] in MDMs either infected with live M. tuberculosis or stimulated with HI M. tuberculosis. Peak production levels were usually observed at 2 days after treatment with M. tuberculosis; thus, we report data obtained at this time point. Secretion of the chemokines CCL2, CCL3, CCL4, and CCL5 (with the exception of CCL22, which was detectable in unstimulated conditions, as described elsewhere [38]) and of the cytokines TNF-α, IL-6, IL-1β, and IL-10 was significantly up-regulated in M. tuberculosis–stimulated and M. tuberculosis–infected MDM cultures. A much stronger effect was exerted by live M. tuberculosis (table 1). In contrast, no evidence of TGF-β, IL-12 p70, LIF, or IFN-α secretion was observed, whereas the low levels of IL-16, MDC, and α-defensin production were not significantly affected by M. tuberculosis stimulation or infection (data not shown). In conclusion, a significant number of HIV-1–enhancing (CCL2, TNF-α, IL-1β, and IL-6) and HIV-1–inhibiting (CCL3, CCL4, CCL5, and IL-10) [19, 36] cytokines and chemokines were up-regulated by incubation of MDMs with live M. tuberculosis or HI M. tuberculosis.

**Connection between M. tuberculosis–mediated inhibition of HIV-1 replication, the release of CCR5-binding chemokines, and the endogenous production of IL-10.** Among the soluble factors released by M. tuberculosis–stimulated MDMs (table 1), CCR5 chemokines [20] were considered to be candidate mediators of the inhibitory effect on HIV-1 replication. Thus,
Figure 3. *Mycobacterium tuberculosis*–mediated inhibition of HIV-1 occurs at a postentry level. Expression of HIV-1 p24 Gag–related products was evaluated in monocyte-derived macrophages after infection with vesicular stomatitis virus G–HIV-1 in the presence of supernatants (SNs) from either control cells (CTR SNs) or heat-inactivated *M. tuberculosis*–treated cultures (HI Mtb SNs). Forward scatter (FSC) and side scatter (SSC) dot plots are shown in the top 2 panels. Analyses from double stainings for the expression of CD14 and HIV-1 Gag–related products are shown in the bottom 2 panels. Percentages of HIV-1–expressing cells also are indicated. Data are representative of 3 independent experiments. FITC, fluorescein isothiocyanate; PE, phycoerythrin.

we neutralized the endogenous CCL3, CCL4, and CCL5 in supernatants from unstimulated and *M. tuberculosis*–stimulated MDMs. We then performed in vitro HIV-1 infection of MDMs, as described above, using these immunodepleted supernatants. Neutralization of the endogenously secreted CCR5-binding chemokines did not significantly modify *M. tuberculosis* inhibition of HIV-1 replication, despite the fact that no detectable levels of these chemokines were demonstrated in the supernatants by ELISA (data not shown). Addition of a pool of rhCCR5–binding chemokines inhibited HIV-1 replication (figure 2A). In contrast, neutralization of endogenous IL-10 reduced *M. tuberculosis*–mediated viral inhibition by ∼30%, but this was not statistically significant (*P > .05*), and the effect emerged after 10 days of viral infection. Exogenous addition of rhIL-10 led to a mild decrease in HIV-1 replication (figure 2B). These results indicate that *M. tuberculosis*–mediated inhibition of HIV-1 replication in MDMs is not caused by the endogenous production of CCR5-binding chemokines and is only partially mediated by endogenous IL-10.

*M. tuberculosis*–mediated inhibition of HIV-1 at a postentry level. To better characterize the interaction between HIV-1 and *M. tuberculosis*, we infected MDMs with HIV-1 pseudotyped with the membrane glycoprotein from VSV, a virus that infects cells by membrane fusion, bypassing the requirement for CD4 receptor and CCR5 coreceptor interaction with gp120 Env. Using intracellular flow cytometric analysis for the
expression of Gag-related products, we observed a significant reduction of the number of HIV-1–expressing cells (91% vs. 65%) as a consequence of treatment with supernatants from M. tuberculosis–stimulated MDMs (figure 3). These data indicate that it is unlikely that M. tuberculosis–mediated inhibition of HIV-1 expression occurs at the level of viral entry. We next evaluated whether the inhibition occurred at the level of reverse transcription. HIV-1 DNA accumulation was determined by semiquantitative PCR performed by use of different pairs of primers detecting early (R/U5 [M667/AA55] and U3 [M665/M666]) and full-length (long terminal region/gag [M667/M661]) reverse transcripts. β-actin polymerase chain reaction product served as an internal control for the amount of DNA. Data are representative of 3 independent experiments.

Lack of inhibition of HIV-1 expression in U1 cells, a chronically infected promonocytic cell line, by M. tuberculosis. To further characterize M. tuberculosis–mediated viral inhibition, we investigated its potential effect on U1 cells, a pro-monocytic cell line chronically infected with HIV-1. These cells are characterized by a state of relative viral latency, in which HIV-1 expression is inducible after activation by PMA and numerous cytokines [34, 35]. In U1 cells, neither reverse-transcription inhibitors [39] nor agents capable of interfering with virion attachment and entry [40] modulate HIV-1 expression. The U1 cells were stimulated with HI M. tuberculosis supernatants and control supernatants in the presence or absence of PMA for 3 days. As shown in figure 5, HI M. tuberculosis supernatants significantly induced HIV-1 expression (P < .01), compared with control supernatants, as described elsewhere [8], with the direct addition of M. tuberculosis. In contrast, HI M. tuberculosis supernatants caused only a mild up-regulation of viral production in U1 cells stimulated with PMA, compared with control supernatants. Taken together, these findings are consistent with the up-regulation of several proinflammatory cytokines that occurs after M. tuberculosis stimulation (table 1) and further supports the hypothesis that M. tuberculosis–mediated viral inhibition occurs at early stages of the HIV-1 cycle, before reverse transcription and proviral DNA integration.

DISCUSSION

In the present study, we demonstrated that M. tuberculosis inhibits HIV-1 replication in MDMs through the release of soluble factors. Although they are strongly up-regulated by M. tuberculosis, endogenous CCR5-binding chemokines were not involved in M. tuberculosis–mediated viral inhibition. It is probable that no other soluble factors clearly linked to inhibition of HIV-1 replication, such as IL-16, LIF, MDC, TGF-β, α-defensins, and IFN-α, were involved, because these were not induced or modulated by M. tuberculosis stimulation or infection. All the data generated led to the conclusion that HIV-1 inhibition occurred after viral entry but before or during early reverse transcription.

Multifactorial effects of M. tuberculosis on HIV-1 replication have been described elsewhere. In particular, it has been shown that M. tuberculosis enhances in vitro HIV-1 replication in cells of the monocytic lineage [6–9, 41] and in acutely infected primary macrophages [6, 41]. However, there are several differences between those previous studies and the present study. First, the procedure of monocyte isolation, obtained by elutriation [9], cell adhesion [9, 11, 13, 41], or, in the present study, positive selection, suggests that the cells have a different status at the time of mycobacterial and viral infection. Second, protocols of cell differentiation after isolation of monocytes differ: we used low concentration of GM-CSF, whereas others used macrophage CSF [11], or others did not add any differentiation-inducing factor at all [9, 13, 41]. Third, the time interval between M. tuberculosis infection and HIV-1 infection...
Figure 5. Mycobacterium tuberculosis supernatants (SNs) induce HIV-1 expression in U1 cells. U1 cells were either unstimulated or stimulated with phorbol 12-myristate 13-acetate (PMA) in the presence or absence of control (CTR) SNs or heat-inactivated M. tuberculosis SNs (HI Mtb SNs) for 3 days. M. tuberculosis significantly increased HIV-1 expression in U1 cells tested by measurement of HIV-1 p24 Gag antigen contents by ELISA. *P* values were calculated for the CTR SN–treated vs. HI Mtb SN– or live M. tuberculosis SN (live Mtb SN)–treated cultures. Error bars indicate the mean ± SE of triplicate experiments (bars do not appear when smaller than symbols). Data are representative of 4 independent experiments.

has differed, from 4 or 6 days between preinfection with M. tuberculosis and HIV-1 infection [9, 41] or to 1 day of preinfection with HIV-1 before M. tuberculosis coinfection [13] to 2 days between preinfection with M. tuberculosis and infection with HIV-1 in the present study. Finally, previous studies used different virus strains, such as HIV-1 LAI or HIV-1 BaL [9, 13, 41], whereas HIV-1 ADA was used in the present study. All of these differences may have influenced the outcome of the experiments. Moreover, we observed an M. tuberculosis–mediated antiviral effect with both live M. tuberculosis and HI M. tuberculosis, whereas a modulation of HIV-1 replication has been reported only with live M. tuberculosis elsewhere [9].

The antiviral effect of M. tuberculosis supernatants was observed using different pretreatment, cotreatment, or posttreatment protocols with respect to in vitro HIV-1 infection, which provides a first line of evidence that M. tuberculosis–mediated viral inhibition occurred after viral entry. This hypothesis was supported by the results obtained with HIV-1 pseudotyped with the membrane glycoprotein from VSV. Because pseudotyped viruses infect cells, thereby bypassing the requirement for the CD4 receptor and CCR5 coreceptor, the observation of significantly reduced levels of viral replication in MDMs stimulated with M. tuberculosis indicated the existence of a postentry level of inhibition. In addition, neutralization of CCR5-binding chemokines did not affect M. tuberculosis–mediated viral inhibition, which further supports the notion that inhibition occurred after HIV-1 entry. Molecular analysis of the HIV-1 life cycle suggested that M. tuberculosis–mediated interference occurred early after entry and likely before reverse transcription, because HIV-1 DNA accumulation decreased early after infection. In addition, in chronically infected U1 cells, M. tuberculosis supernatants did not inhibit HIV-1 expression that was, in fact, significantly up-regulated, which is in agreement with the result of an independent study [8] and consistent with M. tuberculosis–mediated up-regulation of proinflammatory HIV-inductive cytokines, such as TNF-α, IL-1β, and IL-6 [19, 36, 42].
Furthermore, no evidence of suppressor activity was obtained when *M. tuberculosis* supernatants were added to PMA-stimulated U1 cells, which supports the hypothesis that the inhibiting factor(s) were active in acutely HIV-1–infected MDMs at an early, preintegration step of the viral life cycle.

Natural HIV-1 antagonists have been under intense investigation [19, 37, 42], among them, IL-10; one study reported the occurrence of viral inhibition in primary monocytes/macrophages and promonocytic cell lines through a decrease of endogenous TNF-α and IL-6 [43]. In our system, IL-10 played only a marginal role in viral suppression. We do not know the exact mechanisms involved in IL-10–mediated decrease of viral replication, because high levels of endogenous TNF-α and IL-6 were present in *M. tuberculosis* supernatants, as shown in table 1.

Several coinfections have been described to have the potential to inhibit HIV-1 replication, both in vitro (human herpesvirus 6 and influenza virus [44–47]) and in vivo (Orientia tsutsugamushi) and acute measles, in HIV-infected humans, and *Mycobacterium bovis*, in simian immunodeficiency virus–infected macaques [48–50]). In the present study, we demonstrate that *M. tuberculosis* inhibits HIV-1 replication in MDMs at a post-entry level. We do not know the clinical relevance of this in vitro phenomenon for the natural history of *M. tuberculosis* and HIV-1 coinfection. In vivo multifactorial effects may occur simultaneously, such as the interaction of macrophages with different cell types and secretion of soluble factors known to modulate HIV-1 replication. All these events may profoundly alter the effects observed in vitro with a single cell population, as described elsewhere [12].

In conclusion, our in vitro study does not necessarily imply that infection with *M. tuberculosis* should be used as an immune-therapeutic approach. However, the identification of the mechanisms involved in the elicitation of *M. tuberculosis*–induced antiviral immunity may contribute to the design of novel, safe, complementary anti–HIV-1 therapeutic strategies.

**Acknowledgments**

We are grateful to the blood donors; to Schering-Plough, for providing granulocyte-macrophage colony-stimulating factor; to Eugenio Morassi, for graphical assistance; to Enrico Girardi, for statistical assistance; and to Carla Nisii and Francesca Mattioli, for editing the manuscript.

**References**


