Coinfection with *Mycobacterium tuberculosis* and human immunodeficiency virus (HIV) is a serious problem, particularly in developing countries. Recently, *M. tuberculosis* and purified protein derivative (PPD) were demonstrated to induce HIV replication in CD8 T cell–depleted peripheral blood mononuclear cells from HIV-positive, PPD-positive persons but not in cells from PPD-negative persons. The role of endogenous and exogenous cytokines in modulating *M. tuberculosis*–induced HIV replication was evaluated. *M. tuberculosis*–induced HIV replication decreased following simultaneous inhibition of endogenous interleukin (IL)-2, IL-1β, and tumor necrosis factor-α by the addition of soluble receptors and receptor antagonists or following exogenous IL-10 and transforming growth factor (TGF)-β. In contrast, neutralization of endogenous IL-10 and TGF-β augmented *M. tuberculosis*–induced HIV replication by increasing cellular activation. Thus, the balance between IL-2 and proinflammatory and antiinflammatory cytokines plays a major role in *M. tuberculosis*–induced replication of HIV.

At least 8 million new tuberculosis cases and 3 million tuberculosis-related deaths occur each year, predominantly in resource-poor areas [1]. About 9% of global tuberculosis cases in 1995 were among human immunodeficiency virus (HIV)–infected persons, a number projected to increase to 14% by the year 2000 [1–3]. Clinical and epidemiologic observations indicate that HIV-infected persons have an increased likelihood of developing active tuberculosis following infection with *Mycobacterium tuberculosis* [4]. In addition, it has been shown that *M. tuberculosis* increases HIV replication both at local tissue sites [5] and at the systemic level [6] and accelerates the rate of progression of HIV disease [7–9].

A complex network of proinflammatory cytokines is involved in the pathogenesis of *M. tuberculosis* infection [10–12]. Tumor necrosis factor (TNF)-α is critical in granuloma formation in animal models of mycobacterial infection and is essential for immune control of tuberculosis [13]; however, TNF-α has also been demonstrated to promote HIV expression and replication in lymphocytes and macrophages in vitro [14, 15]. In particular, *M. tuberculosis* increases HIV long terminal repeat (LTR)–driven transcription in monocytic cell lines [5, 6] through the release of endogenous proinflammatory cytokines [5]. Additionally, *M. tuberculosis* increases the secretion of antiinflammatory cytokines, such as interleukin (IL)-10 [10, 11, 17] and transforming growth factor (TGF)-β [18, 19], which may contribute to the immune suppression often observed during tuberculosis [20, 21] and to the replication of *M. tuberculosis* in vitro [22]. These antiinflammatory cytokines have been reported to both down-regulate and up-regulate HIV replication [23–28].

T lymphocytes are intimately involved in the immune response and host defense against *M. tuberculosis* [12, 29], and it has been shown that tuberculosis is associated with an increase in cellular activation [30]. Cellular activation is important in vitro to establish productive HIV infection in T cells [31]. In vivo, cellular activation has been associated with an acceleration of viral replication both in HIV-infected patients and in animal models after immunization procedures or infection with pathogenic organisms [32–34].

To study the interaction between *M. tuberculosis*, HIV, monocytes, and lymphocytes, we developed an in vitro system involving peripheral blood mononuclear cells (PBMC) from HIV-infected persons with a history of purified protein derivative (PPD) skin test positivity. CD8 T cells were removed because of their ability to inhibit HIV replication [35]. We previously demonstrated in this system that PPD- and *M. tuberculosis*–induced HIV replication correlates with increases in
cellular activation through a specific recall antigen response mechanism [6].

Given the fact that PPD and M. tuberculosis induce the secretion of IL-2, as well as proinflammatory [10, 12] and antiinflammatory cytokines [10, 17, 20] known to either induce or inhibit HIV replication in vitro [23–28], we evaluated the effect of the modulation of these cytokines on PPD- or M. tuberculosis–induced HIV replication in an in vitro model that mimics antigen-induced viral replication.

Materials and Methods

Reagents. The medium used was RPMI 1640 (BioWhittaker, Walkersville, MD) supplemented with glutamine, penicillin and streptomycin at 100 U/mL (Biofluids, Rockville, MD), and 10% human AB serum (Sigma, St. Louis). Also used were the avirulent form of M. tuberculosis H37Ra (American Type Culture Collection, Rockville, MD); two different preparations of PPD (gifts from Cyanamid, Lederle-Praxis Biologicals, Pearl River, NY, and from F. Collins and S. Morris, Food and Drug Administration, Bethesda, MD); and keyhole limpet hemocyanin (KLH) (Sigma). The following cytokines and antibodies were used at the concentration reported, after preliminary experiments were done to evaluate their biologic effects on our cell system: recombinant (r) IL-10 (5 ng/mL); rTGF-β (2 ng/mL); rIL-1 receptor antagonist (200 ng/mL); soluble rIL-2 receptor (100 ng/mL); soluble rTNF-α receptor I (10 ng/mL) (R&D Systems, Minneapolis); neutralizing mouse monoclonal directed against human IL-10 (10 µg/mL) and human IFN-γ (10 µg/mL); polyclonal rabbit neutralizing anti-human TGF-β (10 µg/mL); mouse and rabbit IgG isotype control antibodies (R&D); antibodies recognizing CD4, CD8, and CD25; and isotype controls (Becton Dickinson, Mountain View, CA).

Patient samples. Samples were collected from two different clinical centers (National Institutes of Health, Bethesda, MD, and Johns Hopkins University, Baltimore). Venous blood or leukapheresis samples were collected from HIV-infected persons with a CD4 T cell range of 300–1100 cells/µL and a history of PPD skin test reactivity.

Virus isolation and culture of cells from HIV-infected subjects. PBMC were isolated by ficoll-hypaque density gradient centrifugation. The cells were washed three times at low speed (300 g for 15 min) to remove platelets. PBMC were incubated with anti-CD8–coated magnetic beads (Dynal, Lake Success, NY) according to the manufacturer’s instructions. Contamination with CD8 T cells was assessed and found to be <1%. CD8 T cell–depleted PBMC were plated in 48-well plates (Costar, Cambridge, MA) at 1.5–2 × 10⁶ cells/well in a total volume of 1 mL. Cell cultures were untreated or stimulated with PPD (10 µg/mL), M. tuberculosis (10⁵ bacilli/mL), or KLH (10 µg/mL) at 37°C in 5% CO₂ and cultured for 2–3 weeks. Supernatants (one-third volume) were removed and replaced with fresh medium 1 day after culture and then every 3 days thereafter. The supernatants were frozen at −80°C to determine reverse transcriptase activity and cytokine secretion. Cells from the cultures were removed between days 5 and 7. Cellular proliferation was evaluated by [³H]thymidine incorporation, and expression of cell surface activation markers was measured by flow cytometric analysis.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>None</th>
<th>KLH</th>
<th>PPD</th>
<th>M. tuberculosis</th>
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<tbody>
<tr>
<td>IL-2</td>
<td>ND</td>
<td>ND</td>
<td>39 ± 13</td>
<td>61 ± 23</td>
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<tr>
<td>IL-1β</td>
<td>ND</td>
<td>3.5 ± 3</td>
<td>160 ± 45</td>
<td>172 ± 59</td>
</tr>
<tr>
<td>TNF-α</td>
<td>ND</td>
<td>4 ± 3</td>
<td>217 ± 64</td>
<td>922 ± 305</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>ND</td>
<td>ND</td>
<td>1475 ± 888</td>
<td>2400 ± 124</td>
</tr>
<tr>
<td>IL-10</td>
<td>ND</td>
<td>ND</td>
<td>62 ± 15</td>
<td>652 ± 125</td>
</tr>
<tr>
<td>TGF-β</td>
<td>688 ± 35</td>
<td>695 ± 55</td>
<td>1302 ± 234</td>
<td>1903 ± 207</td>
</tr>
</tbody>
</table>

NOTE. ND, not detectable (<10 pg/mL); KLH, keyhole limpet hemocyanin. ELISAs were done on cell supernatants harvested at day 1 for IL-2, day 3 for IL-1β, tumor necrosis factor (TNF)-α, IL-10, and transforming growth factor (TGF)-β, and day 4 for interferon (IFN)-γ. Data are pg/mL.

Analyses. Cell proliferation was analyzed by harvesting an aliquot of cells between days 5 and 7, plating in 96-well round-bottom plates (Costar), pulsing with 0.5 µCi/well [³H]thymidine (New England Nuclear, Boston), harvesting (Tomtec, Orange, CT) after 18 h, and counting (Betaplate 1205; Wallac, Gaithersburg, MD). Production of IL-2 was measured by the CTLL cell bioassay; IL-1β, TNF-α, IFN-γ, IL-6, and IL-10 were measured by commercial ELISA kits (Biosource, Camarillo, CA); and TGF-β ELISA was done after acid activation of the samples to release mature TGF-β from the latent form according to the manufacturer’s instructions (R&D Systems). For flow cytometric analysis, CD8 T cell–depleted PBMC were incubated on ice with the appropriate labeled antibodies for 30 min, washed, and analyzed (Epics C flow cytometer; Coulter, Hialeah, FL). Reverse transcriptase activity in culture supernatants was determined as previously described [28].

Statistical analysis. Data were analyzed by the paired t test. P < .05 was considered significant.

Results

Role of endogenous cytokines in the modulation of PPD- and M. tuberculosis–induced HIV replication in CD8 T cell–depleted PBMC from HIV-infected subjects. PPD- and M. tuberculosis–stimulated cultures secreted higher levels of IL-2, IL-1β, TNF-α, and IFN-γ compared with unstimulated or KLH-stimulated cultures (table 1). KLH represents an antigen to which the donors had not been exposed and served as a negative control. To evaluate whether these endogenous cytokines played a role in the induction of HIV replication, we neutralized their activity by adding soluble receptors or receptor antagonists or neutralizing monoclonal antibodies. Selective inhibition of individual endogenous cytokines resulted in only minor decreases in PPD- or M. tuberculosis–induced HIV replication (data not shown), whereas the simultaneous inhibition of IL-2, IL-1β, and TNF-α resulted in a significant inhibition of PPD-induced HIV replication (P < .005) and M. tuberculosis–induced HIV replication (P < .0006) (figure 1). Evaluation
ppd- and \textit{M. tuberculosis}-induced HIV replication is decreased by selective blockade of activity of endogenous IL-2 and proinflammatory cytokines. CD8 T cell–depleted peripheral blood mononuclear cells isolated from HIV-infected, PPD-positive persons were stimulated with either keyhole limpet hemocyanin (KLH) used as negative control, PPD (A), or \textit{M. tuberculosis} (MTB; B) in presence or absence of soluble IL-2 receptor (sIL-2R), IL-1 receptor antagonist (IL-1ra), and soluble tumor necrosis factor-\(\alpha\) receptor I (sTNF\(\alpha\)RI). Cultures were monitored for reverse transcriptase (RT) activity. Data are means \pm SDs for 5 different patients. Cytokine inhibitor treatment resulted in significant inhibition of PPD-induced \((P < .005)\) and \textit{M. tuberculosis}-induced \((P < .0006)\) viral replication. Statistical significance was evaluated at peak of PPD- or \textit{M. tuberculosis}-induced HIV replication.

**Discussion**

This study demonstrates that the induction of HIV replication by \textit{M. tuberculosis} or its products through a recall antigen response mechanism in CD8 T cell–depleted PBMC from HIV-infected, PPD-positive persons is finely regulated by a complex network of endogenous cytokines that includes IL-2 and proinflammatory and antiinflammatory cytokines.

Selective inhibition individually of IL-2, IL-1\(\beta\), or TNF-\(\alpha\) resulted in a partial decrease in PPD- and \textit{M. tuberculosis}-induced HIV replication, whereas the simultaneous inhibition of these endogenous cytokines by soluble cytokine receptors and receptor antagonists, or by the addition of exogenous IL-10 and TGF-\(\beta\), dramatically reduced PPD- or \textit{M. tuberculosis}-induced HIV replication. In addition, neutralization of endogenous \textit{M. tuberculosis}- or PPD-induced IL-10 and TGF-\(\beta\) led to an enhancement of HIV replication. These data highlight the complex interaction of HIV-enhancing and suppressive cytokines produced during an antigen-specific response. Interestingly, the neutralization of IL-2 alone decreased \textit{M. tuberculosis}-induced cellular proliferation (data not shown) without significantly inhibiting HIV replication. Similar to other in vitro antigen-specific systems of HIV replication that are dependent on IL-2 production \cite{38}, this study reinforces the finding that \textit{M. tuberculosis} drives HIV replication by multiple mechanisms, including antigen-specific mechanisms \cite{6, 33}, antigen-
IL-10 and transforming growth factor (TGF)-β decrease PPD- and *M. tuberculosis*-induced HIV replication, an effect that correlates with decrease in cellular activation. CD8 T cell–depleted peripheral blood mononuclear cells isolated from HIV-infected, PPD-positive persons were stimulated with either keyhole limpet hemocyanin (KLH) used as negative control, PPD, or *M. tuberculosis* (MTB) in presence or absence of IL-10 and TGF-β. A, Effect on viral replication. Supernatants were harvested at peak of viral replication between day 6 and day 13 and assayed for reverse transcriptase (RT) activity. B, Effect on cellular proliferation. Aliquots of cultures were harvested on day 6, and [³H]thymidine was measured. C, Effect on cellular activation. CD25 expression on CD4 T cells was measured by flow cytometric analysis. Data are means ± SDs of 5 different patients.

Figure 2. IL-10 and transforming growth factor (TGF)-β decrease PPD- and *M. tuberculosis*-induced HIV replication, an effect that correlates with decrease in cellular activation. CD8 T cell–depleted peripheral blood mononuclear cells isolated from HIV-infected, PPD-positive persons were stimulated with either keyhole limpet hemocyanin (KLH) used as negative control, PPD, or *M. tuberculosis* (MTB) in presence or absence of IL-10 and TGF-β. A, Effect on viral replication. Supernatants were harvested at peak of viral replication between day 6 and day 13 and assayed for reverse transcriptase (RT) activity. B, Effect on cellular proliferation. Aliquots of cultures were harvested on day 6, and [³H]thymidine was measured. C, Effect on cellular activation. CD25 expression on CD4 T cells was measured by flow cytometric analysis. Data are means ± SDs of 5 different patients.

Figure 3. Endogenous cytokines are inhibited by exogenous addition of IL-10 (A) or transforming growth factor (TGF)-β (B). CD8 T cell–depleted peripheral blood mononuclear cells isolated from HIV-infected, PPD-positive persons were stimulated with either PPD or *M. tuberculosis* (MTB) with or without addition of exogenous IL-10 and TGF-β. Supernatants were harvested at day 1 for IL-2 detection, day 3 for IL-1β and tumor necrosis factor (TNF)-α detection, and day 4 for interferon (IFN)-γ detection. Data are means ± SDs of 5 different patients.
Figure 4. Neutralization of endogenous IL-10 and transforming growth factor (TGF)-β increases PPD- and *M. tuberculosis*–induced HIV replication that correlates with increase in cellular proliferation. CD8 T cell–depleted peripheral blood mononuclear cells isolated from HIV-infected, PPD-positive persons were stimulated with keyhole limpet hemocyanin (KLH) used as negative control, PPD, or *M. tuberculosis* (MTB) in presence of either control antibodies (Ab) or neutralizing antibodies against IL-10 and TGF-β. **A**, Effect on viral replication. Supernatants were harvested at the peak of viral replication, and levels of reverse transcriptase (RT) were determined. **B**, Effect on cellular proliferation. Aliquots of cultures were harvested on day 6, and [3H]thymidine incorporation was measured. Data are means ± SDs of 3 different patients.

on the balance of the effects of several endogenous cytokines.

Other authors have investigated the role of endogenous cytokines in the modulation of *M. tuberculosis*–induced HIV replication. Zhang et al. [5] demonstrated that simultaneous inhibition of both endogenous TNF-α and IL-1β dramatically decreased *M. tuberculosis*–induced HIV LTR-driven transcription in transiently transfected THP-1 cells compared to the effect achieved when these cytokines were neutralized individually. In addition, Lederman et al. [39] observed that neutralization of TNF-α led only to a partial decrease in *M. tuberculosis*–induced HIV expression in the chronically infected U1 cell line, and even less inhibition in PPD-stimulated U1 cells; unfortunately, no data were presented on the simultaneous neutralization of multiple endogenous cytokines. Taken together, these studies suggest that *M. tuberculosis* induction of HIV at either a transcriptional [5] or post-transcriptional level [6, 39] is the result of activation events and cytokines acting in autocrine and paracrine loops.

The importance of cytokines in controlling HIV replication and tuberculosis in vivo has been recently demonstrated in clinical trials of AIDS patients with active tuberculosis [40, 41]. Both thalidomide, a specific TNF-α inhibitor, and pentoxifylline, a nonspecific TNF-α inhibitor, caused a decrease in HIV load. Clinical benefit was associated with thalidomide treatment [40], and increased Karnofsky score and hemoglobin levels were associated with pentoxifylline therapy [41]. In contrast, thalidomide treatment was associated with an increase in virus load in HIV-positive patients without tuberculosis [40, 42].

The antiinflammatory cytokines TGF-β and IL-10 exhibit several immunomodulatory functions, including inhibition of T cell and B cell mitogenesis and reduction in proinflammatory cytokine secretion [36, 37]. TGF-β is produced by monocytes infected with mycobacteria in vitro and acts both to promote intracellular mycobacterial replication of the avirulent strain of *M. tuberculosis* (H37Ra) [22] and to enhance the bactericidal activity of IFN-γ on the virulent strain of *M. tuberculosis* (H37Rv) [36]. TGF-β either decreases or increases HIV replication, depending on the culture conditions [23, 24]. In addition, *M. tuberculosis* induces IL-10 secretion [10, 11]. It has been shown that compartmentalization of IL-10 at the site of infection, whether HIV-positive or not, has a reduction in proliferative and type 1 responses to *M. tuberculosis* [5] or post-transcriptional level [6, 39] is the result of activation events and cytokines acting in autocrine and paracrine loops.

The antiinflammatory cytokines TGF-β and IL-10 exhibit several immunomodulatory functions, including inhibition of
least in part by the release of proinflammatory cytokines [40, 41]. This effect may be partly counterbalanced by the production of IL-10 and TGF-β. These cytokines may overcome the effects of proinflammatory cytokines by reducing tissue damage and inhibiting HIV replication. Thus, a delicate balance between IL-2 and proinflammatory and antiinflammatory cytokines may play an important role in determining the course of tuberculosis and HIV disease progression.

Further delineation of these events will be important to better understanding the pathogenesis of both HIV disease and M. tuberculosis infection, as well as to the design of therapeutic strategies aimed at modulating the host factors involved in these pathogenic mechanisms.

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References


