Porcine Thymic Grafts Protect Human Thymocytes from HIV-1–Induced Destruction

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Human immunodeficiency virus type 1 (HIV-1) infection depletes thymocytes and destroys thymic structure. Functional, tolerant human T cells develop in vivo in immunodeficient mice receiving porcine thymus and human fetal liver fragments under the kidney capsule. In this model, we evaluated the potential of porcine thymus to protect human thymocytes from the effects of HIV-1. Compared with that observed in control mice with human thymic grafts, porcine thymus attenuated human thymocyte depletion by the CCR5-tropic isolate JR-CSF without preventing thymocyte infection. Porcine thymus protected human thymocytes from infection and depletion by a CXCR4-tropic HIV-1 isolate without reducing peripheral blood viral loads or T cell infection. Human thymocytes from human but not porcine grafts showed decreased Bcl-2 expression and increased apoptosis after NL4.3 infection. Thus, porcine thymus protects human thymocytes from the cytopathic effect of HIV-1, suggesting a possible approach to achieving immune restoration in patients with acquired immunodeficiency syndrome who have incomplete responses to antiretroviral therapy. The model allows analysis of the mechanisms of HIV-mediated thymic dysfunction.

HIV-1 destroys thymocytes and thymic stromal cells [1, 2]. CD4 T cell deficiency after HIV-1 infection reflects shortened survival and failure to replenish depleted cells [3, 4]. Thymic regeneration can contribute to immune reconstitution after effective highly active antiretroviral therapy (HAART) [5]. Although thymus involution occurs with age, the adult thymus remains active late in life [6, 7]. However, some patients do not achieve adequate immune recovery despite having good antiviral responses [8], and some develop resistance to antiretrovirals [9, 10].

Allogeneic thymic transplants achieved immune restoration in children with DiGeorge syndrome [11], and similar transplants were evaluated in patients with AIDS, without success, during the pre-HAART era [12]. Here, we explore the possibility that porcine thymic xenotransplantation might replace the HIV-1–affected human thymus and restore thymopoiesis. We have previously shown that porcine thymic grafts can replace the host thymus in thymectomized T cell–depleted mice to generate a xenotolerant, functional T cell repertoire [13–15]. Furthermore, porcine thymic grafts support the development of polyclonal, functional, and tolerant human T cells in immunodeficient mice [16]. Because porcine cells resist infection with HIV-1, we hypothesized that porcine thymic transplantation might permit immune restoration in the presence of HIV-1 infection.
We tested this hypothesis in immunodeficient mice receiving thymic xenografts with human hematopoietic progenitors.

MATERIALS AND METHODS

Mice. NOD/SCID mice (purchased from Jackson Laboratories) were maintained in sterile microisolator cages according to National Institutes of Health guidelines and underwent transplantation at 5–7 weeks of age.

Transplantation. Human fetal tissue was obtained from Advanced Bioscience Resources with institutional approval. Porcine fetal tissue was harvested from Massachusetts General Hospital (MGH) miniature swine as described elsewhere [13]. Human (gestation week 17) or porcine (gestation day 66–72) fetal thymic fragments were grafted with human fetal liver fragments (hereafter, HU/HU and SW/HU, respectively) under the kidney capsule as described elsewhere [16]. In later experiments, mice also received 5 × 10^3 human fetal liver–derived CD34 cells from the same donor intravenously to enhance human T cell and non–T cell reconstitution [17, 18]. NOD/SCID mice received whole-body irradiation (1.5 Gy) in a Mark I Cs^137 irradiator (JL Shepard and Associates) 2 h before transplantation.

Isolation of human CD34 cells. Human fetal liver CD34 cells were isolated as described elsewhere [18] using MACS and anti-CD34 microbeads (Miltenyi Biotec), yielding >90% CD34 cells.

HIV-1 infection. Infection was induced 8–16 weeks after grafting. Thymic grafts were injected with supernatant from HIV-1- or mock-infected peripheral blood mononuclear cells (PBMCs) via laparotomy under general anesthesia. HIV-1 isolates included JR-CSF (R5 strain; obtained from the Partners AIDS Research Center), NL4.3 (X4 strain), and JH-580 (dual tropic) (the latter 2 provided by L. Su, University of North Carolina, Chapel Hill). JR-CSF and NL4.3 doses were 2000–4000 TCID50, whereas that for JH-580 was 7 × 10^3 TCID50.

Immunohistochemical staining of thymic tissue sections. Thymic tissue was washed, incubated with horse serum and then with mouse anti–human cytoketin monoclonal antibody (MAb; AE1/AE3; Dako), washed, incubated with peroxidase blocking solution, rinsed, incubated with biotinylated horse anti–mouse IgG and then with avidin-biotinylated enzyme complex, rinsed, and incubated with dianimobenzidine peroxidase substrate. Sections were counterstained with hematoxylin and then rinsed before dehydration in ethanol, followed by clearing with xylene.

Flow cytometric analysis. Single-cell suspensions from thymic grafts and spleens were prepared, and PBMCs were isolated over Ficoll-Histopaque 1077 (BioWhitaker). Splenocytes were treated with ACK erythrocyte-lysing buffer (BioWhitaker). Anti-Fcγ MAb (2.4G2) or human serum was added before staining with MAbs. Intracellular staining for p24 and Bcl-2 expression was performed using the Cytofix/Cytoperm Kit (BD Pharmingen) before staining with MAbs for 30 min at 4°C in Perm/Wash buffer (1×). Anti–human CD8–allophycocyanin, anti–human CD4–phycoerythrin (PE), anti–human CD45–fluorescein isothiocyanate (FITC), rat anti–mouse IgG2a–PE, Ly.5–PE, anti–human CD3–FITC, anti–human CD14–PE, and anti–human CD19–PE were purchased from Pharmingen. Anti–porcine thymocyte/T cell marker CD2 (MSA-4–FITC) was obtained from D.H.S. Anti-p24 MAb KC57–FITC was purchased from Coulter, and anti-Bcl2–PE was purchased from BD Immunocytometry Systems. Stained cells from HIV-infection studies were fixed in 1% paraformaldehyde in PBS before analysis on a FACScalibur (BD Biosciences). Apoptotic cells were detected with annexin V together with 7-amino actinomycin D or propidium iodide to exclude dead cells. Gated lymphocytes (5000–10,000) were analyzed using CellQuest (version 1.0; BD Biosciences) or FlowJo software (version 6.4.5; Tree Star).

Determination of plasma viral load. Blood was anticoagulated in EDTA, and plasma was isolated and stored at −80°C. Viral loads were determined using the reverse-transcription polymerase chain reaction (PCR) Amplicor HIV Monitor Test (Roche) at the MGH Microbiology Laboratory.

Measurement of viral DNA. Nested PCR amplification of thymocyte DNA with gag-specific primers SK100 and SK104 as well as SK38 and SK39 or of human-specific β-actin with control primers was performed as described elsewhere [19]. PCR products were subjected to agarose gel electrophoresis, imaged by ethidium bromide staining, transferred to a nylon membrane, hybridized with either a ^32P-labeled HIV-specific probe (SK19) or β-actin–specific probes [20], and analyzed by phosphoimager (BioRad Molecular Imagier System GS-363). Viral copy quantification was achieved by comparison to standard curves consisting of DNA isolated from known numbers of ACH-2 cells (1 copy of HIV-1 genome per cell) and from uninfected human lymphocytes. The signal from each sample was compared with both β-actin and SK19 standard curves, and the percentage of infected cells was calculated accordingly.

Double-immunofluorescence staining. Thymic grafts were harvested from HU/HU and SW/HU recipients 5 weeks after infection. Frozen thymic sections (8-μm thickness) were fixed in acetone, air-dried, and washed and then permeabilized in 0.1% saponin, blocked with 0.5% bovine serum albumin (Vector Laboratories), and stained with either purified anti–human CD11c MAb (CGR-p150/4G1, mouse IgG2a; Chemicon) for myeloid dendritic cells (DCs) or CD68 MAb (PG-M1, mouse IgG3; Dako) for macrophages and with either PE-labeled mouse anti–HIV-1 p24 MAb (KC57-RD1, mouse IgG1; Beckman Coulter) or IgG isotype–matched antibodies (negative controls).
before incubation overnight at 4°C in the dark. In preliminary titrations, we showed that the specific Mabs did not cross-react on porcine thymus. After being washed, sections were incubated with biotinylated secondary Mabs (biotinylated rat anti–mouse IgG2a MAb R12-3 or rat anti–mouse IgG3 MAb R40-82). Sections were washed, stained with FITC-avidin, washed, and then mounted in 4′-6-diamidino-2-phenylindole–containing Vectastain medium (Vector Laboratories). Fluorescence was examined using a Nikon inverted fluorescence microscope (Nikon Eclipse TE2000-U). The 2-color fluorescence images are the computer-merged (Photoshop) view of both FITC and PE staining.

Statistical analysis. For parametric data, groups were compared using Student’s unpaired t test. For nonparametric data, the Mann-Whitney U test was used. P < .05 was considered to indicate statistical significance.

RESULTS

Resistance of human T cells developing in porcine thymic grafts to the cytopathic effect of the R5-tropic strain JR-CSF. HIV-1 JR-CSF, an M-tropic strain that destroys thymus and thymic epithelial cells in SCID-hu mice [3, 21, 22], was injected into the grafts of mice with 50% human peripheral blood lymphocytes 3–4 months after grafting. Six weeks after infection, grafts infected with heat-inactivated virus maintained normal thymocyte subsets. Live virus–infected HU/HU grafts showed profound depletion of CD4/CD8 double-positive thymocytes and of CD4 single-positive thymocytes with increased total numbers of double-negative thymocytes, compared with the numbers in mock-infected control mice (figure 1A and 1B). In contrast, infected mice with SW/HU grafts, which often have increased numbers of CD4/CD8 double-negative cells that are porcine CD2 thymocytes [16], maintained human thymocyte subsets comparable to those in control mice. No loss of CD4 or CD8 single-positive or double-positive thymocytes and no increase in the number of double-negative thymocytes was observed in infected compared with control SW/HU grafts (figure 1A and 1C). The reduced number of human CD4- and CD8-negative thymocytes in infected versus uninfected SW/HU recipients may reflect unexplained differences in the numbers of porcine thymocytes [16] between the groups.

Cytokeratin staining demonstrated a marked loss of architecture and cytokeratin in infected HU/HU but not in SW/HU grafts (figure 1D). Thus, porcine thymic grafts were protected from the destructive effects of HIV-1 infection.

Infected recipients of HU/HU grafts demonstrated a marked depletion of peripheral blood (mean ± SE, 0.5% ± 0.50%) and splenic (mean ± SE, 0.3% ± 0.005%) (n = 4) T cells, compared with those in mock-infected HU/HU control recipients (mean ± SE, 32.2% ± 0.05% of PBMCs [P = .0003] and 44% ± 1.00% of splenocytes [P = .0005], respectively) (n = 4), at 6 weeks. Although SW/HU control recipients had fewer peripheral blood human T cells than did HU/HU control recipients, HIV-1 infection did not affect their numbers in the peripheral blood (mean ± SE, 6.5% ± 3.40% vs. 4.8% ± 0.05% for control; P = .667) or spleens (mean ± SE, 5.05% ± 2.05% vs. 5.0% ± 0.05% for control; P = .98) of SW/HU recipients. Thus, the loss of peripheral blood human T cell populations was avoided in HIV-1–infected mice with porcine thymic grafts.

Productive infection with HIV-1 JR-CSF in human thymocytes developing in porcine thymic grafts. A wide range of HIV copy numbers (10–10,500 copies/1 × 10^8 thymocytes; mean, 6376 copies/1 × 10^8 thymocytes; n = 8) was detected at 6 weeks in HU/HU grafts, with no evidence of infection in inoculated porcine thymic grafts with porcine fetal liver (SW/SW grafts) (data not shown). Despite the lack of thymocyte depletion, infected SW/HU grafts demonstrated a similarly wide range of viral copy numbers (10–10,000 copies/1 × 10^8 thymocytes; mean, 2406 copies/1 × 10^8 thymocytes; n = 10) to that in infected HU/HU grafts.

At 3 weeks after infection, plasma viremia was detected in 1 of 4 infected HU/HU recipients and in 3 of 5 SW/SW recipients. By 6 weeks, 4 of 4 infected HU/HU recipients (range, 988–6110 copies/mL) and 5 of 5 SW/SW recipients (564–470,000 copies/mL) had measurable plasma HIV RNA, with the highest level in a SW/HU mouse. Thus, no evidence was obtained for a delay or failure to infect porcine thymic grafts, despite the resistance of human thymopoiesis in SW/HU grafts to the effects of HIV-1 infection.

To address the possibility that porcine thymic grafts simply delayed rather than prevented thymic disruption by JR-CSF infection, similar analyses were performed at 8 and 10 weeks after infection. However, many of the uninfected HU/HU grafts had lost their cellularity and peripheral blood T cells were minimal to undetectable by these times, making it difficult to assess the effect of HIV-1 infection. Viral loads were minimal to undetectable in 4 of 6 infected HU/HU recipients, suggesting that the loss of human T cells led to a loss of the viral reservoir. Although only 2 of 6 infected HU/HU grafts harvested at 8 or 10 weeks contained live thymocytes (2.5 × 10^4 and 4 × 10^4 total), 4 of 6 SW/SW grafts at these time points contained live thymocytes (4 × 10^4, 4.1 × 10^4, 4.9 × 10^4, and 109.5 × 10^4), suggesting that they were still protected from the cytopathic effect of HIV-1 despite measurable viral loads in 5 of the 6 mice. However, as shown in table 1, the absolute number of human double-positive thymocytes in uninfected SW/SW grafts at 8 and 10 weeks was significantly higher than that in infected SW/SW grafts, suggesting that a delayed cytopathic effect of HIV-1 JR-CSF may occur in the xenogeneic grafts.

No depletion of infected peripheral blood CD4 T cells in HIV-1 NL4.3–infected SW/SW recipients. CXCR4-using
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Figure 1. Depletion of CD4/CD8 double-positive (DP) and CD4 single-positive (SP) thymocytes in HU/HU grafts (human fetal thymic fragments grafted with human fetal liver fragments) but not SW/HU grafts (porcine fetal thymic fragments grafted with human fetal liver fragments) infected with the R5-tropic HIV-1 strain JR-CSF. A, Examples of representative individual infected and uninfected HU/HU and SW/HU grafts 6 weeks after infection. B and C, Mean ± SE total no. of thymocytes in each subset from groups ($n = 4$) of infected and uninfected HU/HU (B) and SW/HU (C) recipients killed 6 weeks after infection with HIV-1 JR-CSF. Results are inclusive of 2 separate experiments with similar results. D, Loss of thymic architecture and cytokeratin staining in HU/HU but not SW/HU grafts caused by infection with HIV-1 JR-CSF. Immunoperoxidase staining using an anti-cytokeratin antibody that detects both porcine and human thymic epithelial cells is shown. Results are representative of thymic sections from 2 mice per group, which showed similar results. NS, not significant.

(X4) T-tropic strains of HIV-1 cause rapid depletion of thymocytes in SCID-hu thymus/liver-grafted mice [23]. We compared the effects of an X4 strain, NL4.3, in porcine and human thymic grafts. SW/HU and HU/HU recipients were prepared with the addition of intravenous injection of CD34 cells from the human fetal liver donor to enhance the level of human T cell recovery, as described elsewhere [17]. Eight to 10 weeks later, the grafts were injected with 2000 TCID$_{50}$ of NL4.3 or with mock-infected culture supernatants. In 2 separate experiments, all infected SW/HU and HU/HU recipients showed very high plasma viral loads 3, 5, and 7 weeks after infection (>750,000 copies/mL), whereas virus was undetectable in the plasma of mock-infected control mice.

By 3 weeks after infection, significant depletion of human CD3 cells was observed in the blood of infected HU/HU recipients but not of SW/HU recipients (figure 2A), which failed to show depletion even by 7 weeks after infection (data not shown). However, peripheral blood T cells in both infected HU/HU and SW/HU recipients were productively infected, with abundant p24 expression (figure 2B).
Table 1. Absolute no. of human CD4/CD8 double-positive (DP) thymocytes at 8 and 10 weeks after infection.

<table>
<thead>
<tr>
<th>Group</th>
<th>DP cells, median (range), absolute no. (10^4)</th>
<th>Viral load, median, copies/mL</th>
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</thead>
<tbody>
<tr>
<td>HU/HU mock infected (n = 7)</td>
<td>8.5 (0–18,340)</td>
<td>ND</td>
</tr>
<tr>
<td>HU/HU infected (n = 6)</td>
<td>0 (0–125)</td>
<td>ND</td>
</tr>
<tr>
<td>SW/HU mock infected (n = 5)</td>
<td>2088 (372–3694)</td>
<td>ND</td>
</tr>
<tr>
<td>SW/HU infected (n = 6)</td>
<td>240 (0–8979)</td>
<td>3035</td>
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NOTE. For HU/HU [human fetal thymic fragments grafted with human fetal liver fragments] mock infected vs. HU/HU infected, \( P = .1 \); for SW/HU [porcine fetal thymic fragments grafted with human fetal liver fragments] mock infected vs. SW/HU infected, \( P = .04 \); for HU/HU mock infected vs. SW/HU mock infected, \( P = .04 \); and for HU/HU infected vs. SW/HU infected, \( P = .07 \). ND, not detectable.

**Protection of human thymocytes from NL4.3 infection in SW/HU grafts.** When mice were killed 3, 5, and 7 weeks after infection, infected HU/HU grafts showed marked reductions in total thymocyte numbers, including all thymocyte subsets but most markedly in double-positive and CD4 single-positive subsets, compared with the numbers in mock-infected grafts (figure 3A). The lower thymocyte numbers in control groups in this experiment, compared with those shown in figure 1, reflect the biological variation between experiments seen in this model. In contrast to HU/HU grafts, infected SW/HU grafts showed no difference in total thymocyte number or subset distribution compared with those in mock-infected control grafts, even by 7 weeks after infection (figure 3A). Thymocytes from HIV-1–infected HU/HU grafts were productively infected, as indicated by p24 expression, whereas p24 was not expressed by thymocytes from infected SW/HU grafts (figure 3B). Similar results were achieved in 2 experiments.

**Protection of human thymocytes from HIV-1 NL4.3–induced apoptosis conferred by porcine thymus.** One of the hallmarks of HIV-1 infection is the induction of apoptosis among infected thymocytes [2]. Increased levels of apoptosis were detected among all thymocyte subsets in infected HU/HU grafts, compared with those in mock-infected control grafts, most markedly among double-positive thymocytes (figure 4A). In contrast, infected SW/HU grafts showed no increase in the number of apoptotic thymocytes, compared with that in uninfected control grafts (figure 4A). Additionally, levels of expression of the antiapoptotic protein Bcl-2 were markedly reduced, compared with those in control grafts—especially among CD4 single-positive and double-positive thymocytes—in infected HU/HU grafts but were not markedly reduced in infected SW/HU grafts (figure 4B). Thus, disruption of the normal up-regulation of Bcl-2 that protects immature thymocytes from apoptosis was associated with increased apoptosis in infected HU/HU but not SW/HU grafts.

**Similar expression of CXCR4 among thymocytes in both infected HU/HU and SW/HU grafts.** A failure to express the coreceptor CXCR4 could not explain the lack of thymocyte infection in SW/HU recipients, given that high levels of CXCR4 were expressed on almost all double-positive thymocytes and...
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Figure 3. A, Human thymocyte nos. after NL4.3 infection. Infection with the X4 virus NL4.3 resulted in reduced nos. of human thymocytes by 3 weeks after infection in HU/HU grafts (human fetal thymic fragments grafted with human fetal liver fragments), but SW/HU grafts (porcine fetal thymic fragments grafted with human fetal liver fragments) showed no depletion compared with that in uninfected control grafts even by 7 weeks. The top panel shows the nos. in groups of infected and uninfected SW/HU recipients killed at the indicated time points after infection (for HU/HU NL4.3 infected, ; for HU/HU mock infected, ; for SW/HU NL4.3 infected, ; for SW/HU mock infected, ; * and ** np 3 np 4 np 5 np 4 P ! 0.03 P ! 0.007). The bottom panel shows the relative reduction in CD4/CD8 double-positive (DP) and CD4 single-positive (SP) thymocytes and the increased proportion of double-negative (DN) thymocytes in infected HU/HU but not SW/HU grafts (*** and ^^^ , for infected vs. noninfected grafts). Data are mean ± SE values. B, HIV-1 p24 expression among all thymocyte subsets of infected HU/HU grafts but not in any subset in infected SW/HU grafts. The top panel shows the mean percentages of p24-expressing cells among the indicated thymocyte subsets of each group 5 weeks after infection (for HU/HU NL4.3 infected, n = 3; for HU/HU mock infected, n = 4; for SW/HU NL4.3 infected, n = 5; for SW/HU mock infected, n = 4; *P<.03 and **P<.007). All groups are presented in the bar graph, but lack of detectable p24 led to the absence of visible bars for any except the infected HU/HU group. The bottom panel shows gated DP thymocytes from 1 representative mouse in each group. Data are mean ± SE values from 1 of 2 similar experiments.

on high percentages of CD4 and CD8 single-positive thymocytes in infected SW/HU grafts (figure 4C). In contrast, expression of CXCR4 was reduced in these thymocyte subsets in infected HU/HU grafts (figure 4C).

Infection of human myeloid DCs and macrophages by NL4.3 in HU/HU but not SW/HU grafts. In situ immunofluorescence staining 5 weeks after infection revealed human CD11c myeloid DCs (figure 5A, subpanels a, e, i, and m) and CD68 macrophages (figure 5B, subpanels a, e, i, and m) in both SW/HU and HU/HU grafts with or without NL4.3 infection. A significant number of the CD11c DCs in the infected HU/HU grafts were infected. Colocalization of HIV-1 p24–expressing cells with CD11c cells in HU/HU grafts was evidenced by yellow fluorescence of merged colors (figure 5A, subpanels k and l), CD68 macrophages and HIV-1 p24 antigen also colocalized in the infected HU/HU grafts (figure 5B, subpanels k and l). In contrast, there was no colocalization of human CD11c-positive DCs or human CD68-positive macrophages and HIV-1 p24–expressing cells in SW/HU grafts, and p24 expression was faint or undetectable in infected SW/HU grafts (figure 5A and 5B).

DISCUSSION

Disruption of thymopoiesis by HIV-1 contributes to HIV-induced immunodeficiency. Polyclonal, functional, and xenotol-
Figure 4.  A, Increased apoptosis among infected HU/HU grafts (human fetal thymic fragments grafted with human fetal liver fragments) but not among SW/HU grafts (porcine fetal thymic fragments grafted with human fetal liver fragments). The left panel shows total thymocytes from individual representative mice in each group. The right panel shows the mean percentage of apoptotic cells in each thymocyte subset for each group at 5 weeks after infection (for HU/HU NL4.3 infection, $n = 3$; for HU/HU mock infection, $n = 4$; for SW/HU NL4.3 infection, $n = 5$; for SW/HU mock infection, $n = 4$). Data are mean ± SE values from 1 of 2 similar experiments. All groups are presented in the bar graph, but a lack of detectable apoptotic cells led to the absence of visible bars in any except the infected HU/HU group. B, Loss of Bcl-2 expression among CD4/CD8 double-positive (DP) and CD4 single-positive (SP) thymocytes of infected HU/HU grafts but not SW/HU grafts. The left panel shows the mean percentages of Bcl-2–expressing cells among the indicated thymocyte subsets of each group at 5 weeks. Data are mean ± SE values from a single experiment (**$P < 0.0001$). Similar results were obtained from 2 of 2 experiments. The right panel shows gated CD4 SP thymocytes from 1 representative mouse in each group. 7-AAD, 7-amino actinomycin D; APC, allophycocyanin; PE, phycoerythrin.

C, Reduction of CXCR4 expression in CD4 SP, CD8 SP, and DP thymocyte subsets of infected HU/HU grafts but not in any subset in infected SW/HU grafts. The left panel shows the mean percentages of CXCR4-expressing cells among the indicated thymocyte subsets of each group at 5 weeks ($n = 5$ per group; **$P < 0.0064$ and ***$P < 0.0001$). Similar results were obtained from 2 of 2 experiments. Data are mean ± SE values from 1 experiment. The right panel shows CXCR4 expression among gated DP thymocytes from 1 representative mouse in each group. 7-AAD, 7-amino actinomycin D; APC, allophycocyanin; PE, phycoerythrin.
Figure 5. No productive infection with HIV-1 NL4.3 of human myeloid dendritic cells (A) and macrophages (B) in SW/HU (porcine fetal thymic fragments grafted with human fetal liver fragments) thymic grafts. HU/HU (human fetal thymic fragments grafted with human fetal liver fragments) and SW/HU thymus was either mock infected or infected with 2000 TCID$_{50}$ of HIV-1 NL4.3. Five weeks after intrathymic infection, thymic tissues were harvested. Sections were costained for either the myeloid DC marker CD11c (fluorescein isothiocyanate [FITC]; green staining; A) or the macrophage marker CD68 (FITC; green staining; B) and the HIV-1 p24 antigen (phycoerythrin [PE]; red staining). Colocalization of HIV-1–infected cells (CD68 or CD11c and p24; subpanels c, d, g, h, k, l, o, and p) is illustrated by yellow. Slides were examined with a Nikon immunofluorescence microscope (Nikon Eclipse TE2000-U). Analysis of thymic sections from 2 mice in each group showed similar results. Original magnifications were ×20 for all subpanels except d, h, l, and p and were ×40 for d, h, l, and p.
erant human T cells can develop in porcine thymic grafts implanted with human fetal liver tissue under the kidney capsule of immunodeficient mice [16]. Because porcine source animals could provide essentially unlimited numbers of genetically defined and specific pathogen-free thymic tissues, we addressed the hypothesis that porcine thymic grafts might protect developing human thymocytes from HIV-1 infection. We have demonstrated that the porcine thymus protects human thymopoiesis from the effects of an R5 and an X4 HIV-1 isolate.

In the case of the R5 isolate, the porcine thymus protected against the cytopathic effect of HIV-1 without preventing thymocyte infection. Consistent with the findings of previous studies [24–26], HIV-1 JR-CSF depleted double-positive and single-positive populations in HU/HU grafts within 6 weeks, and an increase in the number of double-negative cells occurred. In contrast, no such changes occurred in SW/HU mice. Moreover, thymic cellularity and structure were maintained in infected SW/HU mice, whereas they were obliterated in infected HU/HU mice. The absence of provirus in thymocytes from SW/ SW mice indicates that the pig is resistant to HIV-1 infection. Although the mechanisms of the protective effect of the porcine thymus against the cytopathic effect of R5 HIV-1 infection are unknown, we hypothesize that the porcine thymic microenvironment may resist destruction by HIV-1 [3]. Additionally, species incompatibilities in stromally derived cytokines and other indirect mechanisms involved in HIV-induced cytopathicity [27] may render thymocytes resistant to destruction in porcine thymic grafts. Because the natural life span of the thymic graft was limited, it was difficult to obtain meaningful data later than 6 weeks after infection. Nevertheless, the data obtained in SW/HU grafts at 8 and 10 weeks after infection indicate ongoing human thymopoiesis at these time points but do not rule out a gradual loss of thymopoiesis due to HIV infection.

Thymopoiesis in SW/HU mice was also protected from the disruptive effects of infection with an X4 strain, NL4.3, but by a different mechanism than that involved after infection with the R5 strain. Human thymocytes, macrophages, and DCs, which were abundant in SW/HU grafts, avoided productive infection by NL4.3, whereas all 3 cell types were infected in HU/HU grafts. Infection of thymic macrophages and DCs may lead to thymocyte infection and influence T cell maturation, given their involvement in negative and positive selection [28]. Type I interferon production by thymic pre-DCs after HIV-1 infection may impair the development of functional T cells [29]. The lack of infection of human thymic DCs and macrophages, despite their presence in large numbers in infected SW/HU grafts, suggests that thymic stroma–derived (i.e., nonhematopoietic) factors promoting HIV-1 infection (e.g., species-specific cytokines) in HU/HU grafts may be lacking in SW/ HU grafts. Some cytokines have been suggested to promote HIV-1 infection or replication in thymocytes [30, 31], and some (interleukin [IL]–7, tumor necrosis factor–α, IL-1, and IL-6) have been shown to promote thymocyte infection in vitro [32, 33].

HIV-1–induced apoptosis of T cells is associated with increased expression of apoptosis-inducing caspases and reduced expression of survival factors such as Bcl-2 [34, 35]. Increased apoptosis in association with reduced Bcl-2 expression, especially by the CD4 single-positive and double-positive subsets, was observed in human thymocytes in NL4.3-infected HU/HU but not SW/HU grafts. The results in infected HU/HU thymocytes are consistent with results in a macaque simian immunodeficiency virus model [36]. Although our in vivo thymic infection studies revealed decreased Bcl-2 expression among all thymocyte subsets in HU/HU grafts, in vitro studies have suggested Bcl-2 down-regulation only among double-positive cells, with Bcl-2 up-regulation among CD4 single-positive thymocytes after HIV-1 infection [37]. These differences may be explained by a contribution from the intact thymic microenvironment to indirect cytopathicity in the presence of HIV-1 infection.

Protection from X4 virus infection in porcine thymic grafts was not due to failure to express the CXCR4 coreceptor, which showed a normal expression pattern [19, 38] in human thymocytes of SW/HU grafts, with high levels on double-positive and on CD4 and CD8 single-positive thymocytes. Reduced CXCR4 expression among these subsets in HU/HU grafts probably reflected selective destruction of CXCR4high thymocytes and, hence, enrichment for those lacking CXCR4.

NL4.3-infected SW/HU mice were productively infected, as demonstrated by p24 expression in peripheral blood T cells and high plasma viral load. It is possible that human thymocytes developing in porcine thymic grafts do not become infected until they leave the thymus and enter the peripheral lymphoid tissues, where a peripheral HIV-1 reservoir exists. This reservoir may reside in the monocyte/macrophage and DC lineages, as high levels of reconstitution with these human cells occur in this humanized mouse model [18]. Monocytes, macrophages, and DCs are important in initial M-tropic HIV-1 infection because they express CD4 and the CCR5 coreceptor needed for fusion and entry [39]. Infected monocytes may maintain a long-lasting HIV-1 reservoir as macrophages or migratory DCs [40, 41] supporting high levels of viral replication [26]. However, it is unclear how such a reservoir could be established by an X4 virus, and we favor the hypothesis that mature peripheral blood T cells are infected in SW/HU mice because of capture of HIV-1 by molecules such as DC-SIGN (DC-specific intercellular adhesion molecule–3–grabbing nonintegrin) [42] on uninfected DCs in the periphery. Further studies are needed to address this possibility. Moreover, it is unclear how HIV-1 enters the periphery in these animals; leakage of virus during the intrathymic injection process or emigration of an unidentified
infected cell population from the thymus to the periphery are possibilities.

Preserved thymopoiesis may explain the lack of depletion of peripheral blood human T cells in NL4.3-infected SW/HU mice, despite high viral loads, because T cells continually produced in the porcine thymus may replace peripheral blood T cells that are destroyed. The contrasting rapid depletion of peripheral blood T cells in infected HU/HU mice may reflect the failure of the disrupted thymopoietic process in human thymic grafts to replace T cells destroyed in the periphery.

Thus, human thymocytes developing in porcine thymic grafts can be protected from the cytopathic effect of HIV-1 by 2 different mechanisms, one in which thymocytes and antigen-presenting cells resist infection and another in which thymocytes are infected but are protected from the cytopathic effect. In studies using a very high dose of the highly thymotropic dual-tropic HIV-1 isolate JH 580, no significant protection of human thymocytes was observed in porcine thymic grafts (data not shown), indicating that the protective effect of xenogeneic thymic tissue can be overridden by infection with a virus strain that is exceptionally cytopathic to thymocytes [43]. Further analyses of this model should provide insights into the mechanisms of thymocyte infection and destruction by HIV-1 and may suggest ways of promoting immune restoration in HIV-1–infected persons.

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