Mouse models with human immunity and their application in biomedical research

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Abstract

Biomedical research in human beings is largely restricted to in vitro studies that lack complexity of a living organism. To overcome this limitation, humanized mouse models are developed based on immunodeficient characteristics of severe combined immunodeficiency (SCID) or recombination activating gene (Rag) null mice, which can accept xenografts. Peripheral constitution of human immunity in SCID or Rag null mice has been achieved by transplantation of mature human immune cells, foetal human thymus, bone marrow, liver tissues, lymph nodes or a combination of these, although efficiency needs to be improved. These mouse models with constituted human immunity (defined as humanized mice in the present text) have been widely used to investigate the basic principles of human immunobiology as well as complex pathomechanisms and potential therapies of human diseases. Here, elements of an ideal humanized mouse model are highlighted including genetic and non-genetic modification of recipient mice, transplantation strategies and proposals to improve engraftments. The applications of the humanized mice to study the development and response of human immune cells, human autoimmune diseases, virus infections, transplantation biology and tumour biology are reviewed as well.

Keywords: immunodeficient mice • human immune system • biomedicine • transplantation • animal model

Introduction

The study of human immunobiology in vivo is limited by technical and ethical constraints. Animal models with humanized immune systems would significantly advance our understanding on human immunobiology and immune-related diseases such as autoimmune diseases, virus infections, as well as tumour and graft rejections. Immunodeficient mice with constituted human immunity have been developed to overcome these constraints and are now important research tools for the in vivo study of human haematolymphopoiesis and immune responses. Severe combined immunodeficiency (SCID) or recombination activating gene (Rag) null mice,
lacking T and B cells, were originally used as recipients to re-build human immunity [1]. Recently, more and more genetically modified SCID or Ragnull mice including SCID/beige [2], non-obese diabetic/severe combined immunodeficiency (NOD/SCID) [3], NOD/SCID/H9252 2Mnull [4] and NOD/SCID/H9253 cnull [5], Rag null [6–8], NOD/LtSz-Rag1nullPfpnull [9] and Rag2 null/H9253 cnull [10] mice have been employed to further enhance the reconstitution efficiency of human immune cells in the periphery, due to their deficiency of innate immunity (Table 1) [11, 12].

On the other hand, to improve the engrafting efficiency of human immune cells and/or tissues, different conditional regimens and transplantation strategies have been intensively pursued, including the depletion of host innate immune cells as well as implanting mature human immune cells, foetal thymus, liver tissues, bone marrow and CD34+ haematopoietic stem cells (HSCs), respectively [13]. In the present review, we will concentrate on the selection and pre-treatment of genetically modified SCID or Ragnull mouse recipients, strategies for implanting human HSCs, mature immune cells and/or tissues, as well as the applications of these models in biomedical research. Generally, mice constituted with human cells, tissues, organs or even human genes [14] may all be considered as humanized mice, including models grafted with foetal human lung, kidney, pancreas, stomach, liver, ovarian, endometrium, nervous and skin tissues [15–24]. To avoid confusion, humanized mice are specifically defined in this review as mice with a human immune system reconstituted by engrafting human haematopoietic or mature immune cells and/or immune tissues.

### Table 1 The characteristics of genetically modified SCID or Ragnull mice

| Mouse strains                      | Mechanisms                                      | Adaptive immunity       | Innate immunity          | Refs   |
|-----------------------------------|-------------------------------------------------|-------------------------|--------------------------|--------|
| SCID                              | DNA-repair and VDJ recombination defect due to deficiency of DNA-PK | T and B cell deficiency |                          | [1]    |
| SCID/beige                        | Combined effect of SCID and beige mutations     | T and B cell deficiency | Reduced NK-cell function | [2]    |
| NOD/SCID                          | VDJ recombination defect added to various NOD anomalies | T and B cell deficiency | Impaired complement, macrophage and NK cell function | [3]    |
| NOD/SCID/β2Mnull                  | VDJ recombination defect, no MHC-I and various NOD anomalies | T and B cell deficiency | Partial NK cell deficiency | [4]    |
| NOD/SCID/γcnull                   | Combined effect of SCID and γcnull on NOD background | Severe defect of T and B cell function | Severe defect of NK cell function, impaired DC function | [5]    |
| Ragnull                           | VDJ recombination defect                        | T and B cell deficiency |                          | [6–8]  |
| NOD/LtSz-Rag1nullPfpnull          | VDJ recombination defect and perforin mutation  | T and B cell deficiency | Complement, macrophage and NK cell deficiency | [9]    |
| Rag2null/γcnull mice              | Combined effect of Rag2null and γcnull          | T and B cell deficiency | NK cell deficiency, Impaired DC function | [10]   |

Recipient mouse selection for engrafting xenogeneic human haematopoietic and immune cells

SCID mice are defective in DNA repair because of a mutation in DNA-dependent protein kinase (DNA-PK) in the CB-17 inbred mouse strain [25, 26], therefore they lack productive rearrangement of T cell receptor and immunoglobulin genes, which subsequently results in the deficiency of T and B cells [27]. The lack of functional T and B cells in SCID mice contributes to the acceptance of allogeneic or xenogeneic grafts without severe rejection [1, 28, 29]. However, SCID mice have a radiation repair deficiency and an incomplete block of VDJ recombination, so some old SCID mice may become ‘leaky’ mice depending on antigen stimulation (Fig. 1). In addition, residual innate immunity including complement, natural killer (NK) cells, macrophages and granulocytes remains somewhat intact in SCID mice, which may limit the grafting efficiency of xenogeneic cells and tissues (Table 1) [30, 31]. Many efforts have been made to develop modified SCID mice with more deficient innate immunity such as severely reduced NK cell function and phagocytosis by genetic crossings with inbred or other mutant strains of mice [32, 33].

NOD/SCID mice are created by backcrossing the SCID mutation into the NOD/LtSz mice, which have less residual immunity than SCID mice, since NOD mice have defects in the complement
pathway, macrophage function and NK cell activity [34]. NOD/Shi-SCID mice also have seriously immune functional impairments similar to NOD/LtSz-SCID mice [35, 36]. The innate immune defects in NOD/LtSz-SCID or NOD/Shi-SCID mice might well account for increased human xenograft survival in vivo [37–40]. CD47 can efficiently protect target cells from phagocytosis by macrophages via reacting with signal regulatory protein α (SIRPα) expressed on effector macrophages [41, 42]. However, in widely disparate xenogeneic combinations, haematopoietic cells can be vigorously engulfed by xenogeneic macrophages due to inefficient interaction between CD47 on the xenogeneic target cells and SIRPα on macrophages [41, 43]. It has been reported that human CD47 cross reacts with SIRPα on xenogeneic macrophages [43]. By crossing NOD/SCID/γcnull mice with a NOD genetic background may, at least partially, be due to the functional matching of CD47/SIRPα phagocytosis-inhibiting pathway between human beings and mice in this combination. Furthermore, the NOD/SCIDβ2Mnull mice, which severely lack NK cell activity, are developed and available [4, 45]. NOD/SCIDβ2Mnull mice have higher engraftment of human immune cells compared with other immunodeficient mice such as SCID or NOD/SCID mice after a single i.p. transfer of human peripheral blood lymphocytes (PBLs) (Fig. 2) [46].

γc chain is an indispensable component of receptor heterodimers for many cytokines (IL-2, IL-7, IL-9, IL-12, IL-15 and IL-21) [47]. It can dramatically increase the affinity of these receptors to their ligands respectively. The γcnull mice display a significant absence of both mature T and NK cells [47, 48]. NOD/SCID/γcnull mice were created by impairing γc chain in NOD/SCID mice, and these mice are completely defective in T, B and NK activity [5]. Moreover, CD11c+ dendritic cells (DCs) become functionally defective in NOD/SCID/γcnull mice, resulting in the reduction of IFN-γ production. Therefore, NOD/SCID/γcnull mice are currently considered as the optimal recipients of human HSCs [5] because of their complete deficiency in adaptive immunity and more impaired innate immunity (Fig. 2).

The Rag1 or Rag2 are responsible for the initiation of TCR and immunoglobulin genes’ VDJ rearrangement through the generation of a DNA double strand break, therefore homozygous mutants in mice result in the inability to produce mature T and B cells, leading to a SCID-like phenotype [6–8]. These mice do not become leaky with age due to their complete inability to initiate VDJ recombination and display a longer life span than NOD/LtSz-SCID mice. This is due to a delay or prevention of thymic lymphomas associated with the DNA repair defect (Fig. 1) [49]. However, they could not support efficient human engraftment because of the functional innate immunity (Fig. 2) [46]. NOD mice with targeted mutations of both Rag1 and perforin genes were developed, named NOD/LtSz-Rag1nullPfpnull mice, which lacked mature T, B and NK cells (Table 1). These mice supported a 4- to 5-fold increase in percentages and total numbers of human lymphocytes in the periphery compared with NOD/LtSz-Rag1null mice [9]. By crossing γc and Rag2 mutant mice, Rag2nullγcnull mice were developed producing no T, B and NK cells, which provided excellent immunodeficient setting for human engraftment similar to NOD/SCID/γcnull mouse recipients (Fig. 2) [10].

Thus, the frequency of leakiness in immunodeficient mice is as follows (in descending order): NOD/SCID/γcnull mice, Rag2nullγcnull mice, NOD/LtSz-Rag1nullPfpnull mice, NOD/LtSz-Rag1null mice< Rag2nullγcnull mice< NOD/SCID mice< SCID mice (Fig. 1). Until now, the favoured mouse recipients for grafting human haematopoietic or immune cells were generally as follows: NOD/SCID/γcnull mice, Rag2nullγcnull mice or NOD/LtSz-Rag1nullPfpnull mice > NOD/SCID mice or NOD/LtSz-Rag1null mice> SCID mice (Fig. 2).

**Fig. 1** The frequencies of cell leakiness in various immunodeficient mouse strains with aging.

**Pre-conditioning regimens for establishing humanized mice**

**Depletion of innate immune cells in recipient mice**

The injected xenogeneic human HSCs or mature immune cells could be rejected in so-called immunodeficient recipient mice by innate immune elements including mouse neutrophils, granulocytes, macrophages and NK cells. So, the residual innate immunity of SCID or Ragnull mice became the main factor limiting the extent of human immune cell reconstitution. The strategies to deplete NK cells and certain subpopulations of monocytes and macrophages in recipient mice could improve human HSCs or immune cell
engraftment, especially when limited cell numbers of human HSCs were transplanted [12, 35, 36, 50–52]. Anti-CD122, anti-asialo GM-1 or anti-IL-2R/H9252 chain antibody was used to deplete NK cells, and liposome-encapsulated dichloromethylene-bisphosphonate to deplete macrophages [11]. The neutrophil and granulocyte-depleting mAbs were found to profoundly deplete mouse neutrophils [53] and granulocytes [54], which may control natural immune response of SCID or Ragnull mice against human grafts and be associated with a significant increases in human engraftment (Table 2).

Implication of human growth factors

Certain growth factors, which play critical roles in haematopoiesis and peripheral immune homeostasis, may be species specific or may have mismatched physiological function in xenogeneic settings. The low engrafting efficiency of human HSCs and immune cells in xenogeneic immunodeficient recipient mice may be somewhat improved by offering human growth factors.

Tumour necrosis factor-α (TNF-α), produced by a variety of cell types including thymic stromal cells, was found to be associated with critical events leading to T lineage commitment and differentiation. In mice, TNF-α-induced expression of CD25 on early immature thymocytes was required for thymocyte maturation to single-positive CD4 and CD8 cells [55]. Administration of human TNF-α to irradiated NOD/SCID mice before transplantation of mobilized human PBLs led to rapid augmentation of human T lymphopoiesis, including the emergence of immature human CD4/CD8 double-positive (DP) T cells and mature CD4+ and CD8+ T cells [56].

Because T cell development, proliferation and survival are critically dependent on epithelially derived IL-7 [57], administration of human Fc-IL7 fusion protein greatly enhances the presence of human T cells in spleens and peripheral blood of NOD/SCIDγcnull mice transplanted with human HSCs [58]. IL-15 is shown to have haematopoiesis-promoting effects, including the proliferation of T cells, maturation of B cells and development of NK cells [59–61]. rhIL-15 could significantly improve the engraftment and reconstitution of human T cells in NOD/SCID mice after transfer of human PBLs [62]. Also, administration of IL-12 or IL-18 enhanced the engraftment of human CD4+ and CD8+ T cells in mice, whereas their co-administration prevented grafting due to IFN-γ-dependent apoptosis and regulation of class-switching IgA [63]. In SCID mice grafted with human PBLs or bone marrow cells (BM), injection of recombinant human growth hormone (rhGH) or recombinant human prolactin (rhPRL) strongly promoted human T cell engraftment in the thymus and spleen, as well as enhanced IgG/M levels in sera [64, 65].

Making ‘space’ for donor cells by irradiation or chemical agents

Although the residual immunity of NOD/SCID mice could be depleted by additional pre-treatment with antibodies against NK cells or macrophages, a preparative regimen involving sublethal irradiation or chemical reagents may be required to make ‘space’ for grafting xenogeneic human HSCs or immune cells. These pre-treatments could result in depletion of mouse HSCs, increase concentrations of growth factors and chemoattractants and reserve a certain amount of space for the development and repopulation of human HSCs and immune cells in recipient mice [66]. Generally, human HSC transplantation in NOD/SCID mice requires 2–3 Gy pre-irradiation, and human immune cells could repopulate well in irradiated recipients [9, 67, 68]. Some immunosuppressive and alkylating agents have shown similar effects to irradiation, for example, a single dose (35 mg/kg) of Busilvex enabled equivalent engraftment of human cells compared to 3.5 Gy irradiation [66]. It was recently reported that the treatment with anti-c-kit monoclonal antibody (clone ACK2) could transiently remove a majority of recipient HSCs and make niches where HSCs could home to. Subsequent transplantation of donor HSCs could lead to 90% donor chimerism, and this may become an alternative conditioning regimen for engrafting xenogeneic HSCs (Table 2) [69].

Transplantation strategies to establish humanized mice

Certain levels of reconstituted human immune systems have been achieved in immunodeficient mice by different transplantation strategies including implantation of mature human immune cells, HSCs or a combination of human thymus and HSCs.
Humanized mice achieved by directly implanting mature human immune cells

As early as the 1980s, it was found that peritoneal transplantation of human PBLs into SCID mice resulted in the establishment of a partially functional human immune system in mice, in which mature human T and B cells were found to be present in peritoneal cavity, peripheral blood, spleens or lymph nodes. In addition, some human macrophages were found in lymphoid organs [29]. T and B cells were functionally demonstrated by the in vitro T cell proliferative response and the in vivo antigen-specific antibody production when the transplanted mice were immunized with tetanus toxoid in the early stage after transplantation. However, anti-mouse reactive clones were subsequently selected and the ‘so-called’ established human immune system became non-functional in the late stage. The intrasplenic or intravenous injection of human PBLs into SCID mice caused mature activated T cells to populate the primary lymphoid tissues [70] and a transient and massive expansion of human B cells in the spleen, which permitted the isolation of human antibodies specific for target antigens [71, 72]. The high rate of reconstitution could be achieved after transfer of human PBLs into SCID, NOD/SCID and NOD/SCIDβ2Mnull mice [46]. Furthermore, the engraftment of human immune cells was significantly improved by the treatment with antibodies toward recipient mouse NK cells, macrophages or granulocytes [33, 54, 73]. Compared with SCID mice, Rag2null mice transplanted intraperitoneally with human PBLs showed more limited human engraftment and lower levels of human immunoglobulins [74]. Although there was less leakiness, the intact macrophage function in these mice were likely too high to overcome for transferred human PBLs to migrate beyond the peritoneal cavity [75]. Additional conditioning with anti-asialo GM1 serum and 600 cGy irradiation could increase the efficiency of human engraftment in Rag2null mice [76].

On the other hand, to provide the appropriate support microenvironment for the development of human immune responses, human lymph nodes, in which all the necessary cellular components, including T cells, B cells and antigen presenting cells (APCs), were transplanted into SCID mice. In these mice, human T cells supported human immunodeficiency virus (HIV) replication [77, 78] and human B cells differentiated to plasma cells producing human IgM or IgG [1]. But interaction between human T cells and mouse xenoantigens within the mouse environments caused activation phenotypes of human T cells and graft-versus-host disease (GVHD) in this model.

One of limitations of this mouse model was the low levels of human PBL engraftment [29, 70]. Analysis of antigen-specific cellular immune responses was extremely difficult. Various strategies were explored to improve the efficiency of human PBL engraftment in SCID mice, including an increasing number of transferred cells [29, 79], pre-treatment of recipient mice with low-dose irradiation [32], elimination of recipient mouse NK cells by anti-asialo-GM1 antibodies [80] or a combination of these [81]. All these protocols showed only marginal effects if both the function and distribution of transferred human PBLs in lymph organs of these models were considered.

Another weakness of this model was the weak primary immune response [82–84]. However, it induced substantial immune responses against recipient mouse xenoantigens, which ‘skew’ the human lymphoid repertoire [85]. This xeno-response mediated by human T and B cells not only caused potentially fatal GVHD [79, 84, 86], but also severely limited the ability of human PBLs to respond to exogenous antigens [70, 87]. Thus, the application of this approach in biomedical research is currently somewhat limited.

| Table 2 | The potential strategies to improve humanized mouse model |
|----------------|---------------------------------|
| **Strategies** | **Targets/purposes** | **Refs** |
| Recipient mouse selection | NOD/SCID/γcnull mice or Rag2nullγcnull mice | Deficient in T, B and NK cells | [5, 10] |
| | Anti-CD122, anti-asialo GM-1 or TM-β1 antibody | Depletion of NK cells | [12, 35, 36, 50–52] |
| | Liposome-encapsulated dichloromethylene-bisphosphonate | Depletion of macrophages | [11] |
| Pre-treatments of recipient mice | Specific antibody | Depletion of neutrophils | [106, 125] |
| | Specific antibody | Depletion of granulocytes | [107, 176] |
| | Irradiation | Making space | [66] |
| | TNFα, IL-15, IL-18, rhGH, rhPRL | Growth/Stimulating factors | [56, 58, 62, 65, 177] |
| Transplantation strategies | Co-transplantation of human foetal thymus/liver tissues and CD34+ HSCs | Enhance the resource for human HSCs and immune cells | [13] |
Humanized mice established by engrafting human HSCs

Human bone marrow grafts in SCID mice were histologically comparable to normal foetal bone marrow when fragments of foetal human femur and tibia were implanted into SCID mice [88]. In this model, the human cell populations presenting in the circulation, consisted mostly of B cells and a few of cells belonging to the monocytic-granulocytic lineages. Human IgM, IgG and occasionally IgE were detected in the sera, but no mature human T cells could be observed in the bones or the circulation of these mice [88, 89].

The multi-lineage reconstitution of functional T, B and NK cells was achieved in irradiated NOD/SCID/γcnull mice after transplantation of human CD34+ HSCs without co-transplantation of human immune tissues [90–92]. Human T cells developing in the thymus of these mice migrated into peripheral lymphoid organs. These T cells expressed polyclonal TCR-β and responded not only to mitogenic stimuli, such as PHA and IL-2, but also to allogeneic human cells. These results indicated that functional human T cells could be reconstituted in NOD/SCID/γcnull mice by grafting human CD34+ cells [93].

When human cord blood CD34+ cells were injected into NOD/SCID foetuses, human immune cells could not efficiently self-renew and differentiate in the foetal mouse environment [94]. However, when foetal human liver mononuclear cells or foetal bone marrow-derived CD34+ cells were injected into NOD/SCID foetuses, multilineages of human haematopoietic cells, including B cells, myelomonocytic cells and haematopoietic progenitor cells, but no human T cells, were detected in the chimaeric mice. Although multilineage engraftment occurred in these foetal recipients, both the frequency and the levels of engraftment were lower than those previously reported when human immune cells were transplanted into adult NOD/SCID recipients [95].

Like NOD/SCID/γcnull mice, Rag2null/γcnull mice engrafted with human cord blood CD34+ cells led to multilineage of human haematopoiesis with the production of T cells, B cells and DCs [96]. Primary and secondary lymphoid organs (thymus, bone marrow, spleen and lymph nodes) were reconstituted in these mice with human immune cells. Human immune responses were demonstrated when these mice were immunized with tetanus toxoid or infected with Epstein–Barr virus (EBV) [96]. Low levels of antigen-specific human IgG productions upon immunization indicate that an inefficient class switching is present in this model [90, 92]. In addition, the levels of human T cells in these mice are much lower compared to humanized mice that receive human foetal thymus tissue, suggesting that poor efficacy of human thymopoiesis in xenogeneic mouse thymus may exist [90, 92].

Humanized mice achieved by engrafting human thymus and HSCs

Another important approach to establish a humanized mouse model is transplanting foetal human thymus and liver tissue beneath the kidney capsules of SCID mice, which results in the development of a well vascularized human thymus-like organ [97]. The human HSCs from foetal human liver tissue grafts were observed to migrate into the thymic grafts [1, 97–100]. Human T cells in the grafts were similar to those of normal human foetal thymus, with a hierarchal distribution of CD4+CD8+ cortical thymocytes and CD4 or CD8 single positive (SP) medullary thymocytes. The SP T cells expressed a diverse Vβ repertoire that was indistinguishable from those of the foetal thymic donors prior to implantation [101]. Despite the large numbers of human T cells present in the thymus, the levels of peripheral circulating human T cells were relatively low in this model. These human T cells were responsive to mitogenic stimuli and alloantigens, and produced the types and amounts of cytokines similar to compartment of human beings [100, 102]. However, no detectable mature human B cells were generated in these mice [89, 97]. Increasing the quantity of implanted foetal human thymus and liver tissues somehow enhanced the percentages of human CD4+ and CD8+ T cells in the peripheral blood, spleen and lymph nodes [103]. Simultaneous transplantation of foetal human thymus, liver haematopoietic cells and lymph nodes resulted in the improved differentiation of mature human T and B cells, which were tolerant to recipient mouse antigens [1].

Implantation of foetal human thymus together with foetal bone chips under the SCID mouse kidney capsule supported the proliferation and differentiation of human HSCs into mature myeloid and lymphoid cells including human T cells, B cells and macrophages [104]. These structures of bone and thymus were indistinguishable from age-matched human counterparts. These mice produced human IgM and IgG at levels that were consistently higher than those observed in SCID mice grafted with human thymus and liver tissues from the same donor [89]. Furthermore, all four human IgG subclasses, IgA and IgE were detectable [104]. These results showed that human antibody production and iso-type switching occurred in these mice, indicating that human APCs, T and B cells interacted physiologically in response to environmental antigens.

Importantly, co-transplantation of human foetal thymus/liver tissues and CD34+ HSCs led to the efficient establishment of human haematopoiesis and functional immune systems in NOD/SCID mice, which showed repopulation with multilineage of human haematopoietic cells, including T cells, B cells and DCs in secondary lymphoid tissues [13]. These mice produced high levels of human IgM and IgG antibodies and mediated strong immune responses in vivo as demonstrated by skin xenograft rejection [13]. These humanized mice provided a more powerful model to study human immune function in vivo.

A proposed ideal humanized mouse model

The optimal experimental humanized mouse model, in which all human immune cell lineages can develop and home to specific sites to perform optimal immune response, should be explored.
Firstly, the ideal recipient mice should be deficient of both adaptive and innate immunity. NOD/SCID/γcnull or Rag2null γcnull mice are the best choice at present, because they have no T and B cells, no NK activity and impaired DC function [5, 10]. However, the innate immune in these mice were only partially deficient. Therefore, additional treatments including reagents of depleting neutrophils, granulocytes and macrophages need to be pursued [11, 47, 105–107]. In addition, the more genetically immunodeficient mice with more space to accept xenogeneic human cells and optimal microenvironment for the development, survival and homing of human HSCs and immune cells should be identified. Furthermore, in order to observe the ‘pure’ human immune response, the recipient mice should express no mouse major histocompatibility complex (MHC) or express certain human leucocyte antigens (HLA). On the other hand, if the humanized immunity was achieved by grafting foetal human thymus and HSCs, the recipient mice should be athymic to avoid the mixture of human T cells developing in human and mouse thymi.

Secondly, the strategies for implanting human immune cells or their progenitor cells should be considered. Directly transplanting high doses of mature human T cells and other immune cells may not be an ideal choice, due to the high incidence or possibility of GVHD caused by human anti-mouse xenogeneic immune response. Alternatively, implanting foetal human thymus tissue and HSCs in immunodeficient mice to reconstitute human immunity may be an optimal approach. The co-transplantation strategy of foetal human thymus/liver tissues and CD34+ HSCs could sustain development of multilineage of human immune cells, including T cells, B cells and DCs, which provided a more powerful adaptive and innate immunity [13].

Therefore, a combination of modified recipients (immunodeficiency, athymic, available ‘space’, and proper microenvironments including growth factors and homing molecules) and transplantation strategies (human immune tissues and stem cells) would create an optimal humanized mouse model with an approximation of a complete human immune system (Table 2).

Applications of humanized mouse models in biomedical research

Humanized mice were widely employed in research on self-renewal and multilineage differentiation capacity of human HSCs as well as human immunity to virus infection, tumour and transplantation.

Ontogeny of human HSCs and immunocell lineages

Human umbilical cord blood (UCB), bone marrow and mobilized peripheral blood (mPB) were used as sources of human HSCs for transplantation. Transplantation of CD34+ cells from human UCB resulted in higher engraftment levels in NOD/SCID mice as compared to CD34+ cells from bone marrow or mPB [108]. Human CD34+ population could be segregated into two subpopulations with distinct characteristics (CD34+CD38+ and CD34+CD38−). CD34+CD38− cells repopulated early, whereas in CD34+CD38+ cells repopulation was more robust at late stages and represented a more primitive population and a higher frequency of T cell precursors [109]. The population of human CD34+Lin Thy-1+ progenitor cells could repopulate the thymic grafts. Further, human CD34+ cells or CD34+Lin Thy-1+CD10 populations gave rise to T, B, NK and DC populations in SCID mice [110]. When human CD34+ cells were transplanted into NOD/SCID/IL2Rγnull mice, multilineages of immune cells were reconstituted, including human T cells, B cells, monocytes, macrophages, and DCs [58, 91, 111, 112].

The grafts of human thymus in SCID mice, identical to physiological human thymus, efficiently supported human T cell development. Thymocytes of human thymus grafts in mice had a phenotype comparable to those of freshly isolated foetal human thymocytes, and they comprised all the T cell subsets, with normal ratios of double negative, DP and CD4 and CD8 SP thymocytes [1, 97]. The Vβ repertoire in thymocytes from grafts was comparable to those of the foetal thymus before transplantation, demonstrating generation of human T cell diversity in human thymus grafts [101]. Human T cells maturing in human thymus grafts responded to toxic shock syndrome toxin1 superantigens, EBV infection and mitogens, indicating they were functional [111, 113]. Interestingly, human thymic regulatory T cells (Treg cells) might also be generated via thymic development demonstrated by the expression of CD25 and Foxp3 [96]. Our recent studies also showed human CD4+CD25+Foxp3+ T cells could efficiently develop in human thymus grafts and repopulate in peripheral blood and lymphoid tissues in NOD/SCID mice (BJ Zhang and Y Zhao, unpublished data).

Human B cells underwent normal class switching and matured to produce IgM, IgG and IgA in various immunodeficient mice grafted with human CD34+ cells [13, 90, 112]. These human antigen-specific immunoglobulins were produced after immunization with a T cell-independent antigen [90–92, 112]. Human DCs in humanized mice were developmentally, phenotypically, and functionally similar to the DC subsets found in human beings [114]. Human NK cells mainly repopulated in BM and spleen, and showed cytotoxicity against K562 cells in this model [91].

Autoimmune diseases

Human PBLs obtained from patients with organ-specific and multi-system autoimmune diseases survived in SCID mice for several months and produced IgG and autoantibodies with the same specificities found in the donor [115]. Therefore, this provides a possible way to investigate the pathogenesis and effector phases of human autoimmune diseases in in vivo models.
Pemphigus vulgaris (PV) is a severe autoimmune skin disorder induced by antibodies against desmoglein (Dsg) 3 on epidermal keratinocytes. After transferring PBLs from patients with PV into SCID mice, circulating anti-pemphigus antibodies were found in these mice, and human IgG deposited in their traumatized skin. Allogeneic human skin grafted to SCID mice before reconstitution with patients’ PBLs developed PV-like lesions containing human IgG deposits [116]. These results indicate that humanized mice may serve as models of antibody-mediated human autoimmune skin diseases.

**Virus infections**

Some viruses can only infect human beings, thus, the humanized models were used to study the interactions between viruses and the human immune system, and to evaluate the efficacy of vaccines and therapeutics for human viruses such as HIV, severe acute respiratory syndrome (SARS), porcine endogenous retrovirus (PERVs), varicella-zoster virus (VZV), Dengue virus and others.

HIV infections are largely restricted to *in vitro* or clinical studies because of species tropism, and humanized mice have been extensively used to study HIV pathogenesis and therapy *in vivo* [117, 118]. In SCID mice grafted with human PBLs, HIV infections were limited to a short period because CD4+ T cells were rapidly depleted and lacked replenishment sources [119]. After intrasplenic transfer of PBLs from untreated HIV-infected patients into NOD/SCID mice, a strong HIV-specific antibody response was observed [120]. This, therefore, is feasible for the study of HIV-specific human humoral immune responses and for the isolation of human mAbs against HIV. In humanized mice grafted with human thymus and liver tissues, HIV infection showed preferential tropism for a population of intrathymic CD3+ CD4-CD8- T progenitor cells [121], while other thymocytes were normally generated [122, 123]. After transplantation of human CD34+ C8 cells, the long-lasting viremia after infection with both CCR5- and CXCR4-tropic HIV-1 isolates was detected in spleen, bone marrow or thymi of NOD/SCID/IL-2γc-/- mice. Also, this high rate of viral infection was accompanied by production of anti-HIV-1 Env gp120 and Gag p24 antibodies [90]. In humanized Rag2-/-γc-/- mice constructed with cord blood CD34+ cells, CXCR4 and CCR5 expression on human CD4+, T cells in lymphoid organs closely resembled HIV coreceptor expression in human beings [124, 125]. CXCR4-tropic HIV virus infected all lymphoid organs, while CCR5-tropic HIV virus infection was largely restricted to extrathymic tissues. Both viral strains led to long-term lymphoid organ disseminated infection closely resembling HIV infection in human beings.

Humanized mouse models have been developed for *in vivo* evaluation of antiviral compounds against HIV including azidothymidine [126], dideoxynosine, dideoxycytosine, nevirapine, protease inhibitors [127], non-immunosuppressive cyclosporine derivatives, peptide inhibitors of env-CD4 interactions and ‘zinc-finger’ inhibitors [128]. Humanized mice constructed by human thymus, liver tissues and lymph nodes were permissive for HIV replication [78] and antiviral compounds such as azidothymidine would partially suppress replication in a time- and dose-dependent manner [126]. Exposure to cocaine can enhance HIV infection *in vivo* by activating sigma-1 receptors and modulating the expression of HIV coreceptors. A selective sigma-1 antagonist, BD1047, blocked the effects of cocaine on HIV replication in SCID mice grafted with human PBLs [129]. In addition, humanized mouse models have also been proven useful in evaluating gene therapeutic approaches. Transferring human CD4+ T cells containing the IFN-β retroviral vector drastically reduced the pre-existing HIV infection and enhanced CD4+ T cells survival as well as Th1 cytokine expression [130].

SARS, a kind of corona virus, caused a deadly epidemic in Asia [131]. The humanized mice constructed by human PBLs were used to study novel vaccine candidates against SARS CoV. SARS DNA vaccines induced human cytotoxic T lymphocytes specific for SARS antigens and human neutralizing antibodies against SARS CoV [132]. It was demonstrated that Angiotensin-converting enzyme 2 was a functional receptor for the SARS CoV [133].

VZV appeared to cause viremia by infecting human lymphocytes [134]. Humanized mouse models were used to examine VZV pathogenesis and immunobiology *in vivo* [135–137]. These experiments provided evidence in support of a critical role for T cell tropism in VZV pathogenesis. Eliminating ORF47 kinase activity impaired virus production and envelopment, which would block VZV infectivity for T cells [134].

Dengue virus is an important mosquito-borne flavivirus and it infects human beings causing a range of illnesses from subclinical infection to acute dengue fever to the severe dengue haemorrhagic fever/dengue shock syndrome [138]. A major limitation to the understanding of dengue virus pathogenesis and immunity has been the lack of an ideal humanized animal model. Irradiated NOD/SCID or Rag2nullγcnull mice grafted with human cord blood haematopoietic progenitor cells or foetal liver-derived CD34+ cells achieved replication of dengue viral infection in a physiological setting. These models were found to be susceptible to dengue virus infection, showed signs typical of fever and thrombocytopenia, and were used to evaluate dengue virus pathogenesis [138, 139]. Also, humanized mice were used to investigate pathogenesis and therapeutics of EBV [140], cytomegalovirus (CMV) [135] and influenza infections [141].

Pigs may be suitable donors for human xenotransplantation. The potential transmission of PERVs to human cells was investigated in humanized mice, and some tissues were positive for PERVs’ DNA [142, 143]. However, the infection was non-productive as PERV transcripts were not detectable in those tissues [144], and might be due to microchimerism or pseudotyping with murine viruses [145]. Importantly, it was shown that human cells and porcine cells could co-exist for many weeks without any evidence for infection of human cells by PERVs in NOD/SCID mice transgenic for certain porcine cytokines and transplanted by porcine BMCs, human foetal thymus and liver tissues [146]. However, it remains to be seen whether immunosuppressive therapy would facilitate retroviral infection of human cells.
Immune response or tolerance to allografts or xenografts

Transplantation of allogeneic HLA-mismatched foetal pancreases in SCID mice grafted with human thymus and liver tissues or PBLs resulted in infiltration of human mononuclear cells in the pancreas and subsequent rejection [89, 147]. Human T cells were responsible for the rejection of skin transplants from allogeneic donors in SCID mice [148, 149]. Like skin grafts performed among human beings, the dermal microvessels were destroyed and the skin became necrotic [150].

SCID mice grafted with human PBLs were established models for delayed-type hypersensitivity (DTH) [151]. Human skin was grafted onto the backs and autologous human immune cells were injected into the peritoneal cavity of SCID mice. Injection of tetanus toxoid into the human skin grafts not adjacent mouse skin caused perivascular human T cell infiltrate, which indicates that human T cells specifically recognize human but not mouse skin as homing sites, and that human T cell responses depend on the human microenvironments. Recently, it was found that adaptive CD4+CD25+Treg cells isolated from kidney transplant recipients who are tolerant to the donor, mediated the suppression of donor-specific DTH in humanized SCID mice [152].

SCID mice transplanted with human foetal liver from donor A and foetal thymus from donor B developed a mixed chimeric human thymus [153]. In this model, human T cells reactive to donor A were clonally deleted by selection in the thymus, while T cells potentially responding to donor B were rendered anergic upon interaction with the allogeneic thymic epithelial cells of donor B [154].

GVHD remains a major cause of morbidity and mortality after allogeneic HSC transplantation. Sublethal irradiated immunodeficient mice reconstituted with human T cells would be a valuable tool for investigating the development and pathophysiology of the disease. NOD/SCID/β2M−/− mice conditioned with 300 cGy total body irradiation developed lethal GVHD after injection of human T cells through the retro-orbital venous plexus, in which high numbers of activated human CD4+ and CD8+ T cells expressing CD25 and CD69 were demonstrated in spleen, liver, lung, kidney and bone marrow of the recipients. These T cells secreted large quantities of human IFN-γ, and specific antibodies (IgM and IgG) to the dengue virus were detected in the majority of the mice that showed viremia [67]. This model replicated similar phases and pathogenesis of human acute GVHD in physiological settings and would contribute to development of therapeutic strategies for GVHD. Furthermore, human CD4+CD25+ Treg cells could efficiently prevent xenogeneic GVHD in Rag2−/−,γc−/− mice injected with autologous human peripheral blood mononuclear cells (PBMCs) [155]. Depletion of CD4+CD25+ Treg cells from human PBMCs significantly exacerbated xenogeneic GVHD, whereas co-administration of this subset of Treg cells significantly inhibited the expansion of effector T cells and reduced the lethality of xenogeneic GVHD. Interestingly, the protective role was associated with a significant increase in plasma levels of IL-10 and IFN-γ, indicating the de novo development of Tr1 cells [155].

Humanized mouse models were used to study the immune response to xenogeneic porcine grafts in vivo [156–158]. Circulating human T cells in these mice did not infiltrate pig skin or artery grafts in contrast to the robust immune responses against allogeneic human cells in vitro. However, TNF-treated pig tissue permitted human T cells to infiltrate and injure both pig skin and artery grafts because the actions of this proinflammatory cytokine were not species-restricted and could activate pig endothelial cells to express cell surface molecules that were necessary to recruit a local infiltrate of leucocytes [156, 158]. Thus, by addition of TNF, the humanized mice might be used to study primary human T cell-mediated anti-porcine xenogeneic responses in vivo.

Porcine thymus grafts in SCID mice grafted with foetal porcine thymus and human liver tissues support normal development of polyclonal, functional human T cells from haematopoietic precursors provided by human foetal liver cells [159]. These human T cells were specifically tolerant to donor porcine antigens but normally responded to non-donor porcine xenantigens and alloantigens. Exogenous IL-2 did not abolish tolerance, suggesting that central clonal deletion rather than anergy was the possible tolerance mechanism [159]. Recently, our results showed that human functional CD4+CD25+Foxp3+ Treg cells could develop in xenogeneic pig thymus grafts in NOD/SCID mice (BJ Zhang and Y Zhao, unpublished data).

Chimerism was constructed by human thymus/liver tissues and porcine HSCs in SCID mice [160]. Human T cells developed in this chimerism showed specific non-responsiveness to the porcine donor because it lacked rejection of porcine haematopoietic cells and anti-donor pig MLR responses, as well as the acceptance of donor swine leucocyte antigen (SLA)-matched skin grafts, whereas human T cells in the mixed chimeric mice rejected the third party porcine skin grafts and responded to the third party pig and allogeneic human antigens in MLR assays [160]. The capacity of haematopoietic chimerism to induce T cell tolerance to donor cells largely resulted from intrathymic clonal deletion of maturing donor-reactive thymocytes [161, 162].

Anti-tumour immune response

Malignant tumours can unlimitedly grow, escaping from human immune surveillance. Athymic nude mice, SCID or Rag−/− mice could be successfully engrafted with xenogeneic human tumours including a wide variety of solid human tumours and haematological neoplasms [163, 164], in which human tumour biology, growth, angiogenesis and metastasis have been evaluated.

The ability to engraft human tumours and human immunocompetent cells successfully in immunodeficient mice has spawned the development and use of humanized mouse models to evaluate anti-tumour therapies, because the study of tumour biology in human beings was impeded by access to tissues and to the site of tumour growth as well as ethical concerns. Indeed, an ideal model was humanized mice with a complete human immune system, which will permit evaluating the mechanism of
tumour immunobiology in the context of an intact human immune microenvironment. Humanized mice can be used for the evaluation of therapeutic approaches for the inhibition of human tumour growth, including the use of angiogenesis inhibitors [165], cell-based therapies [166], humanized antibodies [167], traditional immunosuppressive and immunotherapeutic protocols [168] and tumour-growth inhibitors [169–171].

Humanized mice provide an opportunity to evaluate human cytokines and chemokines that augment both innate and adaptive anti-tumour immune responses of human leucocytes, thus providing a model that is more clinically relevant. This model has been utilized to test the anti-tumour efficacy of a wide spectrum of human cytokines [172–174] and cytokine-delivery strategies including the direct injection of soluble cytokines [175], cytokine gene transfer [173, 174] and the use of slow-release polymer particles [175].

Conclusions and perspectives

Humanized mouse models have made tremendous progress in modification of recipient mice and transplantation strategies in recent years. These models are becoming powerful and versatile tools to investigate human haematolymphoid differentiation, autoimmunity, virus infections, transplantation rejection and tolerance induction. However, much effort should be directed in minimizing innate immunity mediated by NK cells and macrophages in SCID or Rag<sup>null</sup> mouse recipients. Some incompatibility of cytokines and adhesive molecules between mice and human beings could descend the human immune cell levels in immunodeficient recipients. Immunodeficient mice with some important human growth factors will be ideal recipients to establish humanized mice. Optimized humanized mouse models will offer a powerful approach for us to study human immunity in vivo, which will significantly advance biomedical research.

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