Minireview

The mouse is out of the bag: insights and perspectives on HIV-1-infected humanized mouse models

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Abstract

Human immunodeficiency virus type 1 (HIV-1), which is the causative agent of acquired immunodeficiency syndrome, is a human-specific virus. Because HIV-1 cannot infect and cause disorders in other animals, it has been an arduous struggle to study the dynamics of HIV-1 infection *in vivo*. To understand and elucidate HIV-1 pathogenesis *in vivo*, several small animal models for HIV-1 infection have been established and improved over the last 20 years. Recently, a novel murine model, 'humanized mouse', has been generated. A humanized mouse has the potential to maintain human hematopoiesis including human $CD4^+$ leukocytes and, therefore, is able to support persistent HIV-1 infection *in vivo*. We herein describe the current state-of-the-art in HIV-1-infected humanized mice and introduce insights and perspectives of their use for HIV-1 studies *in vivo*.

Keywords: humanized mouse, human hematopoiesis, HIV-1

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Introduction

Although there are numerous kinds of viruses in the world, some pathogenic viruses such as human immunodeficiency virus type 1 (HIV-1) and Epstein–Barr virus (EBV) are species-specific and can only infect and/or cause disease in humans. Since such human-specific viruses are unable to induce disorders in other animals, it has been almost impossible to investigate the dynamics of virulence and pathogenesis caused by human-specific pathogens in a non-human *in vivo* model.

In particular, to investigate and simulate HIV-1 infection including acquired immunodeficiency syndrome (AIDS) in humans, rhesus macaque monkeys infected with simian immunodeficiency virus (SIV), an evolutionary analogous virus to HIV-1, have been widely utilized and adopted. Nevertheless, it may be presumptuous to assume that SIV pathogenesis in macaques is identical to HIV-1 pathogenesis in humans. Indeed, it has been recently reported that an anti-HIV-1 vaccination trial for high-risk individuals, which was shown to be elegantly successful in an experimental SIV/macaque model, was prematurely terminated due to an increased frequency of seroconversion in the vaccinees.[1–3] This result serves as a caveat implying that we should consider the potential extent of virulence of human-specific viruses including HIV-1 by using multiple experimental models. Furthermore, for an in-depth understanding of the pathogenesis of HIV-1 itself *in vivo*, novel animal models, which support HIV-1 infection, have been generated. In this review, we briefly introduce the history of the ongoing trial for a system capable of supporting HIV-1 infection in mice that has spanned over approximately two decades. Moreover, we go in-depth to describe the cutting-edge investigations of HIV-1 infection in a novel experimental mouse model, called 'humanized mouse'.

The road to 'humanized mice': the history and the usage

In order to evaluate the effectiveness of anti-HIV-1 drugs and vaccines *in vivo*, extensive attempts have been performed to generate cost-effective and ease of use monitoring of HIV-1 pathogenesis in a small animal model. In 1983, Bosma *et al.*[4] established the CB17-*scid* mouse that has a spontaneous autosomal recessive mutation in the *Prkdc* gene causing severe combined immunodeficiency (SCID), called $Prkdc^{scid}$. By using this mouse strain as the recipient, an initial successful trial was reported in 1988: the CB17-*scid* mice were surgically co-implanted with approximately 1-mm pieces of aborted human fetal thymus and liver under the kidney capsule, and the resulting chimeric

Table 1 Comparison of the established human/mouse chimeric models for HIV-1 infection

General designation (established)*	Source†	Reconstitution of human cells		HIV-1 infection	
		Lineage	Duration	Target	Systemic infection (RNA copies/mL plasma)
SCID-hu thy/liv mice (1988)	Fetal thymus and liver	Thymocytes	More than 1 year	CD4SP and DP thymocytes	
Hu-PBL-SCID mice (1991)	Human PBMCs	Xenoreactive T-cells	A few months	Activated CD4 T-cells	10^5–10^7
Humanized mice (2006)	Human HSCs	Lines of human leukocytes	More than 1 year	CD4 T-cells, macrophages, dendritic cells	10^3–10^5

PBMC, peripheral blood mononuclear cell; HSC, hematopoietic stem cell; SP, single positive; DP, CD4CD8 double positive
*The general designation of the established chimeric mice. The A.D. when the chimeric mice was initially established for the HIV-1 study is indicated with parenthesis
†The source of human organs for the transplantation

mice were called SCID-hu thy/liv mice.[5] The SCID-hu thy/liv mice could support *de novo* generation of human T-cells including $CD4^+$ T-cells for more than one year and were highly susceptible to infection with HIV-1 (Table 1).[5–12] However, since the generation and the maintenance of human T-cells in the chimeric mice were mostly limited to the implanted organoid, direct inoculation of virus solution into the organoid is required. In addition, HIV-1 infection in SCID-hu thy/liv mice was restricted to the implanted organ, making the use of these models limited and exclusive for the studies on intrathymic infection with HIV-1 (Table 1). Moreover, due to the necessities of the surgical techniques and the ethical issues involved in other countries, such as Japan, HIV-1 studies using such thy/liv co-implanted SCID mice were mainly carried out in the USA.

In 1988, Mosier *et al.*[13] established a novel system, the hu-PBL-SCID mouse model. In 1991, the hu-PBL-SCID mice were initially utilized for an HIV-1 study (Table 1).[14] The hu-PBL-SCID mice are transplanted with human peripheral blood mononuclear cells (PBMCs) into the peritoneal cavity of several SCID mice, such as CB17-*scid* mice,[14–18] NOD-*scid* mice[19,20] and NOG mice.[21] Although transplanted human PBMCs including human $CD4^+$ T-cells circulated through the blood stream of recipient mice, because of graft-versus host disease (GVHD), the transplanted human PBMCs were highly activated and could only be maintained for a few months (Table 1).[22]

Humanized mouse models for HIV-1 infection

The variety of established humanized mouse models for HIV-1 studies

In 2004, Manz and colleagues[23] initially succeeded in establishing 'humanized mice' by transplanting human $CD34^+$ hematopoietic stem cells (hHSCs) into BALB/c-$Rag2^{-/-}\gamma_c^{-/-}$ mice. Humanized mice are mice that are transplanted with hHSCs and are therefore able to generate lineages of human leukocytes including $CD4^+$ T-cells *de novo*.

From 2006, lines of humanized mouse models for HIV-1 infection have been reported (Table 1).[24] As summarized in Table 2, the hHSC-transplanted humanized mice are mostly based on NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/Jic (NOG) mice,[25–28] NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice,[29,30] BALB/c-$Rag2^{-/-}\gamma_c^{-/-}$ mice[24,31–33] and H-2^d $Rag2^{-/-}\gamma_c^{-/-}$ mice.[34] NOG mice were established in the Central Institute for Experimental Animals (Kawasaki, Kanagawa, Japan), and it was reported that NOG mice lacked intrinsic murine T-cells and B-cells, and functional natural killer cells.[35] On the other hand, NSG mice were established in the Jackson Laboratory (Bar Harbor, ME, USA). Although both mice are sometimes synonymously called NOD/SCID/*Il2rg*$^{-/-}$ mice, the former has a defect in the transmembrane region of *Il2rg* gene (i.e. deletion of exon 7 of the gene), while the latter has a defect on the entire *Il2rg* gene. Of note, one report clearly showed that human $CD4^+$ T-cells in human PBMC-transplanted SCID mice were abnormally activated, due to GVHD.[21] In contrast, hHSC-transplanted humanized mouse models have the potential to maintain a relatively natural physiological condition for a long period of time (e.g. more than one year[25]). Therefore, the difference in the activation state of human T-cells in the murine system is of particular importance for understanding the significance of hHSC-transplanted humanized mice in comparison to human PBMC-transplanted mice.

In addition to the variety of recipient mice, the ways to construct humanized mice are also various: the age for hHSC transplantation (neonate or adult), the route for the transplantation (e.g. intrahepatic, intravenous or intraperitoneal injection) and the source of hHSCs (e.g. cord blood or fetal liver) (Table 2). Although it is still uncertain whether the construction method for humanized mice affects the efficiency of human hematopoiesis and the functionality of reconstituted human leukocytes, it has been suggested that the human immune system is more efficiently reconstituted in newborn recipients transplanted with hHSCs than that in adult recipients.[23]

As one of the 'up-graded' humanized mouse models, BLT (bone marrow/liver/thymus) mice has been recently established.[36] BLT mice are mice that are surgically implanted with organoids consisting of human fetal thymus and liver and are further transplanted with hHSCs. Although BLT mice are likely to possess the potential to reconstitute human hematopoiesis more effectively than the other 'conventional' hHSC-transplanted humanized mice, how to construct the BLT mouse model includes ethical problems because of the need for organs donated from aborted human fetuses.

Advantages of humanized mice for HIV-1 studies

So, what was the breakthrough in humanized mice for HIV-1 studies? When we set out to investigate HIV-1 infection and

Table 2 Establishment of HIV-1-infected humanized mice model and basic investigations

Reference	Designation*	Recipient mouse			Source of hHSCs**	HIV-1 strain††	Brief observations			PMID†††
		Strain†	Age‡	Route§			Virological‡‡	Pathological§§	Immunological***	
24	-	BALB/c-Rag2$^{-/-}\gamma c^{-/-}$	Neonate	i.h.	CB	YU-2 (R5), NL4-3 (X4)	**A, C**	**F**	**L (partial)**	17038503
25	NOG-hCD34 mice	NOG	Neonate	i.h.	CB	JR-CSF (R5), NL4-3 (X4)	**A, C**	**F, G**	**n/a**	19744686
26	NOG-hCD34 mice	NOG	Neonate	i.h.	CB	JR-CSF (R5)	**A, C**	**F**	**J, L (partial)**	20510741
27	hNOG mice	NOG	6–10 weeks old	i.v.	CB	JR-CSF (R5), MNp (X4)	**A, B**	**F**	**L**	16954502
28	hNOG mice‡‡‡	NOG	6–10 weeks old	i.v.	CB	JR-CSF (R5), MNp (X4), NL4-3 (X4)	**A, B, C**	**F, G**	**n/a**	17881441
29	BLT mice	NSG		Refer to the footnote§§§		LAI (X4)	**A, C, D**	**F, G, I**	**J, L**	17389241
30	BLT mice	NSG		Refer to the footnote§§§		ADA (R5), JR-CSF (R5)	**A, C**	**F**	**J, K, L**	19420076
31	DKO-hu HSC mice	BALB/c-Rag2$^{-/-}\gamma c^{-/-}$	Neonate	i.h.	CB or FL	JR-CSF (R5), NL4-3 (X4), NL-R3A (R5X4)	**A, C, E**	**F**	**n/a**	17132723
32	RAG-hu mice	BALB/c-Rag2$^{-/-}\gamma c^{-/-}$	Neonate	i.h.	FL	BaL (R5), NL4-3 (X4)	**A, B, C, E**	**F, G**	**n/a**	17078891
33	HIS mice	BALB/c-Rag2$^{-/-}\gamma c^{-/-}$	Neonate	i.h.	CB	ADA (R5), C1157 (R5)	**C, D**	**F, H**	**n/a**	17182671
34	HIS-Rag2$^{-/-}\gamma c^{-/-}$ mice	H-2^{d} Rag2$^{-/-}\gamma c^{-/-}$	Neonate	i.p.	FL	NFN-SX SL9 (R5)	**E**	**F**	**L (no)**	17314230

CB, cord blood; FL, fetal liver; n/a, not applicable

*The designation of the constructed humanized mice model named by the authors

†The strain of recipient mouse. NOG, NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug}/Jic (established in Central Institute for Experimental Animals, Kawasaki, Kanagawa, Japan); NSG, NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1WjL}/SzJ) (established in the Jackson Laboratory, Bar Harbor, Maine, USA)

‡The age of recipient mice for the transplantation

§The route for the transplantation. i.h., intrahepatic; i.v., intravenous; i.p., intraperitoneal

**The source of human CD34^{+} hematopoietic stem cells (hHSCs)

††The HIV-1 strains used in each study. The co-receptor usage of each HIV-1 strain was indicated with parenthesis. R5, CCR5-tropic; X4, CXCR4-tropic; R5X4, CCR5/CXCR4 dual-tropic

‡‡Virological observations: **A**, HIV-1 RNA in plasma; **B**, HIV-1 DNA in tissue(s); **C**, HIV-1-infected cells; **D**, HIV-1 antigen(s) in plasma; **E**, HIV-1 replication by *ex vivo* co-culture with the cells susceptible for HIV-1

§§Pathological observations: **F**, loss of CD4^{+} T-cells in peripheral blood (PB) or decrease of CD4/CD8 ratio in PB; **G**, thymopathy; **H**, lymphadenopathy; **I**, HIV-1 infection and depletion of CD4^{+} T-cells in gut-associated lymphoid tissues

***Immunological observations: **J**, expansion/activation of CD8^{+} T-cells in PB; **K**, detection of HLA-restricted HIV-1 antigen-specific CD8^{+} T-cells; **L**, analysis on anti-HIV-1 antigen antibodies in plasma/serum. Note that the extent of the detected antibodies was indicated with parenthesis

†††The PMID of each paper in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/)

‡‡‡This hNOG mice were constructed without irradiation before the transplantation of hHSCs

§§§Fetal liver and thymus were surgically implanted under the kidney capsule of recipient mouse at 6–8 weeks old. After three weeks postimplantation, autologous FL-derived hHSCs were transplantd by i.v. injection

replication in primary $CD4^+$ T-cells *in vitro*, we usually have to activate the primary cells by using mitogens (e.g. anti-CD3 antibody, phytohemagglutinin and ionomycin) to support HIV-1 replication. On the other hand, as described above, hHSC-transplanted humanized mice are able to persistently maintain human hematopoiesis at a physiological condition. In addition, humanized mice possess a series of human $CD4^+$ T-cell subsets, including $CD45RA^+/CD45RO^-$ naïve, $CD45RA^-/CD45RO^+$ memory and $FOXP3^+$ regulatory cells. Moreover, humanized mice can support longitudinal HIV-1 expansion *in vivo* (see below). Taken together, humanized mice have several advantages for HIV-1 studies. When investigating the dynamics of HIV-1 infection, humanized mice have the potential to provide novel insights.

Basic observations in HIV-1-infected humanized mice

By using the constructed humanized mouse models, HIV-1 infection has been widely investigated based on virological, pathological and immunological issues (Table 2). For the investigation of HIV-1 infection *in vivo*, various HIV-1 strains including laboratory molecular clones and/or clinical isolates have been used. The phenotypes of HIV-1 can be distinguished by their co-receptor usage: CCR5 and/or CXCR4. From the clinical point of view, CCR5-tropic (R5) HIV-1 is broadly detected in patients. Prior to and/or during the rapid progression to AIDS in HIV-1-infected individuals, CCR5/CXCR4 dual-tropic (R5X4) HIV-1 can occasionally emerge, and then CXCR4-tropic (X4) HIV-1 can sometimes become predominant.[37] Therefore, in order to recapitulate HIV-1 pathogenesis in patients, it would be of importance to use R5 HIV-1 strains, such as JR-CSF and BaL, rather than X4 HIV-1.

Most HIV-1-infected humanized mice show persistent and longitudinal viremia in the plasma (Table 2), with the longest viremia reported for 30 weeks.[25] Also, virological evidence for HIV-1 infection, such as the detection of proviral DNA and infected cells, and HIV-1 replication by *ex vivo* co-culturing of cells isolated from the infected humanized mice with the cells susceptible for HIV-1 have been reported in previous literature (Table 2). Furthermore, one report revealed that the majority of HIV-1-producing cells (i.e. the cells positive for HIV-1 p24 antigen) in the spleen of infected humanized mice exhibited $CD45RA^-CD45RO^+CCR7^-$ effector memory phenotype and either or both $Ki67^+$ and/or $CD69^+$ activated/proliferating phenotype.[25] The paper also demonstrated the down-regulation of CD4 molecules on the surface of infected cells,[25] which would be caused by HIV-1-encoding proteins, such as Nef (negative factor), Vpu (viral protein U) and Env (envelope glycoprotein), as elucidated in-depth in *in vitro* studies.[38,39] Nevertheless, an extraordinary manner of viremia in HIV-1-humanized mice should be noted. In patients and SIV-infected macaques, viral load is initially fulminated at the acute phase. Then, the level of viral load declines through antiviral immune responses and is stably maintained at a set-point during the chronic phase. However, in HIV-1-infected humanized mice, a high level of viremia is longitudinally maintained without a reduction to the set-point.[24–32] Although the cause of the extraordinary manner of viremia in humanized mice is still uncertain, it is assumed that anti-HIV-1 adaptive immune responses are not sufficiently reconstituted in most humanized mice models (see below in detail).

Several pathological disorders, which are observed in HIV-1-infected patients, have been reproduced in HIV-1-infected humanized mice models (Table 2). For instance, the loss of $CD4^+$ T-cells (or the decline of the ratio of $CD4^+$ T-cells to $CD8^+$ T-cells) in peripheral blood (PB) of infected mice, which is one of the major pathological observations in patients, has been widely analyzed and demonstrated (Table 2). In addition, some papers showed thymopathy, which is mainly due to the depletion of $CD4^+$ thymocytes by HIV-1 infection. Moreover, an HIV-1-infected BLT mouse model demonstrated the infection and the depletion of $CD4^+$ T-cells residing in gut-associated lymphoid tissues, which has been proposed as one of the crucial steps in patients and SIV-infected rhesus macaque during the acute phase.[29]

Immune reaction against HIV-1 in infected mice has also been evaluated (Table 2). Although some papers showed the expansion and/or activation of $CD8^+$ T-cells in PB and tissues, it is yet unclear whether the expansion is triggered by HIV-1-specific human leukocyte antigen (HLA)-restricted reaction (see below in detail). Some papers also focused on humoral immunity against HIV-1; however, most HIV-1-infected humanized mice do not seem to induce the production of anti-HIV-1 antibodies (Table 2). Moreover, only one study demonstrated the HLA-restricted HIV-1 antigen-specific cellular immune response in HIV-1-infected BLT mice.[30]

Applications of HIV-1-infected humanized mice

In following the basic studies of HIV-1 infection in humanized mice (Table 2), some innovative investigations, which emphasized particular aspect(s) of HIV-1 infection *in vivo*, have been documented (Table 3). For instance, Ince *et al.*[40] analyzed the diversification and evolution of the R5 HIV-1 *env* gene in infected mice and detected the emergence of X4 HIV-1 *env* (Table 3a). Also, Jiang *et al.*[41] reported that $FOXP3^+$ regulatory T-cells were acutely and drastically depleted by HIV-1 infection in humanized mice, which suggests that $FOXP3^+$ regulatory T-cells are highly susceptible to HIV-1 *in vivo* (Table 3a). However, it is important to note that the virus used in the study was R5X4 HIV-1,[41] and that the observations reported need to be re-evaluated using R5 HIV-1. Moreover, a number of trials for the development of anti-HIV-1 therapies have been addressed (Table 3b) and will be described in-depth below.

Significance of host factors for HIV-1 infection *in vivo*

From an enormous amount of *in vitro* studies using cell culture systems, it has been well demonstrated that some cellular proteins have the potential to positively or negatively regulate HIV-1 replication and are called 'host factors'.[42] In respect to host factors positive for HIV-1 infection, CD4, CCR5 and/or CXCR4 on the surface of cells are utilized as the receptors for HIV-1 entry.[43,44] In addition, for the budding of nascent HIV-1 virions from the infected cell, HIV-1 hijacks cellular ESCRT (endosomal sorting

Table 3 Applications of HIV-1-infected humanized mice model

Reference	Designation*	Recipient mouse			Source of hHSCs**	HIV-1 strain††	Brief findings	PMID‡‡
		Strain†	Age‡	Route§				
(a) In-depth investigation of HIV-1 pathogenesis *in vivo*								
40	DKO-hu HSC mice	BALB/c-Rag2$^{-/-}\gamma c^{-/-}$	Neonate	i.h.	FL	NFN-SX SL9 (R5)	Mutation and evolution of HIV-1 *env* gene	20042504
41	DKO-hu HSC mice	BALB/c-Rag2$^{-/-}\gamma c^{-/-}$	Neonate	i.h.	FL	R3A (R5X4)	Acute infection and depletion of FOXP3$^+$ regulatory T-cells	18544681
64	NOG-hCD34 mice	NOG	Neonate	i.h.	CB	JR-CSF (R5), JR-CSFδ*vif* (R5)	Remarkable and lethal G-to-A mutation of HIV-1 DNA by APOBEC3 proteins	20610708
(b) Trials for anti-HIV-1 therapies								
65	hu-Rag2$^{-/-}\gamma c^{-/-}$ mice	BALB/c-Rag2$^{-/-}\gamma c^{-/-}$	Neonate	i.h.	FL	JR-CSF (R5)	Suppression of HIV-1 infection and recovery of peripheral CD4$^+$ T-cell loss by post-treatment with antiretroviral therapy	19494021
66	BLT mice	NSG		Refer to the footnote§§		JR-CSF (R5)	Prevention of vaginal HIV-1 transmission and systemic CD4$^+$ T-cell loss by PrEP***	18198941
67	BLT mice	NSG		Refer to the footnote§§		JR-CSF (R5)	Prevention of rectal and intravenous HIV-1 transmission by PrEP	20098623
72	hu-HSC mice	NSG	Neonate	i.v.	CB	BaL (R5)	Suppression of HIV-1 infection and recovery of peripheral CD4$^+$ T-cell loss by T cell-specific delivery of siRNAs against *CCR5*, and HIV-1 *tat* and *vif* genes using scFvCD7-9R†††	18691745
73	hu-BLT mice	NSG		Refer to the footnote‡‡‡		NFN-SX SL9 (R5), NL4-3 (X4)	Suppression of HIV-1 replication *ex vivo* by transduction of shRNA against *CCR5* using LV	20018916
74	-	NSG	Neonate	i.v.	CB	BaL (R5)	Supression of HIV-1 infection and recovery of peripheral CD4$^+$ T-cell loss by knock-out of *CCR5* gene using CCR5-targeted ZFN§§§	20601939

CB, cord blood; FL, fetal liver

*The designation of the constructed humanized mice model named by the authors

†The strain of recipient mouse. NOG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/Jic (established in Central Institute for Experimental Animals, Kawasaki, Kanagawa, Japan); NSG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (established in the Jackson Laboratory, Bar Harbor, Maine, USA)

‡The age of recipient mice for the transplantation

§The route for the transplantation. i.h., intrahepatic; i.v., intravenous; i.p., intraperitoneal

**The source of human CD34$^+$ hematopoietic stem cells (hHSCs)

††The HIV-1 strains used in each study. The co-receptor usage of each HIV-1 strain was indicated with parenthesis. R5, CCR5-tropic; X4, CXCR4-tropic; R5X4, CCR5/CXCR4 dual-tropic

‡‡The PMID of each paper in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/)

§§Fetal liver and thymus were surgically implanted under the kidney capsule of recipient mouse at 6–8 weeks old. After three weeks postimplantation, autologous FL-derived hHSCs were transplanted by i.v. injection

***PrEP, pre-exposure prophylaxis

†††The CD7-specific single-chain antibody conjugated to oligo-9-arginine peptide (scFvCD7-9R), which was complexed to siRNAs, was administrated into the constructed hu-HSC mice by i.v. injection

‡‡‡FL hHSCs were transduced with lentivirus vector (LV) and solidified with Matrigel. Then the solidified LV-transduced FL hHSCs and fetal thymus were surgically implanted under the kidney capsule of recipient mouse at 6–8 weeks old. After 3 weeks postimplantation, the autologous LV-transduced FL hHSCs were transplantd by i.v. injection

§§§The plasmid expressing zinc-finger nuclease (ZFN) speficic for *CCR5* gene were nucleofected into hHSCs before transplantation

complex required for transport) machinery, which consists of TSG101 (tumor susceptibility gene 101),[45–47] CHMP (chromatin-modifying protein/charged multivesicular body protein) family proteins[48] and others.[49–53] On the other hand, APOBEC3 (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3) family proteins are one of the well-known host factors that can negatively regulate HIV-1 replication.[54,55]

APOBEC3s are cellular cytidine deaminases that convert cytosine in the viral minus-strand cDNA to uracil, resulting in the alteration of guanine (G) to adenine (A) in the nascent proviral DNA. Several APOBEC3 proteins are incorporated

into progeny virions and mutate viral cDNA in the invaded cells, which results in the inhibition of viral replication. On the other hand, an HIV-1 accessory protein, viral infectivity factor (Vif), has the ability to counteract the incorporation of certain APOBEC3 proteins such as APOBEC3G and APOBEC3F into progeny virions by degrading these proteins through the proteasome-dependent pathway.[56–59] Lines of *in vitro* investigations have elucidated the mechanisms of G-to-A hypermutation of HIV-1 DNA mediated by APOBEC3s and the counteracting ability of Vif against APOBEC3s, which have shed light on the relevance of host–retrovirus interaction.[60–63] Nevertheless, the physiological balance between intrinsic APOBEC3s and Vif *in vivo* has been poorly understood, and the significance of APOBEC3-mediated mutagenesis for HIV-1 replication *in vivo* has remained unresolved. In this regard, by using a humanized mice model, we have recently demonstrated the predominant accumulation of G-to-A mutations in *vif*-proficient (i.e. wild-type) HIV-1 provirus displaying characteristics of APOBEC3-mediated mutagenesis (Table 3a).[64] Notably, the APOBEC3-associated G-to-A mutation of HIV-1 DNA that leads to the termination of translation was significantly observed.[64] Furthermore, the paper provided a novel insight suggesting that HIV-1 G-to-A hypermutation is independently induced by individual APOBEC3 proteins. Taken together, these results provide evidence indicating that endogenous APOBEC3s are associated with G-to-A mutation of HIV-1 provirus *in vivo*, which can result in the abrogation of HIV-1 infection.

Trials for anti-HIV-1 therapies in humanized mice models

Since humanized mice are able to support persistent HIV-1 infection *in vivo*, humanized mice are also adequate for the evaluation of the efficiency of anti-HIV-1 strategies *in vivo*. In an effort to evaluate anti-HIV-1 trials *in vivo*, some attempts have been performed (Table 3b). Three papers have attempted to evaluate the effectiveness of anti-HIV-1 drugs, which have been already approved and are in clinical use, in HIV-1-infected humanized mice.[65–67] These reports strongly suggest that we will be able to evaluate the effectiveness of the candidates for novel anti-HIV-1 drugs in humanized mice as preclinical studies in the future.

It is well known that individuals who have a genetic defect in the *CCR5* gene, known as *CCR5Δ32*, are resistant to HIV-1 infection.[68–70] Interestingly, it has been recently reported that a bone marrow transplantation from a *CCR5Δ32* donor into a patient with HIV-1 infection and acute myeloid leukemia lead to the suppression of HIV-1 to levels below detection by conventional methods.[71] This result strongly suggests that the attenuation of *CCR5* gene expression (i.e. knock-down) and/or the disruption of the *CCR5* gene itself (i.e. knock-out) are effective and conceivable options for the suppression of HIV-1 expansion *in vivo*. In fact, as another strategy for the suppression of HIV-1 infection *in vivo*, genetic modifications of human hematopoietic cells in humanized mice models have been addressed. To knock-down/out the *CCR5* gene, several approaches have been reported. The first report showed that the administration of scFvCD7-9R (CD7-specific single-chain antibody conjugated to oligo-9-arginine peptide) complexed with siRNAs against *CCR5* and HIV-1-encoding genes efficiently delivered siRNAs into the T-cells of HIV-1-infected humanized mice and successfully suppressed R5 HIV-1 replication, leading to a recovery of $CD4^+$ T-cell loss in PB.[72] Second, the transduction of shRNA against *CCR5* using lentivirus vector into hHSCs prior to the transplantation into the recipient mice successfully attenuated *CCR5* expression in the $CD4^+$ T-cells differentiated in the constructed humanized mice.[73] The authors also showed that R5 HIV-1 replicated less efficiently in the $CD4^+$ T-cells isolated from the *CCR5*-targeted shRNA-transduced humanized mice *ex vivo*.[73] The third report used a zinc-finger nuclease (ZFN) technique that can target and digest specific nucleotide sequences in the cellular genome, which results in the knock-out of the targeted gene.[74] By nucleofection of *CCR5*-targeted ZFN-expressing plasmid into hHSCs before transplantation into the recipient mice, the researchers succeeded in the construction of *CCR5* knock-out humanized mice and showed suppression of HIV-1 infection and the recovery of $CD4^+$ T-cells in PB.[74] Taken together, these trials are crucial for the establishment of novel strategies to treat HIV-1 infection in patients. However, currently, it is still problematic that the efficiency of knock-down/out of *CCR5* gene in humanized mice is low. Therefore, it would be important to improve the technique(s) for knock-down/out of the target gene in the future. Moreover, as described above, there are several host factors that associate with HIV-1 replication other than CCR5. For instance, it has been already demonstrated that APOBEC3s endogenously expressed in $CD4^+$ T-cells can contribute to the diminution of HIV-1 expansion *in vivo*. Therefore, ectopic expression of APOBEC3s may be one of the effective strategies to abrogate HIV-1 infection *in vivo*.

Perspectives and remaining problems

Although humanized mice have several beneficial aspects for studies on HIV-1 infection and pathogenesis *in vivo*, it is also a fact that the humanized mice are still not perfect, particularly in terms of the reconstitution of acquired immunity. As summarized in Table 2, cellular and humoral immune responses observed in HIV-1-infected humanized mice are likely to be poorer than those in humans. It is thought that the inadequate acquired immune response, particularly cellular immunity, in humanized mice would be due to the thymic environment for the education of human T-cell precursors (i.e. thymocytes). In both humans and mice, it is well known that T-cell precursors are selected in the thymus by major histocompatibility complexes (MHCs) expressed on either or both thymic epithelial cells and/or dendritic cells. In humans, thymocytes are educated and are selected by HLAs (note that human MHC is also called HLA) expressed on thymic epithelial cells and/or dendritic cells. On the other hand, it is strongly suggested that human T-cell precursors in hHSC-transplanted humanized mice (with the exception of BLT mice) are selected and maturated in the murine thymic environment. However, it is still uncertain whether human thymocytes are selected by murine MHC molecules expressed on murine thymic cells or by other sources. In

this regard, it has been shown that mature T-cells can develop from cord blood-derived mononuclear cell cultures with supplementation of stem cell factors and interleukin-7 but without thymic feeder cells *in vitro*,[75] suggesting that the development of human T-cells in hHSC-transplanted humanized mice may be differentiated independent of murine MHCs. On the other hand, others have asserted that murine thymus can support human thymocyte differentiation via murine MHCs.[76,77]

To improve the functionality of human acquired immunity in hHSC-transplanted humanized mice, the genetic modification of the recipient mice has been performed. For example, two kinds of HLA-A2 transgenic (Tg) humanized mice have been independently established and reported. One was established by backcrossing NSG mice with HLA-A2.1 Tg mice,[78] while the other was constructed by backcrossing NSG mice with HLA-A2/HHD Tg mice.[79] Since these two humanized mice were able to mimic the human thymic microenvironment to some extent, human T-cells, especially $CD8^+$ T-cells, were successfully and more effectively educated by HLA-A2. In fact, it was reported that the differentiated $CD8^+$ T-cells in these HLA-A2 Tg humanized mice had the ability to elicit HLA-A2-restricted EBV-specific responses.[78,79] Therefore, these gains will lead to the improvement of humanized mouse models and is one of the most important directions to proceed in humanized mouse studies.

Concluding remarks

In conclusion, we herein documented the studies involving HIV-1 infection in humanized mouse models. To investigate the pathogenesis of AIDS, the SIV-infected rhesus macaque model has been widely used and accepted. However, conducting SIV/macaque experiments requires relatively high costs, and also it may be difficult to identify the findings in SIV/macaque models with those in patients with HIV-1 infection. On the other hand, conducting experiments using humanized mouse models demands relatively low cost, and can support and easily allow for monitoring of HIV-1 infection *in vivo*. Nevertheless, as described above, the dynamics of viral infection during the chronic phase in HIV-1-infected humanized mice are likely to be at variance with those in patients and SIV/macaque models. Namely, both the SIV-infected macaque models and the HIV-1-infected humanized mouse models have respective advantages and/or disadvantages. Therefore, to elucidate the *bona fide* HIV-1 pathogenesis *in vivo*, we believe that it would be important to mush-up the accumulated knowledge provided from SIV/macaque studies and novel findings from the studies on HIV-1-infected humanized mouse models.

Taken together, the humanized mice have the potential to investigate the dynamics of host–pathogen interaction *in vivo*. Using the humanized mouse models, we are able to combine strategies from multiple disciplines, which have been provided to us from the fields of molecular biology, clinical medicine and mathematical biology. This will in turn enable us to address unrevealed but essential aspects of human-specific pathogens including HIV-1.

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