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Induction of MHC Class I Expression on Immature Thymocytes in HIV-1-Infected SCID-hu Thy/Liv Mice: Evidence of Indirect Mechanisms

Grigoriy Kovalev,* Karen Duus,† Liping Wang,* Robert Lee,† Mark Bonyhadi,2† David Ho,‡ Joseph M. McCune,3† Hideto Kaneshima,§ and Lishan Su4*

The SCID-hu Thy/Liv mouse and human fetal thymic organ culture (HF-TOC) models have been used to explore the pathophysiologic mechanisms of HIV-1 infection in the thymus. We report here that HIV-1 infection of the SCID-hu Thy/Liv mouse leads to the induction of MHC class I (MHCI) expression on CD45CD8+ (DP) thymocytes, which normally express low levels of MHCI. Induction of MHCI on DP thymocytes in HIV-1-infected Thy/Liv organs precedes their depletion and correlates with the pathogenic activity of the HIV-1 isolates. Both MHCI protein and mRNA are induced in thymocytes from HIV-1-infected Thy/Liv organs, indicating induction of MHCI gene expression. Indirect mechanisms are involved, because only a fraction (<10%) of the DP thymocytes were directly infected by HIV-1, although the majority of DP thymocytes are induced to express high levels of MHCI. We further demonstrate that IL-10 is induced in HIV-1-infected thymus organs. Similar HIV-1-mediated induction of MHCI expression was observed in HF-TOC assays. Exogenous IL-10 in HF-TOC induces MHCI expression on DP thymocytes. Therefore, HIV-1 infection of the thymus organ leads to induction of MHCI expression on immature thymocytes via indirect mechanisms involving IL-10. Overexpression of MHCI on DP thymocytes can interfere with thymocyte maturation and may contribute to HIV-1-induced thymocyte depletion. The Journal of Immunology, 1999, 162: 7555–7562.

Although not well studied during HIV-1 infection, the thymus has been implicated as a site of early viral replication (1–3), and thymic organs from HIV-1-infected fetuses and pediatric patients show profound parenchymal damage and involution (4–6). More significantly, a strong correlation of HIV-1-induced thymus dysfunction to faster AIDS progression has been established in pediatric patients (7). Since the thymus organ is difficult to study in human subjects, a small animal model for the analysis of human thymopoiesis (SCID-hu Thy/Liv mouse) has been constructed by engrafting fragments of human fetal liver and thymus into the immunodeficient C.B-17 scid/scid (SCID) mouse (8, 9). The Thy/Liv organ promotes normal, long term differentiation of human T cells (9, 10). Thymocyte subpopulations are normally represented, a normal TCR V repertoire is displayed (11, 12), and tolerance is induced toward both self MHC Ags and exogenously provided superantigens (13, 14).

After inoculation of the SCID-hu Thy/Liv mouse with HIV-1, replication of pathogenic HIV-1 isolates reaches high levels at 2 wk postinfection (wpi),3 followed by depletion of CD45 thymocytes with an inversion of the CD4/CD8 ratio between 3–4 wpi (15–18). CD45CD8+ (DP) thymocytes, comprising 80–85% of the total thymocytes, are significantly depleted. In addition, a higher rate of replication and thymocyte depletion is observed with rapidly replicating, syncytium-inducing virus isolated from AIDS patients than with slowly replicating, nonsyncytium-inducing virus isolated from the same patients before AIDS development or from long term nonprogressor patients (19). As observed in the SIV-infected rhesus macaque (20), replication of HIV-1 in the SCID-hu mouse is dependent upon an intact nef open reading frame (21). Analysis of the other HIV-1 accessory genes, such as vpr, vpu, and vif, has demonstrated that, unlike in tissue cultures, mutations in these genes significantly slow down the replication and cytopathic effects of HIV-1 (22, 23). Thus, the SCID-hu Thy/Liv mouse provides a relevant in vivo model to evaluate primary HIV-1 replication and pathogenicity.

Both direct and indirect mechanisms of thymocyte depletion have been implicated in HIV-1-infected thymus organs (16, 18, 24, 25). Target cell depletion may be achieved by a number of HIV-1-encoded factors with cytotoxic or cytostatic activities, as demonstrated in T cells in vitro. For example, vpr has been shown to lead to G2/S phase cell cycle arrest in infected target cells (26, 27). Other HIV-1 proteins, such as Tat, Nef, and gpl20/gp41, have also demonstrated cytotoxic activity in various cell culture systems (28–31). Apoptosis has been associated with HIV-1-induced T cell death both in vitro and in vivo (32–36). In the Thy/Liv organ, some thymocytes with condensed nuclei are detected in HIV-1-infected Thy/Liv organs by thin section light microscopy and electron microscopy (16). Biochemically, partial chromosomal loss (detected

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by propidium iodide staining) (16) and DNA strand breaks (detected by terminal deoxynucleotidyl transferase labeling) (24) are associated with HIV-1-induced thymocyte depletion. DNA strand breaks are detected both in HIV-1-infected and in uninfected cells. However, data from a recent report suggest that necrosis by cytolytic infection may be a major mechanism of HIV-1-induced thymocyte depletion, and significant levels of apoptosis are only detected at late times postinfection (25). In addition, the intrathymic T progenitor cells can be directly infected and depleted to lead to thymocyte depletion by blocking T cell development (24).

The thymus microenvironment is essential for T cell development. Destruction of thymic epithelial cells has been reported in the human thymus and in the SCID-hu Thy/Liv organ after HIV-1 infection (4, 17). This may block T cell development and result in thymocyte depletion. Indeed, two recent reports suggest that hematopoietic progenitor cells in HIV-1-infected Thy/Liv organs are preferentially depleted by indirect mechanisms (37, 38). It is not clear, however, whether the HIV-1-infected thymic stromal cells are still functional in supporting de novo human T cell development. Only transient T cell development is reported after efficient inhibition of HIV-1 replication (39).

During T cell maturation in the thymus, MHC class I (MHC-I) is differentially expressed on different subpopulations of thymocytes. Of note, the expression of MHC-I on CD4+CD8+ DP thymocytes is lower than that on more mature CD4+ or CD8+ SP thymocytes (40). Thus, lowered expression of MHC-I on DP thymocytes is associated with and may be important for normal thymocyte maturation. It has been reported that intrathymic transfer of semiallogeneic thymocytes can induce transient allo-tolerance in host-derived T cells (41). Supporting the idea that proper levels of interaction between MHCI and CD8/TCR on developing thymocytes are important for normal thymocyte maturation, ectopic expression of MHCI on thymocytes leads to thymocyte depletion in a transgenic mouse model (42). Similar results are reported in transgenic mice expressing high levels of CD8 on thymocytes (43).

Interference with intrathymic selection signals of immature thymocytes is probably involved. We report here that HIV-1 infection of the thymus organ leads to enhanced expression of MHCI on DP thymocytes by indirect mechanisms in the SCID-hu Thy/Liv mouse and in human fetal thymus organ cultures (HFC-TOC).

Materials and Methods

Reagents

mAbs reactive with CD3, CD4, and CD8 were purchased from Becton Dickinson (San Jose, CA). Anti-MHCI mAb W6/32 was obtained from Biodesign (Kennebunk, ME). HC-10 mAb and a cDNA encoding the MHCI gene (44) were provided by Ed Collins (University of North Carolina, Chapel Hill, NC). Human recombinant IL-10, IL-7, and IL-2 were purchased from Endogen (Woburn, MA). HIV-1 isolates used in this study have been described previously: NL4-3 (45); ID and EW (24); A6/87, A7/88, B5/85, B11/88, and D9/90 (46); 89.6 (47); and JR-CSF (15).

Infection of SCID-hu Thy/Liv mice

Animal transplantation procedures for SCID-hu Thy/Liv construction have been previously described (9). Infection of SCID-hu Thy/Liv mice was performed as previously described (24). Briefly, SCID-hu Thy/Liv mice (4–6 mo after transplantation) were infected with supernatant collected from PHA-activated PBMC containing HIV-1 (mock) or 4 × 105–109 TCID50/ml of HIV-1. Fifty microliters (200–2000 TCID50) were injected into each thymus graft. The Thy/Liv organs were harvested at the indicated times, thymocyte suspension was prepared, and thymocyte subpopulations were analyzed by FACS (CD4-PE, CD8-Tc, MHCI-FITC).

The study was approved by the institutional review boards, and animal experimentation guidelines were followed.

HF-TOC assays

The HF-TOC procedures are essentially as previously described (48). Briefly, human fetal thymus (19–24 gestational wk) were dissected into approximately 2-mm3 fragments containing about two to four intact thymic lobules and transferred into either mock supernatant or virus-containing supernatant. The vials were gently rocked at room temperature for 2 h, and the fragments were transferred to 0.45-μm pore size Nucleopore filters (Millipore, Bedford, MA) atop gelfoam (Upjohn, Kalamazoo, MI) saturated in HF-TOC medium (RPMI, 10% FCS, 50 μg/ml streptomycin, 50 U/ml penicillin G, 1× MEM vitamin solution (Life Technologies, Gaithersburg, MD), and 1× insulin/transferrin/sodium selenite medium supplement (Sigma, St. Louis, MO)) in 6-well tissue culture plates. The fragments were then cultured at 37°C with 5% CO2 for 4–8 days with daily changes of culture medium.

HIV-1 replication and cytokine assays

Measurement of cell-associated p24 production (picograms per 106 thymocytes) was performed using a p24 ELISA kit (DuPont, Wilmington, DE). Semiquantitative DNA PCR analysis was performed essentially as previously described (24). Briefly, 10,000 human thymocytes from Thy/Liv grafts or HF-TOC fragments were assayed by 10-fold dilution of infected cells into uninfected human cells. Genomic DNA was prepared from the mixed cells (Fig. 4, lane 1, 10,000 sample cells; Fig. 4, lane 2, 1,000 sample cells plus 9,000 normal human cells; Fig. 4, lane 3, 100 sample cells plus 9,900 normal human cells). ACH2 cells (1 HIV-1 genome/cell) were used as a standard control. A standard titration represents samples with 100, 1, 0.1, 0.01, and 0%, respectively, ACH2 cells. For detecting HIV-1 Rev+ thymocytes, cells were stained with CD4-PE and CD8-TC, fixed and permeabilized, and followed by intracellular staining of Rev (anti-Rev-FITC) as previously described (49). CEM parental cells or CEM stably expressing an HIV-1 rev transgene was used as the control.

Cell-associated IL-10 was measured with an IL-10 ELISA kit (BioSource International, Camarillo, CA). Briefly, thymocytes were prepared from SCID-hu Thy/Liv or from HF-TOC fragments as described above. Equal numbers of thymocytes were lysed, and standard ELISA was performed as described in the kit instructions. IL-10 levels from mock-infected HF-TOC fragments were used as baselines (1±x) in each experiment.

Western blot and RNA blot analysis

Western blot analysis was performed with total thymocyte cell extracts prepared from mock- or HIV-1-infected Thy/Liv organs between 10–15 days postinfection (dpi). Thymocytes were meshed out of the thymic stroma, and protein extracts from equal number of thymocytes (3×106) were run on SDS-PAGE. The anti-MHCI mAb (HC-10) and the chemiluminescent detection system (Amersham, Arlington Heights, IL) were used for Western detection. Total RNA (7.5 μg) isolated from thymocytes prepared as described above were used to analyze MHCI gene expression by standard Northern blot with an MHCI cDNA as probe (44). RNA samples were confirmed by ribosomal RNA bands and by blotting with a human β-actin cDNA probe.

Results

Induction of MHCI expression on DP thymocytes in HIV-infected Thy/Liv organs

We analyzed phenotypic changes in thymocytes in HIV-1-infected Thy/Liv organs. During human thymocyte maturation, MHCI expression was low in immature CD4+CD8+ DP thymocytes and high in CD4+ or CD8+ SP thymocytes (Fig. 1B). As shown in two representative Thy/Liv organs at 14 dpi with mock or HIV-1, all DP thymocytes were induced by HIV-1 infection to express 11-fold (JD infected; Fig. 1A) or 4-fold (NL4-3 infected; Fig. 1B) higher levels of MHCI. The differences in basal level MCF of MHCI in the two experiments were due to the setting differences of the FACScan cytometer in different experiments. Multiple mock samples in the same experiment always showed similar basal MHCI levels (data not shown). After HIV-1 infection, surface MHCI expression on CD4+CD8+ (DN) and CD4− or CD8− SP thymocytes was also enhanced by 2- to 6-fold and 2- to 3-fold, respectively. This suggests that direct infection of thymocytes is not necessary for MHCI induction.
Aggregate data from multiple experiments using two different HIV-1 isolates are summarized in Fig. 1C and Table I. The induction of MHCI expression on DP thymocytes appeared to precede HIV-1-induced thymocyte depletion (18, 24). At later stages postinfection, even higher levels of MHCI on DP thymocytes were induced (Fig. 1C and Table I). In agreement with their activity in replication and pathogenicity in the thymus, HIV-1 isolates from patients before AIDS (A6/87 and B5/85) or from long term non-progressors (D9/90) showed significantly lower levels of MHCI induction than isolates from the same patients after AIDS progression (A7/88 and B11/88) or other pathogenic isolates, such as NL4-3, JD, and EW (19, 24). All DP cells were affected, and the level of induction correlated with the level of HIV-1 replication, as measured by p24 (Table I and data not shown).

To confirm the induced expression of MHCI in HIV-1-infected thymus organs, total thymocytes from mock- or HIV-1-infected (12 or 15 dpi) Thy/Liv organs were harvested, and MHCI protein was measured by Western blot analysis with the HC-10 mAb, which reacts with the unfolded MHCI protein of HLA-A, -B, and -C alleles. Mock- and HIV-1-infected Thy/Liv organs showed similar percentages of SP and DP thymocytes at 12 dpi and a slight reduction of DP thymocytes at 15 dpi. In agreement with FACS detection of surface MHCI expression on thymocytes, total MHCI proteins in thymocytes were induced significantly in HIV-1-infected samples (Fig. 2A). Thus, HIV-1 infection of the SCID-hu Thy/Liv mouse led to induction of MHCI protein expression in thymocytes.

To test whether HIV-1 induced MHCI gene transcription in thymocytes, MHCI mRNA levels were analyzed using an MHCI cDNA probe (44). Similar levels of induction of MHCI RNA was observed in HIV-1-infected Thy/Liv organs at 14 dpi (7- to 8-fold; Fig. 2B). Therefore, HIV-1 infection of the SCID-hu Thy/Liv mouse induced MHCI mRNA expression in immature thymocytes, probably via increased transcription.

FIGURE 1. HIV-1 infection-induced MHCI expression on immature thymocytes in the SCID-hu Thy/Liv mouse. A, Mock- or HIV-1 (JD)-infected Thy/Liv organs were harvested at 14 dpi and analyzed for surface MHCI expression (W6/32) on each thymocyte subpopulation. Total live cells were gated based on light scatter profiles. The percentage of each subpopulation is shown. MHCI expression of each subpopulation is shown as the mean channel fluorescence (MCF) in the histograms. B, As in A, NL4-3-infected Thy/Liv organs (14 dpi) showed similar induction of MHCI on immature thymocytes. The difference in basal MHCI levels was due to different flow cytometer settings in different experiments. At least five SCID-hu Thy/Liv mice mock infected or infected with each HIV-1 isolate were analyzed in more than three independent experiments, and similar results were observed. C, The kinetics of MHCI induction on DP thymocytes after infection with two HIV-1 isolates from multiple experiments are summarized. JD (diamond) and NL4-3 (square) are pathogenic viruses that replicate to peak levels at 2 wpi and deplete thymocytes after 3–4 wpi (24). Shown is the average fold induction of MHCI on DP thymocytes (MCF from HIV-1-infected/mock samples) derived from 5–10 SCID-hu Thy/Liv mice at each time point.
Induction of MHC class I on DP thymocytes in HIV-1-infected HF-TOC

To rule out the possibility that murine host factors of SCID-hu Thy/Liv mice may contribute to the phenotypic changes, we performed similar experiments in the HF-TOC model (48). As in the SCID-hu Thy/Liv mouse, HIV-1 infection of HF-TOC also led to induction of MHC class I expression on DP cells (Fig. 3 and Table II), before any significant thymocyte depletion. Thus, HIV-1 infection of the human thymus induced MHC class I expression on DP thymocytes. In addition, HIV-1 isolates with different tropism determinants were all able to induce MHC class I expression. CXCR4-tropic isolates (NL4-3 and EW), CCR5-tropic virus (JR-CSF), and dual tropic isolate (89.6 and JD) were able to efficiently induce MHC class I expression. CXCR4-tropic isolates were productively infected, whereas all or most DP thymocytes were induced to express 5- to 9-fold higher levels of MHC class I. Therefore, the majority of the DP cells, although induced to express high levels of MHC class I, were not directly infected by HIV-1.

Indirect mechanisms are involved in MHC class I induction on DP thymocytes

Using FACS-based detection of HIV-1 Rev proteins (49) in HIV-1-infected HF-TOC samples, we showed that <5% of the DP thymocytes were productively infected, whereas all or most DP thymocytes were induced to express 5- to 9-fold higher levels of MHC class I (Fig. 3). Therefore, indirect mechanisms were involved in the induction of MHC class I expression on DP thymocytes.

As previously reported in HIV-1-infected SCID-hu Thy/Liv mice (15, 17, 21-24), analysis of HIV-1 proviral DNA loads at various times postinfection (2-6 wpi) estimated that <10% of total thymocytes were directly infected (Fig. 4A). High levels of MHC class I induction on the majority of DP thymocytes were observed in the HIV-1-infected Thy/Liv organs (4- to 25-fold). Thus, most DP thymocytes with high MHC class I expression were not directly infected, especially at early times postinfection.

When DP thymocytes from HIV-1-infected Thy/Liv organs at 2 wpi were purified, and proviral DNA was measured, <10% (1-10%, assuming one proviral genome per cell) were directly infected (Fig. 4B). No significant thymocyte depletion was detected at this time point, and MHC class I levels on all DP thymocytes were induced by 7-fold in this Thy/Liv organ infected with NL4-3.

When productively infected thymocytes were measured by intracellular staining of HIV-1 Rev, a very low level (1-5%) of total thymocytes was detected (Fig. 4C) at 2 wpi. As shown in a representative Thy/Liv organ at 2 wpi, DP thymocytes (81%) were induced to express 7-fold higher MHC class I. Therefore, the majority of the DP cells, although induced to express high levels of MHC class I, were not directly infected by HIV-1.

Induction of IL-10 production in HIV-1-infected thymus organs and IL-10-induced MHC class I expression on DP thymocytes

To identify possible cytokines induced by HIV-1 infection, we analyzed the production of a number of cytokines in HIV-1-infected Thy/Liv organs or HF-TOC (50). IL-10 production was induced in HIV-1-infected Thy/Liv organs or HF-TOC by a number of HIV-1 isolates (Fig. 5 and data not shown). IL-10 has been reported to be induced in HIV-1-infected patients (51) and in lymphoid organs of feline immunodeficiency virus-infected cats (52). In addition, IL-10 has been reported to, depending on cell type, inhibit or induce MHC class II expression (53). Both NL4-3- and JD-infected Thy/Liv organs showed significant induction (2- to 5-fold) of IL-10 expression at 2 wpi, before significant thymocyte depletion (Fig. 5A). Similar induction was observed in the HF-TOC model. As induction of IL-10 is not directly correlated with levels of MHC class I induction (Fig. 5A), we further tested the effect of exogenous IL-10 on MHC class I expression in the HF-TOC model. When provided exogenously in HF-TOC assays, IL-10 induced expression of MHC class I on DP thymocytes in a dose-dependent fashion (Fig. 5B). Therefore, HIV-1 infection of the thymus organ led to up-regulation of IL-10 production. Increased levels of IL-10 in the thymus can partly contribute to the induction of MHC class I expression. Other cytokines are also likely to contribute to the induction of MHC class I expression in HIV-infected thymus organs.

### Table 1. Correlation of HIV-1 replication/pathogenicity with MHC class I induction on DP thymocytes

<table>
<thead>
<tr>
<th>HIV-1 (N)</th>
<th>HIV-1 Replication</th>
<th>Pathogenicity</th>
<th>Fold Induction (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL4-3 (25)</td>
<td>++/++</td>
<td>++</td>
<td>10 (1.6)</td>
</tr>
<tr>
<td>JD (10)</td>
<td>++/++</td>
<td>++</td>
<td>17 (2.7)</td>
</tr>
<tr>
<td>EW (9)</td>
<td>++/++</td>
<td>++</td>
<td>22 (2.6)</td>
</tr>
<tr>
<td>A6/87 (5)</td>
<td>+/—</td>
<td>+/—</td>
<td>3.7 (0.2)</td>
</tr>
<tr>
<td>A7/88 (4)</td>
<td>++/++</td>
<td>++</td>
<td>22 (2.4)</td>
</tr>
<tr>
<td>B5/85 (5)</td>
<td>+/—</td>
<td>+/—</td>
<td>2.1 (0.5)</td>
</tr>
<tr>
<td>B11/88 (3)</td>
<td>++/++</td>
<td>++</td>
<td>6.8 (2.5)</td>
</tr>
<tr>
<td>D9/90 (6)</td>
<td>+/—</td>
<td>+/—</td>
<td>3.3 (0.4)</td>
</tr>
</tbody>
</table>

a HIV-1 clones/isolates with different replication (p24 production) and pathogenicity (thymocyte depletion) were compared in MHC class I induction. N is the number of SCID-hu Thy/Liv mice (3-4 wk postinfection) analyzed.

Fold induction indicates mean channel fluorescence (MCF) of MHC class I (W6/32) staining on CD4+CD8+ DP thymocytes from HIV-1 infected Thy/Liv organs divided by MCF of DP thymocytes from mock-infected Thy/Liv organs.
Discussion

HIV-1 pathogenesis in the thymus plays an important role in AIDS progression in pediatric patients (7). We report here that HIV-1 infection of the human thymus leads to enhanced expression of MHC-I on immature thymocytes in SCID-hu Thy/Liv mice and in HF-TOC assays via paracrine mechanisms.

In PBMC or T cell lines, it has been reported that the MHC-I level is down-regulated by HIV-1 infection. HIV-1 Tat, Nef, and Vpu have all been reported to reduce surface expression of MHC-I in HIV-1-infected cells (54 –56). In our thymus models, the majority of the thymocytes affected were not directly infected by HIV-1, suggesting that indirect (viral or host) mediators were induced by HIV-1 infection. Therefore, the mechanisms of HIV-1-induced MHC-I expression in immature thymocytes are different from those of MHC-I suppression in PBL or T cell lines directly infected by HIV-1.

In addition to production of viral proteins, HIV-1 infection in the Thy/Liv organ leads to increased production of cytokines such as IL-4, IL-6, IL-10, and TGF-β (50). IFN-γ, which stimulates MHC-I gene expression, was not significantly induced by HIV-1 in the Thy/Liv organ at 2 wpi (50) (data not shown). Among the induced cytokines, IL-4 showed no significant activity on MHC-I induction in HF-TOC (data not shown). IL-10, which was shown to inhibit MHC class II expression in macrophages (53), appeared to induce MHC-I expression on thymocytes in HF-TOC (Fig. 5B).

However, the level of IL-10 induction is not directly correlated to that of MHC-I induction (Fig. 5A and data not shown). Other viral or host factors or a combination of factors may also be involved in MHC-I on immature thymocytes in SCID-hu Thy/Liv mice and in HF-TOC assays via paracrine mechanisms.

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### Table II. HIV-1 replication and MHC-I induction on DP thymocytes in HF-TOC

<table>
<thead>
<tr>
<th>HIV-1 (N)</th>
<th>Replicationa</th>
<th>Coreceptor Usagec</th>
<th>Fold Induction (SE)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL4-3 (11)</td>
<td>+ CXCR4</td>
<td>9 (2.6)</td>
<td></td>
</tr>
<tr>
<td>JD (5)</td>
<td>+ CXCR4/CCR5</td>
<td>19 (5.7)</td>
<td></td>
</tr>
<tr>
<td>EW (4)</td>
<td>+ CXCR4</td>
<td>24 (4.6)</td>
<td></td>
</tr>
<tr>
<td>HXB2 (11)</td>
<td>- CXCR4</td>
<td>1.2 (0.6)</td>
<td></td>
</tr>
<tr>
<td>89.6</td>
<td>+ CXCR4/CCR5</td>
<td>7 (1.9)</td>
<td></td>
</tr>
<tr>
<td>JRCSF</td>
<td>+ CCR5</td>
<td>5 (1.7)</td>
<td></td>
</tr>
</tbody>
</table>

a HIV-1 clones/isolates with different replication (p24 production) were compared in MHC-I induction. N is the number of HF-TOC assays analyzed. HXB2 is unable to replicate in HF-TOC or SCID-hu Thy/Liv mice (23).

b Peak p24 levels of >5000 pg/10⁶ thymocytes are indicated as +; levels of <50 pg/10⁶ thymocytes are indicated as −.

c HIV-1 coreceptor usage was determined by CXCR4- and CCR5-dependent fusion or replication assays. JD and EW were tested for this study (K.D. and L.S., unpublished results).

d Fold induction indicates mean channel fluorescence (MCF) of MHC-I (W6/32) staining on DP thymocytes from HIV-infected HF-TOC divided by MCF on DP thymocytes from mock-infected HF-TOC.
the MHCI induction. IL-10-neutralizing Ab may be employed in the HF-TOC model to confirm that IL-10 is involved in HIV-1-induced MHCI expression on thymocytes, although efficient Ab penetration of the HF-TOC fragments has not been demonstrated.

An important question related to HIV-1-induced thymocyte depletion is whether direct infection is required. The replication level of HIV-1 in the Thy/Liv organ is relatively low, especially at early time points. Less than 10% (maximal estimation) of thymocytes are infected at 2 wpi as measured by PCR detection of proviral DNA (15, 17, 18, 21–24, 57). This is consistent with the lack of significant mutations during HIV-1 infection in the SCID-hu Thy/Liv mouse (57). Our data show that HIV-1 infection leads to the induction of MHCI expression on all DP thymocytes, yet only a small fraction of them are directly infected by HIV-1 (Figs. 1, 3, 4).

**FIGURE 4.** Indirect induction of MHCI expression by HIV-1 infection. A. Semiquantitative PCR assays (24) were used to analyze HIV-1 proviral DNA loads in total thymocytes at various times postinfection (2–6 wpi). A representative mock- or HIV-1-infected sample is shown to demonstrate the relative proviral DNA loads. The fold induction of MHCT on DP thymocytes by HIV-1 infection is indicated. Lane 3 of ACH2 control cells at 2 wpi was from PCR run in the absence of human cell DNA. Lanes 1–3 represent 100, 10, and 1% sample cells mixed with normal thymocytes, respectively. For ACH2 cells, lanes 1–6 represent 100, 10, 1, 0.1, 0.01, and 0% ACH2 cells mixed with normal human thymocytes. A total of 10^4 cells were used in each PCR reaction. Similar results were observed in three independent experiments. B. Purified thymocyte subpopulation from a SCID-hu Thy/Liv mouse infected at 14 dpi (NL4-3). No significant thymocyte depletion was detected at this time point, and MHCI levels on DP thymocytes were induced by 7-fold in this Thy/Liv organ. Thymocyte subpopulations were purified to >90%, and PCR analysis was performed as described above. CD4^+ SP, CD4^-CD8^- thymocytes; CD8^- SP, CD4^-CD8^- thymocytes; DP, CD4^-CD8^- thymocytes. C. The majority of DP thymocytes with enhanced MHCI expression are not productively infected by HIV-1. Mock or HIV-1 (JD)-infected SCID-hu Thy/Liv mice were analyzed at 2 wpi. Total live cells were gated based on light scatter profiles. The percentage of the DP subpopulation is shown (left panel). MHCI expression of DP thymocytes is shown as the mean channel fluorescence (MCF) in the histograms (middle panel). Intracellular HIV-1 Rev expression was detected by FACS, and the percentage of Rev^+ cells is shown (right panel). At least three independent experiments with duplicate samples were performed to confirm the results.
The exact mechanisms and the significance of HIV-1-induced MHCI expression on immature thymocytes are not clear. As in the transgenic mouse models, induction of MHCI expression on DP thymocytes by HIV-1 may contribute to HIV-1-induced thymocyte depletion. MHCI on DP thymocytes may interact with CD8/TCR to interfere with the proper selection signals required for thymocyte maturation and survival. In addition, MHCI itself or in combination with other receptors may transduce signals affecting cell survival (59, 60). Further studies of HIV-1 pathogenesis in the SCID-hu Thy/Liv mouse and in the HF-TOC model will not only help understand the mechanisms of HIV-1-induced thymus destruction, but will also shed light on the mechanisms of thymocyte selection and maturation.

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