Disseminated Human Immunodeficiency Virus 1 (HIV-1) Infection in SCID-hu Mice after Peripheral Inoculation with HIV-1

By Tobias R. Kollmann,* Massimo Pettoello-Mantovani,‡ Xiajun Zhuang,‡ Ana Kim,‡ Moshe Hachamovitch,§ Pam Smarnworawong,* Arye Rubinstein,*‡ and Harris Goldstein*‡

*From the Departments of *Microbiology and Immunology, ‡Pediatrics, and §Gynecology and Obstetrics, Albert Einstein College of Medicine, Bronx, New York 10461*

Summary

A small animal model that could be infected with human immunodeficiency virus 1 (HIV-1) after peripheral inoculation would greatly facilitate the study of the pathophysiology of acute HIV-1 infection. The utility of SCID mice implanted with human fetal thymus and liver (SCID-hu mice) for studying peripheral HIV-1 infection in vivo has been hampered by the requirement for direct intraimplant injection of HIV-1 and the continued restriction of the resultant HIV-1 infection to the human thymus and liver (hu-thy/liv) implant. This may have been due to the very low numbers of human T cells present in the SCID-hu mouse peripheral lymphoid compartment. Since the degree of the peripheral reconstitution of SCID-hu mice with human T cells may be a function of the hu-thy/liv implant size, we increased the quantity of hu-thy/liv tissue implanted under the renal capsule and implanted hu-thy/liv tissue under the capsules of both kidneys. This resulted in SCID-hu mice in which significant numbers of human T cells were detected in the peripheral blood, spleens, and lymph nodes. After intraimplant injection of HIV-1 into these modified SCID-hu mice, significant HIV-1 infection was detected by quantitative coculture not only in the hu-thy/liv implant, but also in the spleen and peripheral blood. This indicated that HIV-1 infection can spread from the thymus to the peripheral lymphoid compartment. More importantly, a similar degree of infection of the hu-thy/liv implant and peripheral lymphoid compartment occurred after peripheral intraperitoneal inoculation with HIV-1. Active viral replication was indicated by the detection of HIV-1 *gag* DNA, HIV-1 *gag* RNA, and spliced *tat/rev* RNA in the hu-thy/liv implants, peripheral blood mononuclear cells (PBMC), spleens, and lymph nodes of these HIV-1–infected SCID-hu mice. As a first step in using our modified SCID-hu mouse model to investigate the pathophysiological consequences of HIV-1 infection, the effect of HIV-1 infection on the expression of human cytokines shown to enhance HIV-1 replication was examined. Significantly more of the HIV-1–infected SCID-hu mice expressed mRNA for human tumor necrosis factors α and β, and interleukin 2 in their spleens, lymph nodes, and PBMC than did uninfected SCID-hu mice. This suggested that HIV-1 infection in vivo can stimulate the expression of cytokine mRNA by human T cells. Our modified SCID-hu mice may provide an improved model for studying the pathophysiology of HIV-1 infection in vivo and for investigating the effects of anti-HIV interventions on the prevention of disseminated HIV-1 infection.

After exposure of an individual to an inoculum of HIV-1, an infectious cycle is initiated that leads to systemic dissemination of HIV-1 and HIV-1–infected cells into lymphoid organs (1). The subsequent disease course may be determined by the sites to which HIV-1 is seeded during the acute stage of infection (1, 2). The generation of immune responses directed against HIV-1 may result in a prolonged period of clinical latency (2). However, a quiescent state of HIV-1 disease in the peripheral blood is often accompanied by ongoing active and progressive HIV-1 infection in lymphoid organs (3–5). Thus, examination of the peripheral blood of HIV-1–infected individuals does not detect the high degree of covert HIV-1 replication taking place in lymphoid tissues (6). Investigation of the pathophysiology of acute HIV-1 infection

J. Exp. Med. © The Rockefeller University Press • 0022-1007/94/02/0513/10 $2.00
Volume 179 February 1994 513–522

in humans is therefore restricted by the limited sampling of lymphoid organs during this stage. SCID mice implanted with human fetal thymus and liver (SCID-hu mice)[1] are an attractive small animal model for studying in vivo HIV-1 infection (7). However, so far, HIV-1 infection of the human fetal thymus and liver (hu-thy/liv) implanted in these mice has only been reported to occur after direct intraimplant injection of HIV-1 and the resultant HIV-1 infection was restricted to the hu-thy/liv implant (8). In this report we describe a modified SCID-hu mouse model in which disseminated HIV-1 infection occurs in the peripheral mouse lymphoid organs and the hu-thy/liv implant after peripheral inoculation with HIV-1.

Materials and Methods

Implantation of Human Thymic and Liver Tissue Into SCID Mice. Human fetal thymic and liver tissue were obtained from 17–21 gestational week fetuses after the elective termination of pregnancy and implanted into SCID mice (6–8-wk-old) within 8 h of availability as described (9). Briefly, the fetal thymus was cut into pieces (~0.3 cm^3) along the grossly visible lines of thymic lobules and the fetal liver was cut into pieces (~0.5 cm^3) and kept in ice-cold PBS until implantation. While kept on ice, the human fetal thymus and liver pieces described above were cut into 1-mm^3 pieces and then approximately 10 pieces of each were loaded into a 16-gauge needle with a rounded tip. The SCID mice were anesthetized with Pentobarbital (40–80 mg/kg), the left and right kidneys were sequentially exteriorized, a 0.5-mm incision was made in each kidney capsule, and the 10 pieces of thymus and liver tissue obtained from the same donor were implanted underneath the capsule using the 16-gauge needle. After surgery, minimal morbidity and mortality was observed. 3 mo after implantation, >95% of the grafts took and increased in size to >5 × 10 × 5 mm. The histological appearance of the graft resembled that of normal human thymus and the expected thymocyte subpopulations were observed by flow cytometry as described (9). All tissue used came from HIV-1 seronegative donors. 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, spleens, and LN of the SCID-hu mice and stained with PE-, FITC, or peridinin chlorophyll protein-conjugated mouse mAb to human CD4 (Leu 3a, Becton Dickinson, Mountain View, CA), human CD8 (Leu 2a, Becton Dickinson), human CD3 (Leu 4, Becton Dickinson), or human CD45 as described (9). TCR V gene expression was analyzed by staining mononuclear cells with PerCP-conjugated mouse mAb to human CD4 (Leu 3a), PE-conjugated mouse mAb to human CD8 (Leu 2a), and FITC-conjugated mouse mAb to either TCR Vβ2, Vβ5a, Vβ5b, Vβ5c, Vβ6, Vβ8, Vβ12, Vβ19, or Vα2 (T Cell Diagnostics, Cambridge, MA). Expression of human CD45, CD3, CD4, or CD8 or human CD4, CD8, and TCR V genes by lymphocytes present in the SCID-hu mice were then assessed by three-color flow cytometric analysis using a FACScan® cell analyzer with LYSIS-II software (Becton Dickinson). Nonviable cells and unlysed RBC were gated out based on their forward and side scatter profiles, and lymphocyte gates were set on the basis of forward and side scatter profiles to correspond to gates set to control human (from healthy adult volunteer) lymphocytes. Cut-off values for the quadrants were set after compensation for PE vs. FITC vs. PerCP emission based on the analysis of 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.

Infection of SCID-hu Mice with HIV-1. The patient isolate of HIV-1 used in this study, HIV-1_{28}, was obtained after coculture of PBMC isolated from a 2-yr-old HIV-1–infected child with PHA-activated donor PBMC as described (10). The initial coculture supernatant was harvested and cocultured with PHA-activated PBMC to expand the quantity of HIV-1_{28}. The secondary coculture supernatant was harvested and aliquots were frozen in liquid nitrogen. The tissue culture infective $dose_{50}$ ($TCID_{50}$) of the supernatant was determined by culturing titered dilutions of a thawed aliquot with PHA-activated donor PBMC (10^6) in a total volume of 2.0 ml of RPMI 1640 with FCS (10% vol/vol) and IL-2 (32 U/ml). After 2 wk of culture, the p24 antigen content of the culture supernatant was measured by using the HIV-1 p24 core profile ELISA assay (Dupont-NEN, Wilmington, DE). The lowest dilution of supernatant that infected at least half of the quadruplicate cultures with HIV-1 was taken as the end point or $TCID_{50}$. SCID-hu mice were infected either by direct injection of 300 $TCID_{50}$ of HIV-1_{28} in a volume of 30 μl into one hu-thy/liv implant or by intraperitoneal injection of 8,000 or 800 $TCID_{50}$ of HIV-1_{28} in a volume of 800 μl.

HIV Viral Culture. The titer of HIV-1–infected mononuclear cells present in the peripheral blood, spleens, thymic implants, and LN of the SCID-hu mice, was determined as described (11, 12). Fivefold dilutions of PBMC ranging from 10^6 cells to 3.2×10^2 were cultured at 37°C in quadruplicate cultures in 24-well culture plates with PHA-activated donor mononuclear cells (10^6) in a total volume of 2.0 ml of RPMI 1640 with added FCS (10% vol/vol) and IL-2 (32 U/ml). After 1–2 wk of culture, the p24 antigen content of the culture supernatant was measured as described above. The lowest number of added PBMC 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 TCID/10^6 PBMC (11).

HIV-1–specific DNA and RNA PCR. The presence of HIV-1 DNA and RNA *gag*–encoded sequences and spliced *tat/rev* mRNA sequences were assessed by PCR as described (13, 14). Briefly, mononuclear cells from the SCID-hu mice were lysed in guanidine isothiocyanate (4 M) buffer, cellular DNA and RNA were separated by cesium chloride (5.7 M) density gradient centrifugation and precipitated with ethanol. For DNA PCR, the HIV-1 DNA (1 μg) was amplified for 35 cycles with a primer pair specific for the *gag* gene segment (SK38/39), electrophoresed through 1.5% NuSieve/0.5% SeaKem agarose (FMC Corp. BioProducts, Rockland, ME) gel containing ethidium bromide, and the amplified product was detected under ultraviolet light. HIV-1 RNA was detected by PCR amplification of reverse transcribed RNA (RT-PCR) as described (13). Briefly, after treatment of the cellular RNA with RNase-free DNase (Boehringer Mannheim, Indianapolis, IN), RNA (7 μg) in 7 μl of ddH_2O was mixed with 4 μl of 5× buffer (250 mM Tris-HCl, pH 8.3/375 mM KCl/15 mM $MgCl_2$), 2 μl dithiothreitol (100 mM), 1 μl of random hexamers (GIBCO BRL, Gaithersburg, MD) and 5 μl mixed dNTPs (2 mM each). Samples were mixed, heated to 65°C for 10 min, cooled on ice for 5 min and then 1 μl (200 U) of Superscript reverse transcriptase (GIBCO BRL) was added. This final reaction mixture was vortexed, briefly spun down, incubated at 37°C for 60 min, and then placed on ice. HIV-1 cDNA was amplified either with SK38/39 or a primer

[1] *Abbreviations used in this paper:* β_2-MG, β_2-microglobulin; hu-thy/liv, human fetal thymus and liver; SCID-hu, SCID mice implanted with human fetal thymus and liver.

pair specific for *tat/rev*–spliced mRNA sequences (TR-5/TR-3). Specificity of the amplified product was confirmed by hybridization of a Southern blot of the amplified DNA and cDNA with a γ-[^{32}P]ATP–labeled internal probe specific for the SK38/39 product (SK19) or the TR-5/TR-3 product (TR-4). A given sample was regarded as positive if PCR amplification resulted in a DNA product of the predicted size that hybridized to the specific internal probe. Positive and negative controls were included in all runs and, to prevent contamination, suggested guidelines for PCR quality control were followed (15). For RT-PCR, the absence of residual DNA template was verified by the absence of an amplified product after PCR amplification of DNase-treated samples that had not been reverse transcribed.

Detection of Human Cytokine Gene Expression by RT-PCR. The pattern of in vivo human cytokine expression of the human cells present in SCID-hu mice was assessed by using a modification of a previously described technique (16). To ensure that the PCR amplification product was of human origin, we designed the primers so that the nucleotide sequence of the 3' end was complementary to a human cytokine cDNA sequence that was not present on the mouse cytokine cDNA. Derivation of the PCR product from mRNA was insured by designing primer pairs that yielded an amplification product that spanned exon-exon junctions. After reverse transcription of total RNA (7 μg) extracted from the hu-thy/liv implant, PBMC, spleen, and LN of the 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 presence of an amplification product of the predicted size after fractionation of the PCR products by electrophoresis and ethidium bromide staining. The primer pairs for each cytokine were selected from published DNA sequences based on previously described guidelines (17). The nucleotide sequences for 5' and 3' primers respectively were: β_2-microglobulin (β2-MG), TCTGGCCTTGAGGCTATCCAGCGT and GTGGTTCACACGGCAGGCATACTC; IL-2, CATTGCACTAAGTCTTGCACTTGCACTTGTCACA and ATTGCTGATTAAGTCCCTGGGTCTT; IL-4, CTCACAGAGCAGAAGACTCTGTGC and AAGCCCGCCAGGCCCCAGAGGTTCCT; IL-6, TACATCCTCGACGGCATCTCAGCCC and CTGGTTCTGTGCCTGCAGCTTCGTCAGC; TNF-α, CGCTCCCCAAGAAGACAGGGGGCC and GATGGCAGAGAGGAGGTTGACCTTGGT; TNF-β, CCCAGGGGCTCCCTGGTGTTG and GTGGGTGGATAGCTGGTCTCCCTGGG. The amplification product for each primer pair was confirmed by demonstrating hybridization of an internal probe to the predicted PCR amplification product after Southern blotting. Human specificity for each primer pair was verified by demonstrating that amplification of the predicted product did not occur after RT-PCR of RNA extracted from mouse mitogen–activated mononuclear cells. All samples were analyzed by RT-PCR for the presence of mouse or human β2-microglobulin to verify the integrity of the sample mRNA and the efficiency of subsequent reverse transcription. Positive and negative controls were included in all runs and suggested guidelines for PCR quality control were followed as described above.

Results

Peripheral Reconstitution of SCID-hu Mice with Human T Cells. We had previously observed a correlation between the number of human T cells present in the peripheral blood of SCID-hu mice and the degree of growth of the implanted hu-thy/liv. Therefore, in order to increase the number of human T cells present in the periphery of SCID-hu mice, we increased the quantity of hu-thy/liv tissue implanted under the renal capsule and implanted hu-thy/liv tissue under the capsules of both kidneys. 3 mo after implantation, the number of human T cells present in the peripheral lymphoid compartment of SCID-hu mice constructed in this fashion was evaluated by three-color flow cytometric analysis of lymphocytes in the peripheral blood, LN, and spleens for the expression of human CD45, CD3, CD4, and CD8 and representative two-dimensional dot histograms are shown in Fig. 1. Examination of human CD4 and CD8 expression by lymphocytes positive for human CD45 in SCID-hu mice constructed in this fashion revealed that in the peripheral blood (n = 25), 4.06 ± 0.49% were CD4$^+$ and 1.34 ± 0.20% were CD8$^+$, in the spleen (n = 7), 4.91 ± 2.04% were CD4$^+$, and 2.93 ± 1.21% were CD8$^+$, and in the LN (n = 7), 8.64 ± 3.93% were CD4$^+$ and 3.47 ± 1.38% were CD8$^+$. To determine whether human T cells were present in the peritoneal cavity of these SCID-hu mice, peri-

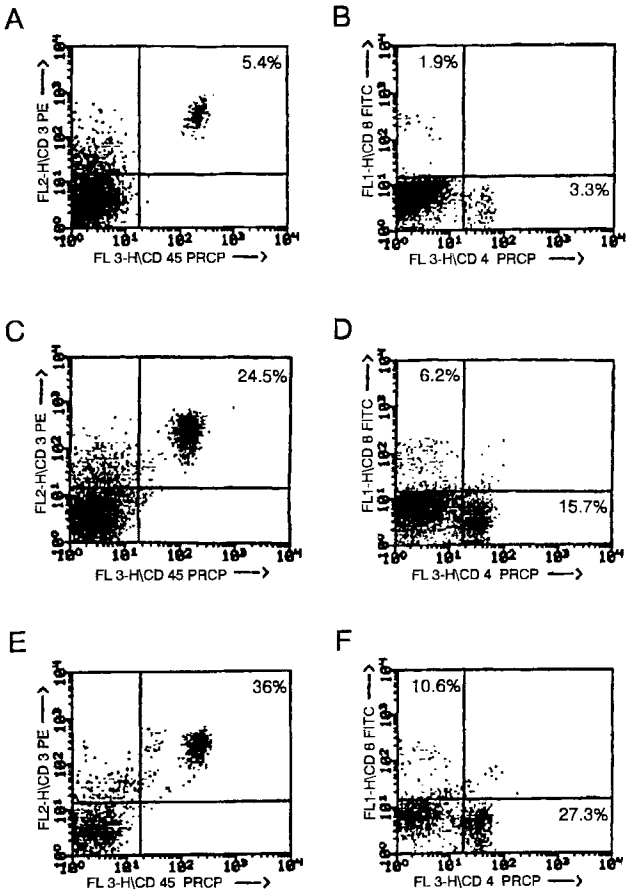

Figure 1. Detection of human T cells in the periphery of SCID-hu mice. Lymphocytes isolated from the peripheral blood (*A* and *B*), spleen (*C* and *D*), and LN (*E* and *F*) of SCID-hu mice were analyzed by three-color flow cytometry for the expression of human CD45 and CD3 (*A*, *C*, and *E*) and CD4 and CD8 (*B*, *D*, and *F*), as described in Materials and Methods. The percentages of cells in each quadrant are indicated.

toneal exudate cells were harvested by peritoneal lavage with cold PBS, assessed for the expression of human CD45, CD4, and CD8, and representative two-dimensional dot histograms are shown in Fig. 2. As determined by three-color flow cytometric analysis of peritoneal exudate cells, 4.13 ± 3.84% of the peritoneal exudate cells in SCID-hu mice (n = 3) were human $CD45^+CD4^+$ lymphocytes and 1.24 ± 1.08% were human $CD45^+CD8^+$ lymphocytes. The diversity of the human T cells present in the periphery of SCID-hu mice was assessed by examining their expression of TCR Vβ genes after staining with a panel of TCR Vβ-specific mAb that recognize about 30% of the peripheral human T cell population. As shown in Figure 3, a diverse population of human TCR Vβ subsets were observed in the periphery of SCID-hu mice. Thus, construction of SCID-hu mice in this fashion results in the significant reconstitution of the peripheral lymphoid compartment with a broad spectrum of human T cells.

Infection of the Peripheral Lymphoid Compartment of SCID-hu Mice with HIV-1. After direct inoculation of the hu-thy/liv implant with HIV-1, the SCID-hu mice were assessed for disseminated HIV-1 infection. Since hu-thy/liv was implanted in each kidney capsule of these SCID-hu mice, we could assess whether HIV-1 directly injected into the hu-thy/liv implanted in one kidney capsule could be systemically disseminated and infect the other hu-thy/liv implanted in the opposite kidney capsule. HIV-1 could be isolated by coculture of thymocytes from both the hu-thy/liv implants, and the splenocytes of SCID-hu mice 1 mo after direct unilateral HIV-1 inoculation into hu-thy/liv implants. The degree of HIV-1 infection present in the HIV-1–injected hu-thy/liv implant, the uninjected hu-thy/liv implant in the opposite kidney, the spleen, and PBMC was determined by quantitative coculture (Table 1). HIV-1 was isolated from as few as 320 thymocytes in all the injected hu-thy/liv implants, indicating the presence of over 3,125 TCID/10^6 cells. A variable range of disseminated HIV-1 infection was observed with over 3,125 TCID/10^6 cells in two of three uninjected hu-thy/liv implants and from 2 to 333 TCID/10^6 cells in the spleens of five SCID-hu mice. In addition, HIV-1 was cocultured from

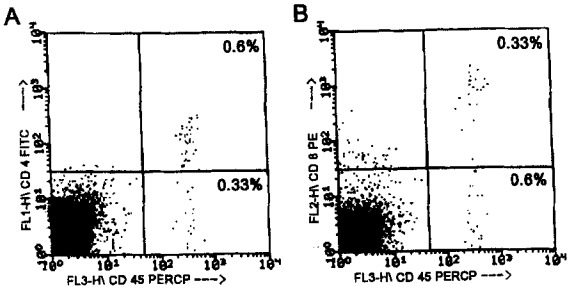

Figure 2. Detection of human T cells in the peritoneal cavity of SCID-hu mice. Mononuclear cells isolated by peritoneal lavage from the peritoneal cavities of SCID-hu mice were analyzed by three-color flow cytometry for the expression of (*A*) human CD45 and CD4 and (*B*) CD45 and CD8, as described in Materials and Methods. The percentages of cells in each quadrant are indicated.

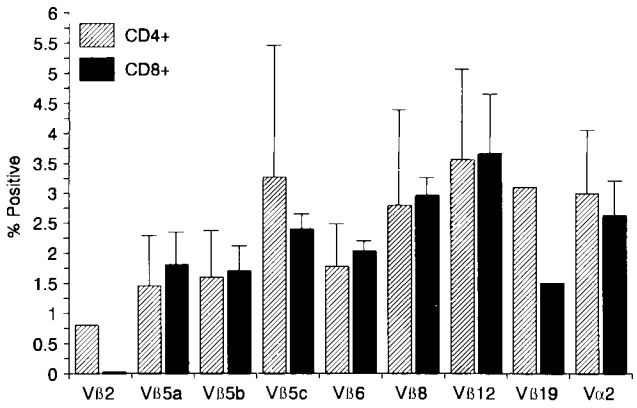

Figure 3. Analysis of TCR Vβ subsets in the periphery of SCID-hu mice. Pooled lymphocytes isolated from the peripheral blood, spleen, and LN of SCID-hu mice were analyzed by three-color flow cytometry for the expression of human CD4, CD8, and the indicated TCR Vβ gene. The mean percentage (±STE) of human T cells in SCID-hu mice (n = 3) expressing CD4 or CD8 and Vβ5a, Vβ5b, Vβ5c, Vβ6, Vβ8, Vβ12, or Vα2, and the percentage of human T cells in a SCID-hu mouse (n = 1) expressing CD4 or CD8 and Vβ2 or Vβ19, are indicated.

PBMC obtained from the peripheral blood of three of these intraimplant-injected SCID-hu mice. Thus, T cells that become infected with HIV-1 in the hu-thy/liv implant can induce disseminated HIV-1 infection of SCID-hu mice that were constructed as described above.

To examine the capacity of HIV-1 to infect SCID-hu mice by other routes, HIV-1 was inoculated into the peritoneal cavity of SCID-hu mice. 1 mo after HIV-1 inoculation, HIV-1 was isolated by coculture from the hu-thy/liv implants of five of five SCID-hu mice injected with 8,000 $TCID_{50}$, one of two SCID-hu mice injected with 800 $TCID_{50}$, and zero of two SCID-hu mice injected with 80 $TCID_{50}$. We confirmed that the HIV-1 isolated was not residual virus from the initial inoculation by demonstrating that no HIV-1 was isolated by coculture from the spleens of unimplanted SCID mice 1 mo after injection with 8,000 $TCID_{50}$ of $HIV\text{-}1_{28}$. Assessment of the extent of HIV-1 infection by quantitative coculture indicated that the HIV-1 infection in the hu-thy/liv implant ranged from 625 to >3,125 TCID/10^6 cells (Table 2), comparable to that which occurred after intraimplant infection of SCID-hu mice. HIV-1 infection ranging from >0.7 to 25 TCID/10^6 cells was also detected in four of five spleens from the inoculated SCID-hu mice. In addition, the peripheral blood of two SCID-hu mice assessed contained >2 and >5 TCID/10^6 cells. Thus, after peripheral inoculation with HIV-1, HIV-1–infected cells or HIV-1 can migrate from the periphery into the hu-thy/liv implant and infect human T cells present in the implant.

Detection of HIV-1 DNA and RNA in HIV-1–infected SCID-hu Mice. The presence of HIV-1 DNA and RNA in the hu-thy/liv implants, and the peripheral lymphoid compartment of the SCID-hu mice infected by inoculation of HIV-1 either into the hu-thy/liv implant or peritoneal cavity were assessed by PCR. HIV-1 *gag* DNA was detected by SK38/39-primed PCR amplification in the hu-thy/liv im-

Table 1. *Infection of SCID-hu Mice after Inoculation of the Hu-thy/liv Implant with HIV-1*

Mouse	HIV-1 titer (TCID/10^6 cells)			
	Injected implant	Uninjected implant	Spleen	Blood
T1	>3,125	ND*	5	ND
T2	>3,125	ND	>2	ND
T3	>3,125	>3,125	333	>2
T4	>3,125	0	2	>1
T5	>3,125	>3,125	>2	>1

3 mo after implantation under the renal capsule of SCID mice, a hu-thy/liv implant in five SCID-hu mice was injected with 300 $TCID_{50}$ of $HIV\text{-}1_{28}$. 1 mo later, the mice were killed, mononuclear cells were isolated from the injected hu-thy/liv, the uninjected hu-thy/liv implanted in the opposite kidney, the spleen, and the peripheral blood, extensively washed and, if sufficient cells were available, quantitative coculture of mononuclear cells with PHA-activated PBMC (10^6) was performed. After 7 d of culture, an aliquot of the supernatant was harvested and assessed for the presence of p24 antigen. The coculture was considered positive if >100 pg/ml of p24 antigen was detected in the supernatant. The data are presented as TCID/10^6 mononuclear cells and ">" indicates that the coculture was positive for the lowest number of cells added.
* Not done.

plant, PBMC, spleen, and LN of SCID-hu mice infected with HIV-1 either by intraimplant injection (Fig. 4 *A*) or by intraperitoneal inoculation (Fig. 4 *B*). In addition, HIV-1 *gag* RNA was detected by SK38/39–primed RT-PCR in the hu-thy/liv implant, PBMC, spleen, and LN of SCID-hu mice infected with HIV-1 either by intraimplant injection (Fig. 4 *C*) or by intraperitoneal inoculation (Fig. 4 *D*). To determine whether ongoing viral replication was occurring in the HIV-1–infected SCID-hu mice, the expression of *tat/rev* spliced mRNA was examined. The presence of *tat/rev* mRNA was detected by RT-PCR in the hu-thy/liv implant, PBMC, spleen, and LN of SCID-hu mice infected with HIV-1 either by intraimplant injection (Fig. 4 *E*) or by intraperitoneal inoculation (Fig. 4 *F*). Fig. 5 shows the results of assessment for the presence of *gag* DNA, *gag* RNA, and *tat/rev* RNA in five SCID-hu mice infected by inoculation of HIV-1 into the hu-thy/liv implant (Fig. 5 *A*) and six SCID-hu mice infected by injection of HIV-1 into the peritoneal cavity (Fig. 5 *B*). No HIV-1 DNA or cDNA was detected in SCID-hu mice that had not been infected with HIV-1 (data not shown). We confirmed that the HIV-1 DNA and cDNA detected was not from the initial inoculation by demonstrating that no

Table 2. *HIV-1 Infection of SCID-hu Mice after Intraperitoneal Inoculation with HIV-1*

SCID-hu mouse	HIV-1 titer (TCID/10^6 cells)		
	Implant	Spleen	Blood
P1	>3,125	0	ND*
P2	>3,125	25	>5
P3	>3,125	>0.7	ND
P4	625	>0.7	ND
P5	>3,125	>2	>2

1 mo after intraperitoneal inoculation of SCID-hu mice with 8.0×10^4 $TCID_{50}$ (*P1*, *P2*, *P4*, and *P5*) or 8.0×10^3 $TCID_{50}$ (*P3*) of $HIV\text{-}1_{28}$, the mice were killed, mononuclear cells were isolated from the hu-thy/liv implant, the spleen, and the peripheral blood, extensively washed and, if sufficient cells were available, quantitative coculture of the mononuclear cells with PHA-activated PBMC (10^6) was performed. After 7 d of culture, an aliquot of the supernatant was harvested and assessed for the presence of p24 antigen. The coculture was considered positive if >100 pg/ml of p24 antigen was detected in the supernatant. The data are presented as TCID/10^6 mononuclear cells and ">" indicates that the coculture was positive for the lowest number of added cells.
* Not done.

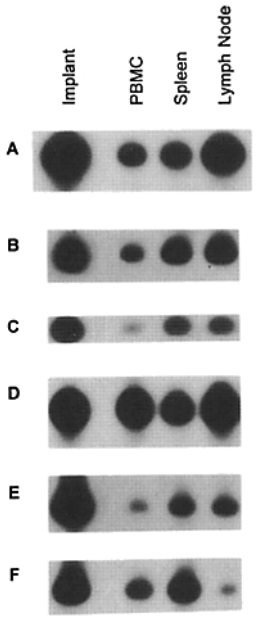

Figure 4. Detection of *gag* DNA and RNA and *tat/rev* RNA in the periphery of HIV-1–infected SCID-hu mice. The presence of *gag* DNA (*A* and *B*), *gag* RNA (*C* and *D*), or *tat/rev* RNA (*E* and *F*) in the hu-thy/liv implants, PBMC, spleens, or LN of SCID-hu mice infected with HIV-1 by intraimplant injection (*A*, *C*, and *E*) or intraperitoneal injection (*B*, *D*, and *F*) was detected by Southern blotting of PCR amplified DNA or cDNA as described in Materials and Methods. The blots shown are representative of data presented in Fig. 5

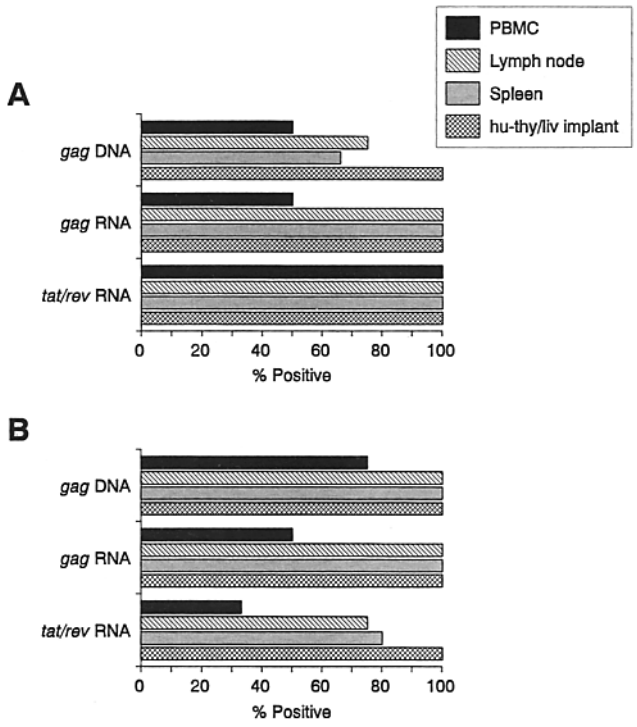

Figure 5. *gag* DNA and RNA and *tat/rev* RNA are detected in the periphery of HIV-1–infected SCID-hu mice. (*A*) After intraimplant injection with HIV-1_{28}, five SCID-hu mice were assessed for the presence of *gag* DNA in the PBMC (n = 4), LN (n = 4), spleens (n = 4), and hu-thy/liv implants (n = 5); *gag* RNA in the PBMC (n = 4), LN (n = 4), spleens (n = 4), and hu-thy/liv implants (n = 5); and *tat/rev* RNA in the PBMC (n = 3), LN (n = 3), spleens (n = 4), hu-thy/liv implants (n = 5) by Southern blotting of PCR amplified DNA or cDNA. (*B*) After intraperitoneal injection with HIV-1_{28}, six SCID-hu mice were assessed for the presence of *gag* DNA in the PBMC (n = 4), LN (n = 4), spleens (n = 4), and hu-thy/liv implants (n = 5); *gag* RNA in the PBMC (n = 2), LN (n = 4), spleens (n = 6), and hu-thy/liv implants (n = 6); and *tat/rev* RNA in the PBMC (n = 3), LN (n = 4), spleens (n = 5), hu-thy/liv implants (n = 5) by Southern blotting of PCR amplified DNA or cDNA. The data are presented as the percent SCID-hu mice wherein the expression of the indicated target DNA or RNA was detected.

HIV-1 DNA and cDNA was detected in the spleens of unimplanted SCID mice 1 mo after injection with 8,000 $TCID_{50}$ of HIV-1_{28} (data not shown). Thus, productive infection with HIV-1 and active viral replication was occurring in the periphery of SCID-hu mice infected by inoculation of HIV-1 either into the hu-thy/liv implant or into the peritoneal cavity.

Since human $CD4^+$ T cells were present in the peritoneal cavity of these SCID-hu mice, we examined whether they became infected with HIV-1 after intraperitoneal injection. After intraperitoneal injection of HIV-1_{28} (8,000 $TCID_{50}$), peritoneal exudate cells were harvested, extensively washed, and their DNA was extracted. As shown in Fig. 5, HIV-1 DNA was detected by SK38/39–primed PCR amplification in the peritoneal exudate cells of SCID-hu mice either 1 wk (lane *1*) or 4 wk (lane *2*) after intraperitoneal injection. No HIV-1 DNA was detected in peritoneal exudate cells harvested from unimplanted SCID mice 1 or 4 wk after intraperitoneal injection with 8,000 $TCID_{50}$ of HIV-1_{28} (data not shown).

Expression of Human Cytokine Genes in HIV-1–infected SCID-hu Mice. Since cytokines such as TNF-α, TNF-β, IL-2, IL-4, and IL-6 can modulate HIV-1 replication in vitro (2), human cytokine mRNA expression in the hu-thy/liv implant, spleen, LN, and PBMC of SCID-hu mice was analyzed by RT-PCR with human mRNA-specific cytokine primers. The detection of human β2-MG mRNA indicated that human cells were present throughout the peripheral lymphoid compartment of HIV-1–infected SCID-hu mice; however, differences in human cytokine mRNA expression by human cells present in different regions were observed (Fig. 6). Analysis by RT-PCR detected the expression of TNF-α mRNA in the hu-thy/liv implant and spleen; TNF-β mRNA in the hu-thy/liv implant, spleen, and PBMC; IL-2 mRNA in the hu-thy/liv implant and spleen; and IL-4 and IL-6 mRNA in the hu-thy/liv implant. To assess the effect of HIV-1 infection on cytokine expression in SCID-hu mice, we compared the expression of human mRNA encoding TNF-α, TNF-β, IL-2, IL-4, and IL-6 in the hu-thy/liv implant, PBMC, spleens and LN of eight SCID-hu mice and seven HIV-1–infected SCID-hu mice. In the seven HIV-1–infected mice, active HIV-1 infection was demonstrated by the detection of *tat/rev* mRNA in seven out of seven of the hu-thy/liv implants, seven out of seven spleens, six out of seven PBMC, and six out of seven LN (Fig. 7). Expression of TNF-α, TNF-β, and IL-2 mRNA was increased in the PBMC (Fig. 7 *B*), LN (Fig. 7 *C*), and spleens (Fig. 7 *D*) of the HIV-1–infected SCID-hu mice. For example, whereas TNF-α, TNF-β and IL-2 mRNA was detected in the spleens of one of eight, five of eight, and one of eight SCID-hu mice, respectively, they were detected in the spleens of five of six, six of six, and three of six HIV-1–infected SCID-hu mice, respectively. Taken together, this data suggests that HIV-1 infection in vivo can stimulate cytokine production by human T cells.

Discussion

Previous reports using SCID-hu mice demonstrated that HIV-1 infection occurred exclusively in the implanted human organs and HIV-1 infection of the hu-thy/liv implant was only observed after direct injection into the implant (8). We demonstrated in our modified mouse model that after intraperitoneal or intraimplant injection of HIV-1 into SCID-

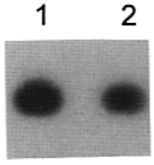

Figure 6. Detection of *gag* DNA in peritoneal exudate cells of intraperitoneally injected SCID-hu mice. Peritoneal exudate cells were harvested from SCID-hu mice 1 wk (lane *1*) or 4 wk (lane *2*) after intraperitoneal injection of 8 × 10^3 $TCID_{50}$ of HIV-1_{28} into SCID-hu mice. The presence of *gag* DNA was detected by Southern blotting of PCR amplified DNA as described in Materials and Methods and the blot shown reflects the results obtained from studying two SCID-hu mice.

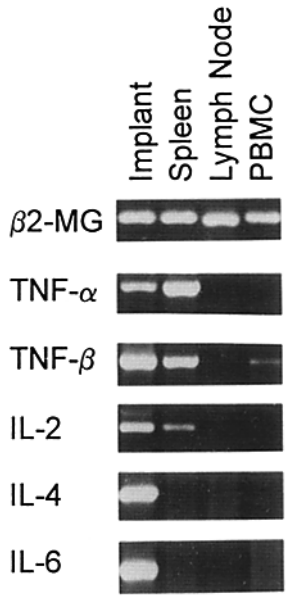

Figure 7. Expression of human cytokine genes in SCID-hu mice. The expression of β_2-MG, TNF-α, TNF-β, IL-2, IL-4, and IL-6 mRNA in the indicated tissue of a SCID-hu mouse infected by intraperitoneal injection of HIV-1 was determined by RT-PCR as described in Materials and Methods. The amplification products of the predicted size were visualized in ethidium bromide stained gels by UV radiation.

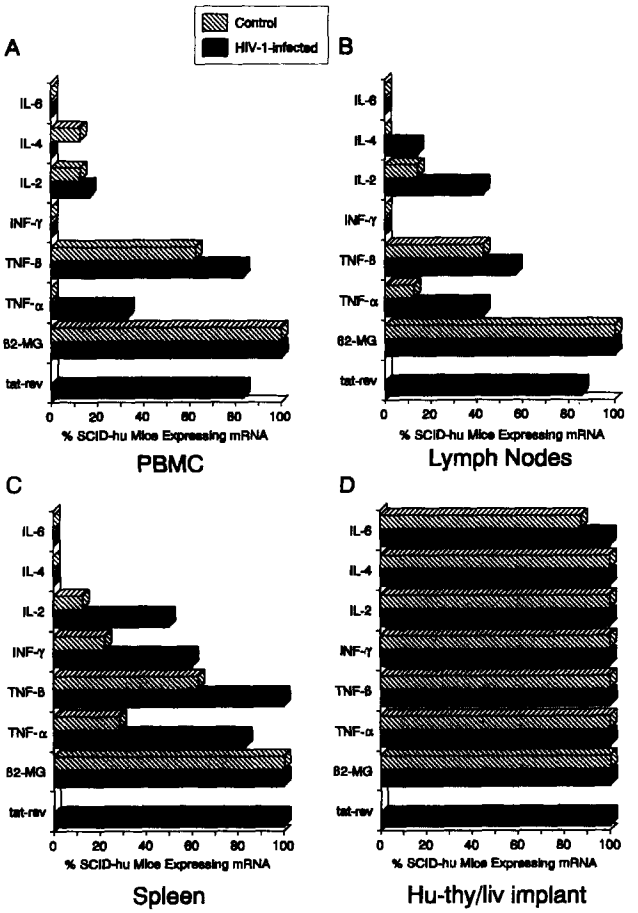

Figure 8. Comparison of human cytokine expression in SCID-hu mice and HIV-1–infected SCID-hu mice. The expression of *tat/rev*, β2-MG, TNF-α, TNF-β, IL-2, IL-4, and IL-6 mRNA in the (*A*) PBMC, (*B*) LN, (*C*) spleens, and (*D*) hu-thy/liv implants of SCID-hu mice (n = 8) and HIV-1–infected SCID-hu mice (n = 7) was determined by RT-PCR as described in Materials and Methods. The data are presented as the percent SCID-hu mice wherein the expression of the indicated mRNA was detected.

hu mice, significant HIV-1 infection occurred not only in the implant, but also in peripheral tissues. Furthermore, the presence of HIV-1 *gag* DNA, HIV-1 *gag* RNA, and spliced *tat/rev* RNA in the hu-thy/liv implants, PBMC, spleens, and LN of HIV-1–infected SCID-hu mice indicated that disseminated, active HIV-1 infection occurred in SCID-hu mice after transmission of HIV-1 by intraimplant injection, as well as by intraperitoneal inoculation.

In the SCID-hu model reported by Krowka et al. (18), peripheral HIV-1 infection after inoculation into the hu-thy/liv implant may have been precluded by the presence of a mean level of only 0.7% of human T cells in the peripheral blood and very low levels of human cells in the spleens of the SCID-hu mice used. The original SCID-hu mice were constructed by implanting one to two fragments of human fetal thymus and liver under the capsule of one kidney in SCID mice (19). To augment the number of human T cells present in the periphery of SCID-hu mice, we increased the quantity of hu-thy/liv tissue implanted under the renal capsule and implanted hu-thy/liv tissue under the capsules of both kidneys. In 25 SCID-hu mice constructed in this manner, a mean of 6.4% of their PBL were human T cells (4.06 ± 0.49% human $CD4^+$ T cells and 1.34 ± 0.20% human $CD8^+$ T cells). It is possible that other variables such as the viability of the implanted fetal tissue or the implantation technique used may have also contributed to the increased engraftment of the peripheral lymphoid compartment that we observed.

After injection of the hu-thy/liv implant in the SCID-hu mice constructed in this fashion with HIV-1, systemic infection of the implants, spleens, LN, and PBMC with HIV-1 was observed. This indicated that human T cells can become infected with HIV-1 while maturing in the thymus, exit the thymus, migrate to the periphery, and then possibly infect T cells present in the peripheral blood, spleen, and LN. The observation that the HIV-1 hu-thy/liv infection on one side was spread to the hu-thy/liv implanted in the opposite kidney suggested that free HIV-1 or HIV-1–infected cells from the periphery can recirculate and infect the hu-thy/liv implant on the opposite side. This assumption was confirmed by our observation that the hu-thy/liv implant could also be infected after intraperitoneal HIV-1 inoculation.

To determine whether free HIV-1 virus could infect the hu-thy/liv implant, we intravenously injected 8×10^4 $TCID_{50}$ of $HIV\text{-}1_{28}$ into these SCID-hu mice (data not shown). No HIV-1 infection occurred in these SCID-hu mice, suggesting that the major route of HIV-1 dissemination occurred via HIV-1–infected cells and not via blood-mediated transfer of HIV-1 during intrathymic injection or via secondary viremia. In our SCID-hu mouse model, human $CD4^+$ T cells were present in the peritoneal cavity and draining LN, and HIV-1 infection in these cells was detected by DNA PCR. Thus, it is possible that the first stage after intraperitoneal HIV-1 inoculation involves local infection of these human $CD4^+$ cells with HIV-1. Subsequently, these

HIV-1–infected cells may migrate throughout the periphery and mediate HIV-1 infection of the spleens, LN, and hu-thy/liv implants. Although the majority of T cell circulation is usually unidirectional from the thymus to the periphery, activated T cells can migrate from the periphery to the thymic medulla (20–22). Since HIV-1 preferentially infects activated T cells (23), an intriguing possibility is that T cells infected with HIV-1 in the periphery migrate back to the thymus and thereby induce extensive HIV-1 infection of the thymus. Taken together, these data suggest that the human fetal thymus may serve as a reservoir for HIV-1 infection and mediate subsequent seeding of the periphery with HIV-1–infected T cells.

The capacity of cytokines such as TNF-α, TNF-β, and IL-6 to stimulate HIV-1 replication in cultured monocytes and T cells has been demonstrated (2). In addition, IL-2 and IL-4 synergistically promote HIV-1 replication in cultured thymocytes (24), and IL-6 secreted by thymic epithelial cells upregulate HIV-1 replication in chronically infected cells (25). To assess the function of peripheral human T cells in SCID-hu mice and the in vivo role of cytokines in HIV-1 replication, the production of human cytokine mRNA in the hu-thy/liv implant and periphery of SCID-hu mice was assessed by RT-PCR. The mRNA for TNF-α, TNF-β, IL-2, IL-4, and IL-6 was detected in all of the hu-thy/liv implants and expression of the mRNA for TNF-α, TNF-β, IL-2, and IL-4 was detected in the spleens, LN, and PBMC of some SCID-hu mice. HIV-1 infection in the hu-thy/liv implant of mice infected with HIV-1 by intraimplant and by intraperitoneal injection was, as shown by quantitative coculture, at least 100-fold greater than that detected in the peripheral murine lymphoid organs of infected SCID-hu mice. The augmented degree of HIV-1 replication in the thymic implant may be related to the increased cytokine expression observed in the implants. After HIV-1 infection, an increased expression of TNF-α, TNF-β, and IL-2 mRNA was observed in the peripheral lymphoid compartment of HIV-1–infected mice. Although HIV-1 infection in vitro may be enhanced by the presence of these cytokines, in vivo HIV-1 replication may not require their presence. This was indicated by the observation that expression of mRNA for TNF-α, TNF-β, IL-2, and IL-4 was not detected in three of six LN wherein the detection of *tat/rev* mRNA indicated that active HIV-1 replication was occurring. Since expression by human T cells of mRNA for TNF-α (26), TNF-β (27), and IL-2 (28) are increased after activation, the data in this report indicated that HIV-1 infection may induce in vivo T cell activation. This is compatible with the recent observation that in vitro HIV-1 infection activates TNF-α and TNF-β gene expression via the HIV-1 transactivating Tat protein (29). Therefore, the use of species-specific cytokine RT-PCR in these SCID-hu mice should provide a valuable model for exploring the in vivo role of cytokines in HIV-1 infection.

Recently it was shown that HIV directly injected into hu-thy/liv implants in SCID-hu mice induces depletion of thymocytes (30, 31). We did not observe this degree of suppression of thymopoiesis 1 mo after infection of the thymic implants of our SCID-hu mice with HIV-1_{28}. This discrepancy most likely is related to the variable pathogenesis of different HIV-1 isolates as observed by Bonyhadi et al. (30). Variability in the behavior of thymic infection with different HIV-1 strains could also account for the observation that only two of three HIV-1–infected thymuses obtained from human abortuses were histologically abnormal (32).

Although the behavior of HIV-1 in SCID-hu mice may differ from that occurring in humans, it is intriguing to apply insights obtained with this mouse model to the controversy regarding whether vertical transmission of HIV-1 in humans occurs in utero, intrapartum, or postpartum. Although HIV-1 was detected in fetal lymphoid organs (33, 34), it is identified in less than half of the peripheral blood of HIV-1–infected newborns (35–37). It is possible that after intrauterine transmission, HIV-1 localizes to lymphoid organs where its presence escapes detection. The high degree of lymphocyte proliferation occurring in the neonatal thymus makes it an attractive environment wherein substantial replication of HIV-1 can occur (1). Various T cell precursor populations in the thymus including the immature "triple negative" $CD3^-$ $CD4^{lo}$-$CD8^-$ T cell precursors and the more mature $CD4^+CD8^+$ thymocytes are susceptible to HIV-1 infection (38). Our SCID-hu mouse model data indicate that human fetal thymus can become infected after peripheral exposure to HIV-1. Furthermore, after becoming infected with HIV-1 in the thymic environment, T cells can migrate from the thymus and mediate peripheral dissemination of the HIV-1 infection. Taken together, these in vivo studies suggest that peripheral cells infected with HIV-1 in utero may home to the thymus where they can infect thymocytes with HIV-1 and thereby mediate subsequent infection of peripheral lymphoid tissues. Examination of peripheral blood immediately after birth may not detect the high degree of HIV-1 replication occurring in the thymus, spleen, or LN. This is comparable to the dichotomy between the very active HIV-1 infection observed in LN and the low degree of HIV-1 infection seen in peripheral blood during the latent phase of HIV-1 infection in adults (4). Therefore, our SCID-hu mice may provide an improved model for studying the role of prenatal and postnatal anti-HIV interventions on the prevention of vertical transmission of HIV-1.

We thank D. Gebhard for assistance in the flow cytometry; A. Watford for assistance in animal care, and J. Berman, F. Lilly, and V. Prasad for critical reading 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 in part by the National Institutes of Health (National Institute of Allergies

and Infectious Diseases Centers for AIDS Research grants AI-27741 and AI-20671), a Pediatric Departmental Research grant, and a Pediatric AIDS Foundation grant from the American Foundation for AIDS Research (500368-14-PG). M. Pettoello-Mantovani was supported in part by grants from the Department of Pediatrics, Naples, and the Istituto Superiore di Sanita', Ministero della Sanita', Italy.

Address correspondence to Dr. Harris Goldstein, Department of Pediatrics, Division of Allergy and Immunology, Albert Einstein College of Medicine, Chanin Building, Room 626, 1300 Morris Park Avenue, Bronx, NY 10461.

Received for publication 13 August 1993 and in revised form 12 October 1993.

References

1. McCune, J.M. 1991. HIV-1: The infective process in vivo. *Cell.* 64:351.
2. Pantalleo, G., C. Graziosi, and A.S. Fauci. 1993. The immunopathogenesis of human immunodeficiency virus infection. *N. Engl. J. Med.* 328:327.
3. Pantaleo, G., C. Graziosi, L. Butini, P.A. Pizzo, S.M. Schnittman, D.P. Kotler, and A.S. Fauci. 1991. Lymphoid organs function as major reservoirs for human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA.* 8:9838.
4. Pantaleo, G., C. Graziosi, J.F. Demerest, L. Butini, M. Montroni, C.H. Fox, J.M. Orenstein, D.P. Kotler, and A.S. Fauci. 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Science (Wash. DC).* 362:355.
5. Embretson, J., M. Zupanic, J.L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A.T. Haase. 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Science (Wash. DC).* 362:359.
6. Temin, H.M., and D.P. Bolognesi. 1993. Where has HIV been hiding? *Nature (Lond.).* 362:292.
7. Namikawa, R., H. Kaneshima, M. Lieberman, I.L. Weissman, and J.M. McCune. 1988. Infection of the SCID-hu mouse by HIV-1. *Science (Wash. DC).* 242:1684.
8. McCune, J.M., B. Peault, P.R. Streeter, and L. Rabin. 1991. Preclinical evaluation of human hematolymphoid function in the SCID-hu mouse. *Immunol. Rev.* 124:45.
9. Kollmann, T.R., M.M. Goldstein, and H. Goldstein. 1993. The concurrent maturation of mouse and human thymocytes in human fetal thymus implanted in NIH-beige-nude-xid mice is associated with the reconstitution of the murine immune system. *J. Exp. Med.* 177:821.
10. Pettoello-Mantovani, M., A. Casadevall, T.R. Kollmann, A. Rubinstein, and H. Goldstein. 1992. Enhancement of HIV-1 infection by the capsular polysaccharide of *Cryptococcus neoformans. Lancet.* 339:21.
11. Ho, D.D., T. Moudgil, and M. Alum. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N. Engl. J. Med.* 321:1621.
12. Dimitrov, D.H., J.L. Melnick, and F.B. Holinger. 1990. Microculture assay for isolation of human immunodeficiency virus type 1 and for titration of infected peripheral blood cells. *J. Clin. Microbiol.* 28:734.
13. Kollmann, T.R., X. Zhuang, A. Rubinstein, and H. Goldstein. 1992. Design of polymerase chain reaction primers for the selective amplification of HIV-1 RNA in the presence of HIV-1 DNA. *AIDS (Phil.).* 6:547.
14. Schnittman, S., J.J. Greenhouse, H.C. Lane, P.F. Pierce, and A.S. Fauci. 1991. Frequent detection of HIV-1 specific mRNAs in infected individuals suggests ongoing active viral replication in all stages of disease. *AIDS Res. Hum. Retroviruses.* 7:361.
15. Krone, W.J.A., J.J. Sninsky, and J. Goudsmit. 1990. Detection and characterization of HIV-1 by polymerase chain reaction. *J. Acquired Immune Defic. Syndr.* 3:517.
16. Yamamura, Y., K. Uyemura, R.J. Deans, K. Weinberg, T.H. Rea, B.R. Bloom, and R.L. Modlin. 1991. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science (Wash. DC).* 254:277.
17. Saiki, R.K. 1990. Amplification of genomic DNA. *In* PCR Protocols. M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, editors. Academic Press, Inc., San Diego, CA. 13.
18. Krowka, J.F., S. Sarin, R. Namikawa, J.M. McCune, and H. Kaneshima. 1991. Human T cells in the SCID-hu mouse are phenotypically normal and functionally competent. *J. Immunol.* 146:3751.
19. Namikawa, R., K.N. Weilbaecher, H. Kaneshima, E.J. Yee, and J.M. McCune. 1990. Long-term hematopoiesis in the SCID-hu mouse. *J. Exp. Med.* 172:1055.
20. Naparstek, N., J. Holoshitz, S. Eisenstein, T. Reshef, S. Rappaport, J. Chemke, A. Ben-Nun, and I.R. Cohen. 1982. Effector T lymphocyte line cells migrate to the thymus and persists there. *Nature (Lond.).* 300:262.
21. Fink, P.J., M.J. Bevan, and I.L. Weissman. 1984. Thymic cytotoxic T lymphocytes are primed in vivo to minor histocompatibility antigens. *J. Exp. Med.* 159:436.
22. Hirokawa, K., M. Utsuyama, and T. Sado. 1989. Immunohistological analysis of immigration of thymocyte-precursors into the thymus: evidence for immigration of peripheral T cells into the thymic medulla. *Cell. Immunol.* 119:160.
23. Zack, J.A., S.J. Arrigo, S.R. Weitsman, A.S. Go, A. Haislip, and I.S.Y. Chen. 1990. HIV-1 entry into quiescent primary lymphocytes. *Cell.* 61:213.
24. Hays, E.F., C.H. Uittenbogaart, J.C. Brewer, L.W. Vollger, and J.A. Zack. 1992. In vitro studies of HIV-1 expression in thymocytes from infants and children. *AIDS (Phil.).* 6:265.
25. Schnittman, S.M., K.H. Singer, J.J. Greenhouse, S.K. Stanley, L.P. Whichard, B.F. Haynes, and A.S. Fauci. 1991. Thymic microenvironment induces HIV expression. *J. Immunol.* 174: 2553.
26. Lindsten, T., C.H. June, J.A. Ledbetter, G. Stella, and C.B. Thompson. 1989. Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. *Science (Wash. DC).* 244:339.
27. English, B.K., W.M. Weaver, and C.B. Wilson. 1991. Differential regulation of lymphotoxin and tumor necrosis factor genes in human T lymphocytes. *J. Biol. Chem.* 266:7108.
28. Hirano, T., K. Fujimoto, T. Teranishi, N. Nishino, K. Onoue, S. Maeda, and K. Shimada. 1984. Phorbol ester increases the level of interleukin 2 mRNA in mitogen-stimulated human

lymphocytes. *J. Immunol.* 132:2165.
29. Buonaguro, L., G. Barillari, H.K. Chang, C.A. Bohan, V. Kao, R. Morgan, R.C. Gallo, and B. Ensoli. 1992. Effects of the human immunodeficiency virus type 1 Tat protein on the expression of inflammatory cytokines. *J. Virol.* 66:7159.
30. Bonyhadi, M.L., L. Rabin, S. Salimi, D.A. Brown, J. Kosek, J.M. McCune, and H. Kaneshima. 1993. HIV induces thymus depletion in vivo. *Nature (Lond.).* 363:728.
31. Aldrovandl, G.M., G. Feur, L. Gao, B. Jamieson, M. Kristeva, I.S.Y. Chen, and J.A. Zack. 1993. The SCID-hu mouse as a model for HIV-1 infection. *Nature (Lond.).* 363:732.
32. Papiernik, M., Y. Brossard, N. Mulliez, J. Roume, C. Brechot, F. Barin, A. Goudeau, J.F. Bach, C. Griscelli, R. Henrion, and R. Vazeux. 1992. Thymic abnormalities in fetuses aborted from human immunodeficiency virus type 1 seropositive women. *Pediatrics.* 89:297.
33. Mano, H., and J-C. Chermann. 1991. Fetal human immunodeficiency virus type I infection of different organs in the third trimester. *AIDS Res. Hum. Retroviruses.* 7:83.
34. Courgnaud, V., F. Laure, A. Brossard, C. Bignozzi, A. Goudeau, F. Barin, and C. Brechot. 1991. Frequent and early in utero HIV infection. *AIDS Res. Hum. Retroviruses.* 7:337.
35. Ehrnst, A., S. Lindgren, M. Dictor, B. Johansson, A. Sonnerborg, J. Czajkowski, G. Sundin, and A.-B. Bohlin. 1991. HIV in pregnant woman and their offspring: evidence for late transmission. *Lancet.* 338:203.
36. De Rossi, A., L. Ometto, F. Mammano, C. Zanotto, C. Giaquinto, and L. Chieco-Bianchi. 1992. Vertical transmission of HIV-1: lack of detectable virus in peripheral blood cells of infected children at birth. *AIDS (Phil.).* 6:1117.
37. Krivine, A., G. Firtion, L. Cao, C. Francoual, R. Henrion, and P. Lebon. 1992. HIV replication during the first weeks of life. *Lancet.* 339:1187.
38. Schnittman, S.M., S.M. Denning, J.J. Greenhouse, J.S. Justement, M. Baseler, J. Kurtzberg, B.F. Haynes, and A.S. Fauci. 1990. Evidence for the susceptibility of intrathymic T-cell precursors and their progeny carrying T-cell antigen receptor phenotypes TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ to human immunodeficiency virus: a mechanism for CD4$^+$ (T4) lymphocyte depletion. *Proc. Natl. Acad. Sci. USA.* 87:7727.