Development of a Novel Transgenic Mouse/SCID-hu Mouse System to Characterize the In Vivo Behavior of Reservoirs of Human Immunodeficiency Virus Type 1–Infected Cells

Emilie-Jeanne Wang,1 Massimo Pettoello-Mantovani,1 Christina M. Anderson,1 Kristin Osielki,1 Devorah Moskowitz,1 and Harris Goldstein1,2

To develop a system in which transgenic and knockout technologies are used to study the in vivo behavior of human immunodeficiency virus type 1 (HIV-1) reservoirs, 2 different mouse models were combined: transgenic mice carrying full-length provirus encoding the monocytotropic HIV-1JR-CSF isolate (JR-CSF mice) and severe combined immunodeficient mice implanted with human fetal thymus and liver tissues (thy/liv-SCID-hu mice). Extensive HIV-1 infection of human thymic implants occurred after injection of JR-CSF mouse leukocytes into thy/liv-SCID-hu mice, indicating that these cells provide an in vivo source of replication-competent HIV-1. In vivo persistence of transferred JR-CSF mouse leukocytes carrying replication-competent HIV-1 in thy/liv-SCID-hu mice was indicated by the emergence of HIV-1 infection in mice that had no detectable HIV-1 infection until after highly active antiretroviral therapy. Thus, thy/liv-SCID-hu mice populated with JR-CSF mouse leukocytes, a persistent cellular reservoir harboring replication-competent HIV-1, present a new in vivo system for characterizing reservoirs of HIV-1 and evaluating therapeutic strategies designed to eliminate them.

Use of highly active antiretroviral therapy (HAART), a combination of drugs that inhibits enzymes essential for human immunodeficiency virus type 1 (HIV-1) replication, dramatically reduces the virus load in the plasma and tissues of most HIV-1–infected persons [1]. The reduction by HAART of plasma HIV-1 loads to undetectable levels is associated with a marked improvement in the clinical course of disease and a significant reduction in the death rate among patients who receive treatment. However, evens after years of treatment with HAART, long-lived cells harboring replication-competent HIV-1 continue to persist in the blood, lymphoid tissues, and male genital tracts of treated individuals [2–8]. These cells may be the source of the virus that causes the reemergence of HIV-1 infection in lymphoid tissues and the rapid rebound in plasma HIV-1 loads that are observed in HIV-1–infected patients after the cessation of HAART [9–13]. Because latently infected T cells capable of producing infectious HIV-1 have a mean half-life of \( \sim 44 \) months, it could take as much as 60 years of HAART to eradicate even a small population of \( 1 \times 10^7 \) latently infected CD4+ memory T cells [5]. This persistent reservoir of HIV-1–infected cells is the major impediment to eradication of HIV-1 infection. Therefore, a major new goal for developmental therapeutics will be to identify and test new treatments capable of intensifying antiretroviral therapies beyond standard HAART, to better target and eliminate HIV-1–infected cells. Identifying the long-lived cells that are capable of harboring replication-competent HIV-1, determining their anatomic localization, and delineating the signals that trigger these long-lived cells to produce HIV-1 would advance the achievement of this goal.

The characterization of cell types that are capable of harboring replication-competent HIV-1 and the delineation of the biological behavior of those cell types would be greatly facilitated by the development of mouse models for studying HIV-1 infection. Although there are differences between the human and the murine immune system, mice have proven to be an extremely useful tool for investigating the pathogenesis of infectious diseases, the regulation of the immune system, and the induction of protective immunity. In particular, the capacity to manipulate the mouse genome, either by introducing new genes that can be specifically expressed in particular tissues or by targeting the disruption of defined genes, provides a powerful in vivo experimental approach for the genetic analysis of a wide range of biological problems. For example, because T cell subsets derived from T cell receptor (TCR) transgenic mice transferred into recipient mice home to the appropriate sites in lymphoid tissues, the in vivo trafficking of T cells can be investi-
HIV-1 in thy/liv-SCID-hu mice are human, these cells cannot be
In addition, because the T cells carrying replication-competent
may be altered by interaction with the mouse microenvironment.
HIV-1–infected human T cells located in the peripheral tissues
the human thymic implant [19] and because the behavior of any
HIV-1 infection in thy/liv-SCID-hu mice was mainly restricted to
ing persistent reservoirs of HIV-1 infection is limited, because
HIV-1–infectible target to detect the in vivo behavior of any
HIV-1 infection of the
liver implant by limiting-dilution coculture.

The experimental approach of adoptive transferral of TCR
transgenic mouse T cells also has been used to demonstrate that
transferred memory T cells can persist in vivo for months with- 
out antigenic stimulation while maintaining the ability to rapid-
ly produce cytokines after reexposure to antigen [15, 16]. Use of
this well-defined system of adoptive transfer of memory CD4+ 
T cells from TCR transgenic mice to investigate the in vivo be-
havior of persistent T cells that harbor replication-competent
HIV-1 was prevented by the inability of HIV-1 to infect mouse
 cells; the HIV-1 envelope protein, gp120, does not bind to the
murine homologues of the human HIV-1 receptors [17]. In vivo
HIV-1 infection did not occur in mice that were transgenic for
the expression of the cellular receptors for HIV-1 and human
CD4 and CCR5, because additional postentry blocks compro-
mise HIV-1 replication in mouse cells [17, 18]. Another approach
taken to circumvent the restricted replication of HIV-1 in mice
was to transplant SCID mice with pieces of human fetal thymus
and liver (thy/liv-SCID-hu mice) that subsequently grew into a
In addition, because the T cells carrying replication-competent
HIV-1 in thy/liv-SCID-hu mice are human, these cells cannot be
manipulated by transgenic technology.

We have recently described a mouse line that is transgenic
for a full-length, proviral clone of a monocyte-tropic HIV-1
isolate, HIV-1JR-CSF (JR-CSF mice), whose T cells, monocytes,
and dendritic cells produce infectious HIV-1 [20]. After being
adoptively transferred into recipient mice, leukocytes from
JR-CSF mice that are capable of producing infectious HIV-
1 could function as a reservoir of HIV-1–infected cells. If thy-
liv-SCID-hu mice were the recipients of the adoptively trans-
ferred JR-CSF mouse cells, then their human thymic implant
would serve as an HIV-1–infectible target to detect the in vivo
production of HIV-1 by the adoptively transferred JR-CSF
mouse leukocytes. Because initiation of HAART immediately
after the inoculation of thy/liv-SCID-hu mice with HIV-1
completely prevents subsequent infection of the implanted hu-
man thymic graft [21], infection of the human thymic graft
by HIV-1 produced by the transferred JR-CSF mouse cells
should be prevented as long as the mice are receiving HAART.
However, discontinuation of HAART would permit persistent
JR-CSF mouse cells to infect the thymic grafts of these re-
cipient mice. In the present study, we examined whether this
approach could be used to develop a new mouse model for
studying the in vivo behavior of cellular reservoirs of HIV-1
infection. Specifically, subpopulations of JR-CSF mouse cells
would be transferred into thy/liv-SCID-hu mice receiving
HAART, and the persistence of JR-CSF mouse cells produc-
ing infectious HIV-1 would be detected by discontinuing
HAART and then evaluating the temporal onset of HIV-1
infection in the human thymic graft. This model could also
be used to evaluate the efficacy of therapeutic interventions
designed to deplete persistent HIV-1 reservoirs. Thy/liv-SCID-
hu mice populated with transferred JR-CSF mouse cells main-
tained on HAART would be treated with a protocol designed
to eliminate HIV-1–infected cells. Successful elimination of
leukocytes carrying replication-competent HIV-1 by the ex-
perimental protocol would be indicated if HIV-1 infection did
not emerge in the human thymic graft after the cessation of
HAART.

Methods

Animals. The JR-CSF transgenic mouse line was constructed
as described elsewhere [20]. In brief, the infectious molecular clone
of HIV-1Jr-csf, PYK-JR-CSF (obtained from the National Insti-
tutes of Health AIDS Research and Reference Reagent Program),
used to construct the transgene was cloned from the lymphocytes
of an HIV-infected patient soon after the initiation of culture. PYK-
JR-CSF contains the full-length genomic sequence of HIV-1Jr-csf,
as well as 0.5 kb of 3′ and 2.2 kb of 5′ flanking sequences, and
produces infectious virions after transfection into cells [22, 23]. The
PYK-JR-CSF plasmid was linearized with EcoRI and then micro-
projected into the pronuclei of fertilized embryos derived from
FVBxC57B6 mouse crosses, as described elsewhere [18]. Trans-
genic mice were identified by polymerase chain reaction analysis
of genomic DNA extracted from tails, using primer pairs specific
for the amplification of HIV-1 gag DNA, as described elsewhere
[20]. The HIV-1 long terminal repeat (LTR) controls the transgene
expression, and HIV transcription occurs in tissues in which the
HIV-1 LTR is active [20]. BALB/c-TgN10Loh mice (DO11.10 mice)
transgenic for the expression of the DO11.10 TCR that recognizes
a single polypeptide (ISQAVHAAAEINEAGR) corresponding to
residues 323–339 of hen egg ovalbumin (OVA323–339) presented
by I-A<sup>d</sup> class II major histocompatibility complex [24, 25]
were purchased from Jackson Laboratory.

Thy/liv-SCID-hu mice. The thy/liv-SCID-hu mouse line (6–8
weeks old) was constructed by implanting human fetal thymic and
liver tissue, obtained from human fetuses at 17–21 gestational
weeks and within 8 h after the elective termination of pregnancy,
under the kidney capsules of SCID mice, as described elsewhere
[26]. In brief, after SCID mice were anesthetized with pentobarbital
(40–80 mg/kg), a total of ~10 pieces of syngeneic human fetal
thymic and liver tissue were implanted under the left and right
kidney capsules. Three months later, the size of implanted tissue
had increased by >20-fold.

Titration of HIV-1–infected mononuclear cells in the human thymic/liver
implant by limiting-dilution coculture. HIV-1 infection of the
human thymic implants from the mice was quantified by measure-
ment of the number of HIV-1–infected thymocytes present in
the graft by limiting-dilution coculture, as described elsewhere [21, 26].
Five-fold dilutions of human thymocytes isolated from the human

that drug consumption could be confirmed by measurement of the thy/liv-SCID-hu mice were housed either singly or in pairs, so that the purity of the sorted cells was determined by flow cytometry and was >95%.

In vivo depletion of transferred CD4+ T cells. Transferred mouse CD4+ T cells were depleted by treating the mice with 2 doses (500 µg/dose) of purified anti-CD4 GK1.5 monoclonal antibody (a gift of Christine M. Grimaldi) by intraperitoneal injection on alternate days. This protocol has been shown to deplete >98% of the CD4+ T cells in treated mice [29].

Results

Productive infection of thy/liv-SCID-hu mice by injection with JR-CSF mouse mononuclear cells. We have previously demonstrated that mononuclear cells from JR-CSF mice produce infectious HIV-1 and infect activated human peripheral blood mononuclear cells (PBMC) in culture [20]. To determine whether cells from the JR-CSF mice are also infectious in vivo, we injected JR-CSF mononuclear cells into thy/liv-SCID-hu mice and examined whether the human thymocytes present in the human thymic graft became infected with HIV-1. JR-CSF mononuclear cells were activated with concanavalin A (ConA; 10 mg/mL) in the presence of IL-2 for 2 days and then introduced into the thy/liv-SCID-hu mice by injection into the human thymic graft, intrasplenic injection, intraperitoneal injection, or intravenous injection. Approximately 1–2 months later, HIV-1 infection was detected in the human thymic implant and was quantified by limiting-dilution coculture of thymocytes isolated from the graft. As shown in table 1, extensive HIV-1 infection of the human thymic implants occurred after JR-CSF mononuclear cells were introduced into the thy/liv-SCID-hu mice by any of these 4 routes. Thus, JR-CSF mononuclear cells carry replication-competent HIV-1 that can introduce disseminated HIV-1 infection in vivo into the thy/liv-SCID-hu mice.
month after transfer into SCID mice. We next examined whether transferred JR-CSF mononuclear cells persist in the recipient SCID mice after intravenous injection. These experiments were facilitated by the availability of the ROSAβ-geo26 (ROSA26) mouse strain, which was created by random retroviral gene trapping of a β-galactosidase reporter gene in embryonic stem cells; the hematolymphoid cells of this strain express β-galactosidase, thereby making the strain useful for chimerism and transplantation studies [31]. We used this transgenic line to facilitate the identification and quantification of transferred JR-CSF mouse cells in the tissues of the recipient mice by crossing ROSA26 mice with JR-CSF mice to obtain JR-CSF/ROSA26 mice. Lymphocytes from these mice could be detected and quantified after transfer into recipient mice by flow cytometry, after incubation of cells with FDG. splenocytes from ROSA26 mice and from JR-CSF/ROSA26 mice were activated with ConA (10 mg/mL) and cultured in the presence of IL-2 for 48 h, washed, and directly injected into SCID mouse spleens. At 2-week intervals after injection, mice (4 mice/group) were killed, and the presence of infectious JR-CSF mouse cells was evaluated by quantitative coculture of splenocytes from the recipient SCID mice with activated human PBMC. JR-CSF mouse cells capable of initiating HIV-1 infection were present in the SCID mice at levels that were relatively constant over the course of a 6-week period (figure 2). These data, therefore, indicate that activated JR-CSF mouse splenocytes persisted in the SCID mice and maintained the capacity to produce infectious HIV-1 for up to 6 weeks after transfer into SCID mice.

Introduction of HIV-1 infection after the cessation of HAART by JR-CSF mouse cells that persist in thy/liv-SCID-hu mice. We have previously reported that HIV-1 infection of thy/liv-SCID-hu mice could be prevented, if HAART was initiated for the mice immediately after inoculation with HIV-1 [21]. As demonstrated earlier (table 1), the human thymic implant present in the thy/liv-SCID-hu mice became infected with HIV-1 after the intrasplenic injection of activated JR-CSF mouse splenocytes. Therefore, we were interested in determining whether immediate treatment of the recipient mice with HAART would prevent in vivo infection of the human thymic graft with HIV-1 after the injection of JR-CSF mouse splenocytes into the thy/liv-SCID-hu mice. JR-CSF mouse splenocytes were activated with ConA (10 mg/mL), cultured in the presence of IL-2 for 48 h, and then injected into the spleens of thy/liv-SCID-hu mice, and HAART administration was started immediately in 1 group of mice. After 1 month, the human thymic graft was biopsied, and its virus load was measured by quantitative coculture. In contrast to the untreated mouse, in which the human thymic graft was markedly infected with HIV-1, no HIV-1 infection was detected in the human thymic grafts of the HAART-treated mice (figure 3A). Thus, treatment with HAART prevented infection of the human thymic graft with HIV-1 after transfer of JR-CSF mouse splenocytes into thy/liv-SCID-hu mice.

To extend the observation presented earlier (figure 2), that the JR-CSF mouse leukocytes that persisted in the SCID mice for >1 month after transfer demonstrated ex vivo infectivity, we examined whether persistent JR-CSF mouse cells also maintained the ability to initiate in vivo HIV-1 infection. To accomplish this, we investigated whether the number of injected JR-CSF mouse cells that persisted in thy/liv-SCID-hu mice after 1 month of treatment with HAART was sufficient to infect the human thymic implant after HAART was stopped. For this experiment, we used the group of thy/liv-SCID-hu mice injected with JR-CSF mouse splenocytes described earlier (figure 3A), in which HIV-1 infection of the human thymic grafts was prevented by treatment with HAART. Anti–HIV-1 therapy was discontinued in these mice at the end of 1 month of treatment with HAART, and, 1 month later, the human thymic implant was rebiopsied, and the level of HIV-1 infection in the graft was quantified by limiting-dilution coculture. As shown in fig-

### Table 1. Infection of thy/liv-SCID-hu mice by injection with JR-CSF mouse leukocytes.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Route of infection</th>
<th>No. of infected thymocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>281b</td>
<td>Intraplant</td>
<td>625</td>
</tr>
<tr>
<td>289b1</td>
<td>Intraplant</td>
<td>3125</td>
</tr>
<tr>
<td>289b4</td>
<td>Intraplant</td>
<td>625</td>
</tr>
<tr>
<td>289h3</td>
<td>Intraplant</td>
<td>3125</td>
</tr>
<tr>
<td>288a3</td>
<td>Intrasplenic</td>
<td>625</td>
</tr>
<tr>
<td>283a</td>
<td>Intraperitoneal</td>
<td>125</td>
</tr>
<tr>
<td>283b</td>
<td>Intraperitoneal</td>
<td>5</td>
</tr>
<tr>
<td>A1</td>
<td>Intra venous</td>
<td>625</td>
</tr>
<tr>
<td>290</td>
<td>Intra venous</td>
<td>25</td>
</tr>
</tbody>
</table>

**Note:** Thy/liv-SCID-hu mice were infected with JR-CSF mouse leukocytes by the indicated route. The no. of human immunodeficiency virus type 1–infected thymocytes per 10^6 thymocytes was quantified 1–2 months later by limiting-dilution coculture, using activated human peripheral blood mononuclear cells.

* Per 10^6 thymocytes.
Figure 1. Persistence of cells from JR-CSF/ROSA\(\beta\)-geo26 (JR-CSF/ROSA26) mouse cells 1 month after transfer into SCID mice. Activated splenocytes from JR-CSF/ROSA26 mice \((2 \times 10^7 - 3 \times 10^8)\) cells) were injected intravenously into SCID mice, and, 1 month later, the mice were killed and the spleens were harvested. The population of \(\beta\)-galactosidase (\(\beta\)-gal)–expressing JR-CSF/ROSA26 mouse splenocytes in the recipient SCID mice was quantified by incubation of splenocytes with the fluorogenic substrate fluorescein di-\(\beta\)-\(\beta\)-galactopyranoside and analysis by flow cytometry. A representative dot plot showing cellular fluorescence vs. forward/side scatter (FSC) of splenocytes from a SCID mouse and from SCID mouse injected with splenocytes from a ROSA26 mouse or a JR-CSF/ROSA26 mouse is shown.

Figure 2. Production of infectious human immunodeficiency virus type 1 by JR-CSF mouse splenocytes that persist after injection into SCID mice. Activated JR-CSF mouse splenocytes \((\text{cells})\) were injected into the spleens of SCID mice, and groups of mice were killed \((4 \text{ mice/group})\) at 2-week intervals. The no. of infectious JR-CSF mouse cells present in the spleens of these mice was quantified by limiting-dilution coculture, using activated human peripheral blood mononuclear cells. Data are shown as the mean TCID/10^6 presentsplenocytes \(\pm\) SEM in each group of mice, analyzed at 2, 4, and 6 weeks after infection.

Figure 3, B, the implants from all of the HAART-treated mice that were not previously infected with HIV-1 now displayed marked HIV-1 infection. This observation indicates that JR-CSF mouse splenocytes transplanted into thy/liv-SCID-hu mice are able to serve as a persistent in vivo reservoir of HIV-1 infection and have the capacity to introduce HIV-1 infection into the human thymus/liver implant, which is suppressed by HAART but emerges after the cessation of HAART.

We next investigated whether in vivo elimination of the HIV-1–producing JR-CSF mouse cells after their transfer into the thy/liv-SCID-hu mice would prevent the development of HIV-1 infection of the human thymic graft. We took advantage of a well-established system for in vivo elimination of mouse T cells that involves treatment of mice with monoclonal antibodies directed against T cell–specific surface markers such as CD4 [29, 32]. Because the recipient mice for these experiments are SCID mice, the CD4^+ T cells present in the mouse circulation and lymphoid tissues of the SCID mice after transfer of JR-CSF mouse leukocytes would be predominantly derived from the donor JR-CSF mice. Consequently, treatment of transplanted thy/liv-SCID-hu mice with antibodies to mouse CD4 should eliminate any mouse CD4^+ T cells derived from the donor JR-CSF mice without affecting the human T cells present in the thy/liv-SCID-hu mice. Therefore, highly purified CD4^+ T cells \((>95\%\) purity) were isolated from the spleens of JR-CSF mice by immunomagnetic sorting and injected directly into the spleens of the thy/liv-SCID-hu mice, and HAART was then initiated in the recipient mice. One group of mice was untreated, and another group of mice was treated the next day and again 2 days later by intraperitoneal injection of purified anti–mouse CD4 GK1.5 monoclonal antibody \((500 \text{ mg})\). One week after intrasplenic injection of the JR-CSF mouse CD4^+ T cells, HAART was discontinued for all of the mice. One month after the cessation of HAART, the level of HIV-1 infection in the human thymic graft of the mice was measured by limiting-dilution coculture. In the thy/liv-SCID-hu mice that were not treated with the monoclonal antibody to mouse CD4, HIV-1 infection was detected in the human thymic implants at a level of 5 TCID/10^6 thymocytes (figure 4), which indicates that CD4^+ T cells from the JR-CSF mice carry replication-competent HIV-1 capable of introducing in vivo infection. In contrast, of the 2 thy/liv-SCID-hu mice that were treated with the monoclonal antibody to mouse CD4 after transfer, no infection was detected in the human thymic implant of one mouse, and the other mouse had only minimal infection, of 1 TCID/10^6 thymocytes.

Sustained in vitro differentiation and growth of JR-CSF mouse antigen-specific Th1 and Th2 T cells. Because HIV-1–infected CD4^+ memory T cells have been implicated as a major infectious reservoir of long-lived cells in HIV-1–infected patients [33], investigation of the in vivo behavior of HIV-1 reservoirs would be furthered by the development of a model in which the in vivo behavior of memory T cells that produce infectious
HIV-1–Transgenic Mouse/SCID-hu Mouse Model

Figure 3. Emergence after cessation of highly active antiretroviral therapy (HAART) of human immunodeficiency virus type 1 (HIV-1) infection of the thymic implants of thy/liv-SCID-hu after injection with JR-CSF mouse leukocytes. Activated JR-CSF mouse splenocytes (4 × 10⁶ cells) were intrasplenically injected into thy/liv-SCID-hu mice, and HAART was immediately initiated in some mice. A. After a 1-month treatment period, the thymic implants were biopsied, and the no. of HIV-1–infected thymocytes in the implants was quantified by limiting-dilution quantitative coculture. B. HAART was then stopped, and, after 1 month, the thymic implants of each mouse were rebiopsied, and the no. of HIV-1–infected thymocytes in the implant was quantified by limiting-dilution quantitative coculture. Data are presented as TCID/10⁶ thymocytes, determined by limiting-dilution coculture.

HIV-1 could be studied. Therefore, we examined whether JR-CSF mice could serve as a source of antigen-specific CD4⁺ memory T cells that could be transferred into thy/liv-SCID-hu mice and would infect the human thymic implant. As the first step, we examined whether the in vitro life span of antigen-stimulated JR-CSF mouse Th1 and Th2 cells would be decreased because these mice are transgenic for a provirus capable of producing HIV-1. Antigen-specific T cell responses have been extensively studied using mice carrying transgenes expressing TCR α and β chains specific for a defined antigen, such as the DO11.10 TCR transgenic mice that express a clonotypic TCR specific for the single polypeptide (ISQAVHAAHAEINEAGR) corresponding to OVA323–339 presented by I-A^d class II major histocompatibility complex [24, 25]. DO11.10 mice were crossed with JR-CSF mice to obtain mice transgenic for both transgenes (JR-CSF/DO11.10 mice). CD4⁺ T cells positively isolated from the spleens of JR-CSF/DO11.10 or from DO11.10 mice by immunomagnetic affinity sorting were maintained in cultures under Th1 or Th2 polarizing conditions. As shown in figure 5, growth of JR-CSF/DO11.10 mouse T cells cultured under Th1 or Th2 polarizing conditions was comparable to that observed for the DO11.10 mouse T cells. After 5 weeks, HIV-1 production by these antigen-specific T cells was determined from an aliquot of supernatant from the Th1 polarized cultures and the Th2 polarized cultures, using the Amplicor HIV-1 Monitor kit (Roche Diagnostic Systems), as described elsewhere [20]. Continued production of HIV-1 was detected for both sets of cultures, using 60,577/10⁶ HIV-1 RNA copies/mL for the Th1 supernatant and 50,885 HIV-1 RNA copies/mL for the Th2 supernatant. After 2 months of culture, the CD4⁺ T cells were analyzed for cytokine production by flow cytometry to determine whether the cells still displayed the Th1 or Th2 phenotype after prolonged culture. As shown in figure 6, after restimulation with OVA323–339, almost one-third of the JR-CSF/DO11.10 cells cultured under Th1 or Th2 polarizing conditions produced interferon-γ or IL-4, respectively. Thus, JR-CSF mouse CD4⁺ T cells can be cultured for prolonged periods under conditions that lead to their differentiation into Th1 or Th2 cells, despite carriage of the JR-CSF provirus as a transgene.

Discussion

Use of transgenic mice permits investigators to manipulate the mouse genome, either by introducing new genes that can...
Figure 5. Effect of expression of the human immunodeficiency virus type 1 (HIV-1) transgene on antigen-specific proliferation of CD4+ T cells under Th1 or Th2 polarizing conditions. CD4+ T cells were positively isolated from the spleens of JR-CSF/DO11.10 or DO11.10 mice by immunomagnetic affinity and stimulated in 24-well plates (∼2.5 × 10⁵ cells/well) with 0.3 mM residues 323–339 of hen egg ovalbumin presented by irradiated syngeneic splenocytes (2.5 × 10⁵ cells/well). The cells were maintained under Th1 polarizing conditions (100 U/mL interleukin [IL]-12 and 10 mg/mL anti–IL-4; A) or Th2 polarizing conditions (200 U/mL IL-4 and 2 ng/mL anti–IL-12; B) and split every week to maintain a cell density of ∼2.5 × 10⁵ cells/well. The no. of T cells in each group was determined at various times, using a hemacytometer, and cellular viability was determined by trypan blue dye exclusion.

be specifically expressed in particular tissues or by targeting the disruption of defined genes. This technology is a powerful tool that provides an in vivo system for the genetic analysis of a wide range of biological problems. Consequently, a host of transgenic mouse lines with defined phenotypes have been developed and used to examine the mechanistic basis for the activation and regulation of the immune system, to identify the correlates of protective immunity against infectious agents, and to delineate the fundamental immunological mechanisms of protection. To use transgenic technology to investigate HIV-1 replication and pathogenesis, we developed the JR-CSF mouse line, which is transgenic for a full-length HIV-1 provirus derived from an M-tropic primary clinical isolate [20]. The transgenic provirus is under the control of the HIV-1 LTR, and T cells from these mice produce infectious HIV-1. The availability of this transgenic mouse line permitted us to cross it with defined transgenic lines to study the effect of HIV-1 expression on lymphocyte function and to determine the feasibility of developing a new mouse system amenable to application of transgenic and knockout technology to investigate the in vivo behavior of reservoirs of HIV-1–infected cells.

In the present study, we demonstrated that activated JR-CSF lymphocytes persisted in recipient SCID mice for >1 month after transfer into HAART-treated thy/liv-SCID-hu mice and were capable of introducing HIV-1 infection into the human thymic graft after HAART was stopped. The transferred JR-CSF mouse leukocytes may directly infect human thymocytes present in the graft by migrating into the human thymic implant. Alternatively, because the peripheral lymphoid tissues of our thy/liv-SCID-hu mice are populated with human T cells [34], it is also possible that the transferred JR-CSF mouse leukocytes home to mouse lymphoid tissues and infect adjacent human T cells that can traffic to and infect the human thymic graft. This scenario is compatible with our previous demonstration that substantial numbers of HIV-1–infected human lymphocytes are present in the peripheral blood and peripheral lymphoid tissues of the thy/liv-SCID-hu mice and introduce HIV-1 infection into the human thymic graft after intraperitoneal injection of HIV-1 [34]. No HIV-1 infection was detected in cocultures of human thymic implants derived from HAART-treated thy/liv-SCID-hu mice that had been injected with JR-CSF mouse leukocytes. Because these cocultures were performed in the absence of antiretroviral drugs, the negative coculture results indicated that insufficient JR-CSF mouse leukocytes were present in the human thymus/liver implant after transfer to initiate in vitro infection. This indicates that the HIV-1 infection detected in cocultures of biopsy specimens from the human thymic implants was derived from HIV-1–infected human thymocytes and not from JR-CSF mouse cells present in the human thymic implant.

A population of latently infected memory CD4+ T cells is established within weeks after the onset of symptoms of primary HIV-1 infection [2], and, because this reservoir exhibits an extremely slow rate of decay, these cells provide a persistent source of infectious HIV-1 [4, 7, 35]. Our goal was to develop an in vivo system in which the lymphoid tissues of thy/liv-SCID-hu mice are populated with memory T cells carrying an HIV-1–infectious provirus. Studies in mice have greatly expanded the understanding of the mechanism of the differentiation, trafficking, and regulation of CD4+ memory T cells [36, 37]. The in vivo transition of CD4+ effector T cells into CD4+ memory T cells has been studied using a system in which Th1 or Th2
HIV-1–encoded proteins that have been reported to interfere with cellular proliferation and viability may mediate this abnormal behavior of HIV-1–infected cells. The HIV-1 vpr gene encodes a 14-kDa protein that is expressed in infected cells and that can induce cell-cycle arrest at the G2 phase of the cell cycle, followed by death of the infected cells by apoptosis [41]. Vpr-mediated arrest of the cell cycle at the G2 checkpoint by does not require productive infection of the cell, as evidenced by the ability of noninfectious virus to induce Vpr-mediated cell-cycle arrest [42]. Another HIV-1 protein, Nef, markedly inhibits proliferation of PBMC stimulated by IL-2 by interacting with cellular proteins involved in cellular signaling, such as p56lck, p53, and p44MAPK [43]. Therefore, to determine whether expression of HIV-1 proteins by the JR-CSF mouse T cells would inhibit cellular growth and differentiation, we crossed the JR-CSF mice with DO11.10 mice that carry a transgene encoding expression of an ovalbumin-specific TCR and generated JR-CSF/DO11.10 mice. After antigen stimulation, JR-CSF/DO11.10 mouse T cells exhibited an in vitro proliferative response that was comparable to that seen in the control DO11.10 mice and, after several rounds of antigenic stimulation and over the course of 1 month of culture, these cells maintained their capacity to differentiate into antigen-specific T cells displaying a Th1 or a Th2 phenotype and continued to produce HIV-1. These in vitro data are compatible with other data presented in the present report (figure 2) that demonstrate that splenocytes from JR-CSF mice persisted and continued to be infectious for >1 month after transfer into SCID mice. Taken together, these data indicate that expression of the JR-CSF transgene does not affect T cell proliferation or differentiation. Why Vpr or Nef production did not affect proliferation or differentiation by the JR-CSF mouse T cells is unclear. It is possible that, although the JR-CSF mouse T cells produce HIV-1, the levels of accessory proteins, such as Vpr or Nef, produced are insufficient to significantly suppress cellular proliferation. Alternatively, Vpr and Nef may require interaction with cellular proteins to exert their suppressive activity, and mouse proteins may differ sufficiently from their human homologues that this interaction does not occur, thereby preventing Vpr and Nef from exerting the functional effects in mouse T cells reported for Tat and cyclin T1 [44].

Differences between the biological behaviors of murine T cells and human T cells may affect the relevancy of findings made with this model for the behavior of the HIV-1 reservoirs in humans. Nevertheless, despite possible disparities between the process of differentiation, homeostatic control, and trafficking in human and mouse effector and memory T cells, studies performed with murine systems have been used to provide insights into the development of immunological memory in humans [15, 36–38, 45]. Therefore, we developed the JR-CSF/SCID-hu mouse model to take advantage of these established murine systems to study the in vivo behavior of HIV-1–infected memory T cells, which are implicated as the primary HIV-1 reservoir [33]. The applicability of findings generated with this model to HIV-1 reservoirs in humans may also be affected, because the behavior of mouse T cells carrying an HIV-1 provirus differs from that of HIV-1–infected human T cells. We sought to minimize these differences by constructing the transgenic mice with an HIV-1 provirus regulated by the native LTR, which was derived from an M-tropic strain isolated from a patient in the early stages of

Figure 6. Exhibition of Th1 or Th2 phenotype by antigen-stimulated JR-CSF mouse T cells after several months of culture. After 2 months of culture under Th1 or Th2 conditions, as described in the legend for figure 5, aliquots of JR-CSF mouse CD4+ T cells were analyzed for the production of interferon (IFN)-γ or interleukin (IL)-4 by flow cytometry, as described in Materials and Methods.
infection [46, 47]. To this end, the JR-CSF mice were constructed with a provirus that contains the full-length genomic sequence of HIV-1JR-CSF, an M-tropic HIV-1 isolate cloned from lymphocytes from an HIV-1–infected patient soon after the lymphocytes were placed in culture [22, 48]. Because the endogenous HIV-1 LTR controls replication of the provirus, viral production by the JR-CSF mouse T cells should be responsive to murine homologues of cellular factors that induce HIV-1 replication in human T cells. This was indicated by our observation that the low level of HIV-1 produced by nonactivated JR-CSF mouse T cells was increased >100-fold after stimulation of the cells with anti-CD3e [20]. Consequently, JR-CSF memory T cells displayed minimal-to-undetectable levels of HIV-1, which is similar to the behavior of latently infected human T cells (data not shown). Therefore, despite the limitations inherent in the use of mouse models for studying HIV-1 infection, we postulate that this model should prove useful in evaluating aspects of the in vivo behavior of reservoirs of HIV-1–infected cells.

Because transferred memory T cells should home to the appropriate anatomic compartments [15, 39], including those in which drug penetration may not be optimal, our mice may provide a system for evaluating the in vivo efficacy of therapeutic approaches designed to target and eliminate persistent cells carrying replication-competent HIV-1. To accomplish this goal, several strategies have been proposed, including the “flushing out” of cells that are latently infected with HIV-1 by broadly activating T cells while the patient continues to receive HAART [49], the specific elimination of HIV-1–infected cells with genetically engineered autologous T cells that express gp120-specific receptors, and the targeted elimination of memory T cells with an anti-CD45RO immunotoxin [50, 51]. Although the pool of latently infected resting CD4+ T cells is considered to be the major cell population harboring replication-competent HIV-1 that contributes to the viral rebound observed after the discontinuation of HAART [33], infectious HIV-1 is also carried in other cellular reservoirs, such as monocyte/macrophage lineage cells [52–54]. The development of new strategies capable of eliminating or diminishing the reservoir of this population of HIV-1 infected cells may be required to prevent the rebound of viremia observed after the cessation of HAART. Our mouse model for studying HIV-1 infection can be used to provide a source of purified subpopulations of cells carrying replication-competent HIV-1 that can be used to evaluate the capacity of different cellular subpopulations, such as monocytes, macrophages, and dendritic cells, to function as reservoirs of HIV-1–infected cells. We propose that the novel mouse model described in this study would provide a valuable preclinical model for studying approaches to targeting and eliminating cells carrying the HIV-1 provirus. This system should also permit us to apply transgenic and knockout technologies to study factors and genes that affect the capacity of defined cellular populations, such as antigen-specific memory T cells and macrophages, to function as reservoirs for HIV-1 infection.

Acknowledgment

We thank Christine M. Grimaldi for her kind gift of purified anti-CD4 GK1.5 monoclonal antibody.

References
