Humanized Mice for Human Retrovirus Infection

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Abstract Inbred mice with specific genetic defects have greatly facilitated the analysis of complex biological events. Several humanized mouse models using the C.B.-17 *scid/scid* mouse (referred to as the SCID mouse) have been created from two transplantation protocols, and these mice have been utilized for the investigation of human immunodeficiency virus type 1 (HIV-1) and human T-lymphotropic virus type I (HTLV-I) pathogenesis and the evaluation of antiviral compounds. To generate a more prominent small animal model for human retrovirus infection, especially for examination of the pathological process and the immune reaction, a novel immunodeficient mouse strain derived from the NOD SCID mouse was created by backcrossing with a common γ chain (γ_c)-knockout mouse. The NOD-SCID γ_c^{null} (NOG) mouse has neither functional T and B cells nor NK cells and has been used as a recipient in humanized mouse models

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for transplantation of human immune cells particularly including hematopoietic stem cells (HSC). From recent advances in development of humanized mice, we are now able to provide a new version of the animal model for human retrovirus infection and human immunity.

Abbreviations AIDS: acquired immunodeficiency syndrome; APC: antigen-presenting cell; ATL: adult T-cell leukemia; AZT: azidothymidine; CCR5: cc-chemokine receptor 5; DC: dendritic cell; ddI: dideoxyinosine; DN: double negative; γ_c; common gamma chain; GVHD: graft-versus-host disease; HAM: HTLV-I-associated myelopathy; HIV-1: human immunodeficiency virus type 1; HSC: hematopoietic stem cell; HTLV-I: human T-lymphotropic virus type I; IL: interleukin; MHC: major histocompatibility complex; PBMC: peripheral blood mononuclear cell; SCID: severe combined immunodeficiency; SP: single positive; TRAIL: tumor necrosis factor (TNF)-related apoptosis-inducing ligand; TSP: tropical spastic paraparesis

1 Mouse/Human Chimeric Models for HIV-1 Infection Using SCID Mouse

The C.B.-17 *scid/scid* (SCID) mouse carries a spontaneously arising autosomal recessive mutation and was found to have severe combined immunodeficiency (SCID) by Bosma and colleagues (Bosma et al. 1983). This strain has a defect of DNA protein kinase and a lack of progenitors to T and B cells (Blunt et al. 1995; Boubnov and Weaver 1995; Kirchgessner et al. 1995; Miller et al. 1995; Peterson et al. 1995). Therefore, these mice are unable to reject the xenograft and they tolerate engraftment of human cells or tissues, followed by subsequent infection with human immunodeficiency virus type 1 (HIV-1). Two of these models are the SCID-hu thy/liv mouse developed by McCune and colleagues (McCune et al. 1988) and, the hu-PBL-SCID mouse developed by Mosier and colleagues (Mosier et al. 1988) (Fig. 1).

1.1 HIV-1 Infection in the SCID-hu thy/liv Mouse

The SCID-hu thy/liv mouse is generated by surgical coimplantation of a piece (1 mm) of human fetal thymus and liver under the murine kidney capsule. The implanted tissues produce a conjoint organ (thy/liv) that appears to reconstitute normal thymus for more than 1 year. The fetal liver provides hematopoietic precursors that are located in islets between the thymic lobes. Thymic epithelial cells are derived from the fetal thymus, whereas the hematopoietic cells including T cells and dendritic cells (DC) are derived from the liver (Vandekerckhove et al. 1992). The generated thymus is composed of more than 70% CD4CD8

Mouse strain	human tissue or cells	References
1) SCID-hu thy/liv mouse		
CB17 SCID mouse	human thy/liv tissue	McCune et al. 1988
2) Human-PBL-SCID mouse		
CB17 SCID mouse	human PBMC	Mosier et al.1991
NOD-SCID mouse	human PBMC	Koyanagi et al. 1997
NOG mouse	human PBMC	Nakata et al. 2005

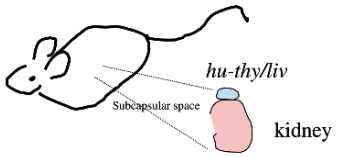


Fig. 1 Mouse/human chimeric models for HIV-1 infection. HIV-1-susceptible humanized mouse models have been reported from two transplantation protocols

double-positive (DP) cells, and the rest are CD4 or CD8 single-positive (SP) and double-negative (DN) T cells. Although these CD4 or CD8 SP cells migrate into the peripheral circulation and express a naive phenotype (>70% $CD45RA^+$), the numbers of these cells are very low (Jamieson and Zack 1999). In addition, no immune response to viral antigen has been found so far. Thus, the reconstitution of the human immune system is not complete in this mouse. Since some skill in the surgical operation to coimplant the thy/liv organ and systematic support on distribution of human fetal organs are required, the SCID-hu thy/liv mouse studies have been carried out in limited numbers of laboratories, mainly in the US.

The human thy/liv implant in SCID mice is highly susceptible to infection with HIV-1, including R5 and X4 HIV-1 (McCune et al. 1991; Namikawa et al. 1988). As the number of circulating human $CD4^+$ T cells is low as mentioned above, direct injection of virus into the implant is performed under anesthesia. The level of HIV-1 replication and potential with CD4 T cell depletion of X4 HIV-1 appears to be higher than that of R5 HIV-1 within a few weeks of infection by evaluation with PCR and flow cytometric analyses (Kaneshima et al. 1994). Immunohistological examination indicated that the infected cells initially appeared in the thymic cortical regions and subsequently spread through the entire organ after HIV-1 infection (Stanley et al. 1993). Flow cytometric analysis indicated that the CD4CD8 DP cells were almost completely eradicated and the ratio of $CD4^+$ and $CD8^+$ thymocytes was reversed after infection (Aldrovandi et al. 1993; Bonyhadi et al. 1993). Furthermore, SCID-hu thy/liv mouse have been also used to assess efficacy of several anti-HIV compounds administrated before infection, including azidothymidine (AZT) and 2′,3′-dideoxyinosine (ddI) (Kaneshima et al. 1991; McCune et al. 1990; Rabin et al. 1996).

1.2 HIV-1 Infection in the hu-PBL-SCID Mouse

The hu-PBL-SCID mouse is created by injection of peripheral blood mononuclear cells (PBMC) from healthy adults into the peritoneal cavity of SCID mice, and human $CD4^+$ and $CD8^+$ T cells circulate through the peritoneal cavity, peripheral blood, and organs such as spleen and liver for more than 1 month after PBMC injection (Mosier et al. 1988). The presence of human $CD4^+$ T cells makes this attractive as a model to study HIV-1 pathogenesis and evaluation of anti-HIV compounds (Mosier et al. 1991; Pastore et al. 2003; Hartley et al. 2004). Although it was initially reported as a model with little graft-versus-host disease (GVHD) in the hu-PBL-SCID mice, the high levels of T cell-reconstituted mice develop symptoms of GVHD within 2 months after injection of PBMC (Sandhu et al. 1995). Therefore, long-term observation may not be possible in this model. In addition, human $CD4^+$ and $CD8^+$ T cells in hu-PBL-SCID mice expressed the CD45RO antigen, a marker found in either activated or memory T cells (Tary-Lehmann and Saxon 1992). This is not similar to the ratio of normal adult PBMC, which contain approximately 50% $CD45RO^+$ and $CD45RA^+$ (naive) cells. Furthermore, human $CD4^+$ cells in the hu-PBL-SCID also express abundant levels of HIV-1 coreceptor CCR5, and accordingly, R5 but not X4 HIV-1 more actively replicates in this system (Mosier et al. 1993; Nakata et al. 2006). The relative ease with which this model can be generated and the high efficiency of R5 HIV-1 infection make this system very attractive to study HIV-1 pathogenesis for researchers who struggle in obtaining fetal organs for generating SCID-hu thy/liv mouse. Importantly, a significantly high level of HIV-1 replication correlates with severe depletion of human $CD4^+$ T cells within 2 weeks after infection, and the replication is dependent on Nef protein (Kawano et al. 1997). Thus, this model appears to be adequate for short-term investigation of HIV-1 replication and pathogenesis. As mentioned above, the reconstituted $CD4^+$ and $CD8^+$ T cells are strongly activated and have memory phenotypes, indicating that these cells are xenoreactive proliferated but anergic T cells (Tary-Lehmann and Saxon 1992; Tary-Lehmann at al. 1995). In this model, neither thymopoiesis nor hematopoiesis is generated (Koyanagi, unpublished observations). Thus, it is assumed that the pathological events of HIV-1 infection in this model include that in mature T cells in humans.

1.3 Human Acquired Immune Responses in the hu-PBL-SCID Mouse

Of interest, some immune responses are induced, including humoral and cellular immune responses in the hu-PBL-SCID mouse with administration of various antigens (Gorantla et al. 2005; Ifversen P and Borrebaeck 1996; Nonoyama et al. 1993; Sandhu et al. 1994). However, there are two major limitations to development of strong human immune responses in the hu-PBL-SCID mice. The first is the lack of

appropriate human antigen-presenting cells (APC) including DC. The second is the lack of a suitable microenvironment such as the presence of normal lymphoid organs and architecture that may facilitate induction and maintenance of immune effector cells (Tary-Lehmann et al. 1995). To overcome the lack of APC, Delhem et al. used autologous skin that contains tissue DC as a source of APC and succeeded in demonstrating the induction of primary MHC-restricted human T cell responses against HIV-1 envelope in the hu-PBL-SCID mice (Delhem et al. 1998). Santini et al. have reported that inactivated HIV-1-pulsed, monocyte-derived, and matured human DC can stimulate human anti-HIV-1 antibody production by B cells from HIV-1-negative donors in the SCID mouse system, and that this immune response is partially protective (Santini et al. 2000). However, there had been no attempts to overcome the lack of a suitable microenvironment in this hu-PBL-SCID mouse until our report (Yoshida et al. 2003).

To overcome the deficiency of a suitable microenvironment in the hu-PBL-SCID mouse system, we attempted to transfer PBMC together with inactivated HIV-1-pulsed autologous monocyte-derived DC directly into the mouse spleen (Yoshida et al. 2003). The intrasplenic inoculation of PBMC was found to reduce excessive GVHD compared to the intraperitoneal transfer method (Tanaka et al., unpublished observations). In addition, with this procedure we could obtain larger yields of human T cells than with the conventional intraperitoneal transfer. Therefore, we reasoned that the microenvironment in the mouse spleen should provide human T cells with optimum conditions for activation (Fig. 2). With this new immunizing protocol, we have succeeded in eliciting a protective $CD4^+$ T cell immunity against R5 HIV-1 infection. We were surprised to see that the immunized mice were totally resistant against challenge with live R5 HIV-1 (Yoshida et al. 2003). The protective immunity was induced equally with either R5 or X4 HIV-1 as an antigen. The sera from the immunized mice contained a soluble R5 HIV-1 suppressive factor that was mainly synthesized by human $CD4^+$ T cells in response to HIV-1 antigen, specific peptides of HIV-1 according to MHC class II haplotypes (Yoshida et al. 2005), but

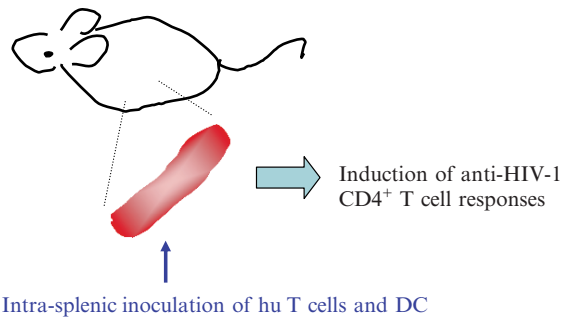


Fig. 2 Human acquired immune responses in the hu-PBL-SCID mouse. Protective $CD4^+$ T cell immunity against R5 HIV-1 infection is induced by PBMC transfer together with inactivated HIV-1-pulsed autologous DC directly into the mouse spleen

not ovalbumin as a control. The factor appears to be unrelated to the currently identified R5 HIV-1 suppressive cytokines examined by neutralization assay using antibodies against CCR5-binding β-chemokines, IL-4, IL-10, IL-12, IL-13, IL-16, MCP-1, MCP-3, IFN-α, IFN-β, TNF-α, and TNF-β.

Our study indicates that a DC-based HIV-1 vaccination can induce HIV-1-reactive human $CD4^+$ T cells producing a yet-undefined R5 HIV-1 suppressor factor. The demonstration made by Lu et al. (Lu et al. 2003) that DC pulsed with AT-2 inactivated simian immunodeficiency virus (SIV) can also stimulate protective anti-SIV specific T cell and antibody responses in rhesus monkeys suggests a rational basis for the DC-based immunization against HIV-1 infection. This idea is further supported by the recent findings by Lu et al. (Lu et al. 2004), who showed the efficacy of a therapeutic DC-based whole HIV-1 virion vaccination for HIV-1 infection.

In order to achieve successful DC-based immunization against HIV-1, a large number of monocyte-derived mature DC with immuno-stimulating activity is expected. A conventional method for generating DC from monocytes has been established, and there are commercially available kits for monocyte purification. In addition, we recently found a simple method to isolate monocytes from bulk PBMC by using antichemokine receptor monoclonal antibody-coated plates (Nimura et al. 2006). These monocytes could be differentiated to Th1-inducing DC and expressed low levels of cell surface CD4 and CCR5. When sensitized with inactivated HIV-1, these DC could induce the R5 HIV-1-suppressing factor in the hu-PBL-SCID mouse (Nimura et al. 2006). Because human monocytes and immature DC are still susceptible to R5 HIV-1 infection, this method, which can induce HIV-1-resistant DC, will be helpful for HIV-1-infected individuals in generation of therapeutic DC against acquired immunodeficiency syndrome (AIDS).

2 Development of Immunodeficient Mouse Strains for Improvement of Human Cell Reconstitution and HIV-1 Infection

2.1 Generation of Novel Immunodeficient Mouse Strains

By introduction of the *scid/scid* mutation in various strains of inbred mice possessing defects in innate immunity, we surveyed congenic mouse strains to find an adequate immunodeficient mouse for transplantation of human cells and HIV-1 infection. From extensive transplantation experiments with human PBMC in immunodeficient mouse strains, the NOD-SCID mouse, which contains some defects in the function of complements and macrophages, was found to be a most suitable strain as a recipient for human cell reconstitution as well as HIV-1 infection (Koyanagi et al. 1997a). The number of human cells in the human PBMC-transplanted NOD-SCID (hu-PBL-NOD-SCID) mice was more than

three- to fivefold higher than that in the conventional hu-PBL-SCID mice. More importantly, the levels of HIV-1 viremia were significantly high in the HIV-1 infected hu-PBL-NOD-SCID mice. Some HIV-1-infected mice exhibited more than 1 ng of p24 antigen per milliliter in plasma, which is a much higher concentration than that in HIV-1-infected patients, and showed severe CD4 depletion after intraperitoneal inoculation with R5 HIV-1. Using this mouse model, we found that tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) but not Fas ligand induced bystander killing of $CD4^+$ T cells in the mouse spleen (Miura et al. 2001). In addition, when these HIV-1-infected mice were treated with a sublethal dose of lipopolysaccharide, HIV-1-infected cells migrated into mouse brain tissue and induced apoptosis in neuronal cells via TRAIL molecules expressed on the HIV-1-infected cells, probably macrophages (Miura et al. 2003). These data suggested that TRAIL should have a pathological role of disease progression for AIDS.

Recently, further genetic manipulation has been performed. A more profoundly severe immunodeficient mouse strain, defective in common γ (γ_c) chain, which is a component of receptors for IL-2, IL-7, and other cytokines and critical for generation of NK and T cells, was generated from the NOD-SCID mouse (Ito et al. 2002) and the recombination activating gene-2 (RAG-2)^{null} mouse. The RAG-2^{null} mouse is also a genetically manipulated immunodeficient strain, which has a defect in the differentiation of T and B cells. NOD-SCID γ_c^{null} (NOG) and RAG-2/γ_c^{null} mouse strains were then generated, and these mice have been confirmed to have neither functional T and B cell nor NK cells and have been used as humanized mouse models by transplantation of human immune cells. The level of human cell reconstitution was greatly improved in NOG as well as RAG-2/γ_c^{null} mice transplanted with human PBMC (hu-PBL-NOG and hu-PBL-RAG-2/γ_c^{null} mice) (Nakata et al. 2005; Koyanagi et al. 1997b). Moreover, a technical improvement should be noted in that it is not necessary for preceding treatment with antibody, an anti-IL-2 receptor β chain antibody or an anti-asialo GM-1 antibody, to protect mouse NK cell differentiation in recipient mice.

2.2 HIV-1 Infection in the hu-PBL-NOG Mouse

Using NOG mice, we indicated the usefulness of the mouse strain for evaluation of an anti-HIV-1 compound (Nakata et al. 2006). HIV-1 suppressive efficacy of a small molecule of CCR5 antagonist was confirmed in the mice 1 day after HIV-1 inoculation. The level of viral loads and HIV-1-induced CD4 depletion was dramatically suppressed after treatment of a CCR5 antagonist, suggesting that the NOG mouse should serve as a small animal model for evaluation of anti-HIV-1 compounds (Nakata at al. 2006). Furthermore, it is noteworthy that this hu-PBL-NOG model provides a greater reproducibility of high viremia levels than the conventional HIV-1-infected SCID mouse models and that the high level of viremia achieved in this mouse model made it possible to monitor the changes in the

viremia levels periodically in the same set of mice without sacrificing them, while most of the previously described SCID mouse models required mice to be sacrificed at each time point of testing (Mosier et al. 1993; Ruxrungtham et al. 1996; Strizki et al. 2001).

2.3 *Development of HSC-Engrafted Mouse for HIV-1 Infection*

Since human immune cells are not fully reconstituted in the chimeric mice transplanted with human PBMC, many researchers have been developing a improved immunodeficient mouse model engrafted with human HSC, which generates human T and B cells and also DC/macrophage myeloid cells for long periods of time and will be amenable to HIV-1 infection (Fig. 3). The HSC-engrafted mouse model allows mechanistic studies of HIV-1-induced disease progression, and possibly would allow analysis of immune responses derived from the human cells.

Using 8- to 10-week-old NOG mice engrafted with HSC or progenitor cells from cord blood, Nakahata and colleagues reported the significantly efficient reconstitution of human B cells as well as T cells in mice for more than 6 months (Hiramatsu et al. 2003). This successful engraftment (designated as the hNOG mouse) encouraged the subsequent HIV-1 infection experiment. We recently showed that in the hNOG mouse exhibiting long-lasting high levels of viremia and CD4 depletion in the peripheral blood were reproduced after both R5 and X4 HIV-1

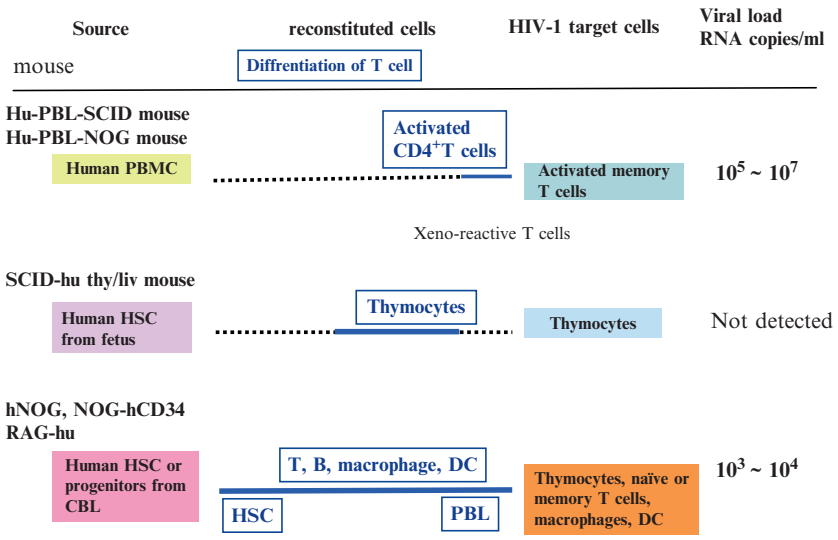


Fig. 3 Comparison of mouse/human chimeric models for HIV-1 infection. There have been three systems of humanized mouse models for HIV-1 infection

inoculation. Large amounts of HIV-1 DNA were detected in the spleen and bone marrow of R5 HIV-1-infected mice, and in the thymus and spleen of X4 virus-infected mice. Furthermore, anti-HIV p24 and gp120 antibodies were found in animals showing a high level of HIV-1 replication, indicating that HIV-1-specific immune response is induced in hNOG mice (Watanabe et al. 2007).

One modification of the CD34 engraftment into mice has been developed. Manz and colleagues reported that the newborn immunodeficient RAG-2/γ_c^{null} mouse strain is an adequate recipient for reconstitution of human immune cells including DC and that human adaptive immunity is clearly reconstituted in these mice (Traggiai et al. 2004). Another group also reported a significant improvement of human immune cell reconstitution with the newborn NOD/SCID/IL2 receptor γ chain^{null} mouse (Ishikawa et al. 2005). More recently, it was reported that efficient HIV-1 replication and CD4 depletion were shown in HSC-engrafted RAG-2/γ_c^{null} mice after HIV-1 inoculation (Baenziger et al. 2006; Berges et al. 2006; Zhang et al. 2007). We also confirmed that the engraftment procedure in newborn NOG mice with human CD34 created an HIV-1-susceptible mouse, and these mice (NOG-hCD34 mouse) after R5 or X4 HIV-1 inoculation possessed a level of viremia similar to that in HIV-1-infected patients. The NOG-hCD34 mice were also susceptible to infection with Epstein-Barr virus (EBV), which is known to be a human-specific γ herpes virus and to infect B cells. Several weeks after EBV inoculation, we could detect EBV DNA in the peripheral blood and spleen cells and could isolate EBV$^+$ lymphoblastoid cells from these mice (Koyanagi, unpublished observations).

3 Application of NOG Mice for HTLV Infection

3.1 HTLV

Human T-lymphotropic virus type I (HTLV-I) is another human retrovirus that causes adult T-cell leukemia (ATL)/ATL lymphoma and can also be involved in certain demyelinating diseases, tropical spastic paraparesis (TSP)/HTLV-I-associated myelopathy (HAM) (Hinuma et al. 1981; Osame et al. 1986). The malignant cells, mostly CD4$^+$ T cells, associated with all phases of ATL express very high levels of IL-2Rα (CD25) (Uchiyama et al. 1985). The median survival duration of all patients with aggressive ATL is about 13 months, and overall survival at 2 years is estimated to be about 30% (Taylor and Matsuoka 2005).

3.2 Model of HTLV-I-Induced Tumorigenicity

SCID mice have been utilized in a study on the pathomechanism and therapeutic strategy of ATL. Imada et al. examined the tumorigenicity of HTLV-I-infected cell

lines in an in vivo cell proliferation model using SCID mice. They found that 4 of 11 HTLV-I-infected cell lines were capable of proliferating in SCID mice after intraperitoneal inoculation. Interestingly, it was shown that some HTLV-I-infected and IL-2-dependent cell lines could be successfully engrafted in SCID mice (Imada et al. 1995). The expression of IL-2 mRNA was not detected in these cell lines growing either in vivo or in vitro. No HTLV-I viral structural proteins were detected in three of four transplantable cell lines proliferating in vivo. Peripheral blood T cells immortalized by introduction of *tax* gene of HTLV-I were found to have no tumorigenic potential in SCID mice. Although these systems were useful, two major drawbacks, namely, the long period of time required for tumor formation and the limitation of its use to certain cell lines, appear to hinder wider use of this animal model. These problems have now been overcome through development of the NOG mouse.

PBMC from patients with ATL were inoculated either intraperitoneally into the abdominal region or subcutaneously in the postauricular region of unconditional NOG mice. All mice developed clinical signs of near death, such as piloerection, weight loss, and cachexia, 6-8 weeks after inoculation of ATL cells in addition to enlargement of lymph nodes, spleen, lungs, and liver, whereas no tumors were found in the postauricular region or abdominal cavity where primary ATL cells were inoculated (Dewan et al. 2006). There was no difference in respect to the successful engraftment of ATL cells either intraperitoneally or subcutaneously inoculated into NOG mice. Histologic analysis of ATL-bearing mice showed massive infiltration of leukemic cells in various organs of NOG mice that were efficiently expressing human CD4 and CD25 molecules. A higher level of IL-2R (CD25) expression was observed on the surface of malignant cells associated with all stages of ATL as well as ATL cells infiltrated into various organs of patients. Thus, results from this model indicated successful engraftment and massive infiltration of primary ATL cells in various organs of NOG mice, like leukemia but without producing tumors at the sites of inoculation.

*3.3 Evaluation of Anti-*ATL *Compounds*

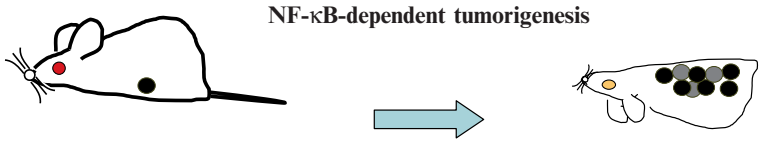
The various chemotherapies so far developed have not significantly increased the survival of patients with ATL (Taylor and Matsuoka 2005). Given the disappointing results using conventional chemotherapy, new approaches for the treatment of ATL are required. HTLV-I-infected cell lines derived from a leukemic cell clone and primary ATL cells failed to express significant amounts of Tax and other viral proteins, suggesting that the expression of viral proteins is not always necessary for leukemic proliferation at the late stage of the disease. However, HTLV-I-infected cell lines and leukemic cells from patients with ATL display constitutive NF-κB binding activity and increased degradation of a specific inhibitor, IκBα (Mori et al. 1999). NF-κB activation has been connected with multiple processes of oncogenesis, including control of apoptosis, cell cycle, differentiation, and cell migration; therefore, inhibition of NF-κB was suggested to be a useful strategy for cancer

therapy. Despite the diversity in clinical manifestations of ATL, strong and constitutive NF-κB activation was reported to be a unique and common characteristic of ATL cells (Mori et al. 1999). Thus, the indispensability of NF-κB for the maintenance of the malignant phenotype of HTLV-I provides a possible molecular target for ATL therapy. To study the effect of an NF-κB inhibitor, ritonavir, on ATL, we injected primary ATL cells from 10 patients subcutaneously into the postauricular region of NOG mice. Beginning 1 day after inoculation, mice were treated with either RPMI-1640 (as control) or ritonavir intraperitoneally daily for 30 days, followed by observation for another 30 days without any treatment. ATL cell inoculation promoted the development of piloerection, weight loss, and cachexia in addition to enlargement of lymph nodes, spleen, lungs, and liver in all control mice 2 months after inoculation. In contrast, ritonavir-treated mice were apparently healthy and had almost no enlargement of these organs. Clinical evaluation of organ invasion 2 months after injection of primary ATL cells showed that ritonavir treatment inhibited their infiltration into lymph nodes, spleen, lungs, and liver (Dewan et al. 2006). Seven of 10 patient samples injected in mice treated with ritonavir presented substantial inhibition of organ invasion, and 2 showed partial inhibition, whereas 1 sample failed to do so. In contrast, all control mice showed formation of new lymph nodes and infiltration with ATL cells into various organs. Organ infiltration of primary ATL cells was analyzed and evaluated by pathological staining and immunostaining of CD4 and CD25. We also performed similar experiments with HTLV-I-infected cell line cells [ED-40515(−), SLB-1, MT-1, TL-Oml, Hut-102, MT-2, and MT-4], using Bay 11-7082, which is another selective inhibitor of TNF-induced phosphorylation of IκBα without affecting the constitutive activation of IκBα phosphorylation, eventually resulting in decreased NF-κB and decreased expression of adhesion molecules (Dewan et al. 2003). Essentially, the same results were obtained in the studies using BAY11-7082 and the ED-40515(−) cell line. Together, these data indicate that NF-κB antagonists significantly inhibit ATL cell growth and infiltration in various organs of NOG mice.

Our NOG ATL model presents many features 6-8 weeks after inoculation of ATL cells such as the clinical signs observed in patients with ATL. Two clinical types, acute and chronic, carry very different prognoses. However, no difference in cell growth, surface phenotype, and NF-κB activity is observed in primary leukemic cells from patients with acute- and chronic-type ATL. Therefore, the same characteristics of freshly isolated ATL cells with acute- and chronic-type were observed in the NOG mouse. Thus, it represents a novel model to evaluate tissue toxicity and the efficacy of therapeutic agents directed toward the treatment of ATL (Fig. 4).

4 Concluding Remarks on Human Retrovirus Infection Model

The humanized mouse model should provide a midway position between the laboratory and clinical studies. From a relevant animal model for HIV and HTLV infections, immunological as well as virological aspects of the disease could be



Clinical signs in NOG ATL model
Development to $CD4^+CD25^+$ lymphoma
Invasion of leukemic cells to multiple organs
Cachexia
No expression of HTLV-I proteins

Fig. 4 NOG ATL model. Model of HTLV-I-induced tumorigenicity is created with NOG mice. $HTLV^+$ T cells as well as primary ATL leukemic cells induce the identical clinical signs observed in patients with ATL

investigated, and such an animal model could be used to examine the disease process and efficacy of antiviral compounds or vaccines. Significant progress in creating refined mouse strains has been achieved. We have now humanized mice, animals that circulate human blood. However, a mouse is not human. Therefore, there are still many limitations for examination of the human defense system. It is expected that the developments of technology in experimental animals and embryology, including methods using human ES or some other progenitor cells, will open the new evolution of humanized animals.

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