Inhibition of acute in vivo human immunodeficiency virus infection by human interleukin 10 treatment of SCID mice implanted with human fetal thymus and liver

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Communicated by Stanley G. Nathenson, Albert Einstein College of Medicine, Bronx, NY, December 29, 1995 (received for review November 2, 1995)

ABSTRACT To improve the usefulness of in vivo models for the investigation of the pathophysiology of human immunodeficiency virus (HIV) infection, we modified the construction of SCID mice implanted with human fetal thymus and liver (thy/liv-SCID-hu mice) so that the peripheral blood of the mice contained significant numbers of human monocytes and T cells. After inoculation with HIV-1S, a primary patient isolate capable of infecting monocytes and T cells, the modified thy/liv-SCID-hu mice developed disseminated HIV infection that was associated with plasma viremia. The development of plasma viremia and HIV infection in thy/liv-SCID-hu mice inoculated with HIV-1S was inhibited by acute treatment with human interleukin (IL) 10 but not with human IL-12. The human peripheral blood mononuclear cells in these modified thy/liv-SCID-hu mice were responsive in vivo to treatment with exogenous cytokines. Human interferon γ expression in the circulating human peripheral blood mononuclear cells was induced by treatment with IL-12 and inhibited by treatment with IL-10. Thus, these modified thy/liv-SCID-hu mice should prove to be a valuable in vivo model for examining the role of immunomodulatory therapy in modifying HIV infection. Furthermore, our demonstration of the in vivo inhibitory effect of IL-10 on acute HIV infection suggests that further studies may be warranted to evaluate whether there is a role for IL-10 therapy in preventing HIV infection in individuals soon after exposure to HIV such as for children born to HIV-infected mothers.

Investigation of the kinetics of the development of resistance by human immunodeficiency virus (HIV) to antiviral agents in HIV-infected individuals has indicated that the clinical course of HIV-mediated disease involves a dynamic interaction between rapidly replicating virus and the immune system (1, 2). The plasma of HIV-infected individuals contains the most recently replicated HIV, therefore, the population dynamics of HIV replication are best evaluated by examining the levels of HIV present in the plasma (3). Thus, the development of an in vivo animal model wherein plasma HIV levels can be monitored would greatly facilitate the ability to evaluate the in vivo efficacy of therapeutic interventions. An attractive small animal model for studying in vivo HIV infection is SCID-hu mice, SCID mice implanted with human fetal thymus and liver (4). The usefulness of this model has been limited by the low numbers of circulating human T cells and the absence of detectable levels of human monocytes in the peripheral blood of these SCID-hu mice (5). We recently described modifications in the construction of SCID-hu mice that significantly increased the numbers of human T cells present in their peripheral blood, spleen, and lymph nodes and permitted the development of disseminated HIV infection after peripheral inoculation with multiple strains of HIV-1 including monocytotropic strains (6, 7). In the present study, we report that the peripheral blood of these modified SCID-hu mice is also populated with significant numbers of human monocytes that can potentially be infected with HIV. Furthermore, we demonstrate that after HIV infection, these mice develop plasma viremia that was markedly inhibited by treatment with interleukin (IL) 10.

MATERIALS AND METHODS

Cytokines and Antibodies. Human recombinant IL-12 was a gift from Stanley Wolf (Genetics Institute, Cambridge, MA) and human recombinant IL-10 was provided by Satwant Narula (Schering-Plough Research Institute, Kenilworth, NJ). Peridinin chlorophyll protein-conjugated monoclonal antibodies to human CD45, fluorescein isothiocyanate-conjugated monoclonal antibodies to human CD4 and CD14, and phycoerythrin-conjugated monoclonal antibodies to human CD8 and CD11b were obtained from Becton Dickinson.

Implantation of Human Fetal Thymic and Liver (hu-thy/liv) Tissue into SCID Mice. The hu-thy/liv tissue was obtained from fetuses at 17–21 gestational weeks within 8 h after the elective termination of pregnancy and implanted into SCID mice (6–8 weeks old) as described (6, 7). Briefly, the hu-thy/liv tissue obtained from one fetus was kept on ice and cut into 1-mm² pieces. After the SCID mice were anesthetized with pentobarbital (40–80 mg/kg), the left and right kidneys were sequentially exteriorized and subcapsularly implanted with at least 10 pieces of hu-thy/liv tissue (resulting in thy/liv SCID-hu mice). This procedure resulted in minimal postoperative morbidity and mortality and the successful implantation of >95% of the mice. The implanted tissue increased by >20-fold from its original size by 3 months after implantation. The consent forms and procedures used in this study were reviewed and approved by the Albert Einstein College of Medicine Committee on Clinical Investigation.

Flow Cytometric Analysis. Mononuclear cells were harvested from the peripheral blood of the thy/liv-SCID-hu mice and stained with phycoerythrin-, fluorescein isothiocyanate-, or peridinin chlorophyll protein-conjugated mouse monoclonal antibodies that were specific for human (and negative for mouse) CD45, CD4, CD8, CD14, or CD11b as described (7, 8).

Abbreviations: HIV, human immunodeficiency virus; thy/liv-SCID-hu mice, SCID mice implanted with human fetal thymus and liver; hu-thy/liv, human fetal thymus and liver; RT-PCR, reverse transcription-coupled polymerase chain reaction; β2-m, β2-microglobulin; IL, interleukin; IFN-γ, interferon-γ; PBMC, peripheral blood mononuclear cell; TCID, tissue culture infective dose; TNF-α, tumor necrosis factor α.

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The peripheral blood of the SCID mice used contained about 2.0 × 10^9 cells per ml and the percentage of human CD4 and CD8 cells given is the percentage of cells present in the lymphocyte gate. The percentage of human CD14 and CD11b cells indicated is the percentage of cells in the monocyte gate. Expression of the human surface proteins by leukocytes present in the SCID-hu mice was assessed by three-color flow cytometric analysis using a FACScan cell analyzer with LYSYS-II software (Becton Dickinson) with expression of human CD45 used to confirm the human origin of the cells. Lymphocyte and monocyte gates were set on the basis of forward and side scatter profiles to correspond to gates set for control human (from healthy adult volunteer) lymphocytes and monocytes. Nonviable cells and unlysed red blood cells were gated out based on their forward and side scatter profiles. Single, double, and triple staining of positive and negative control samples (human adult and C.B-17 mouse mononuclear cells) and of the appropriate mouse IgG isotype controls were assessed and used to compensate for phycoerythrin vs. fluorescein isothiocyanate vs. peridinin chlorophyll protein emission and to set cut off values for the quadrants.

**Detection of Human Cytokine Gene Expression by Reverse Transcription-Coupled Polymerase Chain Reaction Amplification (RT–PCR).** The pattern of in vivo human cytokine expression by the human cells present in thy/liv-SCID-hu mice was assessed by using a modification of a previously described technique (6, 8). Briefly, peripheral blood mononuclear cells (PBMCs) from the thy/liv-SCID-hu mice were lysed in guanidine isothiocyanate (4 M) buffer, and cellular DNA and RNA were separated by cesium chloride (5.7 M) density gradient centrifugation and precipitated with ethanol. RNA (7 μg) in 7 μl of double-distilled H2O was mixed with 4 μl of 5× buffer (250 mM Tris-HCl, pH 8.3/375 mM KCl/15 mM MgCl2, 2 μl of dithiothreitol (100 mM), 1 μl of random hexamers (BRL-Gibco), and 5 μl of mixed dNTPs (each at 2 mM), heated to 65°C for 10 min, and cooled on ice for 5 min, and then 1 μl (200 units) of Superscript reverse transcriptase (BRL-Gibco) was added. This final reaction mixture was mixed, briefly centrifuged, incubated at 37°C for 60 min, and then placed on ice. After reverse transcription of total RNA (7 μg) extracted from the PBMCs of the thy/liv-SCID-hu mice, cDNA was amplified by PCR with human-cytokine-specific primers for 60 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1 min, and extension at 72°C for 1 min. The presence of the target mRNA was indicated by the visualization of an amplification product of the predicted size after fractionation of the PCR products by electrophoresis and ethidium bromide staining. The nucleotide sequences for the 5′ and 3′ primer pairs for each cytokine were selected from published DNA sequences and were designed to be human-specific and mRNA-specific as described (6, 8). All samples were analyzed by RT–PCR for the presence of human β2-microglobulin (β2-m) to verify the integrity of the sample mRNA and the efficiency of subsequent reverse transcription, and positive and negative controls were always included.

**Detection of HIV RNA in the Mouse Plasma.** The thy/liv-SCID-hu mice were infected either by direct injection of 300 tissue culture 50% infective dose (TCID50) units of HIV-1SY in 30 μl into the left hu-thy/liv implant or by i.p. injection of 8000 TCID50 units of HIV-1SY in 800 μl as described (7). The plasma was isolated from the blood of the thy/liv-SCID-hu mice and analyzed for the presence of HIV RNA by RT–PCR using a modification of a previously described technique (6). RNA was extracted from plasma (100 μl) by solubilization in guanidine isothiocyanate (4 M) buffer and precipitation with isopropanol followed by elution with RNAase-free DNase (BRL-Gibco). The RNA was reverse-transcribed as described above, the cDNA was amplified for 35 cycles with a primer pair specific for the gag gene segment (S38/39), electrophoresed through 1.5% NuSieve/0.5% SeaKem agarose (FMC) gel, and subjected to Southern blot analysis with a digoxigenin-labeled internal probe, SK19, specific for the SK38/39 product. The hybridized probe was detected by using the Genius luminescent detection kit (Boehringer Mannheim). A given sample was scored as positive if PCR amplification resulted in a DNA product of the predicted size hybridized to the specific internal probe. Positive and negative controls were always included.

**Titration of HIV-Infected Mononuclear Cells in the hu-thy/liv Implant by Limiting Dilution Coculture.** The titer of HIV-1-infected mononuclear cells present in the hu-thy/liv implants was determined as described (7). Mononuclear cells isolated from the hu-thy/liv implant in 1:5 dilutions ranging from 1 × 10^0 cells to 3.2 × 10^1 cells were cultured in quadruplicate for 1–2 weeks at 37°C in 24-well culture plates with 1.0 × 10^6 phytohemagglutinin-activated donor mononuclear cells in a total volume of 2.0 ml of RPMI 1640 medium containing 10% (vol/vol) fetal calf serum and IL-2 (32 units/ml). The p24 antigen content of the culture supernatant was then measured by using the HIV-1 p24 core profile ELISA assay (DuPont/NEN). The lowest number of added mononuclear cells that infected at least half of the quadruplicate cultures with HIV-1 was taken as the end point or TCID and the data are presented as the number of TCID units per 10^6 mononuclear cells.

**Statistical Analysis of the Data.** The 2 × 2 Fisher exact test and the Fisher’s exact test were used to assess the statistical significance of the data.

**RESULTS**

The Peripheral Blood of These Modified thy/liv-SCID-hu Mice Is Populated with Significant Numbers of Human T Cells and Monocytes. We have shown (6, 7) that by increasing the quantity of tissue implanted in the SCID mice to at least 1-mm^3 pieces of hu-thy/liv tissue and by implanting this amount of tissue under both kidney capsules, we could markedly enhance the peripheral engraftment of thy/liv-SCID-hu mice with human T cells. The peripheral blood of the thy/liv-SCID-hu mice (n = 26) constructed in this fashion was populated 3 months later with 13 ± 2.5% human CD4+ T cells and 3.2 ± 0.4% human CD8+ T cells (mean ± SEM). In addition to providing a source of pre-T cells capable of migrating into and maturing in the adjacent human thymic tissue, the implanted human fetal liver also contains stem/precursor cells that are capable of maturing into the myeloid lineage (9). Therefore, to determine whether myeloid cells differentiating in the implanted fetal liver were capable of populating the peripheral blood of our modified thy/liv-SCID-hu mice with human monocytes, PBMCs of these mice were evaluated for the expression of human CD45 and the monocyte-associated markers CD14 and CD11b. A distinct population of human CD45+ CD14+ and CD45+ CD11b+ monocytes was present in the peripheral blood of the thy/liv-SCID-hu mice (Fig. 1). In eight thy/liv-SCID-hu mice examined, the peripheral blood monocyte gate contained 2.6 ± 1.7% and 3.1 ± 1.9% human CD45+ CD14+ and CD14+ CD11b+ cells, respectively. Thus, the peripheral blood of our modified thy/liv-SCID-hu mice was populated with significant numbers of human T cells and monocytes.

**IL-10 Treatment Inhibits in Vivo HIV Infection.** The results of in vivo studies have indicated a dichotomy between the effects of different cytokines on HIV replication, with IL-10 reported to inhibit HIV replication in monocytes (10, 11) and IL-12 shown to enhance HIV infection in PBMCs (12). Therefore, we used the thy/liv-SCID-hu mice to investigate the effect of IL-10 or IL-12 treatment on the in vivo course of infection after inoculation with a primary patient isolate of HIV, HIV-1S9, previously characterized as being able to infect primary monocytes and lymphocytes and being nonsyncytium inducing (7). To determine whether cytokine treatment could
Immunology: thy/liv-SCID-hu

Whereas plasma viremia was detected in 7 of 10 thy/liv-SCID-hu mice treated with PBS, and 2 of 2 thy/liv-SCID-hu mice treated with IL-12, plasma viremia was detected in none of the 6 thy/liv-SCID-hu mice treated with IL-10 (P = 0.011). IL-10 treatment also markedly decreased the extent of HIV infection detected in the hu-thy/liv implants of the thy/liv-SCID-hu mice 2 days after the last dose. In the PBS-treated i.p. inoculated thy/liv-SCID-hu mice, all of the hu-thy/liv implants were significantly infected with HIV with a mean of 2454 TCID units per 10^6 mononuclear cells (n = 4) compared to the IL-10-treated i.p. injected thy/liv-SCID-hu mice, where no HIV infection was detected in hu-thy/liv implants from three mice and only minimal HIV infection (5 TCID units per 10^6 mononuclear cells) was detected in the hu-thy/liv implants of the fourth mouse. Similar results were observed for intra-implant inoculated thy/liv-SCID-hu mice, where markedly less viral load was present in the thy/liv implants of IL-10-treated mice (mean = 475 TCID units per 10^6 mononuclear cells; n = 2) compared to the PBS-treated mice (mean = 28,601 TCID units per 10^6 mononuclear cells; n = 6).

IL-10 also inhibited HIV infection when treatment was started a short time after inoculation. The thy/liv-SCID-hu mice were infected with HIV by intrateatment inoculation and

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**Fig. 1.** Detection of human monocytes in the peripheral blood of the thy/liv-SCID-hu mice. Mononuclear cells were isolated from the blood of thy/liv-SCID-hu mice at least 3 months after implantation, cells in the monocyte gate were analyzed by three-color flow cytometry for the expression of human CD45, CD14, and CD11b. A representative dot histogram is shown with the percentages of cells in each quadrant indicated.

**Fig. 2.** Detection of HIV RNA in the plasma of PBS-, IL-10-, and IL-12-treated thy/liv-SCID-hu mice after inoculation with HIV. (A) The thy/liv-SCID-hu mice were pretreated on alternate days for two doses with PBS (n = 4) or IL-10 (n = 4), injected i.p. with HIV-1s9 (8000 TCID50 units), and then treated on alternate days for seven doses with PBS or IL-10. (B) After thy/liv-SCID-hu mice were pretreated on alternate days for two doses with PBS (n = 6), IL-12 (n = 2), or IL-10 (n = 2), HIV-1s9 (400 TCID50 units) was injected into the hu-thy/liv implant, and then the mice were treated on alternate days for seven doses with PBS, IL-12, or IL-10. (C) Eleven days after HIV-1s9 (300 TCID50 units) was injected into the hu-thy/liv implants of the thy/liv-SCID-hu mice, the mice were treated with PBS (n = 3), IL-10 (n = 2), or IL-12 (n = 2) on alternate days for seven doses. (D) Six weeks after HIV-1s9 (8000 TCID50 units) was injected i.p. into thy/liv-SCID-hu mice, the mice were treated with PBS (n = 3), IL-10 (n = 3), or IL-12 (n = 2) on alternate days for seven doses. Two days after the last dose of cytokine, the presence of HIV in the plasma of the thy/liv-SCID-hu mice was then assessed.
11 days later the mice were started on treatment with alternate day injections of IL-10 (10 μg per dose), IL-12 (10 μg per dose), or PBS for a total of seven doses. HIV was detected in the plasma of two of three thy/liv-SCID-hu mice treated with PBS, two of two thy/liv-SCID-hu mice treated with IL-12, and in none of the two thy/liv-SCID-hu mice treated with IL-10 (Fig. 2C). Furthermore, while the hu-thy/liv implants of the PBS-treated thy/liv-SCID-hu mice contained a mean of 4191 TCID units per 10^6 mononuclear cells, the hu-thy/liv implants of the IL-10-treated thy/liv-SCID-hu mice contained a mean of 375 TCID units per 10^6 mononuclear cells. However, when treatment of the thy/liv-SCID-hu mice was started 6 weeks after infection with HIV-1sF162, HIV viremia was detected in all of the PBS (n = 3), IL-12 (n = 2), and IL-10 (n = 3)-treated thy/liv-SCID-hu mice (Fig. 2D). Significant infection in the thy/liv implant of the IL-10-treated mice was seen (mean = 14,559 TCID units per 10^6 mononuclear cells) that was comparable to that observed in the PBS-treated mice (mean = 28,944 TCID units per 10^6 mononuclear cells). This indicated that a short course of IL-10 treatment at the doses used was effective in inhibiting HIV infection only when given before or soon after inoculation.

**Effect of IL-10 and IL-12 Treatment of hu-thy/liv-SCID-hu Mice on Human Interferon γ (IFN-γ) Gene Expression.** The presence of significant numbers of human mononuclear cells in the peripheral blood of our thy/liv-SCID-hu mice combined with the development of primer pairs specific for human cytokine gene sequences permitted us to assess the in vivo expression of human cytokine genes by the human PBMCs circulating in these thy/liv-SCID-hu mice (6, 8). After isolation of PBMCs from the peripheral blood of five thy/liv-SCID-hu mice, RNA was extracted and evaluated with human-specific RT–PCR for the expression of mRNA encoding TH1-associated cytokines IFN-γ, IL-2 and tumor necrosis factor α (TNF-α) and TH2-associated cytokines IL-4, IL-5, IL-6, and IL-10 (13). While mRNA for human TNF-α, IFN-γ, IL-5, and IL-2 was detected in five of five, three of five, three of five, and one of five thy/liv-SCID-hu mice, respectively, mRNA expression for IL-4, IL-6, and IL-10 was not detected. No correlation between expression of human cytokine genes and the number of human PBMCs in the peripheral blood was observed. Detection of human IFN-γ mRNA in the PBMCs of only some of the thy/liv-SCID-hu mice allowed us to divide the thy/liv-SCID-hu mice into two groups, one group of mice having circulating human PBMCs that expressed human IFN-γ mRNA and the other group populated with human PBMCs that did not express human IFN-γ mRNA. We took advantage of the fact that in vitro expression of the human IFN-γ gene is regulated by IL-10 and IL-12 (14) to determine, by using qualitative RT–PCR, whether cytokine gene expression in the human PBMCs in our modified thy/liv-SCID-hu mice was responsive to in vitro regulation. The thy/liv-SCID-hu mice were treated daily for 4 days with i.p. injection of PBS, IL-10 (10 μg per dose), IL-12 (10 μg per dose), and the human IFN-γ gene expression of their PBMCs before and after treatment was evaluated. Whereas human IFN-γ mRNA expression remained negative after PBS injection in the PBMCs from seven of eight thy/liv-SCID-hu mice, treatment with human IL-12 stimulated human IFN-γ mRNA expression in the PBMCs from five of five thy/liv-SCID-hu mice (P = 0.005). In contrast, the PBMCs from seven of seven thy/liv-SCID-hu mice that initially expressed human IFN-γ mRNA became negative after treatment with IL-10, as opposed to the PBMCs from one of eight thy/liv-SCID-hu mice injected with PBS (P = 0.005). A representative gel is shown in Fig. 3 demonstrating that human IFN-γ gene expression by human leukocytes present in the peripheral blood of four thy/liv-SCID-hu mice (mice 1–4) was not changed by treatment with PBS. In contrast, treatment with IL-10 suppressed the expression of human IFN-γ mRNA by the PBMCs of four thy/liv-SCID-hu mice.

**DISCUSSION**

Modifications that we introduced into the construction of thy/liv-SCID-hu mice, such as increasing the amount of hu-thy/liv implanted and implanting the hu-thy/liv under both kidney capsules, markedly increased the numbers of human T cells present in the peripheral blood, spleens, and lymph nodes of the implanted mice (6, 7). In the current report, flow cytometric data demonstrated that the peripheral blood of these modified thy/liv-SCID-hu mice was also populated with significant numbers of human monocytes. The presence of human monocytes in these modified thy/liv-SCID-hu mice was suggested by our observation (7) that these mice could be infected by i.p. inoculation with macrophage-tropic strains such as HIV-1ADA_M, HIV-1JR.Fl, and HIV-1SF162. The utility of these modified thy/liv-SCID-hu mice for investigating the pathogenesis of HIV infection is markedly increased by the presence of circulating human monocytes because these mice can be used to evaluate the in vivo effect of therapeutic interventions on HIV infection of monocytes as well as of T cells. This is important in examining the in vivo pathogenesis of HIV infection because macrophage-tropic HIV variants have been reported to initiate infection after sexual, parenteral, and vertical transmission and because of the role played by macrophages in HIV dissemination and persistence (15, 16).

It is likely that the source of HIV plasma viremia observed in these modified thy/liv-SCID-hu mice is from the high numbers of human T cells present in the mouse lymphoid tissue wherein we have detected a high level of HIV replication after inoculation with HIV (6). Because the population of HIV in the plasma fraction represents the most recently replicated
pool of virus (1-3), we could use the presence of HIV plasma viremia to evaluate the effect of cytokine therapy on HIV infection. In vitro studies have indicated that HIV replication is inhibited in monocytes by IL-10 (10, 11, 17-19) and is induced in PBMCs by IL-12 (12). Because the human PBMCs in these modified thy/liv-SCID-hu mice were responsive to exogenous human cytokines, we investigated the effect of treatment with human IL-10 and IL-12 on HIV infection in our modified thy/liv-SCID-hu mice. Although the data indicated that IL-10 inhibited HIV plasma viremia when given soon after infection, the number of mice examined was not large enough to draw a definitive conclusion. However, the observation that no HIV viremia was detected in 6 thy/liv-SCID-hu mice pretreated with IL-10 while HIV viremia was present in 7 of 10 untreated thy/liv-SCID-hu mice was statistically significant (P = 0.011). Furthermore, decreased HIV viral load was observed in the hu-thy/liv implants of the thy/liv-SCID-hu mice pretreated with IL-10 compared to the untreated thy/liv-SCID-hu mice. Thus, to our knowledge, these data are the first demonstration of the in vitro effects of cytokine therapy on HIV infection. Although we demonstrated that IL-10 suppressed IFN-γ mRNA production by human PBMCs in the thy/liv-SCID-hu mice, the inhibitory effects of IL-10 on HIV replication may be independent of its modulation of IFN-γ gene expression. IL-10 has been reported to inhibit in vitro HIV replication by different mechanisms. IL-10 may directly suppress HIV production either by inhibiting viral replication (18) or by interfering with HIV protein processing (19). Alternatively, IL-10 may indirectly suppress in vitro HIV replication by inhibiting the autocrine production of TNF-α and IL-6, cytokines reported to enhance HIV replication (10, 11). This is an intriguing explanation because we have reported that HIV infection stimulated in vivo expression of TNF-α by human PBMCs in our modified thy/liv-SCID-hu model.

Studies delineating the kinetics of HIV replication in HIV-infected individuals have suggested that T cells are the predominant source of plasma HIV (1-3). While in vitro studies have indicated that IL-10 had a significant inhibitory effect on HIV replication in monocytes, it did not have a significant suppressive effect on HIV production by T cells (17, 19). Therefore, it is likely that the IL-10-mediated reduction in plasma viremia and viral load in the hu-thy/liv implant is due to its effects on the acute infection. This was further indicated by the observation that IL-10 administration inhibited HIV production in our modified thy/liv-SCID-hu mice populated with significant numbers of human T cells and monocytes only when given early in the course of infection. This may provide insights into the pathophysiology of early HIV infection by indicating that while the major source of new virus in chronically HIV-infected individuals is from the T-cell population, the predominant location of viral replication and infection soon after infection is in the monocyte population. It is also possible that the initial HIV infection in the hu-thy/liv implant may occur in human monocytes present in the implant. Thus, these observations suggest that therapy aimed at blocking HIV replication in monocytes may be crucial to abort HIV infection soon after exposure. However, because the in vivo effect of IL-10 on HIV replication in T cells may differ from that observed in vitro with mitogen-stimulated T cells, we cannot rule out the possibility that IL-10 is inhibiting HIV replication in T cells in the thy/liv-SCID-hu mice. Although significant HIV infection occurred in the thy/liv-SCID-hu mice, no evidence of a human T-cell immune response directed against HIV was detected (7). Therefore, it is unlikely that IL-10 mediates inhibition of HIV infection in the thy/liv-SCID-hu mice by stimulating an anti-HIV response such as by inducing HIV-specific cytotoxic T cells. However, the absence of human immune responsiveness in these thy/liv-SCID-hu mice may limit their usefulness in determining the effect of treatment with IL-10 on the development of an HIV-specific immune response in HIV-exposed individuals. Although the thy/liv-SCID-hu model described here represents a significant advance over in vitro studies, results obtained using these mice should be interpreted with caution pending confirmation with human studies.

Because accessory cells are involved in T-cell regulation such as the IL-10- and IL-12-regulated control of IFN-γ production by T cells (14, 20), the presence of monocytes and T cells in the peripheral blood of these mice permitted us to investigate the in vivo regulation of cytokine gene expression by human T cells. We demonstrated that human IFN-γ mRNA expression was induced in circulating human PBMCs by the administration of IL-12 and was inhibited by treatment with IL-10. To our knowledge, this is the first demonstration of in vivo regulation of human IFN-γ gene expression by IL-10 and IL-12. The in vivo effects of these cytokines on IFN-γ mRNA expression by the human PBMCs in the thy/liv-SCID-hu mice are compatible with the in vitro capacity of IL-12 to induce IFN-γ production by resting human peripheral blood lymphocytes (21) and neonatal human T cells (22) and IL-10 to inhibit in vitro transcription of cytokine genes by human PBMCs (23). It is likely that signals provided by human monocytes present in our thy/liv-SCID-hu mice were facilitating the regulation of human IFN-γ mRNA because IL-10 indirectly suppresses IL-12 production by downregulating accessory-cell class II major histocompatibility complex expression, interfering with antigen-presenting-cell costimulatory signaling, and inhibiting the production of other cytokines (24). In addition, although IL-12 alone can stimulate IFN-γ production by T cells, IL-12 induction of IFN-γ is markedly enhanced by the costimulatory signal delivered through the interaction between CD28 on the surface of T cells and B7 present on monocytes (20). Although the inhibition of human IFN-γ gene expression by IL-10 may not be related to its ability to inhibit HIV replication, it indicated that human PBMCs in the thy/liv-SCID-hu mice were responsive to the effects of exogenous IL-10.

Therefore, our modified thy/liv-SCID-hu mice that are populated in the peripheral blood with significant numbers of human T cells and monocytes and that develop plasma viremia after infection with HIV should prove to be a valuable model for examining the role of immunomodulatory therapy in modifying HIV infection. Furthermore, since phase I trials to determine the toxicity of IL-10 in humans have been reported (25), our data suggest that further studies may be warranted to determine whether IL-10 treatment, when added to anti-HIV drug therapy, may provide potential benefits especially when given to individuals soon after exposure to HIV such as in needle-stick exposures or children born to HIV-infected mothers. In addition, the ability to sample the plasma population of HIV in these modified thy/liv-SCID-hu mice should permit their use in the study of the in vivo development of resistance to antiretroviral agents as reported in studies of HIV-infected individuals (1, 2).

We thank D. Gebhardt for assistance in the flow cytometry, A. Watford for assistance in animal care, T. C. Chang for performing the statistical analysis, and J. Berman for review of the manuscript. Flow cytometry was performed in the Flow Cytometry Core Facility and oligonucleotides were synthesized in the Oligonucleotide Synthesis Core Facility supported by the Cancer Center Grant 5P30CA13330. This work was supported by the National Institutes of Health (National Institute of Allergy and Infectious Diseases Centers for AIDS Research Grants AI-27741, AI-20671, and HE-53754). M.P-M. was supported in part by a grant (AIDS project) from the Istituto Superiore di Sanita; Ministero della Sanita’, Rome, Italy.

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153, 3751–3756.
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