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T-Cell Dysfunctions in hu-PBL-SCID Mice Infected with Human Immunodeficiency Virus (HIV) Shortly after Reconstitution: In Vivo Effects of HIV on Highly Activated Human Immune Cells

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The state of activation of the immune system may be an important factor which renders a host more receptive to human immunodeficiency virus (HIV) and more vulnerable to its effects. To explore this issue with a practical in vivo model, we developed a modified protocol of HIV infection in hu-PBL-SCID mice. First, we assessed the time course of activation of human peripheral blood lymphocytes (hu-PBL) in the peritoneal cavity of SCID mice. At 2 to 24 h after the intraperitoneal injection into SCID mice, there was a clear-cut increase in the percentage of hu-PBL expressing early activation markers (CD69), concomitant with the release of soluble intercellular adhesion molecule-1 (sICAM-1) and the soluble interleukin-2 receptor (sIL-2R) and with the accumulation of mRNAs for a number of human cytokines. At 2 weeks, virtually all of the hu-PBL expressed the memory phenotype (CD45RO) and HLA-DR antigens as well. Cells collected from the SCID mouse peritoneum at 2 and 24 h after transplantation were fully susceptible to in vitro infection with HIV type 1 (HIV-1) in the absence of either IL-2 or mitogens. The injection of HIV into hu-PBL-SCID mice at 2 h after reconstitution resulted in a generalized and productive HIV infection of the xenochimeras. This early HIV-1 infection resulted in a dramatic depletion of human CD4^{+} cells and in decreased levels of sICAM-1 (in the peritoneal lavage fluid) as well as of sIL-2R and immunoglobulins M and A (in the serum). Enzyme-linked immunosorbent assay and/or reverse transcriptase PCR analysis showed higher levels of IL-4, IL-5, and IL-10 in the HIV-infected animals than in control hu-PBL-SCID mice, while gamma interferon levels in the two groups were comparable. When we compared the current model of HIV-1 infection at 2 weeks after the intraperitoneal injection of the hu-PBL in the SCID mice with the model described here, we found that the majority of immune dysfunctions induced in the 2-h infection of the xenochimeras are not inducible in the 2-week infection. This supports the concept that the state of activation of human cells at the moment of the in vivo infection with HIV-1 is a crucial factor in determining the immune derangement observed in AIDS patients. These results show that some immunological dysfunctions induced by HIV infection in AIDS patients can be mimicked in this xenochimeric model. Thus, the hu-PBL-SCID mouse model may be useful in exploring, in vivo, the relevance of hu-PBL activation and differentiation in HIV-1 infection and for testing therapeutic intervention directed towards either the virus or the immune system.

An in vivo acute or chronic state of activation of the immune system may be an important factor that renders the host more receptive to human immunodeficiency virus (HIV) and more vulnerable to its effects. There is both in vitro and in vivo evidence that supports this concept. Several in vitro data indicate that a particular cellular activation state is required for the establishment of a productive HIV infection (27, 28). Moreover, recent studies on African populations suggest that in vivo immune activation, due to endemic parasitic infections, may be an important cofactor in susceptibility to progressive HIV infection and disease (26, 33). In particular, this persistent immune activation seems to induce an abnormal T-cell response (Th2/Th0) (1) in African individuals that is associated with a worsened prognosis of AIDS in HIV-infected individuals (4).

Studies of human retrovirus pathogenesis have been limited by the lack of suitable animal models. Two animal models involving the introduction of human hematopoietic tissues into severe combined immunodeficient (SCID) mice are currently in use to study HIV pathogenesis. The SCID-hu mouse model developed by McCune and colleagues involves transplantation of various fetal hematopoietic tissues into the SCID mice (12, 17, 23). Mosier and coworkers have described a xenochimeric model of SCID mice stably engrafted intraperitoneally (i.p.) with peripheral blood lymphocytes (PBLs) (hu-PBL-SCID mice) (18). This model proved useful in investigating in vivo human immune development and functions under both normal (18) and pathologic (5, 15) conditions, including HIV infection (19, 21, 22, 32). The model of Mosier and coworkers involves the i.p. injection of HIV into the SCID mice at 2 weeks after the i.p. transplantation of human PBLs (hu-PBLs) (21, 22). Recently, M. Tary-Lehmann and colleagues suggested that a human immune response occurs in hu-PBL-SCID mice within the first 2 to 3 weeks following PBL injection (31). Human lymphoid cells residing in these mice are mature (mainly CD4^{+} and CD8^{+} cells), but the majority of these cells stain for CD45RO and behave as anergic cells 1 to 2 months after i.p. transplantation (29, 30). Therefore, in the present study, we attempted to develop a modified model of the hu-PBL-SCID

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mouse for HIV infection, with the specific aim of exploring the in vivo effects of HIV on highly activated human immune cells. Thus, we first defined the pattern of activation and the functional state of hu-PBLs in the SCID mice. We found that hu-PBLs are highly activated after 2 to 24 h, while they become virtually all $CD45RO^+$ cells at 2 weeks postinjection. HIV infection of hu-PBL-SCID mice shortly after reconstitution (namely, at the time of maximal lymphocyte activation) resulted in efficient virus infection and in marked T-cell dysfunction, while the infection at 2 weeks after reconstitution did not markedly affect the human T-cell functions. Together these findings suggest that the hu-PBL-SCID mouse may be a suitable model for studying the relationships between HIV and the immune system in vivo and for evaluating the effects of antiviral and immunosuppressive therapeutic approaches.

MATERIALS AND METHODS

Subjects. hu-PBLs were obtained from the peripheral blood of healthy donors. All donors were screened for HIV type 1 (HIV-1) and hepatitis prior to donation. The hu-PBLs were obtained by Ficoll-Paque density gradient centrifugation. Twenty million cells were resuspended in 0.5 ml of RPMI 1640 and injected i.p. into the recipient mice.

Animals. CB17 *scid/scid* female mice (Charles River, Milan, Italy) were used at 4 weeks of age and were kept under specific-pathogen-free conditions. SCID mice were housed in microisolator cages, and all food, water, and bedding were autoclaved prior to use.

Recovery of cells from the peritoneal cavity and organs of the SCID mice. At different times after hu-PBL injection (i.e., 2 h, 24 h, and at weekly intervals up to 12 to 16 weeks), SCID mice were sacrificed, and cells were collected from the peritoneal cavity, spleen, thymus, and bone marrow. At each time, a two-step peritoneal lavage was done. The first washing was performed with 1 ml of cold RPMI 1640 medium. The recovered volume was centrifuged, and the supernatant was stored at −20°C, while the cells were pooled with those obtained with a second, 4-ml washing. The spleen and thymus were disrupted with the blunt end of a 5-ml syringe plunger. Connective tissue and debris were allowed to settle, and the single-cell suspensions were washed twice in RPMI 1640 medium. Bone marrow specimens were obtained by flushing of the marrow of both femurs with 1 ml of RPMI medium.

PCR for human DNA and cytokine mRNAs. Amplification and detection of the HLA-DQα gene fragment were performed with the GH26-GH27 primer pair and the corresponding probe RH54 (8).

Total RNA was isolated by use of RNAzol B (Biotecx, Houston, Tex.) and then subjected to phenol-chloroform extraction and isopropanol precipitation. The RNA was resuspended and treated with RNase-free DNase (Boehringer, Mannheim, Germany), further purified, and quantitated by UV absorbance at 260 nm. One microgram of total RNA was incubated for 5 min with oligo$(dT)_{12-18}$ (Pharmacia, Uppsala, Sweden) at 75°C, cooled at room temperature, and reverse transcribed with 200 U of Moloney murine leukemia virus reverse transcriptase (RT) (Bethesda Research Laboratories, Gaithersburg, Md.) for 1 h at 37°C in a final volume of 20 μl. One microliter of single-stranded cDNA was amplified in a final volume of 20 μl (10 mM Tris-HCl [pH 8.3]; 50 mM KCl; 1.5 mM $MgCl_2$; 0.01% gelatin; 200 μM [each] dGTP, dATP, dTTP, and dCTP; 1 μM dUTP-digoxigenin [Boehringer] as a tracer, and 10 pmol of each primer) as follows: 30 cycles of 40 s of denaturation at 94°C, 40 s of annealing at 62°C, and 1 min of extension at 72°C followed by a final extension step at 72°C for 10 min. Ten microliters of each PCR mixture was run on a 2% agarose gel in 0.5× Tris-borate-EDTA buffer. PCR products were visualized by ethidium bromide staining and UV transillumination. Gels were transferred by capillary blotting onto a nylon membrane (Hybond-N; Amersham, Little Chalfont, United Kingdom). Membranes were incubated overnight in 100 mM Tris-HCl (pH 7.5)–150 mM NaCl–10% bovine serum albumin at 37°C and then for 30 min at room temperature with peroxidase-conjugated anti-digoxigenin antibody (Boehringer) diluted 1:1,000. The membranes were then washed as indicated by the manufacturer and incubated for 1 min at room temperature with enhanced chemiluminescence (ECL) detection reagents (Amersham). Chemiluminescence was detected by exposure to ECL Hyperfilm (Amersham). The primers used for human interleukin-1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-10, tumor necrosis factor alpha (TNF-α), TNF-β, gamma interferon (IFN-γ), IL-2 receptor (IL-2R) (p55), intercellular adhesion molecule-1 (ICAM-1), β-actin, and $β_2$-microglobulin sequences are described elsewhere (6, 7, 14). We verified that primers specific for human sequences did not cross-react with murine sequences (i.e., RNA extracts from peritoneal and splenic cells of nonreconstituted SCID mice and from mitogen-stimulated splenocytes of immunocompetent syngeneic CB17 mice). All RT-PCR assays were performed in the linear range (25 to 30 cycles as appropriate). The optimal cycle number for each primer pair was previously determined by comparing amplified serial dilutions of cDNA obtained from mitogen-stimulated peripheral blood mononuclear cells.

ELISA for human cytokines, soluble factors, and Igs. Commercially available enzyme-linked immunosorbent assays (ELISAs) were used for determination of human cytokines, namely, IFN-γ, IL-2, IL-4, and TNF-α (Genzyme, Cambridge, Mass.), IL-10 (Biosource Int., Camarillo, Calif.), and IL-12 (R & D Systems, Minneapolis, Minn.) in peritoneal lavage fluids (PLs) and soluble ICAM-1 (sICAM-1) and soluble IL-2R (sIL-2R) (Genzyme) in PLs and sera of hu-PBL-SCID mice. The detection limits were 100, 45, 15.6, 100, 90, and 500 pg/ml for IL-2, IL-4, IL-10, IFN-γ, sICAM-1, and sIL-2R, respectively. An ELISA system was used to quantitate human immunoglobulin G (IgG), IgM, and IgA in PLs and sera of the chimeras by using a goat anti-human $F(ab')_2$ Ig antibody and peroxidase-coupled goat anti-human IgG, IgM, and IgA (Cappel-Cooper Biomedical, West Chester, Pa.) (2). All ELISAs were performed in duplicate, and laboratory standards were included on each plate. PLs and sera from nonreconstituted SCID mice and CB17 mice were used as negative controls for all of the ELISA determinations.

Flow cytometric analysis. Cells recovered from the peritoneums of the hu-PBL-SCID mice were resuspended in phosphate-buffered saline (PBS) and incubated with the appropriate fluorochrome-conjugated monoclonal antibodies for 30 min. The cells were then washed with 2% PBS–0.1% fetal calf serum–sodium azide and fixed with 2.5% paraformaldehyde. Two-color flow cytometry was performed on a FACSort cytometer (Becton Dickinson, San Jose, Calif.), and stained cells were analyzed by using LYSIS II (Becton Dickinson) software. A total of 5,000 events per sample were collected. Cells were analyzed according to forward- and side-scatter properties to gate live cell population, allowing for the exclusion of erythrocytes, dead cells, and cell and tissue debris.

The monoclonal antibodies used were anti-human leukocyte antigen (CD45) (Becton Dickinson), anti-murine major histocompatibility complex class I molecule ($H2\text{-}K^d$) (Pharmingen, San Diego, Calif.), and anti-human CD4, CD8, CD69, HLA-DR, CD45RA, and CD45RO (Becton Dickinson). None of the monoclonal antibodies to human antigens showed cross-reaction with murine epitopes (not shown).

Cytology. Cell suspensions from the PL, spleen, thymus, and bone marrow were spun on glass slides (Shandon, Cheshire, United Kingdom) as well as attached to L-polylysine-covered glass chamber slides (Lab-Tek, Naperville, Ill.) (9), stained with May-Grunwald-Giemsa stain, and examined under a microscope (Laborlux 12; Leitz).

Histology. The spleen, liver, lymph nodes, lung, and thymus of hu-PBL-SCID mice were fixed in 10% formalin and paraffin embedded, and 4-μm sections were cut, stained with hematoxylin and eosin, and examined under a microscope.

HIV-1 infection. Peritoneal cells (5×10^5) collected from SCID mice at 2 and 24 h after PBL injection were infected with 10^5 50% tissue culture infective doses of viral stock (supernatant of chronically HIV-1 IIIB infected H9 cells, stored at −80°C; titers were determined by the end point dilution method) and cultured in RPMI 1640 supplemented with 15% heat-inactivated fetal calf serum for 25 days. The cultures were inspected daily for syncytium formation, and every 5 days 0.2 ml of medium was harvested to analyze p24 antigen levels and replaced with an equal volume of fresh medium.

For in vivo infection, 10^5 50% tissue culture infective doses of HIV-1 IIIB were injected i.p. in hu-PBL-SCID mice 2 h after reconstitution. The chimeras were sacrificed after 4 weeks and analyzed for HIV-1 infection by (i) cocultivation of cell suspensions from the PL, spleen, thymus, and bone marrow with human C8166 cells; (ii) DNA PCR for virus-specific sequences as described elsewhere (16); and (iii) p24 antigen detection in PL by ELISA (Dupont, Brussels, Belgium). Positivity of cocultivation was determined by the presence of syncytia and/or p24 antigen in culture supernatants.

RESULTS

Phenotypic and functional changes of human cells after transplantation into SCID mice. First we characterized the phenotypes of human cells recovered from the peritoneal cavities of the SCID mice at different times after hu-PBL transplantation. Cells were examined by fluorescence-activated cell sorter (FACS) analysis with monoclonal antibodies to identify human (i.e., $CD45^+$) and mouse ($H2\text{-}K^{d+}$) cells and to evaluate the expression of activation antigens on human cells during the engraftment. As shown in Fig. 1, the vast majority of human $CD45^+$ cells were $CD69^+$ at 24 h after reconstitution. At 1 to 2 weeks, the percentage of $CD69^+$ cells progressively declined, while the percentage of $CD45RO^+$ cells increased, reaching 80 to 100% of the human cells (Fig. 1A). The percentages of HLA-DR and IL-2R (CD25)-positive cells were examined as well. The percentage of $HLA\text{-}DR^+$ cells increased consistently with $CD45RO^+$ cells, while the percentage of $CD25^+$ cells increased only slightly during the first week and slowly declined over time (Fig. 1A). The increase in the expression of activation antigens was associated with a pro-

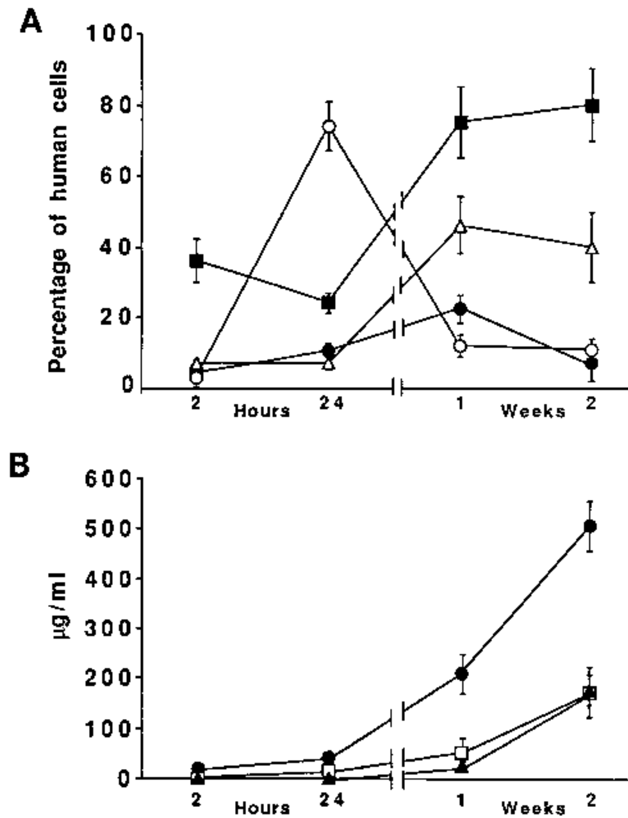

FIG. 1. FACS analysis of CD69 and CD45RO expression on human lymphocytes recovered from hu-PBL-SCID mice and human Ig levels in serum at the same time points. (A) Peritoneal cells collected from hu-PBL-SCID mice at different times after injection were examined for the expression of CD69 (○), CD45RO (■), HLA-DR (△), and CD25 (●) markers by FACS analysis. Percentages were calculated over the total number of human CD45$^+$ cells. (B) Human IgG (●), IgM (▲), and IgA (□) levels in the sera of hu-PBL-SCID mice at the same times after injection as in panel A. For both panels, six mice at each time point were analyzed in three separate experiments. Each point represents the mean ± standard error of the mean.

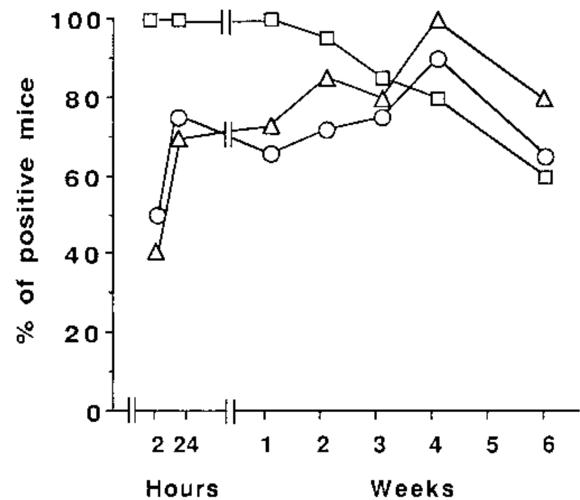

FIG. 3. Detection by PCR analysis of human DNA in organs of hu-PBL-SCID mice sacrificed at different times after reconstitution. DNA was extracted by standard procedures, and HLA-DQα sequences were amplified as previously described (16). The percentages of organs positive for human sequences at the various time points are shown. A minimum of eight animals was analyzed at each time point. The detection limit under our experimental conditions was 1 copy of cellular equivalents per 1.5×10^5 genomes. □, peritoneum; ○, spleen; △, lymph nodes.

gressive increase of human IgG, IgA, and IgM (Fig. 1B). This generalized hu-PBL activation in the SCID mice also included a very early accumulation of a wide spectrum of human cytokine mRNAs in peritoneal cells, including those for IFN-γ, IL-2, IL-4, and IL-10 (Fig. 2), together with the induction of IL-2R and ICAM-1 mRNAs (Fig. 2) and their increased release in PLs and sera (not shown). The levels of human cytokine mRNAs remained virtually unchanged at 1 to 2 weeks, while the levels of both sICAM-1 and sIL-2R in PLs and sera strongly increased at 1 week, dropping down at 2 weeks after PBL transplantation (not shown).

We also evaluated the spread of the engrafted human cells in SCID mice, by amplifying human HLA-DQα sequences with DNA preparations from different organs of the chimeras collected at various times after the i.p. transfer of hu-PBLs. Hu-

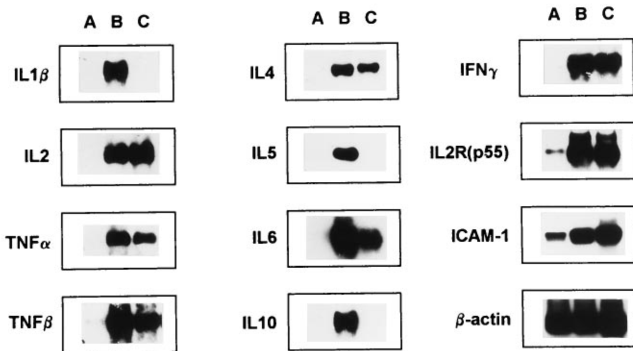

FIG. 2. RT-PCR analysis of human cytokine mRNAs in peritoneal cells from hu-PBL-SCID mice. Total RNA from peritoneal cells was retrotranscribed and amplified by using human-specific primers as described elsewhere (7, 14). Three mice per group were analyzed in three different experiments. The results of one representative experiment are shown. Lanes: A, donor; B, hu-PBL-SCID mouse 2 h after PBL injection; C, hu-PBL-SCID mouse 24 h after PBL injection.

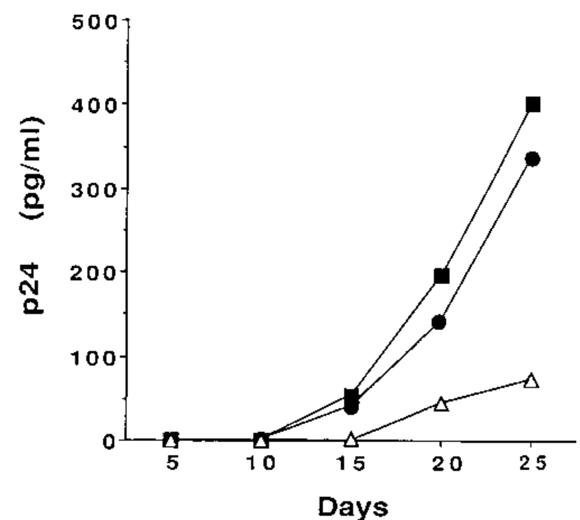

FIG. 4. p24 antigen detection in culture supernatants of peritoneal cells recovered from hu-PBL-SCID mice and infected in vitro with HIV-1. Peritoneal cells were collected from SCID mice at 2 and 24 h after hu-PBL injection. Four different experiments were performed. The results of one representative experiment are shown. △, unstimulated donor PBLs; ●, hu-PBLs 2 h postinjection; ■, hu-PBLs 24 h postinjection.

TABLE 1. Evaluation of HIV-1 infection in hu-PBL-SCID mice at 4 weeks after virus challenge[a]

Organ or cells	% Positive by:	
	PCR	Coculture with C8166 cells
Peritoneal cells	100	85
Spleen	85	66
Lymph node	85	ND[b]
Bone marrow	ND	45

[a] Mice were injected i.p. with HIV-1 at 2 h after hu-PBL transfer. At sacrifice virus was recovered from tissue specimens by coculture and PCR. Twenty mice in three different experiments were analyzed.
[b] ND, not done.

man sequences were detected in the spleen and lymph nodes of the vast majority of the xenochimeras as early as 2 h after injection (Fig. 3). Human sequences were detected up to 6 weeks (Fig. 3), progressively disappearing over time (not shown).

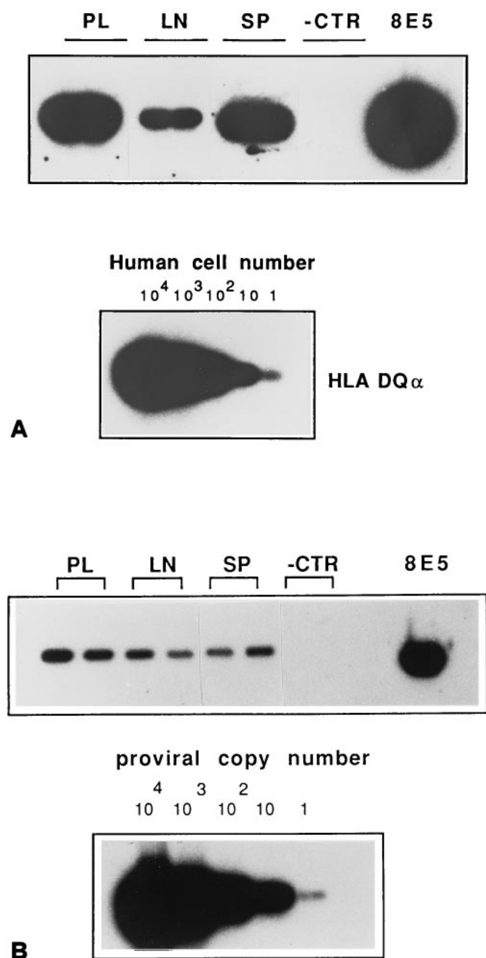

FIG. 5. Detection of HIV-1 proviral sequences in the tissues of virus-challenged hu-PBL-SCID mice. DNA was extracted by standard procedures, and HLA-DQα (A) or *gag*-specific (B) sequences were amplified as previously described (16). The sensitivity of the assay was tested by amplifying DNA prepared from the 8E5 T-cell line (10), which was serially diluted into SCID mouse cell DNA. The number of human cells (A) or proviral copy number (B) is indicated. SP, spleen; LN, lymph nodes; −CTR, reagent control.

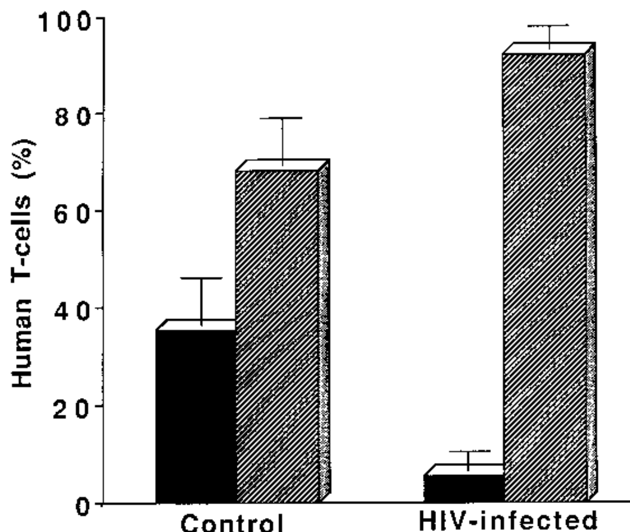

FIG. 6. FACS analysis of $CD4^+$ and $CD8^+$ cells in PLs of hu-PBL-SCID mice at 4 weeks after HIV-1 injection. The histograms represent the mean percentages (± standard errors of the mean) of $CD4^+$ (■) and $CD8^+$ (▨) cells with respect to $CD3^+$ cells in PLs. Twenty mice in three different experiments were analyzed.

HIV-1 infection of hu-PBL-SCID mice shortly after reconstitution. The results described above demonstrate that hu-PBLs undergo an activation process shortly after transplantation into the SCID mice and rapidly migrate from the peritoneum to various organs. To assess the ability of early activated human T cells to support HIV-1 replication, we first performed ex vivo experiments with human cells collected 2 and 24 h after the i.p. injection of hu-PBLs, by infecting the recovered cells in vitro with HIV-1 without the addition of either phytohemagglutinin or IL-2. The input human cell number was standardized by FACS analysis of $CD45^+$ cells (5×10^6 $CD45^+$ cells per well). As shown in Fig. 4, cultures of cells obtained at both 2 and 24 h after injection showed a rise in p24 antigen production in culture fluid 20 to 25 days after virus exposure, suggesting that those cells could efficiently support a productive HIV-1 infection. Only barely detectable levels of p24 antigen were found in cultures of unstimulated donor PBLs (Fig. 4). Therefore, we injected the chimeras i.p. with HIV-1 at 2 h after the engraftment (i.e., time of maximal hu-PBL activation). At 4 weeks postinjection, the animals were sacrificed and analyzed for evidence of infection. The results, presented as percentages of positive HIV-1-specific PCR and coculture assays of various cells and organs, showed that a large percentage of the chimeras exhibited a generalized HIV-1 infection at 4 weeks after virus challenge (Table 1). Figure 5 shows the results of a representative DNA PCR for HLA-DQα (Fig. 5A) compared with HIV-1 (Fig. 5B) sequences in different organs from two HIV-infected hu-PBL-SCID mice. Notably, p24 antigen was directly detectable by ELISA in the PL (data not shown).

Immune dysfunctions in hu-PBL-SCID mice infected with HIV at 2 h after reconstitution. We then compared some immunological parameters in the uninfected and the HIV-1-infected hu-PBL-SCID mice. As shown in Fig. 6, a severe $CD4^+$ lymphocyte depletion was observed at 4 weeks after virus exposure in PLs of HIV-infected xenochimeras, compared with the control mice. At the same time, the percentages of $CD8^+$ cells were roughly equal in infected and control chimeras (Fig. 6). Consistent with this CD4 depletion, we

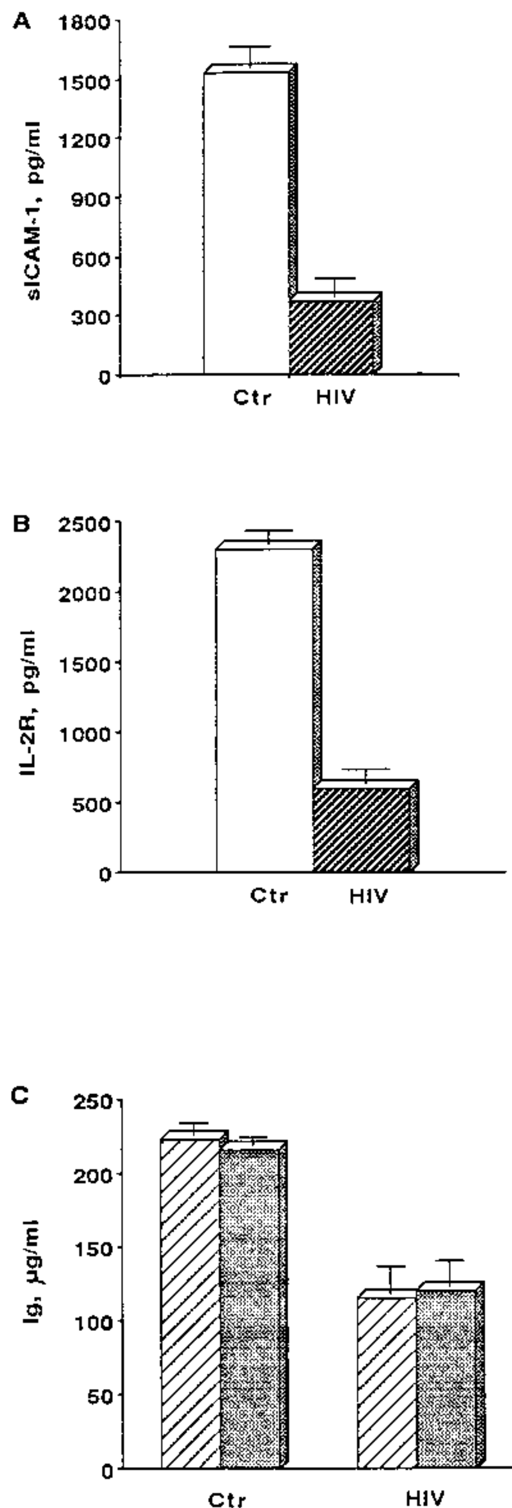

FIG. 7. Human sICAM-1 levels in PLs (A) and sIL-2R (B) and IgM and IgA (C) levels in sera of hu-PBL-SCID mice at 4 weeks after HIV-1 infection, as evaluated by ELISA. Values represent the means ± standard errors of the mean for 20 animals in five separate experiments. ▨, IgM; ▤, IgA. Ctr, control.

found significantly lower levels of human sICAM-1 in PLs, and of human IgM, IgA, and sIL-2R in sera, of the infected chimeras than of the controls (Fig. 7). The levels of human IgG in sera were similar in both groups (not shown). The levels of human cytokines in the PL differed markedly in infected and uninfected animals (Table 2). In fact, the ELISA analysis showed that IL-4 and IL-10 levels were markedly increased in the PLs of HIV-1-infected chimeras, while IFN-γ levels did not differ in infected and control mice (Table 2). IL-2 was undetectable in all samples, and TNF-α was weakly detected in both HIV-1-infected and uninfected xenochimeras (Table 2). The RT-PCR analysis of RNAs extracted from the same PL samples further suggested the presence of a Th0/Th2 profile in the HIV-1-infected hu-PBL-SCID mice (Fig. 8). In particular, mRNAs for both human IL-4 and IL-5 were detected only in the HIV-1-infected xenochimeras. An IL-10 transcript was consistently detectable in HIV-infected mice and occasionally also detected in the control mice (Fig. 8). An IFN-γ transcript was present in both groups, while IL-2 mRNA was undetectable (Fig. 8). Time course experiments showed that at 1 week after HIV-1 infection, the levels of human $CD4^+$ cells, cytokines, and sICAM-1 in PLs, and of human IgA, IgM, and sIL-2R in sera, of HIV-1-infected xenochimeras were not decreased with respect to those found in control mice (not shown).

TABLE 2. Human cytokine production in the peritoneal cavity of hu-PBL-SCID mice at 4 weeks after HIV-1 infection, as evaluated by ELISA

hu-PBL-SCID mice	Concn (pg/ml)[a]				
	TNF-α	IL-2	IFN-γ	IL-4	IL-10
Control	<15	<150	56 ± 17	<45	35 ± 3
HIV infected	<15	<150	46 ± 15	140 ± 46	138 ± 40

[a] Values are the means ± standard errors of the mean for 20 animals in three different experiments.

In a separate set of experiments, we compared the effects of in vivo HIV-1 infection of hu-PBL-SCID mice at 2 h and at 2 weeks after hu-PBL injection. All of the animals were sacrificed at 4 weeks postinfection. As shown in Table 3, the pattern of immune dysfunctions induced by HIV-1 infection at 2 h was not inducible in the 2-week infection. In particular, $CD4^+$ cell depletion was less pronounced, no decrease of sICAM-1, sIL-2R, IgM, and IgA was observed, and there was no increase of IL-4. The only exception was IL-10, which was higher in 2-week-infected animals than in the controls (Table 3). In addition, the percentages of positive HIV-1-specific PCR and coculture assays of various cells and organs showed that while the 2-h infection resulted in a generalized HIV-1 infection of the xenochimeras (100% of spleen, lymph node, thymus, and bone marrow samples were positive for HIV DNA PCR and/or cocultivation), in the 2-week infection the thymus and bone marrow were positive for HIV-1 infection only occasionally (not shown).

DISCUSSION

The hu-PBL-SCID mouse model was originally developed by Mosier and colleagues (18) and proved very useful for in vivo studies on human immune functions under normal and pathologic conditions (32). The HIV-1 infection of hu-PBL-SCID mice described by Mosier and coworkers involves virus injection at 2 weeks postreconstitution (19). This approach provided interesting results on the differential effects of various strains of HIV-1 on the in vivo depletion of human $CD4^+$ cells (19, 21) and on the effectiveness of various strategies in preventing and/or inhibiting HIV replication (20, 25).

In the present article, we characterize a modified model of

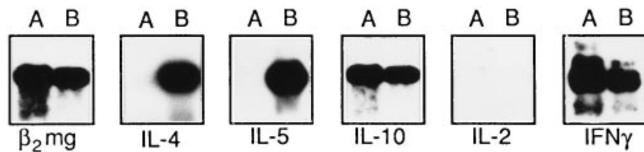

FIG. 8. RT-PCR analysis of human cytokine mRNAs in peritoneal cells of HIV-1-infected (lanes B) and uninfected control (lanes A) hu-PBL-SCID mice. The results for one representative mouse for each group are shown. $\beta_2$mg, β_2-microglobulin.

HIV-1 infection of hu-PBL-SCID mice, which differs from that described by Mosier and colleagues mainly in the state of activation of human cells at the moment of virus challenge. In fact, it has been reported that the SCID mouse environment affects the functional properties of the transplanted hu-PBLs by shaping the repertoire of $CD4^+$ T cells (30), rendering them memory cells at 2 weeks and anergic at 1 to 2 months after transplantation (29, 30). Thus, by injecting hu-PBL-SCID mice with HIV-1 at 2 weeks after reconstitution, Mosier and coworkers studied the effects of HIV infection on memory T cells. Recently, it has been suggested that the human immune system in this xenochimeric model is functional during the first 2 to 3 weeks after hu-PBL transplantation (31, 34). In the present study, we first characterized the kinetics of activation of hu-PBLs soon after their injection into SCID mice. We used the information derived from these studies to develop a modified model of HIV-1 infection of hu-PBL-SCID mice, suitable for the evaluation of the in vivo effects of HIV on highly activated human immune cells. We found that hu-PBLs become highly activated as early as 2 h after the i.p. injection into SCID mice and rapidly spread from the peritoneum to populate other mouse organs. HIV-1 injection at 2 h after reconstitution (i.e., at the time of maximal in vivo activation of human cells) resulted in a diffuse infection of the chimeras and in a marked impairment of human lymphocyte functions, as represented by (i) marked $CD4^+$ cell depletion, (ii) generalized inhibition of the release of soluble factors and Igs, and (iii) a Th0/Th2-type cytokine profile. When we compared the current model of HIV-1 infection at 2 weeks after the i.p. injection of the human PBLs in the SCID mice with the model described here, we found that (i) the majority of immune dysfunctions induced in the 2-h infection of the xenochimeras are not inducible in the 2-week infection, with the exception only of IL-10 induction, and (ii) the 2-h infection resulted in a more generalized spread of the HIV-1-infected cells in the xenochimeras. These results strongly support the concept that the state of activation of human cells at the moment of in vivo infection with HIV-1 is crucial in determining the immune derangement observed in AIDS patients, particularly when lymphocytotropic HIV-1 strains are involved. In fact, Mosier and coworkers have shown that with molecularly cloned viral isolates in the 2-week model of infection, macrophage-tropic strains induced extensive $CD4^+$ T-cell depletion, whereas lymphocytetropic strains caused little $CD4^+$ T-cell depletion (21).

Much importance is now attributed to the state of activation of immune cells in determining the rate of HIV replication and disease progression in HIV-exposed individuals (1, 27, 28). It has been hypothesized that the endemic infectious diseases occurring in African populations may support a chronic state of immune activation characterized mostly by a Th2 and Th0 type of response, which may account for the African pattern of HIV infection and AIDS progression (1). As an additional example, Folks and colleagues (11) reported that preexisting hyperimmune activation of simian immunodeficiency virus-infected rhesus macaques can speed the progression of disease. In the light of all these data, the HIV hu-PBL-SCID mouse model described in this article exhibits some unique features which may allow for certain comparisons of the effects of HIV-1 in certain patients. The general impairment of immune functions observed in the HIV-1-infected xenochimeras (i.e., $CD4^+$ cell depletion and reduced secretion of sIL-2R, sI-CAM-1, IgM, and IgA) together with the up-regulation of the expression of certain cytokines (IL-4, IL-5, and IL-10) is reminiscent of the immune dysfunctions and the Th type of cytokine profile frequently observed in those HIV-1-infected patients with a more rapid progression of the disease and a worse prognosis (4).

The immune dysfunctions observed in the HIV-1-infected hu-PBL-SCID mice seem to be somehow linked to the reduction in the number of $CD4^+$ cells. In fact, at 1 week postinfection the percentage of $CD4^+$ cells did not differ in infected and uninfected mice, and apparently no immune dysfunctions were observed. Evidence indicating that $CD8^+$ T cells can be a source of IL-4 directing the development of a predominant Th2 type of immune response in HIV-1-infected individuals is now available (24). Actually, high percentages of $CD8^+$ cells were constantly detected in the HIV-infected hu-PBL-SCID mice, in spite of the $CD4^+$ cell depletion. Thus, we can speculate that the $CD8^+$ cells may be responsible for the Th2 type of response shown in the HIV-1-infected xenochimeras at 4 weeks after infection.

It has been recently reported that T lymphocytes from HIV-infected individuals are primed to undergo apoptosis after stimulation with anti-Fas antibodies (13). Clerici and coworkers (3) suggested that the programmed cell death observed in $CD4^+$ cells of HIV-infected individuals is not merely due to growth factor (e.g., IL-2) starvation but reflects the change of cytokines produced from a Th1 type to a Th2 type predominance. Thus, experiments can be designed with our HIV hu-

TABLE 3. Effects of HIV-1 infection on human immune parameters in hu-PBL-SCID mice infected with HIV at 2 h and 2 weeks after i.p. injection of hu-PBL[a]

Time after injection and mice	CD4 (%)[b]	CD8 (%)[b]	sICAM-1 (pg/ml)	sIL-2R (pg/ml)	IgM (μg/ml)	IgA (μg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
2 h								
Control	29 ± 8	70 ± 11	1,200 ± 120	1,500 ± 150	210 ± 15	190 ± 12	<45	27 ± 5
HIV infected	5 ± 2[c]	95 ± 10[c]	250 ± 80[c]	510 ± 110[c]	102 ± 22[c]	110 ± 20[c]	101 ± 40[c]	130 ± 35[c]
2 weeks								
Control	31 ± 12	68 ± 14	1,700 ± 250	927 ± 180	800 ± 50	220 ± 15	<45	13 ± 4
HIV infected	24 ± 9	75 ± 8	1,780 ± 290	820 ± 210	1,003 ± 80	450 ± 90[c]	<45	37 ± 6[c]

[a] Values are means ± standard errors of the means for 12 animals in three different experiments.
[b] $CD4^+$ or $CD8^+$ cells as a percentage of human $CD3^+$ cells.
[c] $P < 0.01$ (Student's t test).

PBL-SCID mouse model to define the in vivo role of IL-4 and IL-10 in $CD4^+$ cell depletion.

In conclusion, this study shows that the hu-PBL-SCID mouse can represent a valuable model for exploring the in vivo effects of HIV infection on activated human lymphocytes, including cytokine production. Moreover, this HIV hu-PBL-SCID mouse model might be particularly helpful in evaluating the use of immunosuppressive drugs, alone or in combination with antiviral treatments, in modulating both HIV replication and the effects of HIV infection on the immune response.

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REFERENCES

1. **Bentwich, Z., A. Kalinkovich, and Z. Weisman.** 1995. Immune activation is a dominant factor in the pathogenesis of African AIDS. Immunol. Today **16:**187–191.
2. **Boirivant, M., S. Fais, B. Annibale, D. Agostini, G. Delle Fave, and F. Pallone.** 1994. Vasoactive intestinal polypeptide modulates the in vitro immunoglobulin A production by intestinal lamina propria lymphocytes. Gastroenterology **106:**576–582.
3. **Clerici, M., A. Sarin, R. L. Coffman, T. A. Wynn, S. P. Blatt, C. W. Hendrix, S. F. Wolf, G. M. Shearer, and P. A. Henkart.** 1994. Type 1/type 2 cytokine modulation of T-cell programmed cell death as a model for human immunodeficiency virus pathogenesis. Proc. Natl. Acad. Sci. USA **91:**11811–11815.
4. **Clerici, M., and G. M. Shearer.** 1994. The Th1-Th2 hypothesis of HIV infection: new insights. Immunol. Today **15:**575–581.
5. **Duchosal, M. A., P. J. McConahey, C. A. Robinson, and F. J. Dixon.** 1990. Transfer of human systemic lupus erythematosus in severe combined immunodeficient (SCID) mice. J. Exp. Med. **172:**985–988.
6. **Duplaa, C., T. Couffinal, L. Laba, C. Moreau, J. D. Lamaziere, and F. Bonnet.** 1993. Quantitative analysis of polymerase chain reaction products using biotinylated dUTP incorporation. Anal. Biochem. **212:**229–236.
7. **Ehlers, S., and K. Smith.** 1991. Differentiation of T cell lymphokine gene expression: the in vitro acquisition of T cell memory. J. Exp. Med. **173:**25–36.
8. **Erlich, H. A., and T. L. Bugawan.** 1989. HLA class II gene polymorphism: DNA typing, evolution, and relation to disease susceptibility, p. 202–204. *In* H. A. Erlich (ed.), PCR technology: principles and applications for DNA amplifications. Stockton Press, New York.
9. **Fais, S., V. I. Burgio, M. Silvestri, M. R. Capobianchi, A. Pacchiarotti, and F. Pallone.** 1994. Multinucleated giant cell generation induced by interferon g. Changes in the expression and distribution of the intercellular adhesion molecule-1 during macrophage fusion and multinucleated giant cell formation. Lab. Invest. **71:**737–744.
10. **Folks, T. M., D. Powell, M. Lightfoote, S. Koenig, A. S. Fauci, S. Benn, A. Rabson, D. Daugherty, H. E. Gendelman, M. D. Hoggan, S. Venkayesan, and M. A. Martin.** 1986. Biological and biochemical characterization of a cloned Leu-3− cell surviving infection with the acquired immune deficiency syndrome retrovirus. J. Exp. Med. **164:**280–290.
11. **Folks, T. M., T. Rowe, F. Villinger, B. Parekh, A. Mayne, D. Anderson, H. McClure, and A. A. Ansari.** 1995. Hyperimmune activation and progression to AIDS in rhesus macaques. Eur. Cytokine Netw. **77:**C13. (Abstract.)
12. **Kamel-Reid, S., and J. E. Dick.** 1988. Engraftment of immunodeficient mice with human hematopoietic cells. Science **242:**1706–1709.
13. **Katsikis, P. D., E. S. Wunderlich, C. A. Smith, L. A. Herzenberg, and L. A. Herzenberg.** 1995. Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals. J. Exp. Med. **181:**2029–2036.
14. **Kollmann, T. R., M. Pettoello-Mantovani, X. Zhuang, A. Kim, M. Hachamovitch, P. Smarnworawong, A. Rubinstein, and H. Goldstein.** 1994. Disseminated human immunodeficiency virus-1 (HIV-1) infection in SCID-hu mice after peripheral inoculation with HIV-1. J. Exp. Med. **179:**513–522.
15. **Krams, S. H., K. Dorshkind, and M. E. Gershwin.** 1989. Generation of biliary lesions after transfer of human lymphocytes into severe combined immunodeficient (SCID) mice. J. Exp. Med. **170:**1919–1930.
16. **Locardi, C., P. Puddu, M. Ferrantini, E. Parlanti, P. Sestili, F. Varano, and F. Belardelli.** 1992. Persistent infection of normal mice with human immunodeficiency virus. J. Virol. **66:**1649–1654.
17. **McCune, J. M., R. Namikawa, H. Kanemisha, L. D. Shultz, M. Lieberman, and I. L. Weissman.** 1988. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science **241:**1632–1639.
18. **Mosier, D. E., R. J. Gulizia, S. M. Baird, and D. B. Wilson.** 1988. Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature (London) **335:**256–259.
19. **Mosier, D. E., R. J. Gulizia, S. M. Baird, D. B. Wilson, D. H. Spector, and S. A. Spector.** 1991. Human immunodeficiency virus infection of human PBL-SCID mice. Science **251:**791–794.
20. **Mosier, D. E., R. J. Gulizia, P. D. MacIsaac, L. Corey, and P. D. Greenberg.** 1993. Resistance to human immunodeficiency virus 1 infection of SCID mice reconstituted with peripheral blood leukocytes from donors vaccinated with vaccinia gp160 and recombinant gp160. Proc. Natl. Acad. Sci. USA **90:**2433–2447.
21. **Mosier, D. E., R. J. Gulizia, P. D. MacIsaac, B. E. Torbett, and J. A. Levy.** 1993. Rapid loss of CD4+ T cells in human-PBL-SCID mice by noncytopathic HIV isolates. Science **260:**689–692.
22. **Mosier, D. E., and H. Sieburg.** 1994. Macrophage-tropic HIV: critical for AIDS pathogenesis? Immunol. Today **15:**332–339.
23. **Namikawa, R., K. N. Weibaecher, H. Kaneshima, E. J. Yee, and J. M. McCune.** 1990. Long-term human hematopoiesis in the SCID-hu mouse. J. Exp. Med. **172:**1055–1063.
24. **Paganelli, R., E. Scala, I. J. Ansotegui, C. Ausiello, E. Halapi, E. Fanales-Belasio, G. D'Offizi, I. Mezzaroma, F. Pandolfi, M. Fiorilli, A. Cassone, and F. Aiuti.** 1995. CD8+ T lymphocytes provide helper activity for IgE synthesis in human immunodeficiency virus-infected patients with hyper-IgE. J. Exp. Med. **181:**423–428.
25. **Parren, P. W. H. I., H. J. Ditzel, R. J. Gulizia, J. M. Binley, C. F. Barbas III, D. R. Burton, and D. E. Mosier.** 1995. Protection against HIV-1 infection in hu-PBL-SCID mice by passive immunization with a neutralizing human monoclonal antibody against the gp-120 CD4-binding site. AIDS **9:**F1–F6.
26. **Quinn, T. M., P. Piot, J. B. McCormick, F. M. Feinsod, H. Taelman, B. Kapita, W. Stevens, and A. S. Fauci.** 1987. Serologic and immunologic studies in patients with AIDS in North America and Africa. JAMA **257:**2617–2621.
27. **Schuitemaker, H., N. A. Kootstra, R. A. Fouchier, B. Hooibrink, and F. Miedema.** 1994. Productive HIV-1 infection of macrophages restricted to the cell fraction with proliferative capacity. EMBO J. **13:**5929–5936.
28. **Stevenson, M., T. L. Stanwick, M. P. Dempsey, and C. A. Lamonica.** 1990. HIV-1 replication is controlled at the level of T cell activation and proviral integration. EMBO J. **9:**1551–1560.
29. **Tary-Lehman, M., P. V. Lehman, D. Schols, M. G. Roncarolo, and A. Saxon.** 1994. Anti-SCID mouse reactivity shapes the human CD4+ T cell repertoire in hu-PBL-SCID chimeras. J. Exp. Med. **180:**1817–1827.
30. **Tary-Lehman, M., and A. Saxon.** 1992. Human mature T cells that are anergic in vivo prevail in SCID mice reconstituted with human peripheral blood. J. Exp. Med. **175:**503–516.
31. **Tary-Lehmann, M., A. Saxon, and P. V. Lehmann.** 1995. The human immune system in hu-PBL-SCID mice. Immunol. Today **16:**529–533.
32. **Torbett, B. E., G. Picchio, and D. E. Mosier.** 1991. Hu-PBL-SCID mice: a model for human immune function, AIDS, and lymphomagenesis. Immunol. Rev. **124:**2–26.
33. **Wachter, H., D. Fuchs, A. Hausen, G. Reibnegger, E. R. Werner, and M. P. Dierich.** 1986. Who will get AIDS? Lancet **ii:**1216–1217.
34. **Walker, W., C. W. Roberts, J. M. Brewer, and J. Alexander.** 1995. Antibody response to Toxoplasma gondii antigen in human peripheral blood lymphocyte-reconstituted severe-combined immunodeficient mice reproduces the immunological status of the lymphocyte donor. Eur. J. Immunol. **25:**1426–1430.