Cellular & Molecular Immunology (2012) 9, 208–214
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www.nature.com/cmi

REVIEW

Current advances in humanized mouse models

Ryoji Ito, Takeshi Takahashi, Ikumi Katano and Mamoru Ito

Humanized mouse models that have received human cells or tissue transplants are extremely useful in basic and applied human disease research. Highly immunodeficient mice, which do not reject xenografts and support cell and tissue differentiation and growth, are indispensable for generating additional appropriate models. Since the early 2000s, a series of immunodeficient mice appropriate for generating humanized mice has been successively developed by introducing the *IL-2Rγ*^{null} gene (e.g., NOD/SCID/γc^{null} and Rag2^{null}γc^{null} mice). These strains show not only a high rate of human cell engraftment, but also generate well-differentiated multilineage human hematopoietic cells after human hematopoietic stem cell (HSC) transplantation. These humanized mice facilitate the analysis of human hematology and immunology *in vivo*. However, human hematopoietic cells developed from HSCs are not always phenotypically and functionally identical to those in humans. More recently, a new series of immunodeficient mice compensates for these disadvantages. These mice were generated by genetically introducing human cytokine genes into NOD/SCID/γc^{null} and Rag2^{null}γc^{null} mice. In this review, we describe the current knowledge of human hematopoietic cells developed in these mice. Various human disease mouse models using these humanized mice are summarized.
Cellular & Molecular Immunology (2012) **9,** 208–214; doi:10.1038/cmi.2012.2; published online 13 February 2012

Keywords: animal model; immunodeficient mice; immunology; hematology; humanized mice

INTRODUCTION

'Humanized' mouse models in which various types of human cells and tissues are engrafted and function, as they would in humans are considered extremely useful in basic and applied human disease research.[1–4] For this purpose, highly immunodeficient mice, which do not reject xenografts and support the differentiation and growth of cells and tissues, are indispensable. The support system that maintains human cells and tissues is also a key factor. In addition, technical modifications necessary for the generation of humanized mice are important.

The discoveries of nude and severe combined immunodeficiency (SCID) mice were key advances in the development of immunodeficient mice for xenotransplantation.[5,6] The development of non-obese diabetic (NOD)/SCID mice *via* the introduction of the *Prdkc*^{scid} gene into a NOD inbred strain also contributed to the generation of humanized mice.[7,8] NOD/SCID/β2m^{null} and NOD/Rag1^{null}Pfp^{null} mice were subsequently derived from NOD/SCID and NOD/Rag1^{null} mice.[9,10]

Since the early 2000s, immunodeficient mice appropriate for generating humanized mice have been successively developed by introducing the mutant *IL2rγ* gene into NOD/SCID and RAG1/2^{null} mice by backcross mating, thus resulting in NOD/SCID/γc^{null} mice[11,12] and Rag1/2^{null}γc^{null} mice.[13–16] These mice show multiple immunodeficiencies, including defects in T, B and natural killer (NK) cells, and reduced macrophage (Mϕ) and dendritic cell (DC) function.[11] In these mice, extremely high human cell engraftment rates and increases in well-differentiated human multilineage hematopoietic cells from human hematopoietic stem cells (HSCs), as compared with parent immunodeficient mice, were observed.[17–19] Humanized mice that retain various human immune cells are often termed human immune system mice[3] or human hematolymphoid system mice.[20] The production of humanized mice that are reconstructed with human cells would facilitate analysis of the underlying mechanisms of human disease pathogenesis. Indeed, various humanized models have been developed using these mice.

Various technical modifications have been used when generating humanized mice. These include modifications in the HSC injection route, age of mice, and HSC and irradiation sources, each of which may affect the efficacy of human cell engraftment. In terms of the injection route and age, intrahepatic or intravenous injection (through the facial vein) into newborn mice and intravenous injection (*via* the tail vein) into adult mice were generally used.[11,14,21] Brehm *et al.*[16] examined various parameters, including injection route, injection age and immunodeficient mouse strains. By comparing engraftment rates of human cells from HSCs, they concluded that intrahepatic injection of HSCs into newborn mice enhanced engraftment as compared with adult mice. With respect to the source of HSCs, CD34$^+$ cells from cord blood or fetal liver were typically used. However, CD34$^+$ cells from granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood (PB) or bone marrow (BM) served as additional sources. Lepus *et al.*[22] reported that CD34$^+$ cells from fetal liver were more efficient than those from cord blood or G-CSF-mobilized PB. In addition, Matsumura *et al.*[23] reported that CD34$^+$ cells from cord blood were more effective than those from G-CSF-mobilized PB and BM. Busulfan treatment can also be used in place of irradiation, and results in more effective differentiation of B cells from HSCs as compared with

Central Institute for Experimental Animals, Kawasaki, Japan
Correspondence: Dr M Ito, Central Institute for Experimental Animals, 3-25-12, Tonomachi, Kawasaki-ku, Kawasaki 210-0821, Japan.
E-mail: mito@ciea.or.jp
Received 10 November 2011; revised 5 January 2012; accepted 10 January 2012

irradiation.[24] The use of this drug would be beneficial for research, since special equipment is not required. The engraftment rate of human cells from HSCs varies between reports. A possible explanation for this difference is the quality of HSCs, which is influenced by different isolation techniques and the HSC source (i.e., cord blood or fetal liver).

Despite these efforts, some human cell lineages, such as erythrocytes and neutrophils, have not yet been developed in humanized mice. The differentiation of HSCs into immature human T and B cells and poor interactions between these cells were suggested based on cell phenotype analysis and the rare production of antigen-specific immunoglobulin G (IgG) class antibodies.[25] These results suggest that current immunodeficient mice may be insufficient, and human factors that support the differentiation and maturation of cells and mediate cell-to-cell interactions must be introduced. To this end, a new series of immunodeficient mice has been generated by introducing human cytokine genes into NOD/SCID/γc^{null}, $Rag2^{null}\gamma c^{null}$ mice.

In this review, we describe the current knowledge of human hematopoietic cells developed in NOD/SCID/γc^{null} and $Rag1/2^{null}\gamma c^{null}$ mice and the strains derived from them. In addition, the humanized mouse models used in studies of various human diseases will be summarized.

IMMUNODEFICIENT MICE

Since the discovery of nude and SCID mice,[5,6] humanized mice have been generated by using various immunodeficient mice. SCID mice that received human T and B cells by transplantation of fetal liver and thymus, which were termed *SCID-hu* mice by McCune *et al.*,[26,27] provided an attractive humanized model for research in various fields; however, engraftment rates were not high. In 1998, Goldman *et al.*[13] reported the enhanced engraftment of human cells in $Rag2^{null}$ mice possessing the IL-2Rγ^{null} gene. In the 2000s, a series of immunodeficient mice was developed by combining the IL-2Rγ^{null} gene with conventional SCID and $Rag1/2^{null}$ mice. These strains showed extremely high engraftment rates and differentiation of human cells, resulting in remarkable advances in the development of human disease models. These stains include the NOG (NOD/Shi-*Prkdc*^{scid} *Il2r*γ^{tm1Sug}/Jic) mice reported in 2002;[11] RG (BALB/c-$Rag2^{null}$Il2rγ^{null} (BRG) and C57BL/6-$Rag2^{null}$Il2rγ^{null} (B6RG)) mice reported in 1997, 1998 and 2004;[13,14,28] and NSG (NOD/LtSz-*Prkdc*^{scid} *Il2r*γ^{tm1Wjl}/J) mice reported in 2005.[12] Recently, immunodeficient BALB/c-$Rag1^{null}$Il2rγ^{null},[16] NOD-$Rag1^{null}$Il2rγ^{null},[15] and NOD/SCID-JaK3^{null} mice have been established as alternatives to NOG/NSG mice. Data accumulated to date suggest that NOG/NSG mice are the best recipients for humanized tissue and human cell engraftment occurs in the following order: NSG=NOG>NRG>BRG>NOD/SCID>B6RG.[15,16] The disadvantages of SCID and NOD/SCID mice include the frequent occurrence of thymic lymphoma and the leakiness, in which T and B cells develop in aged mice.[29,30] However, NOG mice show no leakiness or spontaneous thymic lymphoma.[31,32] This may be attributed to inactivation of IL-2Rγ, which is shared by important cytokines, such as IL-2, IL-4, IL-7, IL-15 and IL-21, each of which is important for T- and B-cell growth.[33] These results indicate that NOG/NSG mice are better recipients of human cells and tissues.

The most attractive feature of these humanized mice is the development of multilineage hematopoietic cells by transplantation of human HSCs. In particular, T-cell subpopulations, including CD4 and CD8 single positive cells, which could not be differentiated in NOD/SCID mice, successfully developed in these mice.[14,18,21] These results suggest the usefulness of humanized mice for investigating human immune responses. Indeed, the utility of these humanized mice is well accepted by researchers worldwide. However, these humanized mice are insufficient, since human cells are not fully functional. For example, no or rare antigen-specific IgG production occurs after multiple injections of antigens.[25]

To overcome this issue, various improved strains based on these immunodeficient mice have been developed or are being developed by several groups, including our group.[34,35] In Figure 1, the history of the development of immunodeficient humanized mice and their respective improvements are summarized. These mice have been established in NOG/NSG or BRG backgrounds. Background strain selection is crucial for the improvement of immunodeficient mice. In 1996, we reported human granulocyte–macrophage colony-stimulating factor (hGM-CSF) and interleukin-3 (IL-3) cosecreting transgenic (Tg) B6-SCID mice.[36] These strains were able to maintain human tumor cell lines possessing hGM-CSF and human IL-3 (hIL-3) receptors on the surface;[37] human cells, however, could not engraft in these mice. We recently established hGM-CSF/IL-3 Tg NOG mice by the conversion of strain B6 to NOG by backcross mating. High human cell engraftment rates were observed in hGM-CSF/IL-3 Tg NOG mice. We also compared engraftment of HSCs in NOG, BRG and B6RG mice. Higher engraftment rates were obtained in NOG and BRG, but not B6RG mice. In particular, we were surprised to detect only a few human cells in B6RG mice (unpubl. data). Similar results were reported by Traggiai *et al.*,[14] indicating that immunodeficient mice in a B6 background are not sufficient for generating humanized mice. Recent reports on signal regulatory protein (Sirpα) suggested that NOD mice are superior to other strains, because Sirpα in NOD strains is more similar to that in humans, compared with Sirpα in other mice strains.[38,39] Therefore, BRG transgenic mice with human Sirpα have been established to enhance human cell engraftment efficacy.[20]

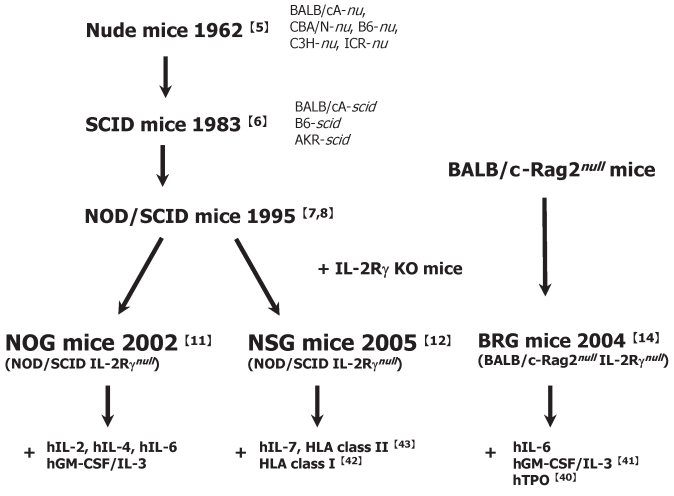

Figure 1 The history of the development on immunodeficient mice for humanized mice model. In retrospect, nude mice or SCID mice were the first immunodeficient strains. Subsequently, their congenic strains were generated to improve engraftment capacities. NOD/SCID mice established in 1995 have been a milestone in this field, because of the severer phenotype than nude and SCID mice. In early 2000s, NOG, NSG and BRG mice were established by introducing the IL-2Rγ^{null} allele into NOD/SCID or BALB/c $RAG2^{null}$ mice. Due to the complete loss of murine immune systems, human hematopoiesis has been enormously enhanced in these mice. Currently, these strains were further improved by introducing human genes for various cytokines or HLA class I and II, so as to recapitulate a human *bona fide* hematopoiesis and immune system. The superscripts represent the respective references. BRG, BALB/c-$Rag2^{null}$Il2rγ^{null}; HLA, histocompatibility leukocyte antigen; NOD, nonobese diabetic; NSG, NOD/LtSz-*Prkdc*^{scid} *Il2r*γ^{tm1Wjl}/J; SCID, severe combined immunodeficiency.

Improved strains were generated by injecting human DNA into pronuclear stage embryos, injecting genetically modified embryonic stem cells into blastocysts (knock-in (KI)), or introducing human genes by backcross mating with established transgenic mice. Specifically, human cell differentiation from HSCs in improved mice produced using the KI strategy has been analyzed by Flavell's group at the Yale University.[20] They reported elevated HSC and myeloid cell numbers in thrombopoietin (TPO) KI mice and increased alveolar Mϕ in hGM-CSF/IL-3 KI mice.[40,41] We also developed transgenic NOG mice that secreted human IL-2 or IL-4 by injecting DNA into NOG embryos. The suppression of graft-versus-host disease (GVHD) and dominant conversion to Th2 cells in hIL-4 Tβ NOG mice and remarkable differentiation of human NK cells in hIL-2 Tβ NOG mice, respectively, were observed (unpubl. data). Shultz's group generated human leukocyte antigen (HLA) class I transgenic NSG mice by backcross mating the respective transgenic mice into NSG mice. The successful generation of antigen-specific cytotoxic T cells[42] was observed. Antigen-specific IgG was produced in HLA class II transgenic NSG mice.[43]

Real humanized mice are expected to be generated in the near future by improving immunodeficient mice.

HEMATOLYMPHOID HUMANIZED MICE

The reconstitution of the human hematopoietic system is one of the most advanced areas in humanized mouse research. The use of NOG/NSG or BRG mice has greatly improved human hematopoiesis, as shown by the development of multiple human cell lineages, including B and T lymphocytes, NK cells, myeloid DC, plasmacytoid DC, Mϕ and erythroblasts. Here, we discuss the current status and perspectives in this field.

Lymphoid cells

Two major subsets of lymphoid cells, i.e., B and T cells, were developed in NOG mice by simply transferring human HSCs after irradiation.

B cells. Human B cells are detected in the PB 1 month after HSC transplantation and gradually increase in number over the following 3 months. In the BM, B-cell differentiation in humanized mice seems to consistently resemble that in humans, since several distinct precursor populations exist:[25] $CD19^-CD38^+CD10^+CD34^+$ early-B cells, $CD19^+IgM^-CD20^-CD34^+$ pro-B cells, $CD19^+IgM^-CD20^-CD34^-$ pre-B cells and $CD19^+IgM^+IgD^-$ immature B cells. The pro-B and pre-B populations were also characterized by the expression of intracellular V_{preB} and Cμ chains. In the spleen, one of the remarkable B-cell phenotypes in humanized mice is high CD5 expression, which is markedly different from genuine human B cells.[23] The significance of this upregulated CD5 expression remains controversial, i.e., whether these are human B-1 cells or transitional 1 B cells.[23,25]

Immunization of humanized mice with various exogenous substances induces antigen-specific IgM responses, suggesting that the B-cell repertoire is diverse and can cover a myriad of antigens.[21,23] Since antigen-specific IgG responses in conventional humanized mice are very weak,[14,21,23] it has been speculated that these B cells have some intrinsic defects in the class switch machineries. Several *in vitro* experiments, however, demonstrated that these B cells do produce IgG in response to stimulation through their antigen receptors and CD40 in the presence of IL-21.[25] In addition, recent reports have shown that new mouse strains that express HLA-DR mounted an antigen-specific IgG response upon immunization.[43] Collectively, B cells in humanized mice maintain the ability to mediate humoral immune reactions.

T cells. Human T cells can develop in humanized NOG/NSG or BRG mice in the thymus and accumulate in the spleen. This is one of the most important points of distinction from NOD/SCID mice, which only partially support the differentiation of human T cells. Typically, 3–5 months is necessary for T cells to colonize the spleen.[44] In the thymus, human thymocytes show typical surface phenotypes,[44] i.e., $CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^-CD8^+$ and $CD4^+CD8^-$ stages, suggesting that they follow the normal differentiation pathway. The thymus is indispensable for T-cell development, and selection is largely mediated by mouse major histocompatibility complex (MHC) molecules, given that Foxn1-deficient NOG mice (NOG *nu/nu*) cannot support T-cell development, and $CD4^+$ and $CD8^+$ T cells do not fully develop in NOG I-$A\beta^{null}$ or NOG $\beta2m^{null}$ backgrounds,[25] respectively.

Although development seems to be relatively normal, the functionality of T cells in humanized mice remains controversial. For example, in studies using Epstein–Barr virus (EBV), humanized mice showed antiviral T-cell responses in which interferon-γ producing $CD8^+$ T cells were differentiated and protected mice from lymphoma development,[14,45] thus supporting the normal functions of human $CD8^+$ T cells. However, *in vitro* experiments have suggested that human T cells have a limited ability to respond to antigenic stimulation.[25] Although the mechanisms underlying abnormal T-cell functions remain unclear, mismatches between mouse MHC and the HLA of donor human cells may be involved. Indeed, HLA-A- or HLA-DR-expressing NSG mice showed normal cytotoxic reactions (cytotoxic T-lymphocyte responses) to viral infection or IgG responses against exogenous antigens, respectively.[43,46,47]

NK cells. Human NK cells also develop from the early stage (4 weeks) of HSC transfer. However, the number of NK cells is not very high (a low percentage of human $CD45^+$ cells), indicating the necessity for growth and differentiation factors in immunodeficient mice.[48] Huntington *et al.*[49] reported that the hIL-15/hIL-15Rα complex induces extensive proliferation and differentiation of $CD16^+KIR^+$ NK cells. Chen *et al.*[50] also reported that administering IL-15 and the Flt-3/Flk-2 ligand by plasmid DNA injection into HSC-transferred mice leads to an increased number of NK cells. In IL-2 Tg NOG mice (Katano *et al.*, manuscr. in prep.), human NK cells predominantly develop prior to B and T cells after HSC transfer, and consist of the largest population in human $CD45^+$ cells. However, their function has not been well characterized.

As an alternative method for studying human NK cell functions, *ex vivo* isolated human NK cells from peripheral blood mononuclear cells (PBMCs) have been transplanted into NOG mice. Although the inoculated NK cells were not maintained in these mice for a long time, the cells exerted effective antibody-dependent cellular cytotoxicity and suppressed the growth of a Burkitt's lymphoma cell line (Daudi), following concomitant administration of an anti-CD20 antibody (rituximab).[51]

Myeloid cells

In conventional humanized mice, although human myeloid cells were shown to be differentiated, the efficiency was poor. Recently, however, the development of various lineages of myeloid cells has been improved by introducing human cytokines.

Monocytes. Human monocytes/Mϕ can be detected in the blood, lymphoid organs (spleen and BM) and some tissue organs (lung and liver) in conventional humanized mice. The frequency of human $CD14^+$ cells among the total human $CD45^+$ cell population is usually not more than 1%–2% in the spleen, while the frequency can reach 8% or 5% in

the lungs or liver, respectively. The delivery of plasmid DNA encoding several human cytokine genes,[50] i.e., IL-15 with Flt-3 ligand (Flt3L) or macrophage CSF, by hydrodynamic injection robustly induces the development of human monocytes/Mϕ in various organs. In addition, in a novel mouse strain in which the TPO gene was replaced with the human homolog, the development of total human myeloid lineage cells was significantly improved.[40] Accordingly, the frequency of monocytes was increased in the blood, but not in the BM. In our studies, the transgenic expression of hIL-3 and GM-CSF genes in NOG (hGM-CSF/IL-3 Tβ NOG) mice also improved the development of whole human myeloid cells, including $CD14^+$ monocytes (Ito *et al.*, manuscr. submitt.).

DCs. In conventional humanized mice, several reports have demonstrated the presence of both myeloid DCs ($CD11c^+HLA\text{-}DR^+CD40^+CD86^+$) and plasmacytoid DCs ($CD123^+HLA\text{-}DR^+BDCA2^+$) in the BM, spleen and liver.[14,50] These $CD11c^+$ or $CD123^+$ DCs were functional, as demonstrated by their ability to induce activation of allogeneic human T cells or to produce interferon-α after stimulation.[14] The frequency of these cells, however, is generally low in the spleen (typically less than 1% in our studies). Chen *et al.*[50] demonstrated that the administration of plasmid DNA encoding human IL-4/GM-CSF/Flt3L or IL-5/Flt3L markedly increased the DC yield.[50] Enhancements in DC development were also observed in our hGM-CSF/IL-3 Tβ NOG mice, in terms of both number and frequency, in various lymphoid organs (Ito *et al.*, manuscr. submitt.).

Granulocytes. Although granulocytes comprise a large fraction of human leukocytes, their frequency in humanized mice is very low (less than 2%–3% of human leukocytes in PB and BM in our studies). To improve differentiation of this population, several groups have attempted to produce novel humanized mouse strains by providing human cytokines. Billerbeck *et al.*[52] created a transgenic NSG strain that expressed the human stem cell factor, GM-CSF and IL-3 genes, and demonstrated a slight increased development of human $CD15^+$ granulocytes in the BM. In TPO KI mice, as mentioned above, a large number of human $CD66b^+$ granulocytes were produced in the BM.[40] Moreover, this strain enabled the development of mature human neutrophils with lobulated nuclei, which has not been achieved before. Additionally, in our hGM-CSF/IL-3 Tβ NOG mice, we confirmed significant increases in $CD66b^+$ granulocyte numbers in the BM and PB. Furthermore, the presence of human basophils and eosinophils in the PB was detected by May–Giemsa staining (Ito *et al.*, manuscr. submitt.). This is the first report to demonstrate the development of these cell populations in humanized mice. Collectively, the development of human granulocytes in humanized mice has been greatly improved by the addition of human cytokines.

Mast cells. Mast cells play an important role in allergic responses by releasing intracellular granules containing histamine or various leukotrienes. Crosslinking of their surface F*c*-epsilon receptor (FcεR) by IgE triggers a series of reactions.[53,54] Although there is little evidence suggesting the development of human mast cells, Kambe *et al.*[55] demonstrated the presence of human mast cells in the skin, spleen and BM of humanized NOG mice. These cells were positive for c-kit and CD203c, but expression of FcεR was not determined. Recently, we detected FcεR positive mast cells in the BM, spleen and several non-lymphoid tissues of hGM-CSF/IL-3 Tβ NOG mice (Ito *et al.*, manuscr. submitt.). These data suggest that IL-3 and/or GM-CSF are important for inducing the differentiation of human mast cells.

HUMANIZED MODELS

Cancer

Due to their supply by the Central Institute for Experimental Animals, NOG mice have been predominantly used in this field. The characteristics of NOG mice include rapid growth of tumors and well-maintained characteristics after multiple passages. In a study by Machida *et al.*,[56] 100 HeLa S3 cells could be successfully engrafted in NOG mice; in contrast, 10^5 and 10^6 cells were required for engraftment in NOD/SCID and C.B-17-SCID mice, respectively. However, primary human tumors do not always engraft in NOG mice, even though these animals show higher engraftment than conventional immunodeficient mice. Some tumors, such as prostate carcinoma, which are difficult to engraft in SCID and nude mice, are also difficult to engraft in NOG mice. The growth of some tumor cell lines appears to be less than in conventional immunodeficient mice. The reason for this is unclear, but it may be explained by the differential adaptation of cell lines to conventional immunodeficient mice. *IL-2Rγ* gene inactivation may influence the growth of some tumors in NOG mice. Another characteristic of NOG mice is a high occurrence of metastasis. Genes responsible for metastasis have been investigated through the use of this characteristic.[57] The high homing capacity of human cells also appears to be maintained in NOG mice. When U266 myeloma cells were intravenously injected into NOG mice, they grew only in the BM, resulting in paralysis.[58]

Various cancer models have been established using these advantages.[51,59] On the other hand, a model that can be used to investigate immune responses to tumors has only recently been developed. Mismatching of HLA between tumor cells and hematopoietic cells from HSCs of different donors may cause severe GVHD or a lack of response. To induce an effective immune response against tumors in mice, HLA matching is required. Recently, Shultz *et al.*[42] reported that antigen-specific cytotoxic T lymphocytes were successfully induced in a newly established HLA class I (A-2) transgenic NSG mouse model by transfer of HLA-matched HSC. The development of these mice may lead to new immunotherapy models for cancer. The injection of human PBMCs (hPBMCs) into immunodeficient mice is known to cause severe GVHD; this provides a good model of GVHD.[60] We recently found that NOG-I-$A\beta^{null}\beta2m^{null}$ mice showed mild GVHD, although high engraftment rates were observed as compared with non-transgenic NOG mice after transfer of hPBMCs (unpubl. data). Cotransplantation of a patient's tumor and hPBMCs into such immunodeficient mice may facilitate analysis of the immunological responses to the tumor.

Infectious diseases

Human lymphocytes, including T and B cells, predominantly develop in humanized mice transferred with HSCs. Therefore, appropriate models are provided for viruses that specifically infect lymphocytes and express their pathology, such as HIV-1, HTLV-1 and EBV. HIV-1 infection models have been widely used for the analysis of disease mechanisms and the development of anti-HIV-1 drugs,[61] as HIV-1 infects human T cells in *SCID-hu* mice.[26,62,63] This research is further accelerated through the use of HSC-transplanted immunodeficient mice, in which multilineage hematopoietic cells can be differentiated.[64–67] In this field, a unique model for HIV-1[68–70] reported by Garcia's group at the University of North Carolina and termed bone marrow–liver–thymus (BLT) mice, has attracted attention. As the name suggests, this model is generated by transplantation of fetal bone marrow, liver and thymus into a subcutaneous region of the kidney. The most attractive feature of BLT mice is reconstitution of human

mucosal immunity; this has not yet been obtained in human immune system mice transferred with HSCs. The human mucosal lymphoid apparatus, including Peyer's patches and gut-associated lymphoid tissue, has been successfully reconstituted in BLT mice, resulting in the development of mucosal immunity. They reported that the *IL-2Rγ* gene was indispensable for development of the mucosal lymphoid system, indicating that mucosal immunity cannot develop in NOG/NSG and BRG mice that contain a mutant *Il2rγ* gene (reported at the Third International Workshop of Humanized Mice (IWHM 2011) held in Pittsburgh in October 2011).

This mouse appears to provide a better HIV-1 model as compared with conventional humanized mice transferred with HSCs. However, this model cannot be investigated from the aspect of humoral immunity involving B cells, and cannot be used in some countries such as Japan because of ethical issues. Additional genetic modifications of current immunodeficient mice may be necessary to overcome this disadvantage.

EBV usually presents in healthy subjects as a latent infection; however, it expresses a variety of pathological features in the healthy, termed EBV-associated infectious mononucleosis, hemophagocytic lymphohistiocytosis, lymphoproliferative disease, Burkitt's lymphoma and Hodgkin's disease in those immunosuppressed, due to HIV-1 infection or BM transplantation.[71] Since the report of EBV-associated lymphoproliferative disease by Traggiai *et al.*[14] using humanized BRG mice, various humanized mouse models of these clinical pathologies have been reported.[72–74]

Humanized models of tuberculosis, salmonellosis, yellow fever and Dengue fever have been investigated.[75–77]

Animal models appropriate for developing a malaria vaccine are eagerly desired, as malaria is one of the most common infectious diseases worldwide.[78] An interesting human malaria model uses immunodeficient mice with transplanted human liver. By injecting human hepatocytes into liver-damaged immunodeficient mice,[79–82] human hepatocytes replace the mouse hepatocytes. In these hu-liver mice, intrahepatic multiplication of *Plasmodium falciparum* has been observed.[83] However, human erythrocytes from human blood must be successively injected into the mice intraperitoneally, because human erythrocytes cannot develop from HSCs.[84] To establish the complete malaria life cycle in mouse models, it is necessary to develop mice in which human erythrocytes persist and flow in mouse peripheral blood. Hu-liver mice provide a good infection model for viruses specific to hepatocytes, including hepatitis C and B viruses.[85–87]

These models provide invaluable tools for analyzing the mechanisms of human infection and for developing chemotherapeutic agents such as antibodies.

GVHD

GVHD is a severe complication with a high mortality rate that often develops in patients who receive allogeneic BM transplantation for the treatment of acute/chronic leukemia, aplastic anemia or congenital immunodeficiency. Approximately 20 years ago, Mosier *et al.*[63] first demonstrated that the induction of xenogenic GVHD was possible in immunodeficient mice (C.B-17-SCID) by transplanting hPBMCs. In this model, the transplanted human T cells may be activated and attack the recipient mouse tissue, thus resulting in the development of allogeneic GVHD-like symptoms.

Although C.B-17-SCID or NOD/SCID mice have been useful in GVHD research, there are several problems. For example, human cell engraftment is relatively low, due to the mouse endogenous innate immune system. It also requires sublethal dose total body irradiation, which results in large variances in disease onset. Furthermore, a relatively large number of hPBMCs have to be administered intraperitoneally, but not intravenously, to induce the disease.[88] This does not reflect BM transplantation, where cells are infused intravenously. van Rijn *et al.*[89] used H-2^{d}-RAG2^{null} IL2rγ^{null} mice in which xeno-GVHD was induced by intravenous injection of hPBMCs; however, this model still depends on the infusion of large numbers of hPBMCs (3×10^{7} cells/head) and total body irradiation. Our xeno-GVHD NOG mouse model has shown significant improvements over other models, such as the rapid onset of disease and uniform death of recipients. In addition, a smaller number of donor cells (2.5×10^{6}) is sufficient with intravenous injection, and total body irradiation is not always necessary.[60] These results were confirmed by other studies using NSG mice.[90]

Collectively, NOG or NSG mice are the most suitable platforms for basic and preclinical GVHD research at this time.

Humanized liver models

Humanized liver models, in which the mouse liver is replaced with a human liver, are useful for evaluating drug metabolism in the human liver, as there are numerous differences in liver enzymes between humans and mice. In the first human liver model developed by Mercer *et al.*,[81] SCID/*bg* mice carrying a urokinase-type plasminogen activator transgene (Alb-uPA) entered a profound hypofibrinogenemic state, which caused hepatocyte death. They transplanted human hepatocytes into the inferior splenic pole and demonstrated that human hepatocytes could be engrafted over 50% in the liver of these mice. To improve xenoengraftment of human hepatocytes, NOG-uPA[82] and FRG (fumarylacetoacetate hydrolase^{null}/RAG2^{null}/IL-2Rγ^{null})[79] mice, in which liver damage is induced by adenovirus-mediated uPA expression, were developed and showed markedly high rates of replacement by human hepatocytes (over 80%). Nevertheless, several problems limit their utility, such as poor breeding efficiency in the mouse colony, development of renal disease, and a very narrow time window for transplantation. Recently, Hasegawa *et al.*[80] established a novel NOG substrain that expresses the herpes simplex virus type 1 thymidine kinase (TK) transgene under the control of a mouse albumin promoter. Administration of ganciclovir, which is non-toxic to human and mouse tissues, ablated TK-expressing liver parenchymal cells. Herpes simplex virus type 1 TK NOG mice allowed high engraftment of human hepatocytes (over 80%) and did not develop systemic morbidity (liver disease, renal disease and bleeding diathesis) as seen in other uPA-dependent models. Stable, long-term humanization of TK NOG mice will facilitate studies of drug metabolism, toxicology and the virology of hepatitis viruses.

FUTURE PERSPECTIVES

Over the last 10 years, remarkable progress has been achieved in humanized mouse models using NOD/SCID/γc^{null}, Rag1/$2^{null}\gamma c^{null}$ mice, especially for hematology and immunology. Various humanized mouse models have been established that enable direct research of human diseases, which was previously impossible in immunocompetent animals. These models will also contribute to the analysis of mechanisms underlying human immune disorders and the development of vaccines against infectious diseases through the use of humanized mice that contain a wide variety of functional human hematopoietic cells.

However, several issues remain to be overcome, such as the rare differentiation of certain cell lineages from HSCs, immature differentiation and insufficient intercellular relationships. To overcome these problems, the inclusion of other immunodeficient mouse genes

or human genes responsible for cell differentiation and interaction has been investigated. These attempts may result in more appropriate immunodeficient humanized mice.

Recently, progress in the field of regenerative medicine has drawn our attention, following the establishment of human embryonic stem and inducible pluripotent stem cells. In the future, artificial human organs or HSCs developed from embryonic stem or inducible pluripotent stem cells may be available. Although these techniques have not yet impacted the field of humanized mice, new models will likely result from transplantation of artificial human organs and HSCs.

ACKNOWLEDGEMENTS

This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), by a grant (22A-9) from the National Center for Child Health and Development, and by a grant from Research on Emerging and Re-emerging Infectious Diseases from Ministry of Health, Labour and Welfare, Japan.

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