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HIV-1 Pathogenesis and Therapeutic Intervention in the SCID-hu Thy/Liv Mouse: A Model for Primary HIV-1 Infection in the Human Thymus

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SUMMARY

The SCID-hu Thy/Liv mouse is a model for the analysis of human thymopoiesis. It has been constructed by engrafting fragments of human fetal liver and thymus into the immunodeficient C.B-17 *scid/scid* (SCID) mouse. The resulting 'Thy/Liv' organ promotes long-term differentiation of human T cells. Given the apparently normal physiology of the SCID-hu Thy/Liv organ, it has been used to explore the pathophysiologic mechanisms of HIV-1 infection *in vivo*, and to test therapeutic modalities such as anti-HIV-1 drugs and haematopoietic stem cell (HSC)-based gene therapy. In this review, I will summarise what we have learned from the SCID-hu Thy/Liv model, with a focus on recent findings in HIV-1 replication and therapy. Unique HIV-1 determinants have been identified which are required for replication in the Thy/Liv organ but not for replication in PBMC or in T cell lines *in vitro*. The mechanism of HIV-1 induced thymus depletion is not clear. It is correlated with high levels of HIV-1 replication. Both direct and indirect mechanisms may be involved. In addition to preclinical evaluation of anti-HIV-1 drugs, the SCID-hu Thy/Liv mouse has also been successfully used to test the feasibility of HSC-based gene therapy.

A number of improved SCID-hu models have been constructed to meet different requirements. Using these SCID-hu Thy/Liv models, current/future efforts will provide insightful information for understanding pathogenesis and designing therapeutic interventions against HIV-1 infection in humans, especially in paediatric patients.

INTRODUCTION

It has become increasingly clear that the pathophysiologic correlates leading to T cell depletion during HIV-1 disease may be clarified by direct evaluation of interactions between the virus and defined haematolymphoid organs.[1,2] Though not well studied during HIV-1 infection, the thymus has been implicated as a site of early viral replication[3–8] and thymic organs from HIV-1 infected fetuses and paediatric patients show profound parenchymal damage and involution.[5,6,9,10] More significantly, a strong correlation of HIV induced thymus dysfunction has been established with faster AIDS progression in paediatric

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patients.[11] In addition, early thymus destruction appears to be a common feature of other lentivirus diseases in FIV-infected cats[12] and SIV-infected monkeys.[13]

In the thymus, where most T cells are derived, CD4 is present not only on mature (CD3+CD4+CD8−) T cells and macrophages, but also on less mature thymocytes (CD3−/low CD4+CD8+) and intrathymic progenitor (CD3−CD4+CD8−) cells.[14,15] Since the thymus organ is difficult to study in human subjects, a small animal model (SCID-hu Thy/Liv mouse) for the analysis of human thymopoiesis has been constructed by engrafting fragments of human fetal liver and thymus into the immunodeficient C.B-17 *scid/scid* (SCID) mouse.[16] The resulting 'Thy/Liv' organ promotes longterm differentiation of human T cells in a manner which appears physiologically normal.[17,18] Thymocyte subpopulations are represented within the organ in expected proportions, a normal T cell receptor V*fl* repertoire is displayed,[19,20] and tolerance is induced towards both 'self' major histocompatibility antigens and exogenously provided superantigens.[21,22]

Given the apparently normal physiology of the SCID-hu Thy/Liv organ, we and others[23–26] have used this model to explore the pathophysiologic mechanisms of HIV-1 infection *in vivo*. In this review, I will summarise what we have learned from the SCID-hu Thy/Liv model, with a focus on recent findings in HIV-1 replication and therapy. A number of reviews of earlier reports have been published.[27,28] A different humanised SCID model, hu-PBL-SCID,[29] which is transplanted with mature human PBMC, will not be covered here.

THE SCID-HU THY/LIV MOUSE AS A MODEL FOR PRIMARY HIV-1 INFECTION AND PATHOGENESIS IN THE HUMAN THYMUS

In contrast to *in vitro* models of HIV-1 infection, the SCID-hu Thy/Liv mouse provides an intact human lymphoid organ for HIV-1 infection. Multiple cell types are present and, most importantly, most target cells are in their physiological resting stage. In addition, normal T cell development and maturation occur in the Thy/Liv organ over 12 months after transplantation.[17,18]

The organ system is permissive for infection with primary HIV-1 isolates.[30] Both macrophages and T cells are infected in the Thy/Liv organ. Most T cell line-adapted HIV-1 strains, however, failed to replicate efficiently.[31] The infection proceeds in a dose- and time-dependent manner and is suppressed by *in vivo* administration of nucleoside analogues such as zidovudine (AZT).[32]

In an attempt to analyse AZT resistant mutants arising during *in vivo* selection, multiple rounds of infection of the Thy/Liv organ in the presence of increasing concentrations of AZT have failed to generate AZT-resistant HIV-1 mutants (J. McCune and H. Kaneshima., personal communication). This is consistent with the finding that very few mutations have accumulated during the infection process.[33] Thus HIV-1 replication in this model reflects a low level, primary infection in the absence of immune selection.

After intra-organ inoculation of the SCID-hu Thy/Liv with HIV-1 (Figure. 1), HIV-1 replication reaches high levels followed by depletion of CD4+ thymocytes with an inversion

of the CD4/CD8 ratio.[23,24,34] CD4+CD8+ thymocytes, which comprise 80–85% of total thymocytes, are significantly depleted about 1 week after HIV-1 reaches peak infection (Figure 2).[24,35] In addition, a more accelerated pace of replication and thymocyte depletion is observed with rapidly replicating, syncytium-inducing (SI) virus isolated from AIDS patients than with slowly replicating, non-syncytium-inducing (NSI) virus isolated from the same patients before AIDS development, or from long term non-progressor patients.[36,37] Thus, the Thy/Liv organ provides a relevant *in vivo* model to evaluate primary HIV-1 replication and pathogenicity.

ANALYSIS OF HIV-1 FACTORS UNIQUELY REQUIRED FOR REPLICATION IN THE THY/LIV ORGAN

Studies of HIV-1 accessory genes in the SCID-hu Thy/Liv model

As is observed in the SIV-infected rhesus macaque,[38] replication and pathologic effects (e.g. thymocyte depletion) of HIV-1 (both NL4–3 and JRCSF) in the SCID-hu mouse are dependent upon an intact *nef* open reading frame[39] Analysis of the other HIV-1 accessory genes such as *vpr*, *vpu* and *vif* has demonstrated that, unlike in tissue cultures, mutations in these genes significantly slowed down the replication and cytopathic effects of HIV-1-NL4-3.[40]

A close correlation between the levels of HIV-1 replication and thymocyte depletion has been established (Table 1). Thus, NL4-3, JD, EW and primary SI isolates replicated to high levels in about 2–3 weeks post inoculation (wpi) and lead to early thymocyte depletion at about 3–4 wpi. JRCSF, NL4-3 mutants (*nef*-, *vpu*- or *vif*-) and primary NSI isolates replicated to peak levels in about 5–6 weeks and significant thymocyte depletion occurred at about 7–8 weeks post infection. In addition, high input HIV-1 accelerated both HIV-1 replication and its associated thymocyte depletion.[40] Therefore, prolonged presence of high viral replication is required to cause thymocyte depletion.

HIV-1 env determinants required for efficient replication in the Thy/Liv organ

Comparison of ‘attenuated’ HIV-1 isolates with ‘pathogenic’ ones *in vitro* and *in vivo* should help to identify important viral determinants for replication and pathogenesis *in vivo*. The Lai/IIIB isolate and its associated infectious molecular clones (e.g. HXB2) were found to infect T cell lines such as H9.[41] When a laboratory worker was accidentally infected by Lai/IIIB, however, HIV-1 was isolated only from inoculation of primary PBMC, but not from T cell lines.[42] The SCID-hu Thy/Liv model was used to study the replication of HXB2 and of HXB2 recombinant viruses with HIV-1 fragments isolated from the infected laboratory worker.[43] HXB2 showed no or very low levels of replication in the Thy/Liv organ. Replacement of its subgenomic fragment encoding the envelope gene with a corresponding fragment from the LW87-1 isolate generated a recombinant virus (HXB2/LW) which replicated actively in SCID-hu mice.

The specific env determinants have been mapped to the V1–V3 regions of the HIV-1 genome. Six unique mutations in the V3 loop region have been identified which contribute to most of its increased replication *in vivo*. These changes affected target cell tropism and/or

overall infectivity of the virus. However, HXB2/LW showed no enhanced replication activity in PBMC. Thus, altered or expanded host cell range may contribute to its enhanced replication in the Thy/Liv organ.

The unique structural determinants in HIV-1 appear to be necessary for infectivity *in vivo*, but not in PBMC or in immortalised T cell lines. Interestingly, the relevant changes did not affect the *nef* gene, previously implicated for pathogenicity of SIV in rhesus macaques,[38] or of HIV-1 in SCID-hu mice.[39,40] The *vpu* and *vpr* genes, which have also been reported to affect HIV-1 replication in SCID-hu Thy/Liv mice,[40] of HXB2/LW also remain defective. Thus, unique features of the V3 region of *env* that are necessary for infection of thymic target cells are revealed by phenotypic and molecular analyses of HIV-1 isolates in the SCID-hu Thy/Liv mouse.

A variation of the SCID-hu Thy/Liv model which transplants more human tissues has been developed.[25] In this model, HIV-1 infection can be initiated by intraperitoneal inoculation.[44] However, the Lai/IIIB isolate appears to infect the Thy/Liv organ in this model and leads to thymus depletion. The difference may be due to its long duration (6 months) of infection and/or to activation of human thymocytes in this model by some unknown mechanisms.

MECHANISMS OF HIV-1 INDUCED THYMUS DEPLETION

HIV-1 replication in the Thy/Liv organ leads to thymocyte depletion with a preference for CD4+ thymocytes. As mentioned above, a close correlation exists between levels of HIV-1 replication and thymocyte depletion (Table 1). Kinetic analysis indicated that thymocyte depletion occurs about 1–2 weeks after HIV-1 peak replication is achieved (Figure 2). This indicates that high levels of viral replication are required to lead to thymus depletion. Both viral encoded proteins and host factors may be involved. The SCID-hu Thy/Liv mouse has been used to address the following questions regarding HIV-1 induced thymus depletion.

How do thymocytes die in response to HIV-1 infection in the Thy/Liv organ?

Apoptosis has been associated with HIV-1 induced T cell death both *in vitro* and *in vivo*.[45–49] It appears to be associated, at least partly, with HIV-1 induced thymocyte depletion in the Thy/Liv organ. Morphologically, some thymocytes with condensed nuclei are detected in HIV-1 infected Thy/Liv organs by thin section light microscopy and by electron microscopy.[24] Biochemically, partial chromosomal loss (detected by propidium iodide staining)[24] and DNA strand breaks (detected by terminal deoxynucleotide transferase labelling)[35] are associated with HIV-1 induced thymocyte depletion, although the characteristic chromosomal DNA ladder associated with most forms of apoptosis is not consistently observed (Su, Bonyhadi, Kaneshima and McCune, unpublished observation). Experiments performed by a different research group using the Thy/Liv model have failed to demonstrate evidence of significant levels of apoptosis during HIV-1 induced thymocyte depletion. Necrosis appears to be the major mechanism of HIV-1 induced T cell death (J. Zack, personal communication). Differences in experimental procedures and in animal maintenance conditions may contribute to the discrepancy.

Are all dead or dying thymocytes directly infected with HIV-1?

Regardless of how thymocytes die after HIV-1 infection, a very important question is whether direct infection is required for the thymocyte to die. The replication level of HIV-1 in the Thy/Liv organ is relatively low. At peak times, about 10% thymocytes are infected as measured by PCR detection of proviral DNA.[23,33–35,39,40,43] This is consistent with lack of significant mutation during infection[33] and failure to generate AZT-resistant mutants (unpublished results), as discussed earlier. Using flow cytometric cell sorting coupled with a semi-quantitative PCR assay for detection of HIV-1 DNA, it was shown that most of the thymocytes within the HIV-1 infected Thy/Liv organ with induced DNA strand breaks in the chromosome were not infected with HIV-1. Thus, both cells with DNA strand breaks and without DNA strand breaks were infected at the same level (about 10% as measured by PCR). Therefore, HIV-1 replication may induce changes in the thymus to cause cell death (or chromosomal breakages) of uninfected, as well as infected, thymocytes.[35] Likewise, it has been recently reported that most apoptotic cells are not productively infected in lymph nodes from HIV-1 infected human patients or from SIV infected monkeys.[48]

What contributes to the thymus depletion following HIV-1 infection?

Both direct and indirect mechanisms of cell death induction may be involved. The plateau and slight reduction of HIV-1 replication during thymus depletion may be interpreted as gradual depletion of direct HIV-1 target cells (Figure 2).[24,35] Thus, HIV-1 infects and depletes the target cells and leads to lower levels of HIV-1 replication. Reduced replication may be achieved by a number of HIV-1 encoded factors with cytotoxic or cytostatic activities as demonstrated in T cells cultured *in vitro*. For example, *vpr* has been shown to lead to G2/S phase cell cycle arrest in infected target cells by a cytostatic mechanism.[50] Other HIV-1 proteins, such as *tat*, *nef* and *gp120/gp41*, have also demonstrated cytotoxic activity in various cell culture systems.[51–54]

Besides evidence discussed above of DNA strand breaks in uninfected cells, there is also evidence showing that, at least in some infected thymocytes, HIV-1 infection does not lead to their immediate destruction. For example, CD3+CD8+CD4− cells from HIV-1 infected Thy/Liv organs have been shown to carry HIV-1 proviral DNA [23,34] (Su, unpublished results). It has recently been shown that the HIV-1+ CD8 single positive cells are derived from HIV-1 infected CD4+ progenitors,[55] possibly CD4+CD8+ and/or CD3−CD4+CD8− cells. Thus, HIV-1 infection of CD4+CD8+, CD3−CD4+CD8− or earlier progenitor cells does not necessarily lead to their immediate cytolysis.

Is thymocyte depletion due in part to destruction of intrathymic T progenitor cells?

Besides the CD4+CD8+ immature thymocytes, the intrathymic CD3−CD4+CD8− T progenitor cell subpopulation constitutes a target for HIV-1 infection *in vivo*. Some HIV-1 isolates infect this population and deplete it; others infect it and do not lead to its immediate depletion.[35] As illustrated in Figure 3, infection of progenitor cells by HIV-1 may lead to the following: (1) generation of a reservoir to transmit the HIV-1 genome to progeny cells; (2) destruction of a population that could provide progeny cells (reduction of progenitor cell pool); and (3) introduction of a maturational block upon normal thymocyte differentiation

processes. Induction of cell death, either by direct or indirect mechanisms, may occur through pathway a or pathway b (Figure 3).

In addition to production of viral proteins, HIV-1 infection also leads to cytokine dysregulation in humans.[56] A large number of cytokines are produced in the thymus which play important roles in modulating T cell development. HIV-1 infection in the Thy/Liv organ leads to increased production of cytokines such as IL4, IL6, IL10. In addition, TNF*a* and TGF*fl* are also induced.[57] The contribution of the increased levels of cytokines to thymocyte depletion is not clear and needs future attention. These viral and host 'virulence factors' may cause thymocyte depletion either directly or indirectly by enhancing HIV-1 replication, as shown in pathway c (Figure 3).

The thymus microenvironment is essential for T cell development. Direct infection and destruction of thymic epithelium cells have been reported in the human thymus and in HIV-1 infected Thy/Liv organs.[5,34] This may lead to blockage in T cell development and result in thymocyte death. Thus, HIV-1 infection of the Thy/Liv organ may cause destruction of the thymus microenvironment to induce thymocyte depletion (pathway d, Figure 3). It is not clear, however, whether the HIV-1 infected thymic stromal cells are still functional in supporting *de novo* human T cell development.

SCREENING ANTI-HIV-1 DRUGS IN THE SCID-HU MODEL

As mentioned above, the Thy/Liv model provides an *in vivo* system to study the human thymus organ in a normal, physiologically relevant state. In addition, the SCID-hu mouse also provides an animal model to test toxicity and bioavailability of therapeutic compounds.[58] The SCID-hu Thy/Liv model has been used to evaluate anti-retroviral drugs since the beginning of its construction. AZT, either administered before or post exposure to HIV-1, appeared to inhibit HIV-1 replication in the model.[32,59] In recent years, the Thy/Liv model has been used to evaluate and screen various compounds for their efficacy, toxicity and *in vivo* formulations.[60,61] This includes AZT, didanosine, nevirapine and bicyclams (singly or in combinations). This will provide useful preclinical information in drug evaluation and clinical trials.

Novel peptide-based therapeutic agents can also be tested in this model. It has been reported that IL10, but not IL12, can inhibit HIV-1 replication in the SCID-hu Thy/Liv mouse.[25]

PRECLINICAL STUDIES OF HSC-BASED GENE THERAPY OR CELL THERAPY FOR AIDS

Gene delivery via the haematopoietic stem cell (HSC) offers an attractive means to introduce antiviral genes into both T cells and macrophages for AIDS gene therapy. HSC can be isolated from a number of tissue sources, including bone marrow and peripheral blood, and are used to reconstitute all haematopoietic lineages in transplant recipients.[62,63] Recently, haematopoietic progenitor cells have been efficiently transduced with murine leukaemia virus-based vectors.[64,65] In addition, a retroviral vector encoding an anti-HIV-1 ribozyme has been shown to inhibit HIV replication in macrophage-like cells derived from

the transduced stem/progenitor cells.[65] Due to the difficulty of deriving human T cells from HSCs *in vitro*, it is difficult to demonstrate efficacy in the T lineage. The SCID-hu Thy/Liv model offers an ideal system to evaluate the potential of HSC-based gene therapy for AIDS.

The HIV-1 *rev* protein is critically required for the transport of unspliced HIV-1 mRNA into the cytoplasm and thus for the expression of HIV structural proteins.[66] A trans-dominant mutant of HIV-1 *rev*, RevM10, has been shown to inhibit HIV-1 replication in PBMCs without affecting the growth and functions of the transduced cells.[67,68] A clinical trial using retrovirally modified PBMCs, however, has demonstrated that RevM10 modified PBMC are short-lived *in vivo*. Thus, HSC-based gene therapy may be necessary to obtain long termanti-HIV-1 PBMCs in AIDS patients. It was unknown, however, whether the transduced gene will express at sufficient levels in T and myeloid cells and what effect (if any) RevM10 expression may have on the ability of transduced HSCs to differentiate into lymphoid or myeloid lineages.

To address these issues, experiments were performed to show that RevM10 could be efficiently transduced into cord blood derived HSC/progenitor cells, which develop into primary T cells[69] or myeloid cells expressing the RevM10 gene in the SCID-hu Thy/Liv model or SCID-hu Bone model,[70] respectively. After reconstitution of the Thy/Liv implants in SCID mice (SCID-hu Thy/Liv) with the transduced HSC/progenitor cells, normal thymocytes were derived and a significant number of donor derived thymocyte cells were found to express the RevM10 gene[69] or a marker gene.[71] It was further demonstrated that sufficient levels of RevM10 expression could be achieved to suppress HIV-1 replication in primary T cells derived from retrovirally transduced human HSCs[69] Thus, the RevM10 gene did not appear to inhibit the differentiation of HSC/progenitor cells into T cells in the Thy/Liv organ. The level of retrovirus-mediated RevM10 expression in T cells derived from transduced HSCs was sufficient to suppress HIV-1 replication.

CONCLUSIONS

I have briefly summarised the most recent findings in HIV-1 infection and therapy using the SCID-hu Thy/Liv mouse. Its application to understanding HIV-1 infection, pathogenesis and therapy has proven that insighful information can be obtained about the HIV-1 disease process *in vivo* and about the feasibility of various therapeutic modalities. Further studies for assessing HIV-1 effects on the thymus environment, mechanisms of HIV-1 replication and associated thymocyte depletion, as well as tests of novel therapeutic agents and protocols are being explored by an increasing number of research groups. The preclinical studies of HSC-based gene therapy in the SCID-hu mouse have helped to launch a Phase I/II clinical trial in HIV-1 infected patients (SyStemix, Inc., 1997).

A number of improved SCID-hu models have been constructed to meet different requirements. For example, human fetal liver, thymus, bone and spleen fragments have been co-transplanted into SCID mice to form a human joint organ with T cell, B cells, myeloid cells and red blood cells.[72] This model will be useful to test HIV-1 pathogenesis and to study HSC differentiation into all lineages in the same organ. In addition, implanting human lung tissues intraperitoneally in the SCID-hu Thy/Liv mouse has created a model to study

HIV-1 infection of the lung (macrophages) and its transmission to the Thy/Liv organ.[26] Using current or improved SCID-hu Thy/Liv models, future efforts are directed to addressing the following outstanding questions.

1. What viral factors (virulence factors) are uniquely required for replication and/or pathogenicity in the Thy/Liv organ?
2. What host factors are involved in HIV-1 induced thymus depletion?
3. What are the distributions of co-receptors (CCR5 and fusin) in the Thy/Liv organ?
4. What target cells are infected in the Thy/Liv organ by different HIV-1 isolates?
5. How significant is HIV-1 induced indirect cell killing in the Thy/Liv model?
6. Can HIV-1 infected/depleted Thy/Liv organs still support *de novo* thymopoiesis from HSC/progenitor cells carrying an antiviral gene or in the presence of antiviral drugs?
7. Are the findings of studying SCID-hu Thy/Liv mice reflective of HIV-1 infection in humans (paediatric infection in particular)?

Answers to these questions will further prove the usefulness of the model and provide insightful information for understanding pathogenesis and designing therapeutic interventions against HIV-1 infection in humans, especially in paediatric patients.

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Abbreviations used

HSC	haematopoietic stem cell
AZT	zidovudine
wpi	weeks post-inoculation
SI	syncytium-inducing
NSI	non-syncytium-inducing

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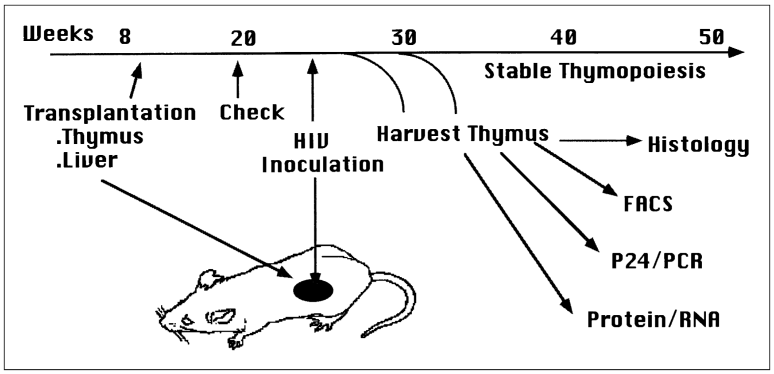

Figure 1.

HIV-1 infection of the SCID-hu Thy/Liv mouse. Human fetal thymus and liver fragments transplanted under renal capsules in SCID mice support stable human thymopoiesis for over a year. HIV-1 inoculation and endpoint (1–6 wpi) analyses are illustrated.

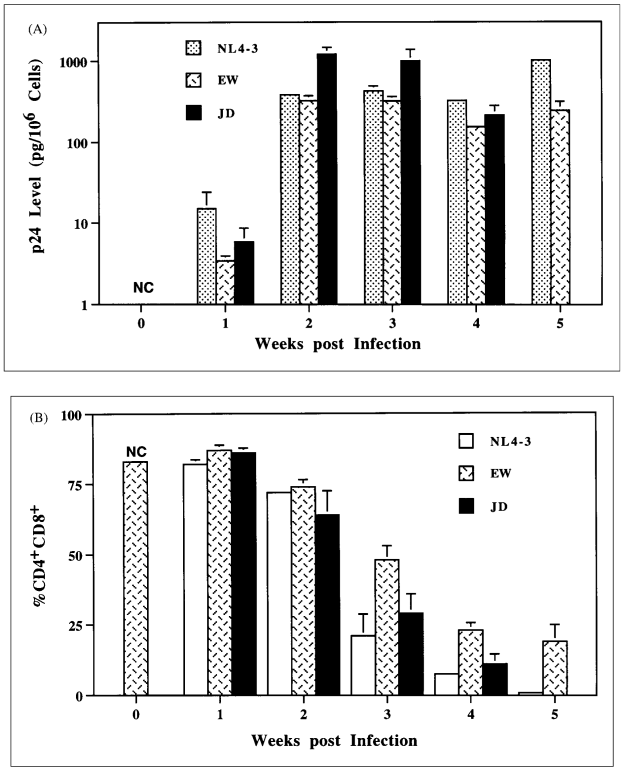

Figure 2.

Kinetics of HIV-1 replication and pathogenesis in the Thy/Liv organ. (A) Production of viral p24 antigen after HIV-1 infection. Thymocyte-associated p24 antigen is measured and standardised as pg/10^6 cells). NL4–3 is a T-tropic HIV-1 clone. EW and JD are two primary patient isolates. No Thy/Liv organs infected by JD are analysed at 5 wpi. NC: Negative controls of mock infected Thy/Liv organs. (B) Depletion of CD4+CD8+ thymocytes after HIV-1 infection. SCID-hu mice are analysed by FACS staining of CD4 and CD8. %CD4+CD8+, which consist of 80%–85% of total thymocytes in normal human thymi and Thy/Liv organs, is a good measure of thymocyte depletion.

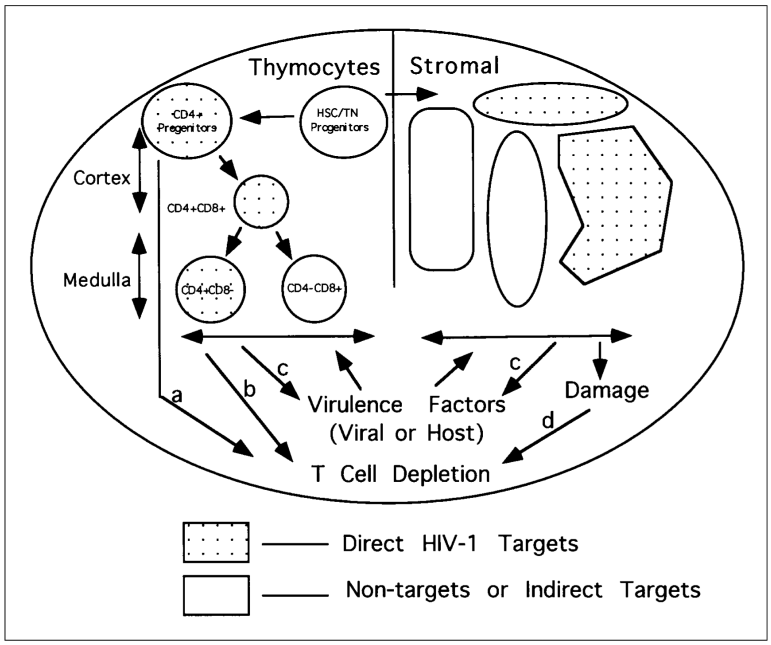

Figure 3.

A model for multiple target cells and pathogenic pathways of HIV-1 pathogenesis in the human thymus. HIV-1 infection may occur in many different types of cells in the thymus (both thymocytes and stromal cells). Infection and destruction of intrathymic T progenitor cells will block the supply of new thymocyte maturation, leading to thymus depletion (pathway a). Direct infection of CD4+ thymocytes may lead to their destruction (pathway b). In addition, virulence factors induced after HIV-1 infection may directly induce thymocyte death or indirectly by enhancing HIV-1 replication in the thymocytes (pathway c). Furthermore, destruction of thymic stromal cells following HIV-1 infection may contribute to thymocyte death induction (pathway d). HSC: haematopoietic stem cell. TN: Triple negative (CD3−CD4−CD8−) thymocytes.

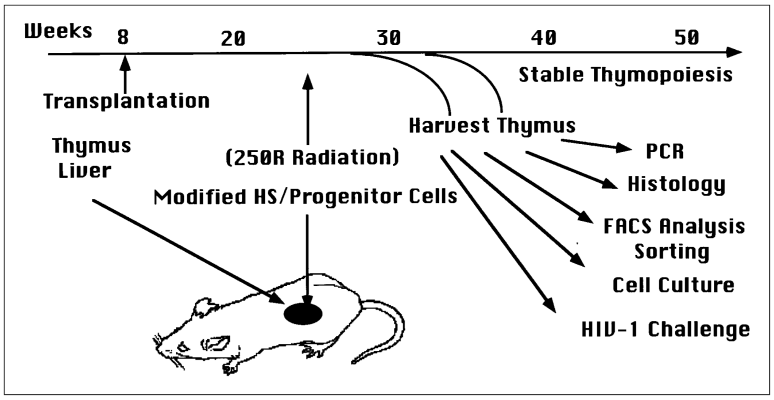

Figure 4.

Preclinical studies of HSC-based gene therapy in the SCID-hu Thy/Liv model. Retroviral-modified HSC/progenitors can be used to reconstitute the Thy/Liv organ which is irradiated to deplete resident thymocytes. Donor cells can be identified by mismatched HLA markers or by retroviral marking. Thymocytes from reconstituted Thy/Liv organs can be isolated and characterised *in vitro* to study gene expression, T cell function and resistance to HIV-1 infection.

Table 1

Correlation of HIV-1 replication and thymus depletion

HIV-1 isolates	PBMC[a]	Thy/Live replication[b]	Thy/Liv depletion[c]
Clones			
NL4–3	+++	+++	+++
NL4–vif	_ [d]	+/−	+/−
NL4–vpr	+++	++	++
NL4–vpu	+++	+	+
NL4–nef	+++	+/−	+/−
JRCSF	+++	++	++
JRCSF-nef	+++	+	NDS[e]
Lai/IIIB	+++	−	−
HXB2	+++	−	−
HXB2/LW	+++	+++	NDS
Primary isolates			
SM	+++	+++	+++
TY	+++	+++	+++
EW	+++	+++	+++
JD	+++	+++	+++
A–NSI	+	+/−	+/−
A–SI	+++	+++	+++
B–NSI	+	+/−	+/−
B–SI	+++	+++	++
LTNP–NSI	+	+/−	+/−

Data from the following reports are summarised. NL4–3[23,33,35,39,40,43] and its mutant derivatives,[39,40] JRCSF[23,24,33,34,39] and JRCSF–nef,[39] Lai/IIIB[31] HXB2 and HXB2/LW.[31,43] SM,[24,34] TY[24] EW[24,35] JD[35] A–NSI, SI and B–NSI, SI.[36] LTNP (long-term non-progressor)–NSI.[37]

[a] Replication in PHA-activated PBMC as measured by p24 or RT production.

[b] HIV-1 replication in the Thy/Liv organ measured by cell- associated p24 or by semi-quantitative DNA PCR analyses.

[c] Thymocyte depletion after HIV-1 infection as analysed by FACS analysis.

[d] NL4–vif mutant replicates in certain T cell lines.

[e] No data shown.