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...
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 anti-viral 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, 4, 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 twice washed 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 RH5 (8). Total RNA was isolated by use of RNAzol B (Biotecx, Houston, Tex.) and subjected to phenol-chloroform extraction and isopropanol precipitation. The RNA was resuspended and treated with RNase-free DNase (Boehringer, Mannheim, Germany) and further purified and quantified by UV absorbance at 260 nm. One microgram of total RNA was incubated for 5 min with oligo(dT)24,28 (Pharmacia, Uppsala, Sweden) at 75°C, cooled at room temperature, and then heated to 90°C for 2 min to inactivate reverse transcriptase (RT). cDNA was produced by using the BD Gentest RT-PCR System (Bethesda Research Laboratories, Gaithersburg, Md.) in a final volume of 25 ml, containing 0.2 pmol of each primer, 250 μM dNTPs, 40 U of SuperScript II reverse transcriptase (Gibco BRL), and 1 × RT-PCR buffer (50 mM KCl, 10 mM Tris-HCl at pH 8.3, 1 mM MgCl2, 15 mM dithiothreitol, 1% Triton X-100, and 15 μg of bovine serum albumin). 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).

**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.

**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.

**ELISA for human cytokines, soluble factors, and IgG.** 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, 100, 100, 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 Bio-medical, 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.

**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-K1+) 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. 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 percentage of HLA-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-

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gressive increase of human IgG, IgA, and IgM (Fig. 1B). This generalised 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-

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**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 analysed in three separate experiments. Each point represents the mean ± standard error of the mean.

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**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 analysed 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.

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**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 analysed at each time point. The detection limit under our experimental conditions was 1 copy of cellular equivalents per 1.5 × 10^6^ genomes. □, peritoneum; ○, spleen; ▲, lymph nodes.

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**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.
Human 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).

**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 phytohemagglutinin or IL-2. The input human cell number was standardized by FACS analysis of CD45<sup>+</sup> cells (5 x 10<sup>6</sup> CD45<sup>+</sup> 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-DQ<sub>α</sub> (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<sup>+</sup> 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<sup>+</sup> cells were roughly equal in infected and control chimeras (Fig. 6). Consistent with this CD4 depletion, we

### Table 1. Evaluation of HIV-1 infection in hu-PBL-SCID mice at 4 weeks after virus challenge<sup>a</sup>

<table>
<thead>
<tr>
<th>Organ or cells</th>
<th>% Positive by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCR</td>
</tr>
<tr>
<td>Peritoneal cells</td>
<td>100</td>
</tr>
<tr>
<td>Spleen</td>
<td>85</td>
</tr>
<tr>
<td>Lymph node</td>
<td>85</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.

<sup>b</sup> ND, not done.
foundsignificantly lower levels of human ICAM-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).

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
HIV-1 infection of hu-PBL-SCID mice, which differs from that described by Mosier and colleagues primarily 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 xenogenic 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 infection 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 IgGs, 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 lymphocyte-tropic 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 lymphocyte-tropic 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, sILCAM-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-

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**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.**

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

b Values are means ± standard errors of the means for 12 animals in three different experiments.

* CD4+ or CD8+ cells as a percentage of human CD3+ cells.

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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