Chapter 10

Application of Humanized Mice in Immunological Research

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Abstract

During the past decade, the development of humanized mouse models and their general applications in biomedical research greatly accelerated the translation of outcomes obtained from basic research into potential diagnostic and therapeutic strategies in clinic. In this chapter, we firstly present an overview on the history and current progress of diverse humanized mouse models and then focus on those equipped with reconstituted human immune system. The update advancement in the establishment of humanized immune system mice and their applications in the studies of the development of human immune system and the pathogenesis of multiple human immune-related diseases are intensively reviewed here, while the shortcoming and perspective of these potent tools are discussed as well. As a valuable bridge across the gap between bench work and clinical trial, progressive humanized mouse models will undoubtedly continue to play an indispensable role in the wide area of biomedical research.

Key words Humanized mice, Immunology, Immune regulation

1 Introduction

During the past century, the application of rodent animal models, especially diverse gene-engineered mouse models, provided indispensable platforms and numerous valuable information for the advances in experimental medicine and biological research. However, the gap between species is still the most challenging obstacle for translation of results from rodents to humans. With the great advancement of technology in molecular biology and gene modification, the attempt to establish "humanized" mouse models has made a leap since 1990s [1–3]. Nowadays, a wide variety of humanized mouse models have been generated and applied in nearly all fields of biomedical research [4]. In this chapter, we briefly review the history and classification of humanized mouse models and then summarize the current situation and recent advancement of their application in biomedical research, especially in the research of immune-related diseases.

1.1   History and Classification of Humanized Mice

In general, the "humanized mice" are composed of three main classes: human gene-expressed transgenic mice (*human gene-transgenic mice*), which are modified by gene knock-in or replacement technology to express one or more human specific genes; humanized mice carrying human tissue, such as the liver (*humanized liver mice*) in which murine hepatocytes are completely or partly replaced by infused human-original hepatocytes; humanized mice equipped with functional human immune system (*humanized immune system mice*), which are established on immunodeficient mice by transplanting human immune organs or cells to reconstitute human immune system in mice and thus referred to special "humanized mice." In the following section, we briefly review the history and current advance of human gene-transgenic mice and humanized liver mice, and then focus on humanized immune system mice.

1.1.1   Human Gene-Transgenic Mice

Human gene-transgenic mice are closer to gene engineered mice rather than "humanized mice." Although the expressions of human gene or protein in transgenic mice provides the platform for studying in vivo role of specific human gene or molecule, the value of these data is limited in translational medicine due to the lack of human microenvironment and signal networks in these mice.

The most widely used human gene-transgenic mice are human leukocyte antigen (HLA)-transgenic mice [5]. These HLA-expressed transgenic mice represent for a useful tool in studying in vivo TCR-restricted immune responses and thus were adopted in the studies of immune-related diseases during the first 10 years of this century. For example, HLA-A0201-transgenic mice were used in inducing CD8+ T cell-restricted type I diabetes (T1D) [6] and experimental autoimmune encephalitis (EAE) [7], while HLA-DRB1-transgenic mice were applied in establishing CD4+ T cell-mediated EAE [8], system lupus erythematosus (SLE) [9] and rheumatoid arthritis (RA) models [10]. More recently, the respective role of HLA-DR2 and HLA-DQ8 in EAE [11] and autoimmune diabetes [12] was also studied through transgenic mice. Meanwhile, transgenic mice with distinct HLA subtype expression favor the study of HLA-related susceptibility on specific diseases, such as EAE [13], experimental autoimmune uveitis [14], arthritis [15–17], allergic bronchopulmonary aspergillosis-like pulmonary responses [18], and celiac disease [19]. Although the application of HLA-transgenic mice has been reduced due to the simplification of diseases into specialized immune responses, the combination of HLA-transgenic technology and reconstitution of human immune system in immunodeficient mice has re-assigned them vitality in biomedical research, which will be discussed in the next section.

Other transgenic mice used in immune-related studies included humanized α1KI mice [20], humanized θ-defensins mice [21], humanized toll-like receptor (TLR) 4/MD2 mice [22],

humanized type I interferon (IFN) mice [23], and humanized tumor necrosis factor (TNF) mice [24]. Similar to HLA-transgenic mice, their combination with humanized-immune system in mice will certainly strengthen their translational capability in the future.

1.1.2 Humanized Liver Mice

Humanized liver mice were established as early as 2001 and mainly applied in the study of drug metabolism, excretion, toxicity [25, 26], and the in vivo activity of enzymes such as human cytochrome P450 [27]. Moreover, the establishment of chimeric mice with humanized liver accelerated the progress of the studies in hepatitis virus B [28, 29], C [30, 31], D [32], and human cytomegalovirus infection [33], which had all been blocked by the lack of optimal animal models in "pre-humanized mice time." On the other side, Chen et al. tried to stabilize the function of cryopreserved human hepatocytes in immune competent mice through a novel system called "human ectopic artificial livers (HEALs)," which involved juxtacrine and paracrine signal in polymeric scaffolds. They claimed that mice transplanted with HEALs exhibited persistent normal liver function for weeks and thus provided a window for drug-related investigation [34]. However, the efficacy and value of humanized liver mice in the development of drug are still on debate due to the proposed side effects such as ongoing liver injury caused by transgenic and the influences on "normal metabolism" mediated by exogenous treatment [35, 36]. Apart from these, the potential application of humanized liver mice in immune-related research also deserves further exploration because liver also represents for a critical component of human immune system.

1.1.3 Humanized Immune System Mice

The development of humanized immune system mice could be divided into three phases corresponding to the establishment of *Prkdc*[scid] (protein kinase, DNA activated, catalytic polypeptide; severe combined immunodeficiency) mutation in CB17 mice, the development of NOD (non-obese diabetic)-SCID mice, and the generation of immunodeficient mice homozygous for mutation at IL (interleukin)-2 receptor γ chain locus [2, 37]. Each break-through mentioned previously significantly improved the engraftment of human immune cells or pluripotent stem cells and stood as milestone on the way to "real humanized mice." The engraftment of multiple human immune components in these mice surpassed conventional human-gene knock-in in breaking the limited viewpoint of studying specific molecules under isolated environment. This unique advantage of humanized immune system mice favors their general application in immune-related studies, and opens a window for researchers to observe the interaction among human immune cells in vivo. In the following content, we focus on the characteristics and application of these mouse models and simply refer them as "humanized mice" if not otherwise specified.

Currently, IL2γc$^{-/-}$ mice established on NOD/scid and recombination activating gene 2 (Rag2)$^{-/-}$ Balb/C background were most widely used strains for the reconstitution of human immune system in vivo [38–40]. Recently, by using bone marrow, liver, thymus (BLT) co-transplantation, Lavender et al. engrafted high levels of multi-lineage hematopoiesis and organized lymphoid tissues in C57BL/6-Rag2$^{-/-}$γc$^{-/-}$CD47$^{-/-}$ triple-knockout mice. These humanized mice sustained human cell and tissue engraftment as long as 29 weeks post-transplantation without the development of chronic graft-versus-host diseases (GVHD), and thus represented for a new advancement in establishment of humanized mice [41].

1.2 Reconstitution of Immune System in Humanized Mice

The reconstitution of functional immune system is the key to evaluate the successful establishment of humanized mice. The graft used for reconstituting human immune system includes stem cells [42], BLT [41], and peripheral blood cells [43] according to specific objectives. Generally, stem cell and BLT transplantation exhibit advantage in establishing stable multi-lineage hematopoietic cells but might need additional treatment for improving development of specific cell subpopulations. On the contrary, humanized mice established by peripheral blood cells provide a ready platform for studying the functions of mature immune cells but the length of window appropriate for research is still limited by chronic GVHD and ongoing reduced engraftment. To maximize the potential of humanized mouse model, some progresses have been made recently. Firstly, pretreatment or gene-engineering of pluripotent stem cell exhibited satisfactory effects on improving engraftment of immune cells [38, 42, 44]. Secondly, human growth factors, cytokines [44, 45] or signal regulatory protein alpha (SIRPa)-expressed [46] immunodeficient mice demonstrated superior engraftment for specific immune cell subpopulations as well. In the following paragraphs, we briefly review current status of the reconstitution of specific immune cell subpopulations in humanized mice.

Lymphocytes are most important components of immune system and thus draw a major attention. Although human peripheral blood mononuclear cells (PBMC) transplantation led to rapid reconstitution of human lymphocytes in humanized mice, it was found that after initial activation and induction of antibody production, human T cell lymphocytes enter an unresponsiveness status due to loss of human professional antigen-presenting cells (APC), which could be reversed by adoptive transfer of human APC [47] or activating organ-resident myeloid dendritic cells (DC) through poly(I:C) treating [48]. Meanwhile, stem cell-transplanted humanized mice displayed diversified T cell repertoire, but the gap between HLA and murine major histocompatibility complex (MHC) molecules prohibited the induction of efficient T cell-mediated primary immune responses in vivo [49, 50].

To overcome these problems, HLA-expressed immunodeficient mice were generated and their efficacy has been confirmed [51]. Another concern origins from Th1 and Th17 immunocompetence in humanized mice [52], which supports the utility of their application as surrogate model in transplantation rejection and autoimmunity but might cause some unwanted immune responses against murine tissue antigen as well.

Distinct from their T cell companion, reconstitution of functional B lymphocytes is generally poor in humanized mice and needed to improve in the future although their primary repertoire were principally unaltered by the differences between mouse and human stromal environments [53] and their ability to produce antigen-specific antibody was partly developed [54].

As described previously, the reconstitution of myeloid cells not only guarantees immune system intact, but also determines the development and function of both adaptive and innate lymphocytes [47, 48, 55]. Unfortunately, monocytes and other myeloid cells usually exhibit immature phenotype and impaired function in humanized mice [56], which could be partly rescued by human colony stimulating factor (CSF)-1 [57]. However, the improvements in their survival, differentiation and even migration and residence [58] are still urgently required. Besides leukocytes, other blood components also play important roles during immune response and regulation. Recently, Hu et al. established the full reconstitution of human platelets in humanized mice after depletion of murine macrophage [59], which represents for an interesting attempt in constructing a more "humanized" circulation in mice.

In summary, the optimization of humanized mouse model is still on the way and the advances in molecular biology, cellular biology, and system biology will definitely bring new era to the development of this useful tool.

2 Applications of Humanized Mice

The applications of humanized mice cover nearly all fields of biomedical research and here we concentrate on immune-related studies, especially those aiming at the mechanisms and translational potentials of immune regulation and suppression. We also briefly summarize the benefits brought by these potent models in tumor, infectious diseases, and vaccine studies.

2.1 Development of Immune System

2.1.1 Lymphocytes

2.1.1.1 T Cells

Benefiting from humanized mouse model established by BLT or CD34$^+$ stem cell transplantation, research on the development of human T cells made a great progress in the past 5 years. In 2011, Choi et al. induced human CD4$^+$CD8$^+$ double-positive (DP) T cells, CD4$^+$ and CD8$^+$ single-positive (SP) T cells, CD34$^+$CD38^{lo}CD1a$^-$ (thymus setting-progenitors, TSP),

CD34+CD38loCD1a− (early T lineage progenitors, ETP), and CD34+CD38+CD1a+ pre-T cells in liver of humanized mice by intrahepatic injection of CD34+ stem cells, establishing a wonderful platform for investigating human T cell development [60]. However, Joo et al. found that human T cells educated by murine MHC in mice without a human thymus differ from normal human T cells marked as higher expression of CD45RO and promyelocytic leukemia zinc figure protein (PLZF) regardless of similar development stages [61]. Correspondingly, Danner et al. generated HLA-DR4-expressed NOD-Rag1−/−γc−/− mice and demonstrated the critical role of HLA class II molecule for development of functional T cells by infusion with HLA-DR-matched human hematopoietic stem cells [62]. Meanwhile, the roles of IL-12 [63] and Notch [64] signals during the development of human CD4+ and CD8+ T cells were evaluated by human hematopoietic stem cell-transplanted mice. Moreover, using a human stem cell factor, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3-expressed NOD/scid-γc−/− mice, Billerbeck et al. found the increased accumulation of human CD4+Foxp3+ T cells in blood, spleen, bone marrow and liver. Most importantly, these CD4+Foxp3+ T cells exhibited potent suppressive capability on T cell proliferation, which made a significant contribution to study of human regulatory T cells (Treg) development in vivo [65].

2.1.1.2 B Cells

As described previously, the development of human B cells in humanized mice is relatively weak compared to T cells. In 2011, Choi et al. evaluated the efficacy of Busulfan, a chemotherapeutic agent, and claimed that it could efficiently improve the reconstitution of human specific antibody-producing B cells, T cells, macrophage, and even DC from CD34+ cord blood cells with less toxic effects [66]. On the other hand, Kim et al. found that co-transplantation of fetal bone tissue with fetal thymus could facilitate the development and reconstitution of human B cells from fetal liver-derived CD34+ cells together with T cells [67].

2.1.1.3 Other Lymphocytes

Besides adaptive lymphocytes like T and B cells, innate lymphocytes development-related factors were also illustrated in humanized mouse model. As early as in 2008, Huntington and Di Santo made a periodic review on the application of humanized mice in the research of NK cell development [68]. In 2011, Pek et al. further confirmed the crucial role of IL-15 in NK cell development in bone marrow and liver with humanized mouse model [69]. We believe that more studies in the development of other innate lymphocytes such as NKT, γδ-T cells and innate-like T cells (ILT) will be reported in the near future.

2.1.2 Myeloid Cells and Other Cells

Myeloid cells are generally regarded as more fragile and difficult to survive in "strange environment", which made it attractive and subtle to improve reconstitution of these sensitive cells. The

addition of human original cytokines such as GM-CSF and IL-4 was generally accepted as an efficient way to improve DC maturation [70]. Similarly, the effects of macrophage colony-stimulating factor (M-CSF) and Fms-related tyrosine kinase (FLT)-3 ligand on promoting the development of macrophage [71], and CD141+ and CD1c+ DC [72] have also been confirmed in humanized mouse model respectively. Moreover, the development of megakaryocytes was replicated and used as index of dengue virus-infection in humanized mouse model recently [73], which also supported the multi-lineage hematopoietic cell development in humanized mice. Finally, transplantation of human stem cells from bone marrow of patients with bone marrow failure syndrome into humanized mice provided invaluable tools for evaluating novel gene-targeted therapy before clinical trial [74].

In summary, the reconstitution of diverse human immune cell populations from their pluripotent progenitors in immunodeficient mice has become a potent platform for investigating the development of human immune system while the next question is how to create a more "humanized" environment in mice for human cells [75].

2.2 Autoimmune Diseases

The advances in the study of autoimmune diseases in humanized mice, especially those T cell-mediated diseases, are always correlated with development of HLA-transgenic technology. In 1999, Bachmaier et al. generated a CD4–CD8– double-knockout mice transgenic for human CD4 and HLA-DQ6 to specifically reconstitute the human HLA-DQ6/CD4 arm in mice and established a dilated cardiomyopathy model [76], which was one of the earliest attempt for applying humanized mouse model in the study of autoimmune diseases. Using similar strategy, Eming et al. established a RA model in a HLA-DR4/human CD4/TCR combined transgenic mice with the stimulation of a RA-related human autogenic protein HCgp-39 in 2002 [77]. However, the lack of human immune system reconstitution in these models constrained their representative for the whole map occurring during autoimmune diseases. On the other side, Shultz et al. established T1D model in NOD/scid-γc$^{-/-}$ mice by co-transplanting with human stem cell and islet cells [78, 79]. Importantly, this group pointed out the potential of HLA-transgenic immunodeficient mice in optimization of these models and provided some interesting preliminary data [78]. Soon after, inflammatory arthritis and type 2 diabetes models were established in HLA-transgenic humanized mice by David [80] and Schultz groups [81] respectively. As we mentioned previously, T cell-mediated immune responses were generally incomplete in humanized mice established on conventional immunodeficient mice, which usually led to insignificant clinical symptoms [82] and thus limited the application of these models. The involvement of HLA not only improves the efficacy of immune responses, but also provides a platform for study of the

relationship between HLA subtypes and specific diseases susceptibility. Nevertheless, the complexity and individuality of HLA phenotypes in healthy donors or patients still remain as the biggest challenge in rebuild of physiopathology process in relatively limited HLA-expressed humanized mice.

Due to relatively weak reconstitution of human B cells in humanized mice, the establishment of B cell or antibody-mediated autoimmune diseases seems to be more difficult than those T cell-mediated autoimmune diseases. Kerekov et al. rebuilt the clinical pathogenesis in humanized mice with cells transferred from SLE patients and evaluated the potential of B cell-targeted therapy with a chimeric molecule containing a monoclonal antibody against human inhibitory complement receptor type I coupled to a decapeptide that mimic DNA antigenicity [83]. In 2012, another group led by Duffield recapitulated systemic vasculitis in humanized mice by treating them with anti-proteinase-3 IgG isolated from patients [84]. With the improvement in reconstitution of multiple components of human immune system in humanized mice, it is predictable that the induction of diverse human B cell-mediated autoimmune diseases in vivo will be accessible soon.

2.3 Transplantation-Related Diseases

Application of humanized mice models in transplantation-related diseases arises as early as the birth of humanized mice but the process is so tortuous till now due to chronic exogenous rejection and ongoing decrease of immune cells [85]. In 2001, Coates established an allogeneic skin rejection model in humanized NOD/scid chimeric mice and examined the therapeutic effects of human myeloid DC transduced with an adenoviral IL-10 gene [86]. In 2006, Marcheix et al. rebuilt a human chronic vascular rejection model in humanized SCID/beige mice with human mesenteric arterial grafts [87]. In 2012, Yi et al. determined the suppressive capacity of in vitro-expanded human CD4$^+$ Treg on porcine islet xenograft rejection in humanized mouse model and found the crucial role of IL-10 in Treg-mediated protection [88]. In above three studies, investigators planted solid grafts into immunodeficient mice before reconstitution of human immune system and induced rejection by infusion of mature human cells. However, the long-term outcome of these models is still not clear.

In order to further mimic clinical situation, human CD34$^+$ stem cells were applied in establishing humanized mice. Using this strategy, three independent groups reported allogeneic islet transplantation [89], xenogeneic islet rejection [90], and xenogeneic skin rejection [91] models during 2010–2012. Unfortunately, insufficient development of immune cell populations in these humanized mice still stayed as an obstacle and even led to the failure of rejection [89]. To solve this problem, some other groups tried to develop a more "mature" human immune system in humanized mice by transplanting human peripheral blood cells.

In 2013, our group reported a novel human allogenic GVHD model established on humanized mice reconstituted with human PBMC [92]. This model reproduced typical clinical process of acute GVHD occurring during allogeneic bone marrow transplantation without apparent interruption of exogenous reactivity. Using this model, we evaluated the protective effects of human CD8+ Treg induced ex vivo by allogeneic CD40-activated B cells and found that human CD8+ Treg could inhibit GVHD and induce long-term tolerance without compromising general immunity and graft-versus-tumor (GVT) activity [92]. The potent regulatory activity of the CD8+ Treg was mainly mediated by the expression of cytotoxic lymphocyte antigen (CTLA)-4 on cell surface, while their alloantigen-specificity and the ability to induce the long-term tolerance favor their clinical application. More importantly, this strategy might reduce clinical dependence on limited HLA-match donors and largely improve the survival chance of millions of patients who are waiting for bone marrow transplantation.

Humanized mouse model undoubtedly brings new hope for transplantation research, but we also need to keep in mind that a lot of questions are still waiting to be answered on this way. As emphasized by Brehm and Shultz, keys to successful humanized mouse model included available immunodeficient mouse strains, the choice of tissue to transplant and the specific human immune cell population that can be grafted [85].

2.4   Other Inflammatory Disease

Besides autoimmune diseases and transplantation-related diseases, humanized mice models are also useful to study some other inflammatory diseases.

In 2002, Hammad et al. compared the Th2 allergic inflammation in the lung of humanized mice reconstituted with PBMC. To induce inflammatory reaction, DCs from home dust mite (HDM)-allergic patients or healthy donors were injected intratracheally and mice were then repeated exposed to aerosol of HDM. In contrast to IFN-γ secretion induced in mice receiving normal DCs, those injected with DCs from patients induced IL-4 and IL-5 production accompanied with the increase of IgE production, which represents characteristics of Th2 response [93]. In 2003, Firouzi et al. used a humanized SCID mouse model confirmed the crucial role of T cells during multiple sclerosis-associated retrovirus particle-caused brain hemorrhage [94], while Sheu et al. found that circulating IgM played the main pathogenic role in skeletal muscle ischemia-reperfusion injury based on their research on hPBL-SCID mice in 2009 [95]. In the meantime, Unsinger et al. established a sepsis model in humanized mice elevated human pro- and anti-inflammatory cytokines as well as a dramatic increase in human T and B cell apoptosis, which was generally found in patients with sepsis [96]. More recently, Vudattu et al. determined the adverse effects of anti-CTLA-4 antibody (ipilimumab) including hepatitis,

lymphadenopathy, and other inflammatory sequelae in humanized mouse model [97].

In addition to immunopathology study, humanized mouse model was also applied in studying the underlying mechanisms of injury repair. By plating retroviral vector-modified human skin on nude mice and adding human keratinocyte growth factor (KGF) to artificial wound in the skin, the re-epithelialization was significantly accelerated [98]. Although this model could not be described as "real" humanized mice because no human immune system was involved in it, this attempt initiated an innovative application of humanized mice.

Compared to satisfactory reconstitution of circulating blood cells, the successful reconstitution of mucosa immunity in humanized mice is still absent till now. Mucosa, especially respiratory and digestive tract surface, plays indispensable role in protection and immune regulation. However, the residence and exchange of immune components in the locus are still difficult to rebuild in animal models because the physiological dynamics remains largely unknown [99]. Another reason is due to their complex gnotobiotic microenvironment. To meet this requirement, Gordon's group firstly established a humanized gnotobiotic mice by transplanting fresh or frozen adult human fecal microbial communities into germ-free C57BL/6J mice and then investigated the effect of diet on human gut microbiome [100]. Similarly, Kashyap et al. determined the relationship among diet, gastrointestinal transit and gut microbiota using the same model [101], while Macrobal et al. further compared the difference between gnotobiotic humanized mice and conventional mice urine and fecal metabolomics profiles [102]. Recently, another exciting breakthrough in immune reconstitution of the gastrointestinal tract was reported by Nochi's group. They developed human gut-associated lymphoid tissue (GALT) in mouse cryptopatches and succeeded in generating functional intestinal immunity marked by human IgA secretion in a BLT-NOD/scid mice model [103]. The combination of this humanized mouse model and gnotobiotics transfer will greatly improve our understanding on intestinal physiology and immune regulation.

2.5 Other Applications

2.5.1 Tumor

The occurrence of humanized mouse model provided a perfect platform for evaluating immunotherapy against tumor. The earliest attempts of inducing antitumor immune responses in humanized mice focused on the generation of specific antibody but the outcome varied due to unstable humanization of models [104, 105]. In the new century, researchers started to pay more attention on developing complete tumorigenicity, especially metastasis process and its relationship with stromal cells, in immunocompetent humanized mice, and made some significant advances in multiple fields like human prostate cancer [106], mixed-lineage leukemia

(MLL) [107], human primary squamous cell carcinoma [108], and human T-cell leukemia virus (HTLV)-induced T cell leukemia [109]. Based on these progresses, some novel immunotherapy strategies were evaluated on humanized mouse models, such as inhibitory receptor Ig-like transcript (ILT)-3 depletion or blockade in melanoma [110] and IL-15-enhanced NK cell-mediated cytotoxicity against human breast cancer [111]. Recently, our group reported a novel application of pamidronate, a phosphoantigen generally used to treat osteoporosis, in treating Epstein-Barr virus (EBV)-induced B cell lymphoproliferative disease in humanized mouse model reconstituted with human PBMC [112]. This "new application of an old drug" was mediated by expanding and activating human Vγ9Vδ2-T cells, a small cell population of human lymphocytes, which might inspire further exploration of currently available resources. More importantly, the established of donor- and tissue-specific humanized mouse tumor models will undoubtedly play an indispensable role during the development of individual therapies in the future [113].

2.5.2 Infectious Disease

2.5.2.1 HIV

The development of humanized mice represents a milestone in the history of human immunodeficiency virus (HIV) study. The new generation of humanized mice not only improved our understanding on transmission, latency, and pathogenesis of HIV [114–119], but also provided unprecedented platform for antiviral study. Besides further exploration of efficient virus-specific neutralization antibodies [120–125] and conventional antiretroviral or antimicrobial therapies [126–128] in these models, the efficacy of vectored immunoprophylaxis [129] and CCR5-targetd treatment [130–132] in preventing HIV transmission were evaluated as well. Meanwhile, the crucial roles of HIV-specific CD8+ T cells [133, 134] and plasmacytoid DC (pDC) [135, 136] in the replication of virus and activation of immune responses, and their potentials in targeted therapy were also investigated. Other novel immunotherapy assays performed in humanized mouse model included blockade of programmed cell death (PD)-1 receptor [137, 138], engineering HIV-resistant T cells from short-hairpin RNA (shRNA)-expressing hematopoietic stem/progenitor cells [139], and inhibition of HIV replication by a chimera containing an RNA aptamer with high binding affinity to the HIV envelop protein gp120 and virus neutralization properties and a small interfering RNA (siRNA) triggering sequence-specific degradation of HIV RNAs [140]. Moreover, a preliminary study on mechanisms underlying viral controlling in HLA-B*57 elite controller or suppressor (ES) was completed in humanized BLT mice and demonstrated that elite suppressors are capable of controlling HIV-1 due to the possession of unique host factors rather than infection with defective virus in vivo [141]. Nowadays, we could even make in-depth study on the cell dynamics in HIV-infected humanized mice model

with the help of intravital microscopy [142]. Therefore, it is countable that the future molecular biology will bring more surprise to the efforts of gene therapy against HIV [143].

2.5.2.2 Other Infectious Disease

Except for application in HIV-related studies, humanized mouse model also brought span-new opportunities for other human infectious diseases [144], especially those blood-borne pathogen-caused diseases such as dengue virus infection [145–149], EBV infection [150–155], HCMV infection [156], HTLV infection [157], and malaria parasite infection [158]. On the other hand, humanized mouse models for Leishmaniasis [159], Salmonella Typhi infection [160, 161], herpesvirus infection [162, 163], Mycobacteria infection [164, 165], and group B Streptococcus (GBS) infection [166] have been established. These efforts fill in the lacks of suitable animal models for those human-specific pathogen-caused diseases and push forward the correlating investigations on development of prevention and treatment, although some technological obstacles like the replication of natural infection and transmission routes are still needed to resolved.

In 2011, our group used PBMC-transplanted humanized mouse model to evaluate a novel therapeutic strategy by targeting the host rather than the virus for treating influenza virus infection. We demonstrated that aminobisphosphonate can control influenza disease through boosting human Vγ9Vδ2-T cell immunity and this beneficial effect is active against viruses of varying subtypes and virulence [43]. Nevertheless, differences in the characteristics of molecules, tissues, and organs between human and mice might impair efficiency of pathogen infection and initiation of specific immune responses [167]. In 2005, Lassning et al. increased the susceptibility of mice on human coronavirus by crossing aminopeptidase N (APN), the receptor for human coronavirus (hCoV)-229E, and transgenic mice into signal transducer and activator of transcription (Stat)-1 null mice [168]. This work, together with HLA- and human cytokines/growth factor-transgenic technology [169], provided successful examples for future studying human infectious agents in humanized mice. In the next stage, improvement of versatility and variability of human immune system in humanized mouse model and application of gene-modified pathogens [170] will definitely enhance translational efficiency of these models.

2.5.3 Vaccine

The usage of humanized mice in the development of vaccines targeting human diseases including EBV, HIV-1, dengue virus, influenza virus, severe acute respiratory syndrome (SARS) corona virus, and carcino-embryonic antigen (CEA) has obtained outstanding achievements during the past decade, while the introduction of HLA transgenic immunodeficient mice further accelerated the advancement in this field [171–173]. With the improvement of immune cell population reconstitution, more and more novel vaccination protocol will be carried out in humanized mice.

3   Perspective

Compared to conventional mice and non-human primate model, humanized mice exhibit great advantage in translational potential, reproductive capacity and data repeatability, economical and ethical concerns. The increasing applications of diverse humanized mice models in biomedical research during the past two decades significantly improved our understanding on human physiological and pathological, especially immunological process at systemic, cellular, and molecular levels. This further accelerated the development of current translational medicine significantly. Nevertheless, there are several major caveats on their development remain to be dealt with, including complete replacement of murine MHC with diversified HLA molecules and efficient methodology to express corresponding growth factors and cytokines at specific time and organs [174]; how to prolong the maintenance of human engraftment, promote the development of myeloid cells and increase relatively weak quantity and quality of immune cells [175]; and the limited development of lymph nodes, inter-organ traffic of immune cells, and the reconstitution of red blood cells and granulocytes [176]. In another word, the most important issue is to find the convenient and cost-effective ways to construct appropriate human-like microenvironment including physical structure, intercellular contact and molecular signals transfer in humanized mice. It is foreseeable that knowledge exchange in the age of big data will bring an even more bright future to this advancing tool than ever.

Acknowledgment

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