Interleukin-12 Decreases Human Immunodeficiency Virus Type 1 Replication in Human Macrophage Cultures Reconstituted with Autologous Peripheral Blood Mononuclear Cells

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In vitro interactions between interleukin (IL)-12, interferon (IFN)-γ, and human immunodeficiency virus (HIV) type 1 infection in human macrophages were examined. Macrophages were infected with HIV-1 and cocultured with autologous monocyte-depleted peripheral blood mononuclear cells (PBMC). The addition of autologous PBMC to HIV-1-infected macrophages resulted in an expansive increase in reverse transcriptase (RT) activity; however, when both autologous PBMC and IL-12 were added, RT activity decreased (75%–90%) and high levels of IFN-γ (9–16 ng/mL) were detected. The addition of anti-IFN-γ antibodies blocked the IL-12–induced decrease in RT activity. Surprisingly, exogenous IL-12 added to HIV-infected macrophage cultures without autologous lymphocytes resulted in a 50%–60% reduction in RT activity and no detectable increase in IFN-γ. The addition of anti-IFN-γ did not inhibit this IL-12–mediated effect. These results suggest that IL-12 is capable of indirectly down-regulating HIV proliferation in macrophage cultures reconstituted with autologous PBMC and of directly suppressing HIV replication in purified macrophage cultures.

Materials and Methods

Preparation of virus stocks. Virus stocks were prepared using a modification of a published method [9]. Briefly, virus containing cell supernatants from PBMC were centrifuged (250 g, 20 min) to remove cell debris. The clarified virus suspension was then ultracentrifuged (41,460 g, 2 h) through a sucrose cushion in 10 mM TRIS hydrochloride (pH 7.4), 1 mM EDTA, and 0.1 M NaCl to ensure that cytokines in the supernatant were removed. The HIV-1 pellet was then resuspended in RPMI with 5% fetal bovine serum (FBS) and maintained at −80°C. Virus stocks were tested (Mycoplasma Rapid Detection Kit; GEN-PROBE, San Diego) and were negative for mycoplasma.

Infection of macrophages with HIV-1. PBMC were isolated from the blood of HIV-1–negative persons by ficoll-hypaque. Monocytes were prepared from PBMC by centrifugation through discontinuous Percoll gradients. Isolation of PBMC-derived monocytes, following Ficoll and Percoll gradients and adherence, results in 98% monocyte purity [10]. The remaining PBMC, depleted of the monocyte fraction (<5% monocytes by FACS; Becton Dickinson, San Jose, CA) were frozen in liquid nitrogen until needed. Monocytes (10⁶ cells/mL [200 µL]/well) were cultured in 96-well flat-bottom plates in endotoxin-free (<10 pg/mL) RPMI 1640 (BioWhittaker, Walkersville, MD) with endotoxin-free (<10 pg/mL) 5% FBS (GIBCO, Gaithersburg, MD) and incubated 5 days (37°C in 5% CO₂) to allow maturation into macrophages. On day 6 after isolation, predetermined groups of macrophages were exposed to HIV₀. (reverse transcriptase [RT] activity 60,000 cpm/mL containing <10 pg/mL endotoxin) or complete medium for 3 h at 37°C (5% CO₂ in air). The virus or medium was then removed and replaced with fresh complete medium. PBMC without mono-

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cytes were thawed and added back to select groups of infected macrophages (0.5–1.0 × 10^6/well).

**Effects of exogenous cytokine on HIV replication.** In some experiments, macrophages or macrophages plus PBMC, plated on a 96-well plate and infected as described above, were given recombinant human (rHu) IL-12 (10 ng/mL; Immunex, Seattle) 3 h after HIV infection. rHuIL-12 was tested for and found to be negative for endoriko. In some experiments, an anti-IFN-γ murine monoclonal antibody (1:50 dilution; Chemicon, Temecula, CA) was added 12 h after infection to some cell cultures with and without IL-12. Cell cultures were maintained by replenishing RPMI 1640 and 5% FBS every 4 days. On days 8, 12, and 14 after infection, 20-μL samples from each culture well were analyzed for HIV RT activity by RT assay [11]. In parallel cultures, a nonspecific murine IgG was added in place of the anti-IFN antibody as an essential control.

**IFN-γ ELISA.** Culture supernatants were harvested 72 h after infection and stored at −80°C until assay. A sandwich IFN-γ ELISA, linear from 0 to 20 ng/mL with a sensitivity to 5 pg/mL, was used to determine the level of IFN-γ within the cell culture supernatants. Antibodies for the assay were purchased from Chemicon and used according to the manufacturer’s instructions.

**Results**

Macrophages are key cells in immune regulation and a primary target for HIV-1 infection [12–15]. We initiated a series of experiments to determine whether the levels of HIV-1 replication in infected macrophage cultures could be altered by the addition of rHuIL-12. Purified monocyte-derived macrophage cultures infected with a monocytotropic HIV isolate (HIV_BaL) had levels of viral infection of 70%–90% of total cells and syncytial formation evident within 5–10 days after infection. As previously documented, a temporal increase in HIV-1 RT activity was observed in infected macrophage cultures at 8, 12, and 14 days after infection [16, 17]. Neither cytopathic effects nor RT levels were detected in uninfected macrophage cultures.

**Effects of IL-12 on HIV-1 replication in infected macrophages reconstituted with autologous PBMC.** Because IL-12 increases the production of IFN-γ, a macrophage-activating cytokine, we did experiments to determine the effects of exogenous IL-12 on HIV-infected macrophages. In the first set of experiments, HIV-infected macrophage cultures were reconstituted with monocyte-depleted autologous PBMC, rHuIL-12 was added, and RT activity was measured 8, 12, and 14 days after infection. As previously observed [16, 17], HIV-infected macrophages had elevated RT activity readily detectable by 8 days after infection (figure 1). During the course of the experiment, HIV-1–infected macrophages reconstituted with PBMC (macrophage-PBMC) had greater RT activity than HIV-infected macrophages without PBMC (figure 1). The addition of rHuIL-12 to infected macrophage-PBMC cultures resulted in 75%–90% suppression of RT activity in all 6 donors examined. This decrease in RT activity was maintained during the 14-day observation period (figure 1).

The ability of IL-12 to induce the production of IFN-γ by T cells and NK cells has been documented [3, 4]. Because of our observation on the down-regulating effects of rHuIL-12 on HIV-1 and the known ability of IFN-γ to activate macrophage-killing mechanisms, the level of IFN-γ in culture supernatants was determined. IFN-γ could not be detected in HIV-1–infected macrophage cultures (figure 2). In the 72-h cell supernatant from macrophage cultures reconstituted with autologous PBMC, we detected levels of IFN-γ of 0–2 ng/mL. The background levels of IFN-γ (<2 ng/mL) observed in 2 of 6 donors was not sufficient to suppress RT activity in these donors (figure 1). In all cultures to which both PBMC and IL-12 were added, higher levels of IFN-γ (10–16 ng/mL) were detected (figure 2). As previously reported, IL-12 also induced IFN-γ in normal PBMC (data not shown) [3, 18, 19].

**Effect of antibody to IFN-γ on the IL-12–induced down-regulation of HIV-1.** IFN-γ can activate macrophages in vitro, resulting in decreased HIV-1 replication in vitro [20]. Because addition of IL-12 to macrophages to which autologous PBMC were added resulted in decreased HIV-1 RT and increased IFN-γ, experiments were done to determine whether the addition of an antibody to IFN-γ would abrogate the antiviral effects of IL-12. A monoclonal antibody to IFN-γ was added to cultures containing HIV-infected macrophage-PBMC and IL-12. Eight days after infection, we analyzed the supernatants for RT activity. The results, presented in figure 3, demonstrate that the down-regulation of HIV RT by IL-12 was blocked by anti-IFN-γ. The IL-12 effect was not inhibited by the addition of the control antibody.

**Effects of IL-12 on HIV-1 replication in macrophage cultures.** Although IL-12 can be produced by macrophages and
B cells and can directly activate T and NK cells, there is little information on the ability of this cytokine to directly alter macrophage function. We did experiments to determine whether IL-12 could directly affect HIV replication in macrophages. rHuIL-12 was added after infection of macrophage cultures, and RT activity was determined on days 8, 12, and 14 after infection. RT activity was decreased in macrophage cultures by the addition of IL-12 (figure 4). To ensure that the reduction in RT induced by IL-12 in macrophage cultures was not the result of IL-12–induced IFN-γ production from contaminating T or NK cells, we added an antibody to IFN-γ in conjunction with IL-12. As seen in figure 4, this antibody was unable to block the IL-12–mediating reduction in HIV-1 RT activity in macrophage cultures. Similarly, the addition of the control antibody did not alter the IL-12 reduction in RT activity (data not shown). Furthermore, in only 2 of the 8 donors could insignificant levels of IFN-γ (<0.1 ng/mL) be detected in the supernatants from HIV-infected macrophage cultures with IL-12 added.

**Discussion**

IL-12 directly binds to IL-12 cell surface receptors, resulting in the up-regulation of NK killing and T cell proliferation [1, 2, 21, 22]. Indirectly, IL-12 subsequently broadens its effect on the immune system by inducing IFN-γ production [1, 18, 19]. IFN-γ then interacts directly with IFN-γ–dependent cells to activate inflammatory pathways and additional immune responses. These up-regulatory functions of IL-12 suggest that this cytokine has antipathogenic functions. In fact, in parasite-infected mice, IL-12 treatment decreases the level of several organisms including *Leishmania major* [23, 24], *Toxoplasma gondii* [25], and cryptococci [4]. In the earlier studies, the reduction in pathogen burden seemed to be linked to the IL-12 induction of IFN-γ that leads to the activation of macrophage functions, including killing mechanisms. Depletion of the IL-12–induced IFN-γ, either by addition of an antibody to IFN-γ or removal of NK cells, blocks the antimicrobial effects of IL-12 [23, 24, 26]. There is also evidence that the antimicrobial properties of IL-12 occur because of an IL-12–induced shift in the Th1-Th2 ratio to that of the Th1 subset, thus augmenting antipathogenic mechanisms.

In addition to an antiparasitic effect, IL-12 may also have a role in regulating the immune system during viral infection. For instance, in mice infected with lymphocytic choriomeningitis virus, addition of IL-12 leads to an increase or a decrease in the virus-specific cytotoxic T lymphocyte response, CD8 T cell
levels, and viral replication, depending upon the amount of IL-12 administered [3]. The dose-dependent effect of IL-12 on other viral pathogens has not yet been determined. However, a recent study of AIDS patients indicated that IL-12 is capable of restoring cell-mediated immune responses against HIV in vitro [7]. In that study, the suppression of T cell proliferation and IL-2 production in response to influenza virus and HIV envelope proteins in asymptomatic persons could be reversed by the addition of IL-12 to these cell cultures.

In the current study, we demonstrated that IL-12 is capable of down-regulating HIV-1 replication in macrophage cultures reconstituted with autologous PBMC by decreasing RT activity. In a previous study of murine macrophages, the addition of autologous cells and IL-12 to macrophage cultures also resulted in an increase in immunologic activity [3]. The activation in the murine model seems to be linked to IL-12 induction of IFN-γ that then energizes the killing mechanisms. In the present study, an increase in IFN-γ concentration coincided with the decrease in HIV RT following the addition of IL-12
to reconstituted human cell cultures. This indicates that the IFN-γ induced by IL-12 in HIV-infected human cell cultures may be responsible for the observed down-regulation in HIV activity. In support of this idea, similar to the observations in murine studies, the addition of an anti-IFN-γ antibody blocked the IL-12 reduction in RT activity in infected macrophage-PBMC cultures. This indicates that an IFN-γ-dependent mechanism is functioning to down-regulate HIV by up-regulating macrophage activity.

The addition of IL-12 to HIV-infected macrophage cultures alone was unexpectedly capable of down-regulating HIV-1 in the absence of autologous PBMC through which IL-12 activates IFN-γ production. Although IL-12 can affect NK and T cells, no direct IL-12 effects have been observed regarding macrophages nor have IL-12 receptors been detected on the macrophage surface [3]. In macrophage cultures to which IL-12 was added, little or no IFN-γ was detected. Also, the addition of an antibody to IFN-γ to the macrophage cultures could not block the IL-12-mediated down-regulation of HIV RT that was observed when the antibody was added to cultures containing macrophage-PBMC and IL-12. The IFN-γ-independent mechanism induced by IL-12 is not known and is being investigated. Although human macrophages are not known to have IL-12 receptors, it is possible that IL-12 receptors may be up-regulated on the surface of macrophages as the direct result of HIV-1 infection. The concept that HIV-1 infection could up-regulate IL-12 receptors or other cytokine receptors is not without precedence: A prior study showed that HIV can control signaling pathways through a selective increase of tumor necrosis factor surface receptors. [27]. It is unlikely that the decrease in HIV replication was indirectly due to an increase of monokines (e.g., IL-6, tumor necrosis factor-α, or granulocyte-macrophage colony-stimulating factor), because these cytokines can increase HIV-1 replication in human macrophages in vitro [28-30]. However, it remains possible that IL-12 may act via other unknown cytokine-effector mechanisms and that macrophages infected with primary HIV isolates or macrophages from HIV-positive subjects may respond differently to IL-12 than macrophages infected with HIV laboratory isolates. Therefore, whether IL-12 induces other monokines or activates other macrophage functions in HIV-infected macrophages from HIV-positive and -negative subjects vaccinated with candidate HIV vaccines is the current focus of our research.

References


