The cytokine interleukin-10 (IL-10; previously called cytokine synthesis inhibitory factor) was initially identified in 1989 as a factor secreted by murine Th2 cell clones that inhibits the secretion of interferon-γ (IFN-γ) and other cytokines by Th1 cells. In addition, IL-10 appears to mediate some of the B-cell stimulatory effects of Th2 cells in the mouse, and this cytokine thus appears to be involved in shifting the balance of an immune response away from cellular immunity and towards humoral immunity. Additional studies have shown IL-10 to have immunomodulatory effects by acting on macrophages and other cell types. In particular, IL-10 suppresses the secretion of various cytokines by activated macrophages, costimulates mast cell growth, and costimulates thymocyte growth in the presence of IL-2 and IL-4. Soon after the discovery of murine IL-10, the human IL-10 (hIL-10) gene was identified and cloned. hIL-10 has been found to be produced by cells of several lineages, including B and T lymphocytes and monocyte/macrophages (M/M). In a number of aspects, the activity of hIL-10 parallels that of mIL-10; it inhibits T-cell responses, suppresses cytokine production by M/M, and stimulates B cells to produce Ig. However, there is recent evidence to suggest that hIL-10 may not be as clearly a cytokine of Th2 cells as is mIL-10. In particular, hIL-10 is produced by both Th1-like and Th2-like T-cell clones and it may inhibit antigen-driven activity of both Th1 and Th2 subsets.

It has recently been reported that the mononuclear cells of human immunodeficiency virus (HIV)-infected patients have increased IL-10 expression and that disease progression is associated with an increase in the capacity of lymphocytes to produce IL-4 and IL-10. However, there are no data yet available on serum IL-10 levels in HIV-infected patients. It has also been found that, in a murine experimental model for acquired immunodeficiency syndrome (AIDS) in which immunodeficiency is induced by the LP-BM5 murine leukemia virus, IL-10 overexpression is associated with B-cell hyperactivation, downregulation of Th1 cytokine secretion, and impaired CD8+ T-cell function. More recently, it has been reported that IL-4-deficient transgenic mice that lack Th2 cytokine responses are relatively resistant to the development of disease when infected with LP-BM5 virus.

Although there are some differences in the target cells of HIV and LP-BM5 and (as noted above) between the murine and human immune system in regard to the delineation of Th1 and Th2 subsets, these observations have led to the concept that both human and murine immunodeficiency syndromes are associated with a switch to a Th2-like immune response with associated IL-10 overproduction. However, there are few data available on the effects of IL-10 on HIV replication.

With this background, we have investigated the effects of hIL-10 on HIV replication in M/M and T-cell lines. We find that, although this cytokine has modest anti-HIV activity in a CD4+ T-cell line, it potently inhibits viral replication in M/M at concentrations that do not interfere with other critical immune responses. Thus, it is possible that IL-10 production may at least in part have beneficial effects in patients with HIV infection.

**MATERIALS AND METHODS**

Cytokines and drugs. Unless otherwise stated, highly purified recombinant hIL-10 (rHIL-10) derived from the supernatants of hIL-
cells were then further enriched for MIM by means of countercurrent centrifugal elutiation as previously described. 25 The resulting cells were greater than 95% monocytoid when examined after Giemsa staining and were greater than 95% nonspecific esterase positive. Cell viability as evaluated by trypan blue exclusion was consistently greater than 95%. More than 93% of the cells were CD11b+ and CD36+ (OMK1 and OMK5, respectively; Ortho Diagnostic Systems, Westwood, MA). Other characteristics of these cells have been described elsewhere. 27 The cells were cultured in 48- or 96-well plates (Costar, Cambridge, MA) in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) supplemented with 20% heat-inactivated, low endotoxin fetal calf serum (defined FCS; HyClone Laboratories, Logan, UT), 2 mmol/L L-glutamine, 50 U/mL penicillin, and 50 µg/mL streptomycin (all from GIBCO Laboratories). No proliferation-inducing cytokines, such as M-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF), were used in the experiments unless specifically stated. Infected and uninfected M/M were cultured in complete medium at a concentration of 1.25 × 10^6 cells/mL in flat-bottomed 96-well culture plates (Costar, Cambridge, MA) for 4 days before infection (starting on day −4), and incubated at 37°C in a humidified 5% CO₂ atmosphere. IL-10 was added at day −2, 0, +5, or +15, and cells were washed extensively to remove the cytokine 48 hours later. On day 0, virus was added to each well (when IL-10 was added at day −2, virus was added just after the cytokine was washed out; when IL-10 was added at day 0, virus was added immediately after the addition of the cytokine). On day 2, cells were washed to remove excess virus (and cytokine when added on day 0). Every 5 days, the supernatant was harvested from each well and replaced with fresh complete medium until day +27 was reached. Supernatants were assayed for HIV-1 p24 antigen. Because peak p24 levels are generally noted at day +12 in control wells, we focused on this time point in evaluating the effects of IL-10.

**M/M phagocytosis.** A modification of the method of Maloney et al 26 was used to determine the effect of rhIL-10 on M/M phagocytosis. Elutriated M/M (2.5 × 10^6 cells/1.0-mL well) were cultured in 48-well plates (Costar) for 4 days at 37°C in a humidified 5% CO₂ atmosphere, with or without rhIL-10. After 2 days, the wells were washed to remove IL-10 and complete medium was added. Twenty-four hours later, the cells were detached using trypsin and suspended at 1.5 × 10^7 cells/50 µL in medium using 10 × 75 mm borosilicate glass tubes (Kimble, Vineland, NJ). Twenty-five microliters of a 1:20 dilution of 0.8-µm latex beads (Sigma) in complete medium was added to each tube, vortexed, and incubated at 37°C for 45 minutes. After incubation, the cell suspension was layered over 1.5 mL FCS and centrifuged at 250g for 10 minutes at room temperature. Serum and nonphagocytosed beads were removed, and the cells were washed twice with PBS containing 2% bovine serum albumin and 0.02% sodium azide. Cells found to have ingested three or more beads on microscopic examination were scored as positive for phagocytic activity.

**Leucine incorporation assay.** Incorporation of [3H]-leucine in monocytes was measured using a modification of the procedure of Bonifacino. 31 Monocytes were cultured on 48-well plates (2.5 × 10^6 cells/1.0-mL well) for 4 days at 37°C in a humidified 5% CO₂ atmosphere. The cells were washed, medium containing purified
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rhIL-10 or control medium was added, and plates were incubated for 48 hours at 37°C in a 5% CO₂ atmosphere. The cells were then washed, 1,000 U/mL M-CSF or 0.02% sodium azide was added to certain wells as a positive and negative control for protein synthesis, and the cells were incubated for 12 hours. The cells were washed with leucine-free medium (RPMI 1640 without leucine; Gibco) supplemented with 20% dialyzed fetal bovine serum and 2 mmol/L L-glutamine (Gibco) and incubated for 3 hours in this medium with 0.2 mL/cm² [³H]-leucine (Amersham, Arlington Heights, IL). After incubation, proteins were precipitated with 1:1 ice-cold 10% trichloroacetic acid (TCA), followed by 30 minutes of incubation at 4°C. Precipitated proteins were collected on glass fiber filters, and the filters were dried and counted in scintillation cocktail. At least three replicate wells were established for each data point.

Human peripheral blood mononuclear cell proliferative responses. Freshly isolated human peripheral blood mononuclear cells were washed and cultured at a density of 1 × 10⁶/mL in 200-μL wells in 96-well round-bottom plates with various concentrations of rhIL-10. Where indicated, 0.5 or 5 μg/mL phytohemagglutinin (PHA; Sigma), 1.0 Lf/mL tetanus toxoid (Commonwealth of Massachusetts Department of Public Health, Jamaica Plain, NY), or 20 ng/mL mouse anti-human CD3 monoclonal OKT3 antibody (a gift of Ortho Pharmaceutical Corp, Raritan, NJ) were added as stimulators. A relatively low concentration of PHA (0.5 μg/mL) was used along with the higher dose of 5.0 μg/mL, because the suppression of PHA-induced proliferation by rhIL-10 has been shown to be more evident with lower doses of mitogen. Triplicate wells were established for each condition examined. Plates were incubated for 3 days for PHA stimulation and 5 days for the other proliferative stimuli at 37°C in a humidified 5% CO₂ atmosphere. The plates were pulsed with 1.0 μCi [³H]-thymidine during the final 18 hours of incubation. The cultures were then harvested onto glass fiber filters and counted in liquid scintillation cocktail.

Assay of HIV replication in cell lines. Uninfected or chronically HIV-infected cell lines in exponential growth phase were washed and cultured at a density of 5.0 × 10⁶ cells/mL in 200-μL wells in 96-well flat-bottom culture plates (Costar). This relatively low cell density was chosen to prevent overgrowth of the culture by the end of the experiment. COS-7 supernatant containing rhIL-10, mock-transfected COS-7 supernatant, rmIL-10 at 20 U/mL, or control medium was added as indicated, followed within 30 minutes by an inoculum of HIV-1 (1,000 × TCID₅₀). The plates were incubated for 5 days at 37°C in a humidified 5% CO₂ atmosphere. Supernatants were then harvested for p24 antigen determination, and the cells were counted by hemocytometer using trypan blue. The percentage of suppression is expressed as (1 - [p24 antigen_control/p24 antigen_experimental]) × 100%.

Inhibition assay for the cytopathic effect of HIV-1. The assay was performed as a modification of the method of Mitsuya et al.²⁴ ATH-8 cells were cultured at a density of 2.5 × 10⁶ cells/mL in 96-well U-bottom plates with human or murine IL-10 at a concentration of 20 U/mL, 20 μg/mL ddL (Sigma), or control medium. After 30 minutes, the cells were inoculated with HIV-1 (3,000 × TCID₅₀) and incubated at 37°C in a 5% CO₂ atmosphere for 7 days. Viable cells were counted using trypan blue exclusion, and the data were expressed as the percentage of viable cells as compared with uninfected, untreated controls.

Statistical analysis. Statistical comparisons were performed with Systat for the Macintosh (Systat, Inc, Evanston, IL). Paired or independent Student’s t-tests were used as appropriate; all P values are two-tailed. No corrections for multiple comparisons were made.

RESULTS

rhIL-10 suppresses HIV replication in M/M. In initial experiments designed to evaluate the effects of rhIL-10 on HIV replication, M/M obtained by elutriation were precultured in complete medium for 4 days and then simultaneously exposed to purified rhIL-10 (expressed in CHO cells) and 400 TCID₅₀ HIVmBaL (day 0). After 2 days, the cells were washed extensively and cultured for another 10 days without additional cytokine or virus. As shown in Fig 1, rhIL-10 (present from day 0 to 2) strongly inhibited p24 production at day 12 in a dose-dependent manner, with an IC₅₀ of approximately 0.1 to 0.03 U/mL. This effect was statistically significant (P < .05) for doses of 0.1 U/mL or greater. A comparable effect was seen when reverse transcriptase in the supernatants was assayed as an alternate measure of viral replication (results not shown). Also, similar results were seen with rhIL-10 as COS-7 supernatant and with purified rhIL-10 produced in E coli (data not shown). In control experiments, rmIL-10 was approximately 30 times less active than rhIL-10 (results not shown).

The time course of p24 production in cultures exposed to rhIL-10 on days 0 to 2 is depicted in Fig 2; a representative experiment is shown for clarity, because HIV replication in primary M/M exhibits a 5- to 7-day interexperimental variability in the time to peak viral production. As can be seen, a 2-day exposure of M/M to 0.1 to 1 U/mL of rhIL-10 resulted in a 7 to 10 day delay in the production of HIV. However, rhIL-10 did not completely abrogate HIV replication in M/M.

Effects of late addition of rhIL-10 to M/M. In subsequent experiments, we explored the effects of rhIL-10 added to cultures of M/M previously exposed to HIV. Under the experimental conditions used, the peak of p24 production generally occurs between days 7 and 15. Of seven experiments performed in which rhIL-10 was added for 2 days at time points starting from day 5 to day 15, five showed suppression of HIV replication by rhIL-10; the two remaining experi-
production was suppressed in wells that were exposed to experiments in which rhIL-10 was added for 2 days starting. Infection of the cultures is already established, and the results suggest that rhIL-10 may suppress HIV production in M/M already infected by HIV. However, it is still possible that this late suppression is the result of rhIL-10 interfering with the spread of infection in these cultures.

In additional experiments (not shown), we investigated the effects of rhIL-10 added to M/M during the 2 days before the addition of HIV, and then washed out at the time of viral exposure. It was found that, under such conditions, much less inhibition of viral replication was noted. Even at the highest dose tested, p24 antigen production at day 12 was suppressed only 37% as compared with control (results not shown).

Effects of rhIL-10 on the viability and function of mononuclear cells. We next asked whether these effects simply resulted from a toxic effect of rhIL-10 on M/M. As seen in Table 1, a 2-day exposure of M/M to rhIL-10 at concentrations of up to 1 U/mL had essentially no effect on the number of viable cells when examined by trypan blue exclusion. Moreover, concentrations of rhIL-10 up to 1 U/mL had no effect on the ability of M/M to phagocytose latex beads. Similar results were seen with HIV-infected M/M (Table 1). We also examined the effect of rhIL-10 on [3H]-leucine incorporation, a measure of protein synthesis in uninfected M/M. Slight inhibition was observed at 1 U/mL of rhIL-10 (63.1% of control, which was 44,300 cpm), but this did not reach statistical significance (P = .25). No evidence of decreased incorporation was observed at lower concentrations of rhIL-10 (108.4% and 114.9% of control at 0.1 and 0.01 U/mL, respectively). Thus, HIV replication in M/M was suppressed by rhIL-10 at concentrations that had little or no effect on the number of viable cells, their phagocytic activity, or their protein synthesis. Moreover, the lack of cytotoxicity in HIV-infected M/M suggests that selective killing of this population is not a mechanism of action of rhIL-10 in suppressing HIV.

It has been reported that secretion of various cytokines (including IL-1β, IL-6, and TNF-α) by endotoxin-stimulated M/M is inhibited by relatively high concentrations of human IL-10. We were interested to learn whether the lower concentrations of rhIL-10 found here to inhibit HIV replication in M/M also inhibited production of these cytokines. M/M were placed in either control medium or medium containing rhIL-10, and immediately stimulated with 1 μg/mL LPS. Twelve hours later, the supernatants were harvested and assayed for IL-1β, IL-6, and TNF-α. As shown in Table 2, IL-1β and TNF-α production were marginally decreased by rhIL-10 at the highest concentration used (10 U/mL); however, this effect was not statistically significant. IL-6 production was
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Table 1. Effects of rhIL-10 on M/M Viability and Function

<table>
<thead>
<tr>
<th>HIV noninfected M/M</th>
<th>rhIL-10 (U/mL)</th>
<th>No. of Viable Cells (%)</th>
<th>Phagocytic Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 2</td>
<td>Day 7</td>
<td>Day 17</td>
</tr>
<tr>
<td>HIV noninfected M/M</td>
<td></td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>1</td>
<td>95.0</td>
<td>110.7</td>
<td>93.5</td>
</tr>
<tr>
<td>0.1</td>
<td>105.0</td>
<td>103.6</td>
<td>ND</td>
</tr>
<tr>
<td>0.01</td>
<td>80.0</td>
<td>111.7</td>
<td>ND</td>
</tr>
<tr>
<td>HIV infected M/M</td>
<td></td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>1</td>
<td>100.0</td>
<td>88.2</td>
<td>ND</td>
</tr>
<tr>
<td>0.1</td>
<td>108.0</td>
<td>95.4</td>
<td>ND</td>
</tr>
<tr>
<td>0.01</td>
<td>132.0</td>
<td>96.0</td>
<td>ND</td>
</tr>
</tbody>
</table>

M/M preincubated for 4 days at a concentration of 1.25 x 10^5 cells/mL were exposed to rhIL-10 (0.01 to 1.0 U/mL) and/or HIV-1_lai, at 400 x TCID_50 for 2 days (days 0 to 2). The cells were then harvested for determination of viability on day 2 or cultured for 5 or 15 additional days (medium replaced every 5 days) and then harvested. For phagocytosis of latex beads, the cells were cultured for 24 hours after the exposure to rhIL-10. Absolute values of controls are as follows: number of viable cells: 6.7 x 10^4 cells/mL HIV noninfected and 4.2 x 10^4 cells/mL HIV infected on day 2; 8.8 x 10^4 cells/mL HIV noninfected and 6.8 x 10^4 cells/mL HIV infected on day 7; and 9.0 x 10^5 cells/mL HIV noninfected on day 17. Phagocytically active cells: 98% in HIV noninfected and 94% in HIV infected. Results shown are the mean of triplicate observations. For determination of cell viability, the day-17 experiment was separate from the others. No differences reached statistical significance (P = .05).

Abbreviation: ND, not done.

Table 2. LPS-Induced Cytokine Secretion in rhIL-10-Treated M/M

<table>
<thead>
<tr>
<th>IL-10 (U/mL)</th>
<th>LPS Stimulation</th>
<th>IL-1β (pg/mL)</th>
<th>TNF-α (pg/mL)</th>
<th>IL-6 (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>&lt;3.9 (0.00)</td>
<td>&lt;15.6 (0.00)</td>
<td>&lt;2 (0.00)</td>
</tr>
<tr>
<td>—</td>
<td>+</td>
<td>&lt;140.0 (36.40)</td>
<td>&lt;223.0 (36.25)</td>
<td>&lt;50.0 (13.08)</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>56.33 (45.97)</td>
<td>104.92 (30.90)</td>
<td>37.32 (12.02)</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>128.54 (24.27)</td>
<td>138.96 (57.51)</td>
<td>43.50 (11.87)</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>89.85 (12.68)</td>
<td>230.78 (39.71)</td>
<td>39.73 (12.53)</td>
</tr>
<tr>
<td>0.01</td>
<td>+</td>
<td>126.04 (84.36)</td>
<td>165.54 (18.10)</td>
<td>64.03 (6.72)</td>
</tr>
<tr>
<td>0.001</td>
<td>+</td>
<td>188.86 (63.68)</td>
<td>252.09 (44.85)</td>
<td>45.83 (10.67)</td>
</tr>
</tbody>
</table>

Various concentration of rhIL-10 or control medium were added to M/M that had been preincubated for 4 days, and stimulation of cytokine secretion with LPS (1 μg/mL) was immediately performed. Twelve hours later, supernatants were harvested for determination of cytokine levels. Results shown are mean (SEM) of triplicate determinations. No difference from the results without IL-10 reached statistical significance.

not affected at any of the doses tested. In additional experiments, we found no effect on the LPS-induced production of IL-6, IL-1β, and TNF-α immediately after or days after a 2-day pulse of rhIL-10 at concentrations up to 10 U/mL (data not shown). Therefore, rhIL-10 inhibited HIV replication in M/M at concentrations that had little or no effect on the capacity of monocytes to secrete stimulatory cytokines.

As another measure of the immunosuppressive actions of rhIL-10 at these lower concentrations, we evaluated the effects of rhIL-10 on mononuclear cell proliferation induced by tetanus toxoid, PHA, and OKT3 monoclonal antibody (anti-CD3). In these experiments, the mononuclear cells were continuously exposed to rhIL-10 throughout the incubation period, starting at day 0. Even so, there was no significant inhibition of cellular proliferation as measured by [3H]-thymidine incorporation until an rhIL-10 concentration of 10 U/mL was attained (Table 3). These data suggest that the effects of IL-10 on HIV replication in monocytes may occur at a different range of concentrations than those that cause a downregulation of cellular immune functions.

Effect of rhIL-10 on HIV replication in T-cell and monocytoid-cell lines. To further characterize the range of cell types in which rhIL-10 had anti-HIV activity, we examined its ability to suppress HIV replication in the CD4+ T-cell lines H9 and MOLT-4 as well as in the monocytoid lines THP-1 and U937. As seen in Table 4, a 5-day continuous exposure to 20 U/mL rhIL-10, starting at the time of infection with HIV_Lai, had no effect on HIV p24 production by MOLT-4 or H9 CD4+ T cells. By contrast, 20 U/mL rhIL-10 inhibited HIV production by acutely infected U937 promonocytic cells by 63%. This effect occurred in the absence of any decrease in cell proliferation. rhIL-10 had little or no effect on THP-1 monocytoid cells acutely infected with HIV production (23% decrease). Also, rhIL-10 had a borderline effect (30% decrease) on constitutive HIV production by U937 cells chronically infected with HIV_Lai. Thus, rhIL-10 can partially inhibit HIV replication in U937 at the relatively high concentration of 20 U/mL.

Finally, we assessed the activity of rhIL-10 in ATH-8, an HTLV-I-immortalized tetanus-toxoid-specific CD4+ T-cell line that is profoundly sensitive to the cytopathic effect of HIV-1.23,24 In the absence of rhIL-10, HIV_Lai infection reduced the number of viable ATH8 cells to 8% of control over 7 days of culture (Table 5). In the presence of 20 U/mL of rhIL-10, some protection from HIV-induced cytopathicity was observed (viable cells averaged 39% of uninfected control). This concentration of rhIL-10 caused no inhibition of cell proliferation in the absence of HIV infection (113% of control), suggesting that the anti-HIV effect was not simply caused by cellular toxicity. In contrast to hIL-10, rmIL-10 had little or no effect on HIV-induced cytopathicity in this system. Although it should be stressed that the protection afforded by rhIL-10 in this T-cell line is substantially less than that induced by ddI, a didoxycycline anti-HIV agent (an average of 91% suppression at 20 μmol/L), the results were still suggestive of a modest protective effect.

DISCUSSION

In this study, we have shown that HIV replication in monocytes is inhibited by rhIL-10 at concentrations that cause little or no cellular toxicity and that do not suppress cytokine secretion in response to LPS. This inhibition was observed with a 2-day exposure to IL-10, starting at the time of viral inoculation as well as 5 or 15 days after viral infection. rhIL-10 also caused partial suppression of viral production in certain monocytoid or T-cell lines, albeit at higher concentrations. These data indicate that one activity of IL-10 is the suppression of HIV replication in cells of the monocyte lineage.
In the short period of time since the identification of murine and human IL-10, a number of effects have been found to be mediated by this cytokine. In particular, IL-10 appears to suppress certain T-cell functions and to decrease parasites escape cell-mediated immunity. It has been speculated that induction of IL-10 production may be a means by which the extracellular killing of Toxoplasma gondii and the extracellular killing of Schistosoma mansoni by macrophages has been found to retain certain biologic activities of IL-10. However, there remain many unanswered questions as to whether IL-10 production is beneficial or deleterious in various infectious states. In the case of parasitic infections, it has been noted that IL-10 inhibits the production of reactive nitrogen oxides, which are involved in the elimination of intracellular Toxoplasma gondii and the development of an apathogenic chronic carrier state.

It has also been speculated that Epstein-Barr virus (EBV) may use IL-10 to its advantage. The BCRFI gene of EBV has extensive sequence homology to IL-10 and has been found to retain certain biologic activities of IL-10. Also, certain EBV-infected cell lines have been found to produce IL-10-like activity by EBV-infected cells may confer some survival advantage to the virus, either by directly enhancing viral infection or by aiding the development of an apathogenic chronic carrier state. More recently, serum IL-10 levels as high as 2,000 pg/mL (roughly 1 to 20 U/mL) have been reported in patients with non-Hodgkin's lymphoma. Interestingly, some of the patients with elevated IL-10 levels were seronegative for EBV, and the source of the IL-10 elevations in patients with lymphoma remains to be determined.

In a murine retroviral model of AIDS, there is evidence to suggest that overexpression of IL-10 may contribute to

<table>
<thead>
<tr>
<th>Table 3. Effect of IL-10 on Mononuclear Cell Proliferative Responses</th>
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</thead>
<tbody>
<tr>
<td><strong>Stimulus</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Medium alone</td>
</tr>
<tr>
<td>0.01 U/mL rhlL-10</td>
</tr>
<tr>
<td>0.1 U/mL rhlL-10</td>
</tr>
<tr>
<td>1.0 U/mL rhlL-10</td>
</tr>
<tr>
<td>10 U/mL rhlL-10</td>
</tr>
<tr>
<td>100 U/mL rhlL-10</td>
</tr>
</tbody>
</table>
| Freshly isolated normal human peripheral blood mononuclear cells were cultured at a density of 1 x 10^6/mL in 200-µL wells in 96-well flat-bottom plates with various concentrations of rhlL-10. PHA at 0.5 or 5.0 µg/mL, tetanus toxoid at 1.0 µL/mL, or OKT3 antibody at 20 ng/mL were added as stimulators. Plates were incubated for 3 days for PHA stimulation and for 5 days for the proliferative stimuli. They were then pulsed with 1.0 µCi/well ³H-thymidine 18 hours before completion of the incubation period. The cultures were then harvested onto glass fiber filters and counted. Results are expressed as mean cpm/well (SEM) of sextuplicate observations.

* Statistically significant differences from control (P < .05).

<table>
<thead>
<tr>
<th>Table 4. Effect of IL-10 on HIV Replication in Acutely and Chronically Infected Cell Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Line</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>H9</strong></td>
</tr>
<tr>
<td>Chronically infected (CD4⁺ T)</td>
</tr>
<tr>
<td><strong>MOLT-4</strong></td>
</tr>
<tr>
<td><strong>U937</strong></td>
</tr>
<tr>
<td>Chronically infected (Promonocytic)</td>
</tr>
<tr>
<td><strong>THP-1</strong></td>
</tr>
</tbody>
</table>
| Cells from the indicated cell lines at a density of 1.0 x 10^6 per 200-µL well were cultured in either rhlL-10, rmlL-10, or control medium and incubated for 5 days. Acutely infected cell lines were exposed to HIVΔm at a dose of 3,000 x TCID₅₀ at the beginning of the culture period; chronically infected lines constitutively produced viral proteins. Supernatants were harvested and assayed for HIV p24 antigen. The number of viable cells was determined for each line by trypan blue exclusion; in no case did the exposure to rhlL-10 or rmlL-10 induce a greater than 10% change in the number of viable cells. Results shown are the average of two experiments, each with triplicate observations.

<table>
<thead>
<tr>
<th>Table 5. Reduction of HIV-Induced Cytotoxic Effect by rhlL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number Viable Cells (% control)</strong></td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
</tr>
<tr>
<td>20 U/mL rhlL-10</td>
</tr>
<tr>
<td>20 U/mL rmlL-10</td>
</tr>
<tr>
<td>20 µmol/L ddl</td>
</tr>
</tbody>
</table>

ATH-8 cells (5 x 10^6 per 200-µL well) were cultured in medium alone, or with rhlL-10, rmlL-10, or ddl, and exposed to HIV∆m at a dose of 3,000 x TCID₅₀. The cells were cultured for 7 days, at which time the number of viable cells was determined by trypan blue exclusion. Shown are percentages of viable cells compared with the uninfected, untreated control wells in two separate experiments (9.2 ± 10^6 cells/well in experiment 1, and 8.4 ± 10^6 cells/well in experiment 2).

Abbreviation: ND, not done in these experiments.
SUPPRESSION OF HIV-1 REPLICATION BY IL-10

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...disease pathogenesis by simultaneously suppressing cellular immunity and stimulating B-cell hyperactivity. However, the results of the present study suggest that IL-10 may provide a means of defense against HIV by suppressing viral replication in M/M. This suppression occurs at concentrations of IL-10 that have little or no effect on either T-cell proliferation to antigen or mitogen or on the secretion of cytokines by M/M. Although CD4+ T cells are the most frequently infected cells in vivo, infection of M/M by HIV is also important in the pathogenesis of AIDS. Indeed, there is some recent evidence to suggest that infection of monocytes by HIV may be more important than infection of lymphocytes in the pathogenesis of HIV-induced immunodeficiency. Moreover, HIV-infected M/M can efficiently infect CD4+ T cells, and monocyte-derived cells are the predominant target cells for HIV infection in the central nervous system. Thus, control of the infection of M/M is likely to be an important step in the overall containment of HIV infection.

It is possible that endogenous production of IL-10 in HIV-infected patients has effects on the disease process. As noted above, it has been observed that stimulated peripheral blood mononuclear cells obtained from patients with advanced HIV infection have increased IL-10 production compared with those taken in earlier stages of disease. If IL-10 is similarly produced in vivo, it may thus serve to suppress HIV replication in M/M and retard disease progression. It is also possible that IL-10 has a role in the shift from monocytotropic to lymphocytotropic HIV in patients by providing selective pressure against replication in monocytes and forcing outgrowth of proportionately greater numbers of virus particles from lymphocytes. Further research will be needed to sort out these issues.

By what mechanism does IL-10 act to suppress the infection of M/M by HIV? The results here with late addition of IL-10 suggest that it may act at one of the late stages of viral replication (ie, after the formation of the provirus). One possible mechanism for the anti-HIV activity of IL-10 may be downregulation of cytokine production (such as TNF-α, GM-CSF, or IL-6), which, in turn, stimulates the later stages of HIV replication. Indeed, Poli et al have recently reported that, in a system using adherent macrophages, HIV replication could be inhibited by rhIL-10, but, at the same time, this inhibition was reversed by the addition of TNF-α. However, under the culture conditions used in the present experiments, rhIL-10 acted to suppress HIV replication at lower concentration than those required to suppress TNF-α production. Also, under these culture conditions, HIV-infected M/M do not produce detectable amounts of TNF-α or IL-6 (A. Foli and R. Yarchoan, unpublished observation). However, it is still conceivable that very small amounts of these cytokines produced by M/M act in an autocrine manner to stimulate HIV infection. Another mechanism for the action of IL-10 in suppressing HIV may be through transcriptional activators such as NF-κB or, alternatively, increased ribonucleolytic activity (proposed by Bogdan et al as the mechanism for IL-10-induced downregulation of cytokine release).

A third mechanism may be through an effect on nucleoside pools or other factors that affect early steps of HIV replication. Although IL-10 had some ability to suppress HIV replication in chronically infected M/M or monocytoid lines, the effects were most pronounced in the setting of acute infection. The ability of HIV to undergo reverse transcription and form a provirus is dependent on the state of activation of the target cell as well as intracellular nucleoside pools. IL-10 may thus influence the rate of proviral formation through its protean effects on M/M activation. However, it should be noted that the suppression of HIV replication is not simply the result of a toxic effect of IL-10 on M/M; as seen in these experiments, the anti-HIV effects occurred at concentrations of IL-10 that had little or no effect on cellular viability, protein synthesis, cytokine secretion, phagocytosis, or the ability to present antigen (tetanus toxoid).

Given its ability to suppress HIV replication in M/M, might IL-10 be worth considering as a candidate for antiviral therapy? An additional benefit of IL-10 in this setting may be its ability (at higher concentrations) to suppress the production of TNF-α and IL-6, cytokines that contribute to the pathogenesis of AIDS and its complications. However, there would appear to be a number of substantial obstacles to its clinical use in this setting: it acts predominantly in M/M; it enhances immunosuppression; and may promote the development of lymphomas. Additional studies will be needed to sort out these issues and to determine whether this compound might conceivably have a clinical role, possibly in combination with other drugs or in select subsets of patients. Finally, as we learn about the mechanism for its anti-HIV activity, it may be possible to engineer a separation of this effect from the other immunologic activities of this cytokine.

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