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CD8+ Cell Depletion Accelerates HIV-1 Immunopathology in Humanized Mice

Santhi Gorantla,* Edward Makarov,* Jennifer Finke-Dwyer,*† Catherine L. Gebhart,‡
William Domn,‡ Stephen Dewhurst,‡ Howard E. Gendelman,*† and Larisa Y. Poluektova*

Stable engraftment of human lymphoid tissue in NOD/scid-IL-2Rγcnull mice after CD34+ hematopoietic stem cell reconstitution permits the evaluation of ongoing HIV-1 infection for weeks to months. We demonstrate that HIV-1-infected rodents develop virus-specific cellular immune responses. CD8+ cell depletion, 2 or 5–7 wk after viral infection, resulted in a significant increase of HIV-1 load, robust immune cell activation, and cytopathology in lymphoid tissues but preserved CD4/CD8 double-positive thymic T cell pools. Human CD8+ cells reappeared in circulation as early as 2–3 wk. These data support a role of CD8+ cells in viral surveillance and the relevance of this humanized mouse model for the studies of HIV-1 pathobiology and virus-specific immunity. The Journal of Immunology, 2010, 184: 7082–7091.

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the importance of this rodent model for the studies of HIV-1 immunobiology.

Materials and Methods

Animals

NOD/scid-IL-2Rγ−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and bred under specific pathogen-free conditions in accordance with ethical guidelines for care of laboratory animals at the University of Nebraska Medical Center, as set forth by the National Institutes of Health.

CD34+ cell isolation and transplantation

Human cord blood was obtained, with parental written informed consent, from healthy full-term newborns (Department of Gynecology and Obstetrics, University of Nebraska Medical Center). After density gradient centrifugation, CD34+ cells were enriched using immunomagnetic beads according to the manufacturer’s instructions (CD34+ selection kit; Miltenyi Biotec, Auburn, CA). Purity of CD34+ cells isolated was evaluated by flow cytometry and was >90%. Cells were either frozen or immediately transplanted into newborn mice at 10⁶ cells per mouse intrahepatically in 20 µl PBS using a 30-gauge needle. Before cell transplantation, newborn pups were irradiated at 1 Gy using a C9 [³²⁵⁰C] source (Picker Corporation, Cleveland, OH). Cells were transplanted between 4 and 12 h after irradiation. Ranging from two to seven, littermates were reconstituted with one cord blood sample derived from one donor. The number of animals reconstituted was dependent on the number of CD34+ cells isolated from cord blood. Mice were weaned at 3 wk of age and randomly distributed between different experimental groups.

Viral stocks

The CCR5 coreceptor-utilizing HIV-1ΔΔA strain was propagated in human monocye-derived macrophages (36). Viral preparations were screened and found to be negative for endotoxin (<10 pg/ml) (Associates of Cape Cod, Woods Hole, MA) and Mycoplasma (Gen-Probe II; Gen-Probe, San Diego, CA). The viral titers were assayed on human monocye-derived macrophages and determined to be 10⁸ tissue culture-infectious doses50 (TCID50) per milliliter.

HIV-1 infection

HIV-1ΔΔA was injected i.p. at 10⁶ TCID50. The levels of viral RNA copies per milliliter in plasma were analyzed using the automated COBAS Amplicor system (Roche Molecular Diagnostics, Basel, Switzerland) with a detection limit of 50 viral RNA copies per milliliter. Mouse plasma samples (20 µl each) were diluted up to the volume of 700 µl with normal human serum for assay use, which made the detection limit 1750 copies per milliliter. HIV-1 infection was confirmed by virologic and histologic examinations. Reconstituted noninfected animals of similar age served as controls.

CD8+ cell depletion

The chimeric mCT807 mAb was obtained from the National Center for Immunodeficiencies, as set forth by the National Institutes of Health. The doses of cM-T807 were selected to create a sufficient and sustained concentration of Ab for elimination of CD8+ cells due to the C5a component of complement deficiency and to prevent rapid clearance of chimeric Abs in immune-deficient mice (37). Two different time points were used for depletion: 2 and 5–7 wk postinfusion. Information for all of the animals used in the study (age, period of infection, time after depletion, and levels of IgM/IgG) is shown in Table I and in Supplemental Table I.

Flow cytometry

Peripheral blood samples were collected into EDTA-coated tubes from the facial vein by using lancets (MEDIpoint, Mineola, NY) or by cardiocentesis at the end of observation. Four- or seven-color flow cytometry on whole blood samples were performed to monitor changes in T cell populations. In brief, 100 µl aliquots of whole blood were incubated with respective Abs for 30 min at 4°C, and RBCs were first lysed with FACS Lysing Solution (BD Biosciences, San Jose, CA) and then washed twice with PBS containing 2% FBS. Spleen was divided into two halves; one was used for flow cytometry, and the other for immunohistochemistry. Blood leukocytes and splenocytes were tested for human pan-CD45, CD3, CD4, CD8, CD11c, CD14, CD19, and HLA-DR markers as four- or seven-color combinations. Abs and isotype controls were obtained from BD Pharmingen (San Diego, CA), and staining was analyzed with a FACS DIVA (BD Immunocytometry Systems, Mountain View, CA). To reproducibly rule the depletion of CD8+ cells in the presence of CM-T807, anti-CD8-RPE clone DK25 (DakoCytomation, Carpinteria, CA) was used. Depletion was confirmed by showing an absence of CD3+CD8+ and CD3+CD8+ lymphocytes. Results are expressed as percentages of total number of gated lymphocytes. The gating strategy was human CD45 → CD3 → CD4/CD8, CD45 → CD19, and CD45 → CD14.

Intracellular cytokine staining procedure

Splenocytes were collected and enriched for human CD45-positive cells using magnetic bead separation (Miltenyi Biotec). Enriched cells were cultured in RPMI 1640 supplemented with 10% FCS and penicillin/streptomycin, at 2 million cells per milliliter, in the presence of either HIV-1 gag or envelope peptide pools (AIDS Research and Reference Reagent Program, Division of AIDS, National Institutes of Health). Peptide pools were used at a concentrations of 1 µg/ml each. Cells were incubated at 37°C for 18 h with brefeldin A (100 ng/ml) added during last 6 h. Cells incubated in the presence of peptide diluents served as a nonstimulated control. At the end of incubation, cells were collected and stained for surface Ags—human CD45, CD3, CD4, and CD8—followed by intracellular staining with Abs to human IFN-γ and IL-2. All of the Abs and reagents for intracellular staining were obtained from eBioscience (San Diego, CA). Stained cells were analyzed using a LSR II (BD Biosciences) and gated for human CD45 → CD3 → CD4 or CD8 → IFN-γ or IL-2.

Cytometric bead array

Mouse plasma samples were diluted five times. The concentrations of human cytokines IFN-γ, IL-2, IL-4, IL-6, IL-10, and TNF-α were determined by FACSA (BD Biosciences) according to the manufacturer’s instructions (BD Biosciences).

Immunohistochemistry

Spleen and lymph node samples were fixed with 4% paraformaldehyde overnight and embedded in paraffin. Five-micrometer-thick sections were stained with mouse HLA-DR (clone CR3/43, 1:100), CD8 (clone 144,

Table I. Profiles of HIV-1 infected and CD8+ cell depleted animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (wk)</th>
<th>Weeks Postinfection</th>
<th>CD45+ (%), Spleen</th>
<th>IgM (µg/ml)</th>
<th>IgG (µg/ml)</th>
<th>HIV RNA Copies/ml</th>
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<tr>
<td>Uninfected control</td>
<td>26.5</td>
<td></td>
<td></td>
<td>30.4</td>
<td>103.4</td>
<td>208.0</td>
</tr>
<tr>
<td>n = 14</td>
<td>23–36</td>
<td>10</td>
<td>3.8–50.5</td>
<td>&lt;3.1–962.0</td>
<td>7.8–402.0</td>
<td></td>
</tr>
<tr>
<td>HIV-1-infected</td>
<td>29</td>
<td>7</td>
<td>28.8</td>
<td>168.0</td>
<td>93.5</td>
<td>114,800</td>
</tr>
<tr>
<td>n = 15</td>
<td>26–36</td>
<td>5–13</td>
<td>3.1–72.9</td>
<td>18.0–1019.6</td>
<td>7.8–453.8</td>
<td>13,335–1,526,000</td>
</tr>
<tr>
<td>HIV-1-infected CD8+ cell-depleted</td>
<td>29</td>
<td>7</td>
<td>20.2</td>
<td>154.2</td>
<td>70.1</td>
<td>382,550</td>
</tr>
<tr>
<td>n = 12</td>
<td>25–33</td>
<td>4–9</td>
<td>3.1–52.6</td>
<td>48.4–523.0</td>
<td>7.8–856.7</td>
<td>24,745 to &gt;35,000,000</td>
</tr>
<tr>
<td>HIV-1-uninfected CD8+ cell-depleted</td>
<td>33</td>
<td></td>
<td>9.4</td>
<td>225.8</td>
<td>175.3</td>
<td></td>
</tr>
<tr>
<td>n = 7</td>
<td>29–35</td>
<td>9.4</td>
<td>3.1–80.6</td>
<td>123.4–1005.1</td>
<td>7.8–576.3</td>
<td></td>
</tr>
</tbody>
</table>

*End point data are shown as median and the range (minimal and maximal values).

 Period of HIV-1 infection before the animals were euthanized.

 Detection limit was 1750 copies/ml. Detection was performed using 20 µl plasma diluted to a total volume of 700 µl as a requirement for automated evaluation by AmpliCor kit with detection limit of 50 copies/ml. Productive infection was confirmed by detection of HIV-1 p24-positive cells in lymphoid tissues.
1:50), CD20 (clone L26, 1:50), CD68 (clone KP-1, 1:50), or HIV-1 p24 (clone Kal-1, 1:10); all were obtained from DakoCytomation. Mouse mAbs to Ki-67 (clone DVB-2, 1:50), CD14 (clone 7, 1:50), and CD4 (clone 1F6, 1:40) were purchased from Biocare Medical (Concord, CA) and Novocastra (Norwell, MA), respectively. The polymer-based HRP-conjugated anti-mouse EnVision systems (DakoCytomation) are used as secondary detection reagents, and 3,3′-diaminobenzidine (DakoCytomation) was used as the chromogen. All of the paraffin-embedded sections were counterstained with Mayer’s hematoxylin. Deletion of primary Ab or mouse IgG served as controls. Images were obtained by Optronics (Goleta, CA) digital camera fixed to an Eclipse E800 (Nikon Instruments, Melville, NY) using MagnaFire 2.0 software.

**Ig measurements**

The plasma levels of IgM and IgG were determined by ELISA (Bethyl Laboratories, Montgomery, TX).

**HIV-1–specific ELISA**

Plasma collected at different time points were analyzed for HIV-1–specific human IgGs by using Con A-based ELISA, as previously described (38). HIV-1ADA envelope protein was expressed in a baculoviral system from a plasmid obtained from Dr. Lee Ratner (39). Purified envelope protein was used to coat the ELISA plate at 0.5 mg per well. HRP-conjugated secondary Abs to either human IgM or IgG were used (Bethyl Laboratories) and finally developed with 3,3′,5,5′-tetramethylbenzidine substrate. Plasma samples from uninfected humanized mice were used as negative controls, and HIV-1–seropositive human serum was used at a 1:10,000 dilution as a positive control.

**Statistical analysis**

Data were analyzed using Prism (GraphPad, San Diego, CA) and Excel software; statistical tests employed were nonparametric Mann-Whitney U test, Wilcoxon test and one-way ANOVA for comparisons of multiple groups. A p value <0.05 was considered statistically significant. For statistical analyses, viral load values of <1750 and >35,000,000 were set to 1750 and 35,000,000 (which represent the lower and upper thresholds for the assay). Because a Wilcoxon test was used to compare the distributions, setting these interval values to their lower and upper limits, respectively, had no effect on the results.

**Results**

**HIV-1 infection in chimeric mice**

Newborn NSG mice were reconstituted with human HSCs (hCD34+) isolated from umbilical cord blood. Phenotypic development of human lymphoid tissue was evaluated by flow cytometry analyses of human cells in peripheral blood (CD45, CD3, CD4, CD8, CD19, and CD14) to determine the relative abundance of immune cell groups. Presence of human Abs in peripheral blood was analyzed by antihuman IgG/IgM ELISA (Table I, Supplemental Table I). At the age of ∼5 mo, chimeric mice showed partial maturation of a reconstituted human immune system. The number of human cells in circulation became stable at 5–7 mo of age, with the majority being CD3+ cells (Fig. 1). At this stage, humanized mice were infected with HIV-1ADA (a macrophage-tropic CCR5-utilizing virus) by administering 10⁶ TCID₅₀ i.p. Progression of viral infection was determined by measuring plasma viral load at different time points. Virus was rarely detected in the serum as early as 2 wk postinfection, but by 5–6 wk all of the tested animals were infected. Fig. 1 demonstrates the percentages of various phenotypes of human immune cells in spleens from uninfected and HIV-1–infected animals, as analyzed by flow cytometry. The total number of human cells (CD45+) in the spleen was unaffected by viral infection. However, in HIV-1–infected animals, CD4⁺ T cell numbers declined, and a parallel reduction of the CD4/CD8 ratio was noted when compared with that in uninfected animals (n = 10 per group; p = 0.032 and p = 0.026, respectively).

Peripheral viral load and number of CD4⁺ and CD8⁺ human cells in circulation were analyzed in three animals biweekly (Fig. 2) and for the rest of animals at weeks 4–8 or at time of sacrifice (weeks 8–13).

**FIGURE 1.** Percentages of human cells in spleen. Human cells in spleens of uninfected and HIV-1–infected mice were determined by FACS at the time of sacrifice. Individual numbers (percentages) per animal, ratio of CD4/CD8, cells and medians are shown. The p values between groups analyzed by nonparametric Mann-Whitney U test are shown.

**FIGURE 2.** Peripheral blood profiles in HIV-1–infected humanized NSG mice. To determine the dynamics of viral replication and immune cell numbers in peripheral blood, mice were bled biweekly starting 3 wk after HIV-1 infection. Only three animals were frequently bled; because facial vein bleeding with short intervals was affecting animal behavior, we did not follow the same procedure for all of the animals.
In prospectively studied animals, the peak of infection occurred at 5–7 wk following viral inoculation, when it reached 5.5 (±149) and 6.5 log_{10} (±159) copies of viral RNA, and the viral load declined by 1.5 (±149) and 0.5 log_{10} (±159) at 9–10 wk. With the selected infectious dose of 10^5 TCID_{50}, which provides successful infection of ~50% of animals, the peak of viral load was observed at 5–6 wk postinfection, and the median reached 5.31 log_{10} with a range of 4.72–6.00 range (n = 6). By 8–10 wk postinfection, viral load declined to 4.80 log_{10} with a range of 4.12–5.39 (p = 0.023 by Mann-Whitney U test, n = 6).

Cellular immune responses

To confirm CD8+ cell control of HIV-1 replication in this model and to characterize the cellular immune antiviral responses, we infected and sacrificed five additional humanized animals at 5 wk postinfection. To detect cellular immune responses, splenocytes were enriched for human CD45+ cells by magnetic bead positive selection for in vitro stimulation with HIV-1 Ags. Three uninfected animals similar in levels of reconstitution served as controls. To evaluate the presence of cellular responses and their functional properties, we...
used intracellular cytokine staining (ICS). The number of IFN-γ, IL-2, or dual cytokine-producing cells was analyzed following stimulation with HIV-1 gag and envelope peptide pools. Representative FACs plots for three infected mice and one uninfected mouse are shown in Fig. 3. Four of five infected animals efficiently responded to HIV-1 gag peptide pool stimulation compared with HIV-1 envelope peptide pool stimulation and had detectable levels of CD8+IFN-γ+ cells, and 30–87% of these cells were double-positive for IFN-γ and IL-2. All five mice showed a virus-specific CD4+ T cell response, as demonstrated by CD4+IL-2+ cells; a subset of these CD4+ cells was double-positive for both IFN-γ and IL-2. These data confirm that HIV-1 infection elicited Ag-specific human cellular immune responses.

Effect of CD8+ cell depletion on HIV-1 replication

To understand what would happen if we depleted the CTL control of viral replication in this humanized mouse model of HIV-1 infection and to provide a proof-of-concept that this animal model can be used for such immune manipulations, we used CD8 depletion strategies. CD8+ cell depletion was achieved by using two sequential injections of cM-T807 Abs (s.c. and i.p. within 3 d). Two schemes of depletion were used: 1) 5–7 wk postinfection, as a model of established HIV-1 infection, and 2) 2 wk postinfection to prevent the development of CD8+ cell-mediated antiviral responses and to accelerate the development of neuropathy (S. Gorantla, E. Makarov, J. Finke-Dwyer, A. Castanedo, A. Holguin, C.L. Gehhart, H.E. Gendelman, and L. Poluektova, submitted for publication). To assure that CD8+ cells were not detected in the presence of the chimeric anti-CD8 RPE clone DK25 Ab was used (27).

Dynamics of CD4+ and CD8+ cells and viral load in blood from two representative uninfected and HIV-infected/CD8 cell-depleted animals engrafted with the same donor cells are shown in Fig. 4A. CD8 cell depletion at 5 wk postinfection increased viral load in circulation and reduced temporarily CD4+ cell number. In spleens of these animals, the percentage of CD4+ cells was 8.7 ± 3.9% and was not different from the number of CD4+ cells in the spleens of HIV-1–infected animals analyzed at 8–10 wk postinfection (11.2 ± 3.2%, n = 5). CD8+ cell-depleted/uninfected animals of similar age and similar total number of human CD45+ cells in spleen (25.2 ± 2.6%, n = 4) analyzed at 1–3 wk post depletion did not experience loss of CD3+CD4+ cells, but a number of CD8+ cells were significantly reduced to 0.38 ± 2.6% (range 0–1.4%). CD8+ cells reappeared in circulation by 3 wk post depletion (Fig. 4B) with appearance of a significant number of CD4+CD8+ cells.

Changes in CD4+ and CD8+ cell numbers and the viral load in peripheral blood of four HIV-infected/CD8 cell-depleted mice (at 5–7 wk postinfection and 2 wk after CD8 depletion) and five HIV-1–infected/nondepleted control mice analyzed at the first peak of viremia 5–6 wk and 2 wk later were compared (Table II). When CD8+ cells were depleted in animals with established infection, an increase in viral load was detected in all of the mice with Δlog10 ranging from 0.57–2.3 (p = 0.008, Mann-Whitney U test). In non-depleted mice, declines could be associated with a considerable decrease in CD4+ T cell count in blood. However, in depleted animals, even with lowered CD4+ T cell numbers, a substantial increase in viral load was observed.

In contrast to the small changes in viral load that were caused by depleting CD8+ cells in mice with established HIV infection (5–7 wk postinfection), depletion of CD8+ cells at 2 wk postinfection had a more profound effect on viral load (Fig. 5B). Only one out of five mice showed significant levels of virus at 2 wk postinfection (m341 had 6.50 log10 viral copies per milliliter and was excluded from statistical analysis). After CD8 depletion, all of the animals exhibited substantial levels of virus in circulation (geometrical mean 5.13 versus 3.70 for all of the nondepleted animals, p = 0.006). The summary of the peripheral viral load values in non-depleted versus CD8+ cell-depleted animals is shown in Fig. 5.

To investigate the other possible mechanisms in addition to CTL loss for the increase of viral replication, we evaluated the levels of human cytokines (IL-2, IL-4, IFN-γ, TNF-α, IL-6, and IL-10) in plasma after i.p. injection of Abs by cytometric bead array. Increased viral replication in HIV-1–infected/CD8+ cell-depleted animals could also be associated with the homeostatic proliferation of CD4+ cells and increased production of cytokines in the lymphoid tissue, which are capable of stimulating HIV-1 replication. We collected plasma from seven animals at 3 d before the first s.c. injection and 3 d after the second i.p. administration of anti-CD8 Abs. Only human IL-2 levels were increased from 67.2 ± 25.9 to

**FIGURE 4.** Effect of CD8+ cell depletion on peripheral viral load and T cell numbers. A, Two uninfected/CD8+ cell-depleted (upper panels, m306 and m303) and two HIV-1–infected (m304–305) are shown. Dynamics of viral load in peripheral blood were determined by the Amplicor kit, and the detection limit was 1750 copies per milliliter. The numbers of human CD4+ and CD8+ T cells were analyzed by FACs, and the percentages shown are from human CD45 gated cells. CD8+ cell depletion was performed at 5 wk postinfection. B, Representative flow cytometric plots from #305 showing human CD4+ and CD8+ cells in peripheral blood before and after 1 and 3 wk of depletion. The inset at 3 wk postdepletion shows CD3+CD8+ cells reappearing in circulation. Similar recovery of CD3+CD8+ cells in the spleen collected at the end point (3 wk after depletion) is also shown. All of the animals were reconstituted with the same sample of cord blood–derived HSCs.
Dynamics of T cells and viral load in peripheral blood of HIV-1–infected mice with established infection with and without CD8+ cell depletion

Table II.  Dynamics of T cells and viral load in HIV-1–infected mice with established infection and without CD8+ cell depletion.

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>CD8 (%)a</th>
<th>CD4 (%)</th>
<th>VL (Copies/ml)</th>
<th>VL (log10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>104</td>
<td>1.2</td>
<td>0</td>
<td>4.9</td>
<td>0.5</td>
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<td>105</td>
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<td>304</td>
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<td>348</td>
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<td>159</td>
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<td>7.7</td>
</tr>
<tr>
<td>p</td>
<td>0.5</td>
<td>0.014</td>
<td>0.443</td>
<td>0.343</td>
</tr>
</tbody>
</table>

aPercentage of human cells in peripheral blood at the time of viral load analysis.
Mice were analyzed at 5–7 wk postinfection before CD8+ cell depletion and 2 wk after depletion.
Data from nondepleted mice represent the first detected peak of viremia (before) and 2 wk after.
p Values were determined by Mann-Whitney U test.
nd, no data.

CD8 cell depletion and lymphoid tissue morphology

Effect of CD8-depleting Abs on lymph node pathologies and double-positive CD4/CD8 thymocyte differentiation in thymus was studied. Two weeks after depletion, the formation of syncytia in lymph nodes was noted (Fig. 6, HIV-1 p24 staining). CD8+ cell depletion resulted in a significant increase of HIV-1 p24+ cells, changes in B cell distribution (CD79a staining), and increased activation (HLA-DR) in the lymph nodes. To test the ability of chimeric mice to restore the CD8+ cell pool after depletion, we analyzed thymic tissues by immunohistochemistry and flow cytometry. Thymic tissues of uninfected/nondepleted animals were populated by human CD45RO+CD4+CD8+ thymocytes in a significant increase of HIV-1 p24+ cells, changes in B cell distribution (CD79a staining), and increased activation (HLA-DR) in the lymph nodes. To test the ability of chimeric mice to restore the CD8+ cell pool after depletion, we analyzed thymic tissues by immunohistochemistry and flow cytometry. Thymic tissues of uninfected/nondepleted animals were populated by human CD45RO+CD4+CD8+ thymocytes (Fig. 7A). Cortical areas had single-positive human CD4+ cells and fewer CD8+ cells. Murine epithelial claudin-3–positive cells (data not shown) and a small number of human HLA-DR+ cells with dendritic cell morphology were also seen in thymi. Four thymi (two control and two HIV-1–infected mice that were depleted for 10 wk. Viral loads were not affected and were in the range of 4.36–5.77 with a median of 5.25 log10. The chimeric 1129 Ab contains 3% of murine sequences directed to the Ag-specific epitope and “grafted” into the CDR region of human V region. It is human IgG. Anti-CD8 cM-T807 contains complete H and L chain V region genes isolated from a murine hybridoma and ligated to the human γ1 H chain and κ L chain genes and are 30% mouse in origins. We used chimeric 1129 as a control Ab; however, the level of “humanization” of these Abs is highly discordant (data not shown).
Discussion

We previously demonstrated that BALB/c-Rag2<sup>2-/-</sup>γc<sup>-/-</sup> mice could be repopulated with human cells within the lymphoid tissue structures following human CD34<sup>+</sup> cell transplantation. These mice developed a functional human immune system that was susceptible to HIV-1 infection (4). In the current report, NSG mice that are more readily engrafted with human HSCs were used. Animals with an established human immune system were infected with a macrophage-tropic HIV-1 virus, resulting in chronic virus infection and CD4<sup>+</sup> T cell depletion. Viremia reached significant levels by 5–6 wk postinfection. We observed the presence of HIV-1 gag- and envelope-specific IFN-γ– and IL-2–producing human CD8<sup>+</sup> and CD4<sup>+</sup> cells in the spleens of these mice, using ICS. Most of the HIV-specific CD8<sup>+</sup> cells reacted with gag, and the number of IFN-γ/IL-2 double-producers exceeded the number of single IFN-γ–producing cells. Most of the virus-specific CD4<sup>+</sup> cells produced only IL-2, although some CD4<sup>+</sup> cells that produced both IFN-γ and IL-2 were also detected. These results support the notion that humanized NSG mice can generate polyfunctional cellular immune responses to HIV-1, as was shown for human subjects (40–42). We did not find strong humoral immune responses in evaluated animals. In 10 evaluated animals, eight had detectable levels of HIV-1–specific IgM that declined by 10–11 wk postinfection without the development of IgG responses (data not shown).

CTL-mediated protection against HIV-1 infection at different disease stages has not been completely elucidated (43–47). The best evidence for the role of CD8<sup>+</sup> CTLs in the control of

FIGURE 6. Immunohistochemical staining analyses of lymphoid tissue in CD8<sup>+</sup> cell depletion. Immunohistochemical staining of cervical lymph nodes from uninfected and HIV-1–infected/CD8<sup>+</sup> cell-depleted animals. Serial sections of paraffin-embedded tissue were stained for human cell markers, CD8, HIV-1 p24, CD79α, HLA-DR, and Ki-67. Images were captured under an objective lens at original magnification ×10. Lymph nodes from HIV-1–infected animals were analyzed at 2 wk following CD8<sup>+</sup> cell depletion performed at 4 wk postinfection. Commonly seen HIV-1 p24 Ag-positive cells and syncytia, altered B cell distribution, and increased activation (HLA-DR) were detected.

FIGURE 7. Thymus morphology in uninfected and HIV-1–infected/CD8<sup>+</sup> cell-depleted animals. A, Representative sections of paraffin-embedded thymi tissues were stained for human cell markers, CD8, HIV-1 p24, CD79α, HLA-DR, and Ki-67. Images were captured under an objective lens at original magnification ×20; all other panels, original magnification ×400. B, Representative FACS plots of thymic cells from an uninfected control and HIV-1–infected/CD8<sup>+</sup> cell-depleted mice. Compared to the control thymus, CD8<sup>+</sup> cell-depleted thymi, the numbers of double-positive cells were reduced, and the proliferative activity was increased as detected by Ki-67 staining. HLA-DR–positive cells with dendritic cell morphology were present in all of the thymi with claudin-3–positive mouse epithelial cells (data not shown).
immunodeficiency virus infection was obtained in SIV-infected rhesus macaques that were subjected to the immunodepletion of CD8\(^+\) cells. In the current model, depletion of CD8\(^+\) cells 2 wk postinfection led to the acceleration in viral replication during the acute phase of infection. CD8\(^+\) cell depletion at 5–7 wk postinfection also enhanced the viral replication and prevented the small decline observed in nondepleted mice. CD8\(^+\) cell depletion was associated with a significant reduction in the number of human CD4/CD8 double-positive cells in thymus. However, the pool of CD8\(^+\) T cells started to return at 2–3 wk postdepletion. Overall, observations made with CD8\(^+\) T cell depletion using cM-T807 Ab in our humanized mice partially were reminiscent of previously published data from nonhuman primate SIV or SIV/HIV model systems. The durable levels of depletion were achieved, and a strong correlation between disease outcome and viral-specific CTL restoration was observed by use of the mouse–human chimeric mAb (cM-T807) during early stages SIV infection in rhesus macaques (26). In humanized NSG mice, earlier depletion of CD8\(^+\) cells accelerated viral replication. A significant reduction in CD4\(^+\) cell number, observed in our study in blood, was also reported in the SIV model (33). Our data are also concordant with the recently published observations that CD8\(^+\) lymphocyte depletion can induce a transient CD4\(^+\) T cell increase, thus providing increased number of targets for SIV in animals during acute SIV infection without an influence on the disease outcomes (35).

The generation of HIV-1–specific human CTLs in “humanized” mice is influenced by issues of T cell maturation, receptor repertoire, interactions with murine stromal cells, and more. Nonetheless, virus-specific human CD8\(^+\) T cells have been observed in previous studies using immune-deficient mice reconstituted with human PBLs (3, 48–54). A more sophisticated BLT mouse model, where NOD/scid or NSG mice were transplanted with fetal thymus/liver tissue combined with i.v. transplantation of CD34\(^+\) stem cells (8, 55–57), also showed the generation of Ag-specific human immune responses. Our results do not contradict those that have been previously published by Brainard et al. (8). They showed peak viremia at 6 wk postinfection regardless of the strain and amount of inoculated virus (ADA or JR-CSF). They injected 70,000 TCID\(_{50}\) dose of virus, which guaranteed 100% infection and peak viremia at 6 wk postinfection followed by a small spontaneous decline by 8–12 wk postinfection. Unfortunately, when they applied the lower inoculums of JR-CSF 2,000 TCID\(_{50}\), no observation after 7 wk postinfection was shown. We used 5–7.5 times lower inoculums of HIV-1\(_{ADA}\) (10,000 TCID\(_{50}\) dose, which did not guarantee 100% infection of mice). This likely prevented rapid exhaustion of CD8 cell functional properties. By 2 wk postinfection, a significant increase in program death receptor 1 Ag expression on CD8\(^+\) cells were found, which reflects the negative effect of higher infection levels on functional properties of CTLs. Human T cell selection may be another factor that could affect the adaptive immune responses. In our model, human T cell development and selection occur in murine thymus with possible elimination of mouse-reactive TCR clones, whereas in the BLT model the selection takes place in human thymus. However, the biology of thymic selection in the murine environment needs further investigation.

Our data show that in NSG mice reconstituted at birth with HSCs the thymus is highly populated with human CD45RO\(^+\) thymocytes, and we also observed that the T cell repertoire, analyzed by staining for variable β-chain of TCR, is preserved (data not shown). The presence of double-positive T cells in HIV-1–infected/CD8\(^+\) cell-depleted peripheral blood of mice and reduction of viral load suggest that these cells may play a role in the restoration of the lymphocyte pool following CD8\(^+\) cell depletion and contribute to control the viral replication (58). We cannot exclude that this is an example of immunopathology induced by combination of significant levels of HIV-1 replication and CD8 depletion. This observation needs further investigation.

We have direct evidence that our chimeric mice developed cellular immune response against HIV-1. However, we cannot overlook the possibility that this increase in viral replication following CD8\(^+\) T cell depletion may be due to, at least in part, to increased production of IL-2/IL-4 and homeostatic expansion of CD4\(^+\) T cells (59). Nonetheless, our results clearly show that the NSG/hCD34 mouse model can be effectively employed to study the progression and pathogenesis of HIV-1 infection in a live animal host and to dissect the role of specific immune subsets in the control of infection. This is expected to enable future investigations of viral diversity, developmental therapeutics, and antiretroviral immunity.

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Disclosures
The authors have no financial conflicts of interest.

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