Current humanized mouse models for studying human immunology and HIV-1 immuno-pathogenesis

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A robust animal model for “hypothesis-testing/mechanistic” research in human immunology and immuno-pathology should meet the following criteria. First, it has well-studied hemato-lymphoid organs and target cells similar to those of humans. Second, the human pathogens establish infection and lead to relevant diseases. Third, it is genetically inbred and can be manipulated via genetic, immunological and pharmacological means. Many human-tropic pathogens such as HIV-1 fail to infect murine cells due to the blocks at multiple steps of their life cycle. The mouse with a reconstituted human immune system and other human target organs is a good candidate. A number of human-mouse chimeric models with human immune cells have been developed in the past 20 years, but most with only limited success due to the selective engraftment of xenoreactive human T cells in hu-PBL-SCID mice or the lack of significant human immune responses in the SCID-hu Thy/Liv mouse. This review summarizes the current understanding of HIV-1 immuno-pathogenesis in human patients and in SIV-infected primate models. It also reviews the recent progress in the development of humanized mice models with a functional human immune system, especially the recent progress in the immunodeficient mice that carry a defective gammaC gene. NOD/SCID/gammaC−/− (NOG or NSG) or the Rag2−/−/gammaC−/− double knockout (DKO) mice, which lack NK as well as T and B cells (NTB-null mice), have been used to reconstitute a functional human immune system in central and peripheral lymphoid organs with human CD34+ HSC. These NTB-hu HSC humanized models have been used to investigate HIV-1 infection, immuno-pathogenesis and therapeutic interventions. Such models, with further improvements, will contribute to study human immunology, human-tropic pathogens as well as human stem cell biology in the tissue development and function in vivo.

Humanized mouse models, HIV-1, NOD/SCID/gammaC−/−, Rag2−/−/gammaC−/−


1 HIV-1 and AIDS

About 25 million people have died from acquired immune deficiency syndrome (AIDS) since its description in 1981. Worldwide, there are still 33 million people living with human immunodeficiency virus (HIV), the etiologic agent of AIDS. The number of new infections continues to increase, with over 2 million new cases reported in 2008 alone [1]. Infection with HIV can truly be considered a pandemic, with no country, class, race, gender, or creed spared from its devastating impact.

HIV is transmitted from person to person through exchange of bodily fluids. Two common means of transmission are sexual and intravenous drug use, while vertical transmission remains a particular concern in developing countries. Fortunately, HIV can not be transmitted through
inhalation of aerosol or through skin to skin contact. In fact, HIV is very inefficiently transmitted through sexual contact as human barriers to infection and stochastic inefficiencies provide sufficient hurdles to make transmission, on a single-event basis, surprisingly unlikely [2]. Concurrent sexually transmitted diseases, tuberculosis, or helminthic parasites make transmission more likely, presumably by providing activated T cells for HIV replication or by physically reducing intrinsic epithelial barriers [3].

HIV-1 infects human cells that express the CD4 receptor [4,5] and either the CCR5 [6–8] or CXCR4 [9] coreceptor. Cells that meet these criteria include CD4⁺ helper T cells, macrophages, microglial cells, dendritic cells, and the majority of thymocytes. The defining feature of disease progression is the gradual, but eventual, depletion of CD4⁺ helper T cells from the blood. These helper T cells are essential for proper generation of an adaptive immune response to foreign pathogens. Accordingly, once the number of these cells drops below a certain level, the ability of the host to fend off foreign infection is likely to be severely impaired. While the rate of disease progression is highly variable between individuals, on average it takes about a year for the CD4⁺ cells to drop to about 200 cells/μl, at which point there is an increased risk of opportunistic infections and other complications of AIDS [10].

During the years of clinical latency, homeostatic mechanisms are able to retain a relatively normal level of T cells, as discussed below. At some point, these homeostatic mechanisms fail and there is a precipitous drop in both CD4⁺ and CD8⁺ T cells [19]. Around this time, opportunistic pathogens are able to cause widespread and fulminant infection and the patient is diagnosed with AIDS. Over 100 distinct opportunistic infections have been described in the context of AIDS and these are typically responsible for death [20]. A number of cancers may also arise, perhaps due to activation of latent oncogenic viruses no longer held in check by immune surveillance. In addition, significant morbidity due to HIV-associated cognitive disorders, including AIDS-associated dementia, has been reported [21].

2 HIV-1 viral pathogenesis or AIDS

The majority of research directed at understanding HIV pathogenesis has focused on chronic disease during clinical latency. When HIV was shown to replicate at high levels throughout this period, it was hypothesized that direct infection and continual destruction of CD4⁺ T cells could account for their depletion and progression to AIDS [13–15]. In this “tap and drain” model, CD4⁺ T cells are constantly depleted by direct viral infection and are constantly replaced by increased thymic output and augmented homeostatic proliferation of peripheral naive and memory T cells. Eventually, renewal sources become exhausted, resulting in final, and specific, elimination of CD4⁺ T cells.

Several clinical observations suggested an alternative explanation. First, increased activation of both CD4⁺ and CD8⁺ T cells is observed throughout infection [22]. Second, ex vivo culture of both CD4⁺ and CD8⁺ T cells from patients results in spontaneous apoptosis relative to uninfected controls [23–25]. Third, labeling studies in vivo reveal a rapid decay in both CD4⁺ and CD8⁺ T cells, indicating rapid death of most proliferating cells during infection [26–29]. Fourth, both naïve CD4⁺ and CD8⁺ T cells decrease in frequency during disease progression [30]. Finally, the frequency of infected cells is insufficient to directly account for the number of apoptotic and activated cells observed [31,32]. These data have led to the emergence of the current, predominant view for HIV pathogenesis during chronic infection. In this view, HIV replication induces a chronic state of immune activation that indirectly results in depletion of the homeostatically maintained T cell pool [33]. HIV replication, per se, does not cause T cell depletion. Rather, a constant immune response against a pathogen that cannot be eliminated eventually results in immune exhaustion [33].

While mechanisms of pathogenesis during chronic infection have been extensively studied, primarily by observation of the peripheral blood, only recently has the contribution of acute infection been examined in detail. It has been determined that during primary infection, many CCR5⁺CD4⁺ memory T cells in the gut-associated lymphoid tissue (GALT) appear to be directly depleted by HIV-1 [34,35]. The contribution of this early and massive immune insult to subsequent viral pathogenesis and disease progression is now only receiving attention. It is likely that primary HIV-1 infection plays a much greater role in the pathogenesis of disease than has previously been appreciated and that the impact of memory T cell depletion before relative control of viral replication by the adaptive immune system determines, in part, the subsequent rate of immune activation and disease progression.

3 Modeling HIV-1 infection and AIDS pathogenesis in humanized mice

Relative to the rapid progress in our understanding of mouse immuno-biology, human immunology and immuno-pathogenesis are not clearly understood. Some immu-
nological findings in mice do not translate directly to the human immune system. In addition, many human pathogens only infect human cells, thus making human patients the only model for investigation. As a result, many conflicting reports have dominated the literature regarding human immunology and infectious diseases. These conflicting reports in patients highlight the importance of studying the interaction between human pathogens and human immune system in relevant models. The confusion is due to at least the following reasons: (i) Basic immuno-biology (phenotypes, development, homing and function) of human immune cells is poorly investigated; (ii) Human cells from blood as analyzed in most studies may not reflect the majority of immune cells in lymphoid tissues; (iii) Human immune cells may be differently affected at different disease stages; and (iv) Individual difference in age and genetics within and between study populations. A robust animal model that addresses these limitations is urgently needed to study the basic human immunology and infection.

4  A brief history of mouse models of human immunology and HIV-1 disease

A small animal model for “hypothesis-testing/mechanistic” research concerning HIV-1 infection and immuno-pathogenesis is needed and has to meet the following criteria. First, it has well-studied hemato-lymphoid organs and target cells similar to those of humans. Second, HIV-1 establishes infection, and induces anti-HIV immune response and HIV infection leading to AIDS-like disease. Third, it is genetically inbred and can be manipulated via genetic, immunological and pharmacological means. HIV-1 fails to infect murine cells due to the blocks at multiple steps of the HIV life cycle. Heroic efforts by several groups have failed to genetically humanize the mice or to adapt HIV-1 to replicate in murine cells. The mouse with a transplanted human immune system thus has become the best candidate. A number of human-mouse chimeric models have been developed, but most with only limited success, due to the selective engraftment of xeno-reactive human T cells in hu-PBL-SCID mice [36–38] or the lack of significant human immune responses in the SCID-hu Thy/Liv mouse [31,39].

The SCID-hu Thy/Liv and hu-PBL-SCID models. Two human-mouse chimera models with human lymphoid organs implanted in immunodeficiency mice have been initially constructed to study HIV-1 infection in vivo. These models have been used to study HIV-1 infection and T cell depletion in vivo. Although both high profile papers have human immunity claimed in the reconstituted mice, further studies have revealed the limitations in studying human immune responses in the models. The SCID-hu Thy/Liv mouse has an intact human thymus organ, thus allowing the investigation of HIV-1 pathogenesis in the thymus [39,40]. However, as no human B or myeloid cells and very low levels of human T cells are detectable in the peripheral organs or blood, no significant primary human immune responses are observed in the model. The hu-PBL-SCID mouse is limited due to its lack of human hematolymphoid organs and its selective engraftment of xeno-reactive human T cells [36–38].

Significant improvements have been achieved in humanized mouse models. The discovery that mutation in the common cytokine receptor gammaC impairs NK cell development in mice has led to the humanization of NOD-SCID/gammaC and Rag/gammaC mutant mice with remarkable success. Further transplan of SCID-hu Thy/Liv mice with autologous human HSC has significantly improved the engraftment of human immune cells in the NOD-SCID mouse. All three models have been used to study HIV-1 immuno-pathogenesis (Table 1).

5  Current mouse models of human immunology and HIV-1 diseases

5.1  The NOG-hu HSC or NSG-hu HSC mouse model

The NOD-SCID/gammaC mutant mouse lacks T, B lymphocytes and NK cells (and impaired macrophage function in NOD mice), and serves as better hosts for engraftment of human cells/tissues. Infusion of human HSC has led to efficient reconstitution of a stable human immune system [41–43]. In addition, the new model can be infected with HIV-1 that establishes long-term persistent infection [44]. However, only limited anti-HIV T or B immune responses have been detected. In a similar NSG-hu HSC model (an independently derived mutant C allele), stable engraftment of human immune cells has also been observed [42,45].

5.2  The DKO-hu-HSC mouse model

The Rag2−/−gammaC−/− double knockout (DKO) mouse also lacks T, B lymphocytes and NK cells. In addition, the new model has injected CD34+ human HSC (hematopoietic stem cells) directly into the liver of new born DKO mice [46]. The new born liver environment appears to support efficient human HSC engraftment and reconstitution of the mouse with a functional human immune system in central and peripheral lymphoid organs. Remarkably, long term human T cell development occurs efficiently in the mouse thymus, and normal human T, B, NK and dendritic cells (both mDC and pDC) are readily detected in peripheral lymphoid tissues such as spleen, lymph nodes (LN) and peripheral blood (PB). Human T cells developed in the DKO-hu mouse are tolerated to both human and mouse antigens, indicating efficient negative selection by both murine and human APC. Significantly, de novo human B and T cell responses are elicited in the DKO-hu-HSC mouse by standard immunization (human TT-specific IgG induction) or infection with the
human tumor virus EBV (expansion of EBV-specific CD8 T cells). These EBV-reactive T cells respond to EBV antigens in a human MHC-dependent fashion, suggesting human T cells are positively selected by human MHC as well as by murine MHC. Interestingly, DKO mice in C57/B6 background are not as efficiently engrafted as in Balb/C background, suggesting other host factors still contribute to the rejection of human immune cells.

5.3 The NOD-SCID-hu BLT mouse model.

SCID-hu Thy/Liv mice have a human thymus organ developed under the kidney capsule for over 50 weeks. However, B cells and myeloid cells normally developed in the bone marrows are not present in significant numbers in these mice. In addition, few T cells are detectable in the peripheral organs possibly due to the impaired emigration or survival of human naïve T cells. The heroic effort to build a “full house” humanized mouse has led to the mouse with transplanted human bone, thymus, mLN and epithelial tissues which generate an efficient human T/B immune response [47]. Due to its high degree of difficulty, this model has not been widely used following its initial report. It has been reported that the transfer of NOD-SCID-hu Thy/Liv mice with human HSC isolated from the same fetal liver tissues leads to efficient reconstitution of human T, B, and myeloid cells in all lymphoid organs [48]. Both innate and adaptive immunity are induced in this SCID-hu BLT model. The gut associated lymphoid tissues (GALT) are also efficiently repopulated in this model [51].

6 Functional human adaptive immunity is developed in the new humanized mice

In the humanized NTG-hu mice, long term human T cell development efficiently occurs in the mouse thymus, and normal human T, B, NK and dendritic cells (both mDC and pDC) are readily detected in such peripheral lymphoid tissues as spleen, lymph nodes (LN) and peripheral blood (PB). Human T cells developed in the DKO-hu mouse are tolerated to both human and mouse antigens, indicating efficient negative selection by both murine and human APC. de novo human B and T cell responses are elicited in the DKO-hu-HSC mouse by standard immunization (human TT-specific IgG induction) or infection with the human tumor virus EBV (expansion of EBV-specific CD8 T cells). These EBV-reactive T cells respond to EBV antigens in a human MHC-dependent fashion [46].

Table 1 Humanized mouse models of HIV-1 infection and immuno-pathogenesis

<table>
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<tr>
<th>Model</th>
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<th>huTEC</th>
<th>HIV/iv</th>
<th>HIV/im</th>
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<tr>
<td>Hu-PBL-SCID</td>
<td>*T/B/APC</td>
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<tr>
<td>SCID-hu Thy/Liv</td>
<td>**T/B/APC</td>
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<td>SCID-hu BTSLN</td>
<td>T/B/APC</td>
<td>+</td>
<td>LN/skin</td>
<td>+?</td>
<td>+?</td>
<td>[47]</td>
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<tr>
<td>NSG-hu or NOG-hu HSC</td>
<td>T/B/APC</td>
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<td>DKO-hu HSC</td>
<td>T/B/APC</td>
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<tr>
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a) #, SCID: severe combined immunodeficiency; DKO: rag2-IL2R double null mice; PBL: peripheral blood lymphocytes i.v.; Thy/Liv: human fetal liver and thymus fragments transplanted under renal capsules; HSC: CD34+ human hematopoietic stem cells. BTSLN: human fetal bone, thymus, skin and lymph node; BLT: human fetal thymus/liver fragments under renal capsule and CD34+ HSC i.v. *, T/B/APC: T/B cells/Macrophages-DC, but mostly xeno-reactive T cells in the hu-PBL-SCID model; **, T/B/APC: T/B cells/Macrophages-DC, but mostly restrained in the thymus in the SCID-hu Thy/Liv model. +, HIV infection: iv-intravenously; im-intramucosally; it-intrathymically; +, productive HIV infection; –, no infection; ?, not done or reported.
lymphocyte response (MLR) between splenocytes from DKO, A1 and B DKO-hu mice, and between “syngenic” A1 and A2 DKO-hu mice. However, either A1 or A2 cells react strongly with the “allogenic” cells from the cohort B DKO-hu mice (Unpublished results).

Immunization of DKO-hu mice with Ova protein induces Ova-specific human T and B cell responses (Su, unpublished data). In the immunized DKO-hu HSC mouse, Ova-specific human IgG is readily detected. This is consistent with the report that human T and B cell immune responses are induced by EBV infection and by immunization. Therefore, normal human T and B cells are generated in the DKO-hu HSC mouse.

7 HIV-1 infection and pathogenesis in NTB-hu HSC mice

With a stable functional human immune system, functional human immune cells are developed in normal proportion in all lymphoid organs in DKO-hu mice. It has been shown that HIV-1 infection is efficiently established and persistently detectable in the PB of DKO-hu HSC mice with X4, R5, or X4/R5 dual-tropic isolates. Human CD4+ T cells are gradually depleted during HIV infection in a dose-dependent manner [44,50,53,54]. Multiple human CD4+ cells, such as T cells, macrophages and pDC, are directly infected by HIV-1. Therefore, the DKO-hu HSC mouse, as well as the NSG-hu HSC and SCID-hu BLT mice, will serve as crucial models to investigate the mechanisms of HIV immunopathogenesis.

In the NTB-hu mouse models, HIV-1 isolates with CCR5. CXCR4 or dual tropism establish productive infection in various lymphoid organs and lead to gradual CD4 T cell depletion. More significantly, CXCR4-tropic HIV-1 isolates usually show faster CD4 depletion kinetics, and the pathogenic HIV-1 isolated from rapid disease progressors shows highly rapid CD4 depletion in the model. Thus, NTB-hu HSC mice provide good models to study HIV-1 infection and pathogenesis in vivo.

We have reported that both CCR5 and CXCR4 are expressed on human T and myeloid cells in DKO-hu mice. DKO-hu HSC mice allow efficient HIV-1 infection with high plasma viremia. High levels of productive infection occur in the thymus, spleen and lymph nodes. Interestingly, both CD45RO+ (memory/effector) and CD45RO− (naive) CD4T cells are productively infected as stained for HIV gag p24. Human CD4+ T cells are rapidly depleted by a pathogenic HIV-1-R3A, associated with undetectable viremia in the blood [52,53,55–57]. However, HIV-1 infection persists in these infected DKO-hu HSC mice for at least 19 weeks, with infectious HIV-1 recoverable in lymphoid tissues [53].

We also infected DKO-hu mice with the less pathogenic HIV-1 isolate JRCSF (CCR5-tropic). As reported in this model, high levels of HIV replication were detected at 1 to >50 weeks post infection [54,58,59]. CD4+ T cells in the blood were only slowly decreased but maintained at steady levels. When lymphoid organs were analyzed from R3A-(2 wpi) or JRCSF-(4 wpi) samples, R3A infection almost completely depleted human CD4+ T cells in the spleen and mLN, whereas JRCSF infection did not significantly deplete human CD4 T cells in the spleen or mLN. Interestingly, the CD8 levels relatively increase in the mLN for both infections (Zhang & Su, unpublished data). Thus, the DKO-hu HSC mouse may serve as a relevant in vivo model in investigating the mechanisms of HIV-1 infection and immunopathogenesis. HIV-R3A may be used to study acute HIV infection with a rapid CD4 depletion, and JRCSF may be used to study acute HIV infection, immuno-response and chronic HIV infection and immunopathogenesis.

8 Development of the GALT and mucosal transmission of HIV-1 in humanized mice.

The PP and GALT are not efficiently developed in the NTB-hu HSC mice, possibly due to the defective gammaC gene implicated in organizing such lymphoid tissues. As a result, only a small number of human CD45+ leukocytes have been detected in the gut of NTB-hu mice [60]. However, inoculation of HIV-1 intra-rec tally or intra-virginally in the DKO-hu HSC mouse has led to efficient transmission of HIV-1, but only after mucosal damage is introduced ([61] and Zhang & Su, unpublished results). In a different report, HIV-1 inoculation via the mucosal routes has failed to lead to infection, although it is not clear if mucosal damage is induced prior to HIV-1 inoculation [60].

In the NOD-SCID-hu BLT mouse, on the other hand, PP and GALT are well-developed with similar human leukocyte subpopulations including CD4CCR5+ memory T cells as in human GALT. HIV-1 is efficiently transmitted via either rectal or vaginal route. However, the infection is also initiated by introducing mucosal damages. It is not clear if HIV-1 can be transmitted through an intact mucosal tissue in the humanized mouse. When SCID-hu BLT mice are infected with HIV-1, significant infection and CD4 T cell depletion is observed in the GALT as in SIV-infected Rhesus monkeys [49,50]. Importantly, similar HIV-1 pathogenesis in the GLAT has been documented in this model. These include efficient infection and depletion of human CD4+ CCR5+ memory T cells. It is not clear if similar HIV-induced damage to the gut epithelia is occurring during HIV-1 infection.

Inhibition and development of drug-resistant HIV-1: As reported with other humanized mouse models, HIV-1 infection in NTB-hu HSC mice can be prevented by pre-exposure treatment with ART. Interestingly, when treated after the establishment of HIV-1 infection in DKO-hu mice, ART treatment can lead to emergence of HIV-1 recombinants that are resistant to ART [59]. This is in contrast to similar
treatment in the SCID-hu Thy/Liv model, where no such drug-resistant HIV-1 recombinants are detected, even after virologic breakthrough in the presence of ART [62]. Differences in replication levels and target cells/organisms probably contribute to the different outcomes. Nonetheless, the NTB-hu HSC mouse provides an excellent model to study drug treatment and resistance in vivo.

Although human T and B cell responses are detected in the NTB-hu models, HIV-1 infection only leads to weak HIV-specific antibody response, and not clear anti-HIV T cell response due to lack of MHC-peptide reagents. When SCID-hu BLT reconstituted with human donors that are HLA-A2+, HIV-specific CTL are readily detected with A2-gag peptide tetramers, and antibody responses are also readily detected in HIV-infected SCID-hu BLT mice [63]. This may be due to education of human T cells in the human thymus, which allows survival and immune responses of human T cells to human cell-tropic HIV-1 infection.

9  Treg cells vs. HIV-1 infection and pathogenesis in vivo

FoxP3+CD4+CD25+ regulatory T (Treg) cells have been implicated in a number of pathologic processes including elevated levels in cancers and infectious diseases, and reduced levels in autoimmune diseases. Conflicting findings are reported regarding relative levels of Treg cells during HIV-1 infection and disease progression. The role of Treg cells in HIV-1 diseases (aberrant immune activation) is poorly understood due to the lack of a robust model [64].

HIV-1 infection and replication in Treg cells in vivo: CD4+CD25+ Treg cells express both CXCR4 and CCR5 coreceptors for HIV-1 infection. Given that Foxp3+ Treg cells are thought to be functionally anergic in vitro, characterized by the repression of the T cell activation-dependent IL-2 gene, it was surprising to find Treg cells support higher levels of infection by HIV-1 or FIV compared to Foxp3-CD4+ T cells in vitro and in vivo [52,64,65].

The role of Treg in HIV-1 infection and immunopathogenesis: The role of Tregs in establishing chronic versus acute diseases has been established for several pathogens, and this cell population in HIV-1 infection is of great significance. The best approach to define the function of Tregs in HIV infection is through genetic manipulation in relevant HIV infection models. The question remains if depletion of Treg prior to HIV infection will lead to elevated anti-HIV immunity and reduced acute viremia, and whether depletion of Treg in chronic HIV-1-infected patients contributes to uncontrolled immune activation and accelerates AIDS progression. Thus determining the kinetics and functional response of Tregs is of great importance.

We have directly addressed the role of Treg cells in HIV infection in the BALB/c-DKO-hu mouse model [52]. CD4+FoxP3+ T cells developed in all lymphoid organs display normal Treg phenotype and function. These FoxP3+ Treg cells in lymphoid organs are preferentially infected and depleted by a pathogenic strain of HIV (NL-4-R3A) and depletion of Treg cells is related with induction of their apoptosis in vivo. To assess the function of Treg cells in the control of viral replication during acute infection, Treg cells were depleted with the IL2-toxin fusion protein (ONTAK) prior to infection. The result was a significant impairment of HIV replication and infection, possible due to depletion of HIV-1 target Treg cells, as well as a robust anti-HIV immune response. This has been demonstrated by reduced levels of productively infected cells in lymphoid organs and lower plasma viremia. We see increased inflammatory cytokines (IFN-γ, TNF-α by intracellular staining) in ONTAK-treated HIV infected lymphoid organs compared to mock, supporting the presence of increased antiviral T cell response in the absence of Treg cells [52]. It will be equally interesting to determine the significance of Tregs in the chronic phase of HIV-1 infection by depleting Treg during chronic infection in future experiments.

10 New improved humanized mouse models and perspectives

The current humanized mouse models have established a functional, but sub-optimal, human immune system. A lot of effort has been directed at further optimize the human immune functions in humanized mice. Three general approaches have been taken to achieve such goals.

First, human-specific cytokines have been used to enhance human immune cell types. Reconstitution of natural killer (NK) cells and myeloid cells is generally poor in the humanized mouse models using NSG or BALB/c-DKO mice as recipients. In SCID-hu BLT mice, human NK cells are absent despite significant reconstitution of lymphocytes, dendritic cells (DCs) and monocytes. Like lymphoid cells, NK cells and myeloid lineage cells are also derived from HSCs. During their differentiation, specific cytokines are required. However, due to evolutionary divergence between humans and mice, many mouse cytokines do not function on human cells. For example, mouse IL-15 has no effect on human NK cells and precursors. We found that human CD34+ precursor cells isolated from the BM of humanized mice were stimulated in vitro to differentiate into NK cells, DCs, monocytes/macrophages and erythrocytes if appropriate human cytokines were added into the cultures. More significantly, when appropriate human cytokines are expressed in the humanized mice by hydrodynamic delivery of cytokine-encoding plasmid DNA, significantly elevated levels of NK cells, DCs, monocytes and erythrocytes are induced. Specifically, expression of human IL-15 and Flt3L enhanced NK cell reconstitution and other myeloid cells; expression of human GM-CSF, IL-4 and Flt3L enhanced DC reconstitution; expression of human M-CSF enhanced...
monocyte/macrophage reconstitution, and expression of human IL-3 and erythropoietin enhanced erythrocyte reconstitution. The cytokine-induced NK cells are functional as demonstrated by in vitro and in vivo functional assays [66]. Thus, the poor reconstitution of NK cells and myeloid cells in humanized mice is due to the lack of appropriate human cytokines normally required for their differentiation and maintenance. Introduction of appropriate cytokines leads to a dramatic increase in reconstitution levels of these human blood cell lineages. Similarly, by injecting recombinant human IL-15 into humanized mice, human NK cells are significantly enhanced [67]. The ability to enhance reconstitution of specific human blood cell lineages in humanized mice by expression of human cytokine genes will facilitate the study in mice of these human cell types in immunity and disease processes. Future effort will be to express human cytokines by transgenic approaches in NSG or DKO recipient mice. It will be also possible to genetically modify human HSC with lentiviral vectors (cDNA or shRNA). Such “transgenic” NSG-hu HSC mice will be a contributing factor study genetic regulation of human hematopoiesis, human immune development and functions. In addition, human gene therapy approaches will be modeled prior to clinical applications.

Second, human immune-relevant genes are expressed in NSG mice by transgenic constructs expressing human HLA-A2 or HLA-DR. The human MHC expressed in thymus will positively select human T cells that are restricted by human MHC. In addition, human MHC expression on APC in the peripheral lymphoid organs will support homeostatic proliferation and survival of human T cells as well as efficient human MHC-restricted T cell responses [68,69].

Third, other human tissues, such as liver, brain or lung, will be co-transplanted into humanized mice with a functional human immune system. Human tissue-tropic virus or bacteria infection and their immuno-pathogenesis will be studied in such mouse models. Engraftment of human liver cells has been achieved in NOG or NSG and hepatocyte-deficient mice [70,71]. However, due to technical difficulty, humanized mice with both human liver and human immune system have not been reported yet.

Finally, it will be exciting to model directed human tissue-specific differentiation from human ES and/or iPSC cells in humanized mice. These models will provide critical in vivo assays for tissue-specific human stem/progenitor cells. Equally important, the humanized mouse models will be critical to study human diseases mediated by human immune responses as well as human pathogens.


Chen Q, Khoury M, Chen J. Expression of human cytokines dramatically improves reconstitution of specific human-blood lineage cells in humanized mice. Proc Natl Acad Sci USA, 2009, 51: 21783–21788


