Chapter 10
Human T-Cell Biology in a Mouse Environment

Nicolas Legrand and Hergen Spits

Abbreviations

BLT	Bone marrow, liver, thymus
BRG	BALB/c Rag2$^{-/-}$ IL-2R$\gamma_c^{-/-}$
BRGS	BALB/c Rag2$^{-/-}$ IL-2R$\gamma_c^{-/-}$ SIRPα^{NOD}
DC	Dendritic cells
FDC	Follicular dendritic cells
FL	Fetal liver
FRC	Fibroblastic reticular cells
GvHD	Graft-versus-host disease
HIS	Human immune system
hHSPC	Human hematopoietic stem and progenitor cells
hTSPC	Human thymus seeding progenitor cells
ISP	Immature single positive
LN	Lymph node
LTi	Lymphoid tissue inducer
NOD	Nonobese diabetic
NOG	NOD/Shi-scid/γc^{null}
NSG	NOD.Cg-Prkdc^{scid} *IL2rg^{tmlWjl}*/Sz
SCID	Severe combined immuno-deficiency
T_{FH}	T follicular helper
UCB	Umbilical cord blood

N. Legrand (✉)
AXENIS, Paris, France
e-mail: nicolas.legrand@pasteur.fr

H. Spits
Tytgat Institute for Liver and Intestinal Research, AMC-UvA, Amsterdam, The Netherlands
e-mail: hergen.spits@amc.uva.nl

L. Y. Poluektova et al. (eds.), *Humanized Mice for HIV Research,*
DOI 10.1007/978-1-4939-1655-9_10

10.1 A Large Collection of Mice Humanized for the Immune System

The experimental, prospective analysis of human hematopoietic development and function is complex, for technical, ethical, and practical reasons. Human immunology mostly relies either on in vitro systems, or on observations in clinical settings. Tackling these limitations, and facing the lack of appropriate models to study HIV, several pioneering groups developed in the late 1980s chimeric mouse models of human immunity based on the transplantation of human hematopoietic cells into various immunodeficient mouse recipients [1–3]. The history of humanized mouse models of hematopoiesis has already been reviewed before, providing an extensive overview of the incremental optimization strategies that have been applied over the past two decades to meet desired features for such "human immune system" (HIS) mice [4–10].

In brief, three main criteria have been demonstrated to strongly impact on the stability of the human hematopoietic xenograft in HIS mice: (i) the degree of immunodeficiency of the mouse recipients (the inactivation of mouse Rag2 and IL-2Rγ_c gene expression being particularly beneficial, as it fully abrogates the mouse T/B and NK cell development, respectively); (ii) their genetic background (the nonobese diabetic (NOD) and BALB/c strains being for instance particularly permissive, in contrast to the C57BL/6 strain); and (iii) the age at which the mice receive the xenotransplant (newborns being more permissive than adult animals). Additional many technical variations (e.g., site of transplantation, origin of the human hematopoietic cells, coengraftment of human tissues, and treatment of the recipient mice with exogenous products) have been applied, each with specific advantages and drawbacks. Among those, HIS mouse models generated through the transplantation of human hematopoietic stem and progenitor cells (hHSPC) are particularly attractive, as they should provide systems supporting multilineage human hematopoietic reconstitution in a durable fashion, allowing long-term studies in a more physiological environment. The past decade has seen the emergence of two major families of HIS mouse models: One based on the transplantation of hHSPC suspensions alone and another on hHSPC transplantation together with human fetal thymic tissues. Both families of models make use of various immunodeficient mouse genetic backgrounds, mainly NOD-based and BALB/c-based.

10.2 Humanized Mouse Generation with Hematopoietic Progenitors Only

NOD background-based mouse strains, either originating from the Jackson Laboratories in the USA (NSG [NOD.Cg-*Prkdc*^{scid} *IL2rg*^{tm1Wjl}/Sz] mice) or from the Central Institute for Experimental Animals in Japan (NOG [NOD/Shi-scid/γc^{null}] mice), are widely used to generate HIS mice. Human hematopoietic reconstitution was

demonstrated in both newborn and adult NSG/NOG mice, although newborn animals are more permissive to the xenograft [11–15]. A common strategy nowadays involves conditioning of newborn animals by sublethal irradiation followed by a single injection with a suspension of 10^5–10^6 human umbilical cord blood (UCB) or fetal liver (FL) $CD34^+$ cells, a cellular fraction known to be enriched for hHSPC. Transplantation of as few as ten purified $CD34^+CD38^-CD90^+CD45RA^-Lin^-$ hHSPC resulted already in human hematopoietic reconstitution in NSG or NOG mice [16, 17]. The particularly high efficiency of xenotransplantation in the NOD background can be explained by the combination of defective function of mouse complement, mouse plasmacytoid dendritic cells (DC), and/or mouse phagocytes [18–20]. Of note, due to the *scid* mutation, NSG/NOG mice exhibit an ineffective DNA repair machinery and are therefore particularly radio-sensitive, which may explain the relatively short life-span of NSG mice (<6 months) after completion of the humanization procedure [21].

We and others have shown that newborn (≤5-day-old) BALB/c $Rag2^{-/-}$ IL-$2R\gamma_c^{-/-}$ (BRG) mice can also serve as a recipient for a hHPC xenograft, following the aforementioned straight-forward reconstitution strategy [22, 23]. Human hematopoietic reconstitution is directly dependent on the transplantation age, since 1-week and 2-week-old recipient BRG animals exhibit a severely reduced capacity to support the generation of a human hematopoietic ($hCD45^+$) cell graft, which is virtually absent when starting from adult BRG animals [22, 24]. Of note, human hematopoietic reconstitution is inefficient in C57BL/6 $Rag2^{-/-}$ IL-$2R\gamma_c^{-/-}$ recipients, both with newborn or adult animals [24, 25].

10.3 Humanized Mouse Generation with an Additional Human Thymus Organoid

The second major family of HIS mice—known as BLT (for "bone marrow, liver, thymus") mice—is reminiscent of the original SCID-hu Thy/Liv mice [1]. In this model, xenotransplantation of human fetal thymus and fetal liver fragments is performed under the kidney capsule of immune-deficient C.B-17 SCID mice, a natural immune-deficient mouse strain in which T- and B-cell development is inhibited due to a defective V(D)J recombination process—although T and B cells are detectable in some mice, a phenomenon known as "T/B cell leakiness" [26–28]. As a result, a human thymus organoid engrafts and becomes the major site of human thymopoiesis. Still, only a minority of the SCID-hu Thy/Liv animals (~1 out of 3) contain a detectable population of human hematopoietic cells, which all belong to the T cell lineage and only represent a minor fraction (~0.7%) of all leukocytes [29]. Partly because mouse T and B cells may arise and mouse NK cells are normal in the C.B-17 SCID background, the human xenograft is eventually lost over time, probably due to immune rejection. To resolve these issues, a similar technical strategy has later been applied to NOD-based immuno-deficient mouse strains to generate BLT mice [30–32]. Similarly to SCID-hu Thy/Liv mice, the BLT model is intrinsically

more challenging to construct due to specific technical constrains—e.g., dependency on human fetal tissues, requirement for delicate surgery, but it exhibits attractive features—in particular a relatively large human peripheral T-cell population which has permitted major achievements in the HIV field [33]. Nevertheless, it was recently reported that BLT mice develop over time a wasting syndrome due to graft-versus-host disease (GvHD), a feature that may limit the use of BLT mice in long-term settings [34–36].

10.4 Human T-Cell Development in HIS Mice

The most recent, aforementioned HIS mouse models (NSG/NOG, BRG, and BLT) all support de novo, multilineage human hematopoiesis, including human T-cell development. BRG-HIS and NSG/NOG-HIS mice rely on the mouse thymus for the generation of human T cells, as illustrated by the absence of human T cells in thymus-deficient nude BRG-*nu/nu* mice after hHSPC inoculation [37]. In contrast, human T-cell development in BLT mice mostly takes place in the ectopic human thymic organoid deriving from the human fetal tissues engrafted under the kidney capsule. This fundamental distinction obviously imposes major differences on their relative capacity to support human T-cell production, as human thymopoiesis is likely to be more efficient in a human microenvironment.

It is currently unknown whether the hHSPC established in HIS mice are able to generate human thymus seeding progenitor cells (hTSPC) as efficiently and using the same differentiation pathway as in human individuals. Furthermore, different hTSPC might colonize a mouse thymus (NSG/NOG; BRG) vs. a human thymic organoid (BLT), an issue that requires further investigations. It has been described that $CD34^+CD38^{low}$ human thymocytes contain the earliest immature thymocyte population, since they mostly lack TCR gene rearrangements and contain T cell, DC, and NK cell precursor activities [38–41]. This cell population resembles $CD34^+CD45RA^+CD7^+CD38^{low}$ cells found in human UCB, which are also able to give rise to T cells [42, 43]. Of note, it has been shown that human fetal bone marrow $CD34^+CD45RA^+CD7^+$ cells can be actively recruited to immuno-deficient mouse (NOD.*scid*) thymic lobes, where they commit to the T cell lineage [44].

Overall, one can therefore speculate that hTSPC are generated in HIS mice from the transplanted $CD34^+$ hHSPC and are then able to colonize the murine thymus. In BLT mice, hTSPC populate both the murine thymus and the human thymic organoid of BLT mice—potentially resulting in a more effective engraftment. It is likely that different sources of human hHSPC, which significantly differ in their developmental status (e.g., FL, UCB, and adult bone marrow) result in major differences in the degree of hTSPC generation and thymus engraftment. For instance, we have consistently observed that thymus colonization occurs at least two weeks earlier in FL $CD34^+$ hHSPC-injected BRG mice, as compared to UCB $CD34^+$ hHSPC-injected BRG mice (unpublished observation), and similar kinetic differences have been reported for total human hematopoietic reconstitution [45]. These observations

might be due to intrinsic differences when considering the differentiation capacity of each of these hHSPC sources. It is also possible that the frequency of pluripotent $CD34^+CD38^-$ hHSPC present in the $CD34^+$ cell population used as the original inoculum determines the degree of colonization, since this frequency is significantly higher when using FL samples (30–50 % vs. < 1 % in UCB).

Colonization of the mouse thymus in BRG-HIS or NSG/NOG-HIS mice is initiated 2–4 weeks after the hHSPC transplantation, and leads to limited accumulation of human thymocytes in situ. NSG/NOG-HIS mice tend to exhibit lower human thymocyte numbers ($0.1–0.5 \times 10^6$ cells) than BRG-HIS mice ($1–5 \times 10^6$ cells) [11–13, 15, 22, 23, 46, 47]. In contrast, the size of the human thymic organoid in NSG-BLT mice after hHSPC transplantation is much higher ($\sim 70 \times 10^6$ human cells), with high-interindividual variability [48]. Thymic tissues (mouse and human organoid) get organized with cortex- and medulla-like regions, which mostly contain immature, $CD4^+CD8^+$ double-positive thymocytes and mature, $CD4^+$ or $CD8^+$ single-positive thymocytes, respectively—as seen in mouse or human thymus control tissues [11, 14, 23, 49–51].

Altogether, the human thymocyte differentiation pattern in HIS mice is similar to what is reported from normal human thymus samples with respect to the major cell surface markers [52]. As in the human thymus, the rare $CD34^+CD1a^-$ cells observed in the thymus of HIS mice may contain dual T/NK cell precursors, which would correlate with local, relatively high accumulation of discrete human NK cell sub-populations [53, 54]. Acquisition of CD1a expression marks commitment towards the T-cell lineage, and $CD34^+CD1a^+$ cells subsequently differentiate into $CD4^+$ immature single positive (ISP) cells, which contain precursors of both αβ and γδ T cells [52]. The heterogeneous $CD4^+CD8^+$ double-positive immature thymocyte population expresses variable levels (mostly from negative to dim) of CD3ε on cell surface, whereas mature $CD4^+$ or $CD8^+$ thymocytes express high levels of surface CD3ε and have lost the expression of CD1a. Most of the mature thymocytes (~99 %) belong to the Tαβ lineage. A fraction (1–5 %) of $CD4^+$ mature thymocytes resemble "natural" regulatory T cells ($CD25^+GITR^+FOXP3^+$), both phenotypically and functionally [55–59].

10.5 Human Peripheral T-Cell Homeostasis in HIS Mice

Once they reach a mature stage, human thymocytes (including γδT cells and T_{reg} cells) colonize lymphoid organs and peripheral tissues of HIS mice, with kinetics that differ from other human hematopoietic lineages. Sequential analysis of human cell content in peripheral organs of BRG-HIS mice shows initial colonization by immature/naive B cells, followed by gradual T-cell accumulation, which correlates with accumulation of mature/memory-like B cells and serum human IgG over time [46, 60]. At early time points after reconstitution, reconstitution of human cells in HIS mice therefore mimic the situation of young infants or lymphopenic patients, e.g., shortly after hHSPC transplantation, who exhibit relatively immature, partially functional immune systems [61].

The relative proportion of human hematopoietic cell subsets in peripheral lymphoid organs of all HIS mouse models is reminiscent of a mouse situation, with high proportion of lymphocytes and low frequency of granulocytes, in contrast with normal human blood. Overall, the number of human leukocytes found in adult BRG-HIS mice is relatively low as compared to what is observed for murine leukocytes in immuno-competent mice (e.g., for BRG-HIS mice: 20–30 % of BALB/c leukocyte numbers in the bone marrow, 1–2 % in the thymus, and 1–5 % in the spleen) [22–24, 46]. Transplantation of hHSPC into NOD-based immuno-deficient mice results in higher numbers of human hematopoietic cells (two to tenfold depending on the secondary lymphoid organ) than in BRG-HIS mice [24, 45]. This difference is apparent only when the actual cell numbers are determined by counting, as similar relative frequencies of human ($hCD45^+$ vs. $mCD45^+$) leukocytes have been reported in several NOD-based and BRG-HIS mice.

Major differences have been reported between BRG, NSG/NOG, and BLT mice regarding the actual frequency and phenotype of human T cells in the secondary lymphoid organs of hHSPC-reconstituted animals. In the spleen, T cells usually represent less than 10 % of human leukocytes in adult (~14-week-old) BRG-HIS mice [22–24, 46], around 10–20 % in NSG/NOG-HIS mice [11–15] and >50 % of human leukocytes in BLT mice [30–32]. Large numbers of naive ($CCR7^+CD45RA^+$ or $CD27^+CD45RO^-$) T cells are observed in BLT and NSG/NOG-HIS mice, whereas BRG-HIS mouse T cells mostly exhibit a phenotype of activated, cycling ($Ki67^+$ or BrdU-incorporating), apoptosis-prone cells [24, 55]. BRG-HIS mice also tend to show the accumulation in peripheral lymphoid organs of $CD4^+CD8^+$ double-positive T cells, which may correspond to a population of human activated T cells usually observed in inflammatory conditions [24, 62].

The major difference in reconstitution of human hematopoietic cells between NOD-based and BRG-HIS mice is likely to be caused by mouse phagocytic activity against human xenografts in the BRG-HIS mouse model [19, 21, 24]. Depletion of mouse phagocytes using clodronate-containing liposomes has been shown to enhance human hematopoietic cell accumulation in various HIS mouse settings [4, 63]. Further evidence for a major role of phagocytic cells in limiting human cell engraftment in BRG mice comes from analysis of the phagocytosis-inhibiting receptor SIRPα. CD47, the ligand for SIRPα, is broadly expressed on hematopoietic and nonhematopoietic cells [64, 65]. Mouse phagocytes in the BRG mice are unable to integrate signals from human CD47, whereas in NOD mice these phagocytes express a human CD47-compatible allele of the phagocyte-inhibiting SIRPα receptor [19].

Full inactivation of antihuman phagocytic activity through optimal hCD47/$SIRP\alpha^{NOD}$ interactions thus explains the relatively high degree of human hematopoietic cell accumulation in NSG/NOG mice. We and others have demonstrated that a similar level of human cell accumulation can be obtained in the BRG recipient mice, either by transgenic expression of human SIRPα [21], enforced expression of mouse CD47 DNA into hHSPC prior to transplantation, or by using congenic BRG $SIRP\alpha^{NOD}$ (BRGS) mice [24]. We have shown that human T cells, NK cells, and hHSPC are particularly sensitive to phagocyte-mediated cell removal and that

compatible CD47/SIRPα interactions leads to the selective accumulation of naive, resting T cells in the BRG background and severe reduction of the frequency of activated, inflammation-associated $CD4^+CD8^+$ T cells in secondary lymphoid organs [24]. Of note, similar genetic engineering or congenic approaches also allow for efficient human hematopoietic reconstitution in the "nonpermissive" C57BL/6 mouse background [24, 66].

Both NSG/NOG-HIS mice and BLT mice share a compatible CD47/SIRPα mediated tolerance of mouse phagocytes, and the difference between these two models in terms of peripheral human T cell accumulation should be caused by another feature. Considering the size of the thymopoiesis-supporting organ in BLT mice, it is likely that human mature T cell export to peripheral organs is higher in these mice. It is estimated that ~ 1–2 % of thymocytes are exported to peripheral lymphoid organs each day [67–69], and that reduced thymopoiesis—as seen for instance in BRG-HIS and NSG/NOG-HIS mice—can only be partially compensated by an enhancement of thymus cell export [70]. Furthermore, the human thymic organoid in BLT mice is a nonnegligible source of human IL-7, a central factor in the development and peripheral maintenance of naive T cells that is expressed by bone marrow stromal cells and thymus epithelial cells, as well as various cell types in secondary and tertiary lymphoid tissues [71–73]. Mouse IL-7 interacts with the human IL-7 receptor, but less efficiently than human IL-7. Therefore mouse IL-7 does not fully compensate for human IL-7 absence in HIS mice, and human IL-7 supplementation in humanized mice indeed potentiates human T-cell development and/or peripheral survival [6, 14, 47, 74].

10.6 Human Hematopoietic Cell Colonization of Lymph Nodes in HIS Mice

Human hematopoietic cell reconstitution of mouse lymph nodes (LN) is described as particularly limited in several HIS mouse models making use of the IL-2Rγ_c deficiency. This feature might be due to the inability of human hematopoietic cells (in particular human T cells) to migrate to these tissues, or to defective LN microarchitecture characteristics impairing entry/retention of hematopoietic cells. It is well documented that LN organogenesis requires signaling via the IL-7 receptor—composed of IL-7Rα and IL-2Rγ_c chains—and IL-2Rγ_c-deficient hosts therefore lack LN, with the notable exception of mesenteric LN. Deficiency in IL-7 signaling in IL-2Rγ_c-deficient mice consequently leads to defective generation of bone marrow-derived $CD4^+CD3^-IL\text{-}7R\alpha^+$ lymphoid tissue inducer (LTi) cells [75]. When coinjected to NOD.*scid* BLT mice—i.e., a HIS mouse model without LN organogenesis defect—human and mouse $CD4^+$ T cells can colonize mesenteric LN with similar efficiencies, whereas entry of human $CD4^+$ T cells to peripheral LN is 50 % less efficient [76]. A less efficient entry of human hematopoietic cells into mouse peripheral LN combined to specific microenvironment architecture defects in IL-2Rγ_c-deficient immuno-deficient mouse strains might thus explain the relatively

poor accumulation of human immune cells in such tissues. Chemokines promoting recruitment of hematopoietic cells to LN—such as CCL19, CCL20, or CCL21—are mostly expressed by nonhematopoietic tissues and show limited sequence identity (65–75%) between mouse and human [10, 77]. Such interspecies incompatibilities may limit the efficacy of human hematopoietic cell chemo-attraction to mouse peripheral LN.

Interestingly, studies reporting improved LN colonization by human hematopoietic cells are obtained in HIS mouse models generated with optimized humanization protocols. For instance, more systematic peripheral lymph node reconstitution as been described in BRG-HIS mice cotransplanted with UCB $CD34^+$ cells and autologous $CD2^-CD3^-CD34^-$ "support cells" [46], or in BRGS-HIS mice generated with FL $CD34^+CD38^-$ hHSPC [24]. As the most up-to-date HIS mouse models allow for enhanced accumulation of human hematopoietic cells, the potential emergence of discrete human hematopoiesis-derived subsets, such as human LTi-like cells, is also reported [24, 78]. It is not clear yet whether such human cells could impact on mouse LN organogenesis and/or organization over time, an interesting issue that deserves further investigations. Of note, BRG-HIS mouse LN reconstitution was recently described as a long-term, T-cell-dependent phenomenon, since LN containing only human B cells were never observed in such mice [46]. Overall, it can therefore not be excluded that long-term cross-talk between human hematopoietic cells and mouse stromal/epithelial components leads to beneficial features on LN tissue microarchitecture—a feature that may now be possible to explore in the latest generation of HIS mice.

10.7 Human T-Cell Repertoire and Selection in HIS Mice

The analysis of the T-cell repertoire available in various HIS mouse models has been performed, either by flow cytometry for the relative representation of the various TCR-Vβ families or by TCR-Vβ CDR3 length monitoring. CDR3 length analysis provides a fair indication of TCR-Vβ repertoire diversity. Overall, TCR-Vβ repertoire in the thymus or peripheral lymphoid organs of BLT or NSG/NOG mice was indistinguishable from the repertoire of control, human PBMCs, whereas BRG-HIS mice exhibited a more restricted, oligoclonal repertoire [11, 23, 30, 48, 51, 57, 79–81]. This observation was consistent with the notion that the NOD-based HIS mouse models are more permissive to the accumulation of human hematopoietic cells than the BRG strain, as discussed earlier. Furthermore, oligoclonality of the TCR-Vβ repertoire in BRG-HIS mice is probably further reinforced by the fact that human T cells are cycling in an extensive manner in this model [55], a feature that is corrected in presence of compatible CD47/SIRPα interactions, like in BRGS-HIS mice [24].

The exact nature of MHC restricting elements for HIS mouse human T cells remains a matter of debate. It is usually considered that positive selection of developing thymocytes is mostly mediated by cortical thymic epithelial cells. Still, there are experimental conditions in which alternative positive selection pathways might

exist, based on interactions with hematopoiesis-derived cells. For instance, *nu/nu* H-2^b mice receiving a H-2^k thymus graft or a rat thymus xenograft mostly mount a H-2^b-restricted anti-LCMV cytotoxic T cell response [82]. Furthermore, *nu/nu* H-2^b mice receiving a H-2^k thymus graft and partially reconstituted with T cell incompetent (Rag1$^{-/-}$) H-2^k bone marrow progenitor cells were able to mount T cell responses restricted to both host and donor MHC molecules [82].

These results tend to argue in favor of flexibility during the process of T-cell selection, and the mouse/human chimeric composition of the hematopoietic compartment in HIS mice should deliver a mixed H-2/HLA restriction of the human T-cell repertoire. Detection of H-2 vs. HLA-restricted antigen-specific T-cell responses further depends on other aspects, such as the niche in which the antigen is presented (e.g., specific cell subsets supporting the replication of a live pathogen) and the technical tools used to detect a T-cell response (e.g., tetramers, peptide pools), which might be not ideal in some specific HIS mouse situations. HLA-restricted, antigen-specific human CD8$^+$ T-cells responses can be detected in BLT or HLA-expressing HIS mice infected with human-specific, lymphotropic viruses such as HIV, EBV, or dengue virus [31, 83–86]. It remains unclear though whether the HLA molecules expressed by the mouse recipients should match with the HLA haplotype of the hHSPC donor cells for maximized efficacy.

Last, the question of T cell negative selection in HIS mice also remains particularly elusive. In theory, deletion of human T cells reactive to mouse tissue antigens should be occurring in the mouse thymus, based on interactions with AIRE-expressing medullary thymic epithelial cells. Such a feature should not be observed in the human thymic organoid of BLT mice, which would by definition only permit negative selection based on expression of human genes, therefore fitting with the relative dichotomy between BLT and BRG/NSG/NOG HIS mouse models as far as GVHD incidence is concerned [34–36]. Still, functional tolerance could also be imposed through the generation of human regulatory T cells, for instance in the thymus via the interactions with mouse and/or human DC [36, 87, 88], but this point remains to be clarified.

10.8 Improving Human T-Cell Biology in HIS Mice

All current HIS mouse models share similar limitations that provide leads for the identification of the next HIS mouse optimization increment. In steady state conditions, the number of human hematopoietic cells present in central and peripheral organs is relatively low, lymph node organogenesis is limited but appears to be connected to T-cell reconstitution levels. The concentration of plasma immunoglobulins is low, very variable between individual HIS mice and usually represents ~1–10% of normal mouse and human levels. Last, immune responses in HIS mice are most of the time faint, with high-interindividual variability. In particular, B-cell responses are characterized by weak immunoglobulin isotype switch to IgG and low frequency of somatic hypermutations. High inter- and intramodel variability could in part be due to the very diverse reconstitution protocols used, when consid-

ering hHSPC origin, potential coinjection of support cell population and cytokine-based precultures, as well as housing conditions for mouse colonies.

In this context, a more careful identification and description of the various T cell subsets present in HIS mice would be of importance to determine for instance to what extent T_{H17} [89] or follicular helper T (T_{FH}) cells are actually present and functional in humanized mice. The T_{FH} subset is of particular interest, as it plays a critical role in the triggering of the germinal center formation, antigen-specific B-cell activation and antigen-specific maintenance [90, 91]. The development of T_{FH} cells is strictly dependent on the expression of the inducible T-cell costimulator (ICOS) and the cytokine IL-21, which expression pattern might be suboptimal in HIS mice. Fine-tuned supplementation of HIS mice with ICOS and IL-21 might be a way to ensure the proper generation of T_{FH} cells around immunization procedures. The T_{FH} cells express the B cell follicle homing receptor CXCR5, and it is also a possibility that the appropriate chemokine ligand CXCL13 (in theory of hematopoietic cell origin) is not properly expressed in HIS mice.

Mice and humans hematopoietic systems differ significantly and exhibit profound interspecies incompatibilities that directly impact on the construction of HIS mouse models [6, 10, 92]. In some cases, specific murine mouse products may limit per se the mouse colonization by human cells and therefore have to be invalidated. For instance, deletion of the mouse Flt3/Flk2 receptor strongly reduces the number of murine DC, which cannot outcompete their human counterparts anymore, and renders human DC selectively reactive to exogenous treatment with the cross-reactive ligand FLT3-L [93].

Supplementation of HIS mice with human gene products might also be particularly helpful to optimize human T-cell development and/or function. Several supplementation strategies have been tested already for a variety of human cytokines, such as using exogenous products for injections [14, 47, 54, 94, 95], genetic engineering of the transplanted hHSPC [47], transgenic expression of human genes [96], replacement of mouse genes by their human equivalent [97–99], or hydrodynamic delivery of human DNA-encoding plasmids [74, 100]. All these approaches are valuable to provide support to human T cells during their development or triggering of an immune response, and could be integrated into vaccination protocols as immuno-stimulatory strategies to improve T-B-DC interplay. This would for instance be the case with human IL-15 agonist [80], human IL-12 [101], or human GM-CSF/IL-4 [102]. On a more general level, optimization of immunization procedures to potentiate cytotoxic and/or helper T-cell responses is required in the field of humanized mice, with a nonexhaustive list of parameters that might be addressed, such as antigen formulation and dose, immunization site, adjuvant type, sequence of successive immunizations, or time frames between immunizations.

In parallel, efforts are required to further improve humanization of the nonhematopoietic compartments in the mouse recipients used to generate HIS mice. This could be achieved via genetic approaches (which may be particularly laborious) or by exogenous supplementation of specific cell subsets using suspensions of purified human cells. Apart from the aforementioned LTi cells, lymphoid microarchitecture limitations might also explain the relatively defective germinal center reaction that can be observed in HIS mice. At least two nonhematopoietic cell populations might

have to be supplemented in HIS mice to permit the optimal initiation of human immune responses. First, it is very likely that the nonhematopoietic follicular dendritic cells (FDC) of HIS mice are of murine origin and might therefore be unable to efficiently crosstalk with human leukocytes. Considering the critical role of FDC in the organization of lymphoid microarchitecture and support to B cell memory [103, 104], it seems rather critical to establish a human FDC compartment in HIS mice. Of note, the murine FDC precursor was very recently identified as a perivascular progenitor cell expressing the platelet-derived growth factor receptor β [105], and we expect that the identification and inoculation of human FDC progenitor cells into HIS mice will be a valuable approach to obtain improved human immune cell functions. Last, the fibroblastic reticular cell (FRC) network, which is known to be critical for the migration and maintenance of T cells in lymphoid organs, is probably heavily disturbed in HIS mice, as observed in lymphopenic HIV-infected patients [106]. Treatment of the animals with lymphotoxin-β (LTβ), a key factor for FRC network maintenance, might represent a valuable strategy to positively impact on lymphoid micro-architecture in HIS mice. Alternatively, the in vitro generation of FRC from mesenchymal stem cells [107] could represent a valuable source for in vivo supplementation of HIS mice.

10.9 Concluding Remarks

Development and function of the human hematopoietic xenograft, in particular human T cells, in HIS mouse models have been strongly improved over the past decade. It is now possible to detect antigen-specific T- and B-cell responses using a variety of assays. Nevertheless, the frequency, intensity, and quality of these responses are still very weak when compared to responses in humans and supplementary optimization increments are required to obtain immune responses that are systematic, robust, and accurate. Considering the recent progress made in the field, one can be optimistic about what the coming years will deliver in further refinements and improvements of HIS mouse models to a point that they can serve as a robust and useful preclinical platform to address human unmet medical needs.

Acknowledgments We thank Dr. Mireille Centlivre for valuable suggestions and critical reading of the manuscript.

References

1. McCune JM, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science. 1988;241(4873):1632–9.
2. Kamel-Reid S, Dick JE. Engraftment of immune-deficient mice with human hematopoietic stem cells. Science. 1988;242(4886):1706–9.

3. Mosier DE, Gulizia RJ, Baird SM, Wilson DB. Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature. 1988;335(6187):256–9.
4. Legrand N, Weijer K, Spits H. Experimental models to study development and function of the human immune system in vivo. J Immunol. 2006;176(4):2053–8.
5. Legrand N, Ploss A, Balling R, Becker PD, Borsotti C, Brezillon N, et al. Humanized mice for modeling human infectious disease: challenges, progress, and outlook. Cell Host Microbe. 2009;6(1):5–9.
6. Manz MG. Human-hemato-lymphoid-system mice: opportunities and challenges. Immunity. 2007;26(5):537–41.
7. Manz MG, Di Santo JP. Renaissance for mouse models of human hematopoiesis and immunobiology. Nat Immunol. 2009;10(10):1039–42.
8. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nature reviews Immunology. 2012;12(11):786–98. (Epub 2012/10/13).
9. Ito R, Takahashi T, Katano I, Ito M. Current advances in humanized mouse models. Cell Mol Immunol. 2012;9(3):208–14. (Epub 2012/02/14).
10. Rongvaux A, Takizawa H, Strowig T, Willinger T, Eynon EE, Flavell RA, et al. Human hemato-lymphoid system mice: current use and future potential for medicine. Annu Rev Immunol. 2013;31:635–74. (Epub 2013/01/22).
11. Hiramatsu H, Nishikomori R, Heike T, Ito M, Kobayashi K, Katamura K, et al. Complete reconstitution of human lymphocytes from cord blood $CD34^+$ cells using the NOD/SCID/gammacnull mice model. Blood. 2003;102(3):873–80.
12. Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood. 2002;100(9):3175–82.
13. Yahata T, Ando K, Nakamura Y, Ueyama Y, Shimamura K, Tamaoki N, et al. Functional human T lymphocyte development from cord blood $CD34^+$ cells in nonobese diabetic/Shi-scid, IL-2 receptor gamma null mice. J Immunol. 2002;169(1):204–9.
14. Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol. 2005;174(10):6477–89.
15. Ishikawa F, Yasukawa M, Lyons B, Yoshida S, Miyamoto T, Yoshimoto G, et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor {gamma} chainnull mice. Blood. 2005;106(5):1565–73.
16. Notta F, Doulatov S, Dick JE. Engraftment of human hematopoietic stem cells is more efficient in female NOD/SCID/IL-2Rgc-null recipients. Blood. 2010;115(18):3704–7. (Epub 2010/03/09).
17. Majeti R, Park CY, Weissman IL. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. Cell Stem Cell. 2007;1(6):635–45.
18. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, et al. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol. 1995;154(1):180–91.
19. Takenaka K, Prasolava TK, Wang JC, Mortin-Toth SM, Khalouei S, Gan OI, et al. Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells. Nat Immunol. 2007;8(12):1313–23.
20. Ito R, Katano I, Ida-Tanaka M, Kamisako T, Kawai K, Suemizu H, et al. Efficient xenoengraftment in severe immunodeficient NOD/Shi-scid IL2rgammanull mice is attributed to a lack of $CD11c^+B220^+CD122^+$ cells. J Immunol. 2012;189(9):4313–20. (Epub 2012/09/29).
21. Strowig T, Rongvaux A, Rathinam C, Takizawa H, Borsotti C, Philbrick W, et al. Transgenic expression of human signal regulatory protein alpha in $Rag2^{-/-}gamma(c)^{-/-}$ mice improves engraftment of human hematopoietic cells in humanized mice. Proc Natl Acad Sci U S A. 2011;108(32):13218–23. (Epub 2011/07/27).
22. Gimeno R, Weijer K, Voordouw A, Uittenbogaart CH, Legrand N, Alves NL, et al. Monitoring the effect of gene silencing by RNA interference in human $CD34^+$ cells injected into newborn $RAG2^{-/-}$ $gammac^{-/-}$ mice: functional inactivation of p53 in developing T cells. Blood. 2004;104(13):3886–93.

23. Traggiai E, Chicha L, Mazzucchelli L, Bronz L, Piffaretti JC, Lanzavecchia A, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. Science. 2004;304(5667):104–7.
24. Legrand N, Huntington ND, Nagasawa M, Bakker AQ, Schotte R, Strick-Marchand H, et al. Functional CD47/signal regulatory protein alpha (SIRP(alpha)) interaction is required for optimal human T- and natural killer- (NK) cell homeostasis in vivo. Proc Natl Acad Sci U S A. 2011;108(32):13224–9. (Epub 2011/07/27).
25. Mazurier F, Fontanellas A, Salesse S, Taine L, Landriau S, Moreau-Gaudry F, et al. A novel immunodeficient mouse model–RAG2 x common cytokine receptor gamma chain double mutants—requiring exogenous cytokine administration for human hematopoietic stem cell engraftment. J Interferon Cytokine Res. 1999;19(5):533–41.
26. Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature. 1983;301(5900):527–30.
27. Bosma GC, Fried M, Custer RP, Carroll A, Gibson DM, Bosma MJ. Evidence of functional lymphocytes in some (leaky) scid mice. J Exp Med. 1988;167(3):1016–33.
28. Bosma MJ. B and T cell leakiness in the scid mouse mutant. Immunodefic Rev. 1992;3(4):261–76. (Epub 1992/01/01).
29. Krowka JF, Sarin S, Namikawa R, McCune JM, Kaneshima H. Human T cells in the SCID-hu mouse are phenotypically normal and functionally competent. J Immunol. 1991;146(11):3751–6.
30. Melkus MW, Estes JD, Padgett-Thomas A, Gatlin J, Denton PW, Othieno FA, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med. 2006;12(11):1316–22.
31. Brainard DM, Seung E, Frahm N, Cariappa A, Bailey CC, Hart WK, et al. Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. J Virol. 2009;83(14):7305–21.
32. Lan P, Tonomura N, Shimizu A, Wang S, Yang YG. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and $CD34^+$ cell transplantation. Blood. 2006;108(2):487–92.
33. Denton PW, Garcia JV. Mucosal HIV-1 transmission and prevention strategies in BLT humanized mice. Trends Microbiol. 2012;20(6):268–74. (Epub 2012/04/17).
34. Greenblatt MB, Vbranac V, Tivey T, Tsang K, Tager AM, Aliprantis AO. Graft versus host disease in the bone marrow, liver and thymus humanized mouse model. PloS One. 2012;7(9):e44664. (Epub 2012/09/08).
35. Covassin L, Jangalwe S, Jouvet N, Laning J, Burzenski L, Shultz LD, et al. Human immune system development and survival of NOD-scid IL2rgamma (NSG) mice engrafted with human thymus and autologous hematopoietic stem cells. Clin Exp Immunol. 2013; 174(3):372–88. (Epub 2013/07/23).
36. Lockridge JL, Zhou Y, Becker YA, Ma S, Kenney SC, Hematti P, et al. Mice engrafted with human fetal thymic tissue and hematopoietic stem cells develop pathology resembling chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2013;19(9):1310–22. (Epub 2013/06/29).
37. Scheeren FA, Nagasawa M, Weijer K, Cupedo T, Kirberg J, Legrand N, et al. T cell-independent development and induction of somatic hypermutation in human $IgM^+IgD^+CD27^+$ B cells. J Exp Med. 2008;205(9):2033–42.
38. Dik WA, Pike-Overzet K, Weerkamp F, de Ridder D, de Haas EF, Baert MR, et al. New insights on human T cell development by quantitative T cell receptor gene rearrangement studies and gene expression profiling. J Exp Med. 2005;201(11):1715–23.
39. Res P, Martinez-Caceres E, Cristina Jaleco A, Staal F, Noteboom E, Weijer K, et al. $CD34^+CD38$dim cells in the human thymus can differentiate into T, natural killer, and dendritic cells but are distinct from pluripotent stem cells. Blood. 1996;87(12):5196–206.
40. Blom B, Spits H. Development of human lymphoid cells. Annu Rev Immunol. 2006;24:287–320.

41. Blom B, Verschuren MC, Heemskerk MH, Bakker AQ, van Gastel-Mol EJ, Wolvers-Tettero IL, et al. TCR gene rearrangements and expression of the pre-T cell receptor complex during human T-cell differentiation. Blood. 1999;93(9):3033–43.
42. Haddad R, Guardiola P, Izac B, Thibault C, Radich J, Delezoide AL, et al. Molecular characterization of early human T/NK and B-lymphoid progenitor cells in umbilical cord blood. Blood. 2004;104(13):3918–26.
43. Hao QL, Zhu J, Price MA, Payne KJ, Barsky LW, Crooks GM. Identification of a novel, human multilymphoid progenitor in cord blood. Blood. 2001;97(12):3683–90. (Epub 2001/06/05).
44. Haddad R, Guimiot F, Six E, Jourquin F, Setterblad N, Kahn E, et al. Dynamics of thymus-colonizing cells during human development. Immunity. 2006;24(2):217–30.
45. Lepus CM, Gibson TF, Gerber SA, Kawikova I, Szczepanik M, Hossain J, et al. Comparison of human fetal liver, umbilical cord blood, and adult blood hematopoietic stem cell engraftment in NOD-scid/gammac$^{-/-}$, Balb/c-Rag1$^{-/-}$gammac$^{-/-}$, and C.B-17-scid/bg immunodeficient mice. Hum Immunol. 2009;70(10):790–802.
46. Lang J, Kelly M, Freed BM, McCarter MD, Kedl RM, Torres RM, et al. Studies of lymphocyte reconstitution in a humanized mouse model reveal a requirement of T cells for human B cell maturation. J Immunol. 2013;190(5):2090–101. (Epub 2013/01/22).
47. van Lent AU, Dontje W, Nagasawa M, Siamari R, Bakker AQ, Pouw SM, et al. IL-7enhances thymic human T cell development in "human immune system" Rag2$^{-/-}$IL-2Rgammac$^{-/-}$ mice without affecting peripheral T cell homeostasis. J Immunol. 2009;183(12):7645–55.
48. Denton PW, Nochi T, Lim A, Krisko JF, Martinez-Torres F, Choudhary SK, et al. IL-2 receptor gamma-chain molecule is critical for intestinal T-cell reconstitution in humanized mice. Mucosal Immunol. 2012;5(5):555–66. (Epub 2012/05/10).
49. Sun Z, Denton PW, Estes JD, Othieno FA, Wei BL, Wege AK, et al. Intrarectal transmission, systemic infection, and CD4$^+$ T cell depletion in humanized mice infected with HIV-1. J Exp Med. 2007;204(4):705–14.
50. Joo SY, Chung YS, Choi B, Kim M, Kim JH, Jun TG, et al. Systemic human T cell developmental processes in humanized mice cotransplanted with human fetal thymus/liver tissue and hematopoietic stem cells. Transplantation. 2012;94(11):1095–102. (Epub 2012/12/12).
51. Huntington ND, Alves NL, Legrand N, Lim A, Strick-Marchand H, Plet A, et al. Autonomous and extrinsic regulation of thymopoiesis inhuman immune system (HIS) mice. Eur J Immunol. 2011;41(10):2883–93. (Epub 2011/07/09).
52. Spits H. Development of alphabeta T cells in the human thymus. Nat Rev Immunol. 2002;2(10):760–72.
53. Pek EA, Chan T, Reid S, Ashkar AA. Characterization and IL-15 dependence of NK cells in humanized mice. Immunobiology. 2011;216(1–2):218–24. (Epub 2010/07/16).
54. Huntington ND, Legrand N, Alves NL, Jaron B, Weijer K, Plet A, et al. IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. J Exp Med. 2009;206:25–34.
55. Legrand N, Cupedo T, van Lent AU, Ebeli MJ, Weijer K, Hanke T, et al. Transient accumulation of human mature thymocytes and regulatory T cells with CD28 superagonist in "human immune system" Rag2($^{-/-}$)gammac($^{-/-}$) mice. Blood. 2006;108(1):238–45.
56. Onoe T, Kalscheuer H, Danzl N, Chittenden M, Zhao G, Yang YG, et al. Human natural regulatory T cell development, suppressive function, and postthymic maturation in a humanized mouse model. J Immunol. 2011;187(7):3895–903. (Epub 2011/08/31).
57. Kalscheuer H, Danzl N, Onoe T, Faust T, Winchester R, Goland R, et al. A model for personalized in vivo analysis of human immune responsiveness. Sci Transl Med. 2012;4(125):125ra30. (Epub 2012/03/17).
58. Billerbeck E, Barry WT, Mu K, Dorner M, Rice CM, Ploss A. Development of human CD4$^+$FoxP3$^+$ regulatory T cells in human stem cell factor-, granulocyte-macrophage colony-stimulating factor-, and interleukin-3-expressing NOD-SCID IL2Rgamma(null) humanized mice. Blood. 2011;117(11):3076–86. (Epub 2011/01/22).

59. Jiang Q, Zhang L, Wang R, Jeffrey J, Washburn ML, Brouwer D, et al. FoxP3^{+}CD4^{+} regulatory T cells play an important role in acute HIV-1 infection in humanized Rag2$^{-/-}$gammaC$^{-/-}$ mice in vivo. Blood. 2008;112(7):2858–68.
60. Becker PD, Legrand N, van Geelen CM, Noerder M, Huntington ND, Lim A, et al. Generation of human antigen-specific monoclonal IgM antibodies using vaccinated "human immune system" mice. PloS one. 2010;5(10).
61. Vuyyuru R, Patton J, Manser T. Human immune system mice: current potential and limitations for translational research on human antibody responses. Immunol Res. 2011;51(2–3):257–66. (Epub 2011/11/01).
62. Parel Y, Chizzolini C. CD4^{+} CD8^{+} double positive (DP) T cells in health and disease. Autoimmun Rev. 2004;3(3):215–20.
63. Rozemuller H, Knaan-Shanzer S, Hagenbeek A, van Bloois L, Storm G, Martens AC. Enhanced engraftment of human cells in RAG2/gammac double-knockout mice after treatment with CL2MDP liposomes. Exp Hematol. 2004;32(11):1118–25.
64. Brown EJ, Frazier WA. Integrin-associated protein (CD47) and its ligands. Trends Cell Biol. 2001;11(3):130–5.
65. Sarfati M, Fortin G, Raymond M, Susin S. CD47 in the immune response: role of thrombospondin and SIRP-alpha reverse signaling. Curr Drug Targets. 2008;9(10):842–50.
66. Yamauchi T, Takenaka K, Urata S, Shima T, Kikushige Y, Tokuyama T, et al. Polymorphic Sirpa is the genetic determinant for NOD-based mouse lines to achieve efficient human cell engraftment. Blood. 2013;121(8):1316–25. (Epub 2013/01/08).
67. Berzins SP, Godfrey DI, Miller JF, Boyd RL. A central role for thymic emigrants in peripheral T cell homeostasis. Proc Natl Acad Sci U S A. 1999;96(17):9787–91.
68. Gabor MJ, Scollay R, Godfrey DI. Thymic T cell export is not influenced by the peripheral T cell pool. Eur J Immunol. 1997;27(11):2986–93. (Epub 1997/12/12).
69. Scollay RG, Butcher EC, Weissman IL. Thymus cell migration. Quantitative aspects of cellular traffic from the thymus to the periphery in mice. Eur J Immunol. 1980;10(3):210–8. (Epub 1980/03/01).
70. Almeida AR, Borghans JA, Freitas AA. T cell homeostasis: thymus regeneration and peripheral T cell restoration in mice with a reduced fraction of competent precursors. J Exp Med. 2001;194(5):591–9.
71. Alves NL, Huntington ND, Mention JJ, Richard-Le Goff O, Di Santo JP. Cutting edge: a thymocyte-thymic epithelial cell cross-talk dynamically regulates intrathymic IL-7 expression in vivo. J Immunol. 2010;184(11):5949–53.
72. Mazzucchelli R, Durum SK. Interleukin-7 receptor expression: intelligent design. Nat Rev Immunology. 2007;7(2):144–54.
73. Huang HY, Luther SA. Expression and function of interleukin-7 in secondary and tertiary lymphoid organs. Semin Immunol. 2012;24(3):175–89. (Epub 2012/03/27).
74. O'Connell RM, Balazs AB, Rao DS, Kivork C, Yang L, Baltimore D. Lentiviral vector delivery of human interleukin-7 (hIL-7) to human immune system (HIS) mice expands T lymphocyte populations. PloS One. 2010;5(8):e12009.
75. Cupedo T, Mebius RE. Cellular interactions in lymph node development. J Immunol. 2005;174(1):21–5.
76. Murooka TT, Deruaz M, Marangoni F, Vrbanac VD, Seung E, von Andrian UH, et al. HIV-infected T cells are migratory vehicles for viral dissemination. Nature. 2012;490(7419):283–7. (Epub 2012/08/03).
77. Bromley SK, Mempel TR, Luster AD. Orchestrating the orchestrators: chemokines in control of T cell traffic. Nat Immunol. 2008;9(9):970–80. (Epub 2008/08/20).
78. Bernink JH, Peters CP, Munneke M, Te Velde AA, Meijer SL, Weijer K, et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nat Immunol. 2013;14(3):221–9. (Epub 2013/01/22).
79. Pham HP, Manuel M, Petit N, Klatzmann D, Cohen-Kaminsky S, Six A, et al. Half of the T-cell repertoire combinatorial diversity is genetically determined in humans and humanized mice. Eur J Immunol. 2012;42(3):760–70. (Epub 2011/11/23).

80. Huntington ND, Alves NL, Legrand N, Lim A, Strick-Marchand H, Mention JJ, et al. IL-15 transpresentation promotes both human T-cell reconstitution and T-cell-dependent antibody responses in vivo. Proc Natl Acad Sci U S A. 2011;108(15):6217–22. (Epub 2011/03/30).
81. Marodon G, Desjardins D, Mercey L, Baillou C, Parent P, Manuel M, et al. High diversity of the immune repertoire in humanized NOD.SCID.gamma c−/− mice. Eur J Immunol. 2009;39(8):2136–45. (Epub 2009/07/03).
82. Zinkernagel RM, Althage A. On the role of thymic epithelium vs. bone marrow-derived cells in repertoire selection of T cells. Proc Natl Acad Sci U S A. 1999;96(14):8092–7.
83. Sato Y, Nagata S, Takiguchi M. Effective elicitation of human effector CD8(+) T cells in HLA-B*51:01 transgenic humanized mice after infection with HIV-1. PloS One. 2012;7(8):e42776. (Epub 2012/08/11).
84. Strowig T, Gurer C, Ploss A, Liu YF, Arrey F, Sashihara J, et al. Priming of protective T cell responses against virus-induced tumors in mice with human immune system components. J Exp Med. 2009;206(6):1423–34.
85. Shultz LD, Saito Y, Najima Y, Tanaka S, Ochi T, Tomizawa M, et al. Generation of functional human T-cell subsets with HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL2r gamma(null) humanized mice. Proc Natl Acad Sci U S A. 2010;107(29):13022–7.
86. Jaiswal S, Pearson T, Friberg H, Shultz LD, Greiner DL, Rothman AL, et al. Dengue virus infection and virus-specific HLA-A2 restricted immune responses in humanized NOD-scid IL2rgammanull mice. PloS One. 2009;4(10):e7251. (Epub 2009/10/06).
87. Jung KC, Park CG, Jeon YK, Park HJ, Ban YL, Min HS, et al. In situ induction of dendritic cell-based T cell tolerance in humanized mice and nonhuman primates. J Exp Med. 2011;208(12):2477–88. (Epub 2011/10/26).
88. Watanabe N, Wang YH, Lee HK, Ito T, Cao W, Liu YJ. Hassall's corpuscles instruct dendritic cells to induce $CD4^{+}CD25^{+}$ regulatory T cells in human thymus. Nature. 2005;436(7054):1181–5.
89. Rajesh D, Zhou Y, Jankowska-Gan E, Roenneburg DA, Dart ML, Torrealba J, et al. Th1 and Th17 immunocompetence in humanized NOD/SCID/IL2rgammanull mice. Hum Immunol. 2010;71(6):551–9. (Epub 2010/03/20).
90. King C. New insights into the differentiation and function of T follicular helper cells. Nature reviews Immunology. 2009;9(11):757–66.
91. Rodriguez-Bayona B, Ramos-Amaya A, Bernal J, Campos-Caro A, Brieva JA. Cutting edge: IL-21 derived from human follicular helper T cells acts as a survival factor for secondary lymphoid organ, but not for bone marrow, plasma cells. J Immunol. 2012;188(4):1578–81. (Epub 2012/01/18).
92. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004;172(5):2731–8.
93. Di Santo JP Mention JJ. Dendritic cell-boosted humanized immune system mice. PCT/US2010/029800. WO 2010/115115 A1. Patent. 2010.
94. Bjorkstrom NK, Riese P, Heuts F, Andersson S, Fauriat C, Ivarsson MA, et al. Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. Blood. 2010;116(19):3853–64.
95. Wils EJ, Rombouts EJ, van Mourik I, Spits H, Legrand N, Braakman E, et al. Stem cell factor consistently improves thymopoiesis after experimental transplantation of murine or human hematopoietic stem cells in immunodeficient mice. J Immunol. 2011;187(6):2974–81. (Epub 2011/08/24).
96. Takagi S, Saito Y, Hijikata A, Tanaka S, Watanabe T, Hasegawa T, et al. Membrane-bound human SCF/KL promotes in vivo human hematopoietic engraftment and myeloid differentiation. Blood. 2012;119(12):2768–77. (Epub 2012/01/27).
97. Willinger T, Rongvaux A, Strowig T, Manz MG, Flavell RA. Improving human hemato-lymphoid-system mice by cytokine knock-in gene replacement. Trends Immunol. 2011;32(7):321–7. (Epub 2011/06/24).

98. Willinger T, Rongvaux A, Takizawa H, Yancopoulos GD, Valenzuela DM, Murphy AJ, et al. Human IL-3/GM-CSF knock-in mice support human alveolar macrophage development and human immune responses in the lung. Proc Natl Acad Sci U S A. 2011;108(6):2390–5. (Epub 2011/01/26).
99. Rongvaux A, Willinger T, Takizawa H, Rathinam C, Auerbach W, Murphy AJ, et al. Human thrombopoietin knockin mice efficiently support human hematopoiesis in vivo. Proc Natl Acad Sci U S A. 2011;108(6):2378–83. (Epub 2011/01/26).
100. 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 U S A. 2009;106(51):21783–8.
101. De Giovanni C, Nicoletti G, Landuzzi L, Romani F, Croci S, Palladini A, et al. Human responses against HER-2-positive cancer cells in human immune system-engrafted mice. Br J Cancer. 2012;107(8):1302–09. (Epub 2012/08/30).
102. Chen Q, He F, Kwang J, Chan JK, Chen J. GM-CSF and IL-4 stimulate antibody responses in humanized mice by promoting T, B, and dendritic cell maturation. J Immunol. 2012;189(11):5223–9. (Epub 2012/10/24).
103. Vinuesa CG, Linterman MA, Goodnow CC, Randall KL. T cells and follicular dendritic cells in germinal center B-cell formation and selection. Immunol Rev. 2010;237(1):72–89.
104. Cyster JG. B cell follicles and antigen encounters of the third kind. Nat Immunol. 2010;11(11):989–96. (Epub 2010/10/21).
105. Krautler NJ, Kana V, Kranich J, Tian Y, Perera D, Lemm D, et al. Follicular dendritic cells emerge from ubiquitous perivascular precursors. Cell. 2012;150(1):194–206. (Epub 2012/07/10).
106. Zeng M, Paiardini M, Engram JC, Beilman GJ, Chipman JG, Schacker TW, et al. Critical role for CD4 T cells in maintaining lymphoid tissue structure for immune cell homeostasis and reconstitution. Blood. 2012;120:1856–67. (Epub 2012/05/23).
107. Ame-Thomas P, Maby-El Hajjami H, Monvoisin C, Jean R, Monnier D, Caulet-Maugendre S, et al. Human mesenchymal stem cells isolated from bone marrow and lymphoid organs support tumor B-cell growth: role of stromal cells in follicular lymphoma pathogenesis. Blood. 2007;109(2):693–702. (Epub 2006/09/21).