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Experimental Models to Study Development and Function of the Human Immune System In Vivo

Nicolas Legrand,* Kees Weijer,*† and Hergen Spits†*

The study of development and function of the immune system in vivo has made intensive use of animal models, but performing such work in humans is difficult for experimental, practical, and ethical reasons. Confronted with this scientific challenge, several pioneering groups have developed in the late 1980s mouse models of human immune system development. Although these experimental approaches were proven successful and useful, they were suffering from limitations due to xenograft transplantation barriers. By reviewing the characteristics of the successive models over the last 20 years, it becomes apparent that screening of potentially interesting mouse strains and usage of combinations of genetic deficiencies has led to major advances. This is particularly true for human T cell development in the murine thymus. This review will focus on these advances and the potential future improvements that remain to be accomplished. The Journal of Immunology, 2006, 176: 2053–2058.

During the last decades, there have been constant efforts to design animal models permitting an in vivo approach of the human immune system. Confronted with the exploding HIV pandemic in the 1980s, the need for a small, easy-to-handle animal model of human lymphocyte development arose (1).

The discovery of the scid mutation in the C.B-17 mouse strain (2) opened the door to extensive research for the design of such animal models. These C.B-17 mice harbor a mutation of the prkdc gene involved in rearrangement of TCR and Ig genes and therefore mostly lack mature T and B lymphocytes (3). The first report of xenogeneic transplantation of human PBL in C.B-17 SCID mice was published in 1988, and only the transferred mature T and B cells survived during a limited period of time in this human (hu)–PBL-SCID model (4). Obviously, the presence of human pluripotent progenitor cells is required to ensure development of multiple hemopoietic lineages. Therefore, human fetal thymic lobes and pieces of fetal liver (FL) were transplanted under the kidney capsule in C.B-17 SCID mice (5). In this difficult experimental setting, the developing human PBL found were almost exclusively T cells. These cells migrated from the human thymus graft to the periphery after 3–4 wk. However, only one-third to one-half of the animals had a detectable population of human cells in the blood (0.7% huCD45+ cells on average in the lymphocyte gate) 3–5 mo posttransplant (6). This SCID-hu (thymus (Thy)/liver (Liv)) model has been extensively used for the analysis of human hemopoiesis, especially T cells, and as an animal model of HIV infection (1).

In another approach, sublethally irradiated C.B-17 SCID mice were repopulated with progenitor cells from human bone marrow (BM) or umbilical cord blood (UCB). Such progenitors contain cells with SCID repopulating ability (SRC), and this system was therefore referred to as hu-SRC-SCID model (7, 8). Similarly, human myeloid differentiation was obtained after transfer of human total BM cells into athymic NK-deficient beigel nude/lsxid SCID mice (9). Although UCB cells appeared to be more efficient, only low efficiencies and levels of engraftment were achieved. Furthermore, T cell development was always extremely limited, supporting the original observation of a virtual absence of human cells in the murine thymus of SCID-hu (Thy/Liv) mice (5).

The genetic background of the recipient animals appears to determine the success of engraftment (for review, see Ref. 10). For example, NOD/SCID mice were a more receptive strain for human cell engraftment than the C.B-17 SCID mice (11, 12). Mice in the NOD genetic background exhibit deficits in 1) NK cell activity, at least partially due to impairment of the activating receptor NKG2D (13); 2) complement activation, due to C5 deficiency (14); and 3) LPS-induced production of IL-1 by macrophages (15). Unfortunately, NOD/SCID mice show an elevated tendency to develop thymic lymphomas, hindering the feasibility of long-term studies (16). When NOD/SCID mice were engrafted with CD34+ cells from human UCB or adult BM, human T cell progenitors could be identified and isolated from the BM of the animals, and these cells gave rise to T cells in fetal thymic organ cultures (17). Although T cell development occurred only rarely in NOD/SCID mice, ablation of NK cells by using the CD122/IL-2Rβ-specific Ab TM-B1 significantly increased the frequency of thymopoiesis in animals reconstituted with human UCB cells (18). Since the host murine NK cells appeared to be particularly limiting, human T cell

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2 Abbreviations used in this paper: hu, human; FL, fetal liver; BM, bone marrow; HIS, human immune system; HSC, hematopoietic stem cell; i.h., intrahepatic; SRC, SCID repopulating cell; UCB, umbilical cord blood; γc, common γ-chain; β2m, β2-microglobulin; SCF, stem cell factor; Thy, thymus; Liv, liver.
Identification of human progenitors that repopulate SCID mice and influence of cytokines

SCID mouse models have been extensively used to assay human hematopoietic stem cells (HSC) candidates in an in vivo setting (for reviews, see Refs. 23 and 24). Using the SCID-hu (Thy/Li) model, it was shown that human fetal bone marrow CD34<sup>high</sup> cells, which represent around 1% of BM cells (25), can generate T, B, and myeloid lineages (26). It was later described that only purified CD34<sup>+</sup>CD38<sup>−</sup> from human BM, and not their CD38<sup>+</sup> counterpart, representing 0.01% of BM or UCB (25) and up to 2.5% of FL (23) respectively, contain a SRC fraction that ensures long-term engraftment in NOD/SCID mice (27). Furthermore, HSC can be separated into short-term and long-term (the SRCs) repopulating cells. Short-term repopulating cells mainly give rise to myeloid and erythroid lineage cells, observed in a 2- to 8-wk window after engraftment, and are extremely NK sensitive since their engraftment and differentiation is only seen in NK cell-deficient (B2m<sup>−/−</sup>, γc<sup>−/−</sup>) or NK-depleted (anti-IL-2Rβ) NOD/SCID mice (28–30). In contrast, long-term repopulating cells transplanted into NOD/SCID mice do not disappear after 6–8 wk and develop predominantly into highly dividing B cells with a poor capacity of terminal differentiation. Indeed, the BM of such mice contains mostly CD34<sup>+</sup>CD19<sup>+</sup> pro-B cells and the spleen CD19<sup>+</sup>IgM<sup>+</sup> immature B cells unable to undergo class switch recombination (31–33).

The use of SCID models in combination with IL treatments also gave insight into the requirements for xenogeneic engraftment of human progenitors. HuIL-3 treatment, but not IL-2, IL-7, Flt-3 ligand, nor stem cell factor (SCF; kitL) alone, was shown to enhance the engraftment by human BM progenitors in beige/nude/xid mice from <1% huCD45<sup>+</sup> cells in the blood up to 4% (9, 34). Similarly, cotreatment with erythropoietin, SCF, and/or GM-CSF/IL-3 fusion protein in BM-transplanted C.B-17 SCID mice enhances the proportion of human cells from <1% to 10–15% in treated animals, with development of multiple hematological cell lineages (7). Such a treatment was ineffective when UCB cells were used instead of BM cells, since UCB already showed a high engraftment capacity (15% huCD45<sup>+</sup> cells in BM) in the absence of cytokines (8). Overall, the outcome of cells with SCID repopulating capacity appears to be highly heterogeneous, depending on the SCID model used, and supplementation by human cytokines may improve engraftment in these models.

**The human immune system (HIS) Rag2<sup>−/−</sup> γc<sup>−/−</sup> model**

Since C.B-17 scid/scid mice are not consistently devoid of T and B cells (35), this mouse model is suboptimal for the establishment of a complete HIS in vivo. Introduction of the NOD genetic background in the C.B-17 scid/scid mice resulted in improved engraftment. However, many of these mice develop thymic lymphomas in 5 mo, posing problems with long-term experiments. By deleting genes essential for the proper development of T, B, and NK cells, new mouse strains of interest were created. Of these, Rag2<sup>−/−</sup> γc<sup>−/−</sup> mice appeared to be of major interest because they lack T, B, and NK cells (36).

Initial work done by reconstituting adult Rag2<sup>−/−</sup> γc<sup>−/−</sup> mice with human stem cells from cord blood or FL was proven not successful as far as T cell development was concerned. Similar poor thymopoiesis (6 vs 18% animals with active human T cell development) was observed in BALB/c Rag2<sup>−/−</sup> γc<sup>−/−</sup> and NOD/SCID mice (18, 37). Treatment of CD34<sup>+</sup> UCB cell-reconstituted C57BL/6 Rag2<sup>−/−</sup> γc<sup>−/−</sup> mice with a mixture of IL-3, GM-CSF, and erythropoietin showed limited improvement of human engraftment (38). These observations suggested that the BM environment in Rag2<sup>−/−</sup> γc<sup>−/−</sup> mice lacks the capacity to produce growth factors necessary for optimal engraftment. A not mutually exclusive possibility is that the murine macrophages hinder the engraftment, because of noxious effects on human progenitors and their mature progeny, or niche occupancy.

Recently, Manz and coworkers (39) and our group (40) have administered human HSC to sublethally irradiated BALB/c Rag2<sup>−/−</sup> γc<sup>−/−</sup> (BALB-Rag/γ) newborn mice (Fig. 1). This approach has been proven successful and reliable, since >80% of the obtained HIS mice exhibit 10% or more human CD45<sup>+</sup> cells in peripheral blood and other lymphoid organs. Animals

**FIGURE 1.** Protocol for HIS mice production. Progenitor CD34<sup>+</sup> cells from various human origins (fetal, neonate, or adult) were isolated, sorted, and inoculated to sublethally irradiated newborn BALB/c Rag2<sup>−/−</sup> γc<sup>−/−</sup> mice. The obtained HIS mice exhibit de novo T cell differentiation in the murine thymus, as shown by CD4/CD8 flow cytometry plot on gated huCD45<sup>+</sup> thymocytes 8 wk after inoculation (2–10 × 10<sup>6</sup> human thymocytes).
reconstituted with CD34⁺ UCB cells have been referred to as “human adaptive immune system Rag₂⁻/⁻ γ⁺⁻ mice” (huAIS-RG) by the group of Manz and colleagues (41). All major immune cell subsets are produced de novo in the HIS (BALB-Ragγγ) mice within 6 wk, e.g., T, B, NK cells, monocytes, conventional, and plasmacytoid dendritic cells. The efficiency of reconstitution by i.p. injected HSC is extremely age dependent, since 1-day-old animals give rise to ~80% human cells in the blood 8 wk after reconstitution, whereas only ~30% human cells are obtained with 1-wk-old animals and <10% with 2-wk-old mice (40). We compared the effect of the route of injection of CD34⁺ FL cells, namely, i.p. (40) vs intrahepatic (i.h.) (39). When CD34⁺ FL cells were inoculated i.h., reconstitution was more reliable, but the effective level of human chimerism was not different between i.p. and i.h. routes (our unpublished observations). Routinely, the percentage of human cells in HIS (BALB-Ragγγ) mice 8 wk after reconstitution reaches >95% in thymus, >60% in blood and liver, and >50% in spleen and BM, although variability is observed. This variability is not due to the source of the CD34⁺ cells, namely, from 14 to 17 wk of gestation FL (40) or from UCB (39), but rather directly linked to the number of injected progenitor cells. As compared with the previously available models (Table I), reconstitution of newborn mice represented a major improvement, and this was also recently observed with NOD/SCID/γ⁺⁻ mice (which show no signs of lymphoma development) and, to a lesser extent, NOD/SCID/β²m⁻/⁻ mice (42, 43).

There are at least two possible explanations for the success of using newborn mice for reconstitution: 1) it is well documented that the size of the thymus decreases with age. This process of thymic involution might occur at an accelerated rate in adult Rag₂⁻/⁻ γ⁺⁻ mice, resulting in thymic rudiments unable to support human T cell development. 2) Another possibility is that phagocytic cells present in newborn mice, e.g., macrophages or neutrophils, are less limiting than those in adults, resulting in less resistance in newborns. The latter possibility is supported by observations showing efficient reconstitution in adult BALB/c Rag₂⁻/⁻ γ⁺⁻ mice with FL CD34⁺ cells (our unpublished observations) or CD34⁺ UCB cells (44), only if the recipient mice were previously treated with clodronate (Cl2MBP; dichloromethylene-bisphosphonate)-containing liposomes. These liposomes can be used as a tool to deliver drugs to phagocytic cells, especially macrophages, and the accumulation of clodronate leads to irreversible metabolic damages, which will eventually result in apoptosis (45). Treatment of adult BALB/c Rag₂⁻/⁻ γ⁺⁻ mice with clodronate-containing liposomes was already shown to improve the speed and efficiency of engraftment by human PBL (46). These observations indicate that phagocytic cells, presumably macrophages, are a particularly limiting factor for xenogeneic engraftment in adult Rag₂⁻/⁻ γ⁺⁻ mice. This could explain the positive results previously obtained in NOD/SCID/γ⁺⁻ and NOD/SCID/β²m⁻/⁻ mice, which combine both myeloid defect of the NOD background and NK cell deficiency (19, 20).

**Influence of murine genetic background on repopulation efficiency**

The aforementioned HIS mice used BALB/c Rag₂⁻/⁻ γ⁺⁻ animals as recipients for the human HSC (39, 40). Similarly, the original C.B-17 SCID mice were made in a BALB/c background (5). As already mentioned, the impact of the scid mutation on human cell engraftment was tested in different genetic backgrounds (11), including the C3H strain with defective macrophage response to LPS, the complement C5-deficient DBA/2 strain and the C57BL/6j strain, in which NK cells can be depleted by anti-NK1.1 Ab treatment (10). Only NOD/SCID mice combining complement, macrophage, and NK deficiencies showed an improved capacity for xeno-engraftment. It is likely that the presence of fully competent complement, NK cells, and/or macrophages in the other tested SCID mouse strains limited such a comparison (11). The use of “nonleaky” Rag₂⁻/⁻ γ⁺⁻ mice could therefore give information about the relative strength of the innate immune system, in relation to

Table I. Summary of major SCID mouse models and their properties

<table>
<thead>
<tr>
<th>Mouse Model</th>
<th>Grafted HSC</th>
<th>Engraftment[a]</th>
<th>M⁺</th>
<th>B</th>
<th>T</th>
<th>Thymus[b]</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>bg/nu/sid-hu</td>
<td>BM or CD34⁺ BM</td>
<td>&lt;1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9, 34</td>
</tr>
<tr>
<td>C.B-17 SCID-hu (Thy/Liv)</td>
<td>10⁵ FL</td>
<td>&lt;2% (0–15%)</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td></td>
<td>5, 6, 56</td>
</tr>
<tr>
<td>hu-SRC C.B-17 SCID</td>
<td>2–4 × 10⁵ BM</td>
<td>&lt;1% (BM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>hu-SRC C.B-17 SCID</td>
<td>15–50 × 10⁶ UCB</td>
<td>15% (BM)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td>8, 12</td>
</tr>
<tr>
<td>NOD/SCID</td>
<td>10⁴–5 × 10⁶</td>
<td>5% ± 11%</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td></td>
<td>11, 12, 18, 27, 38, 37</td>
</tr>
<tr>
<td>FL cells</td>
<td>40 ± 40% (BM)</td>
<td>29% ± 26%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>NOD/SCID</td>
<td>-35% 79 (BM)</td>
<td>0.5–8 × 10⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19, 58, 59</td>
</tr>
<tr>
<td>NK depleted (TM-β1)</td>
<td>0.4–2 × 10⁶</td>
<td>-45%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOD/SCID/γ⁺⁻</td>
<td>35–75% (BM)</td>
<td>-70%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>Newborn</td>
<td>CD34⁺ UCB</td>
<td>5–13% (BM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOD/SCID/β²m⁻/⁻</td>
<td>2 × 10³ CD34⁺ UCB</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>20, 58</td>
</tr>
<tr>
<td>Newborn NOD/SCID/β²m⁻/⁻</td>
<td>5 × 10⁵ CD34⁺ UCB</td>
<td>30% (BM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42, 43</td>
</tr>
<tr>
<td>BALB/c Rag₂⁻/⁻ γ⁺⁻</td>
<td>21 ± 16% (BM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>BALB/c Rag₂⁻/⁻ γ⁺⁻</td>
<td>3.8–12.16 × 10⁵ CD34⁺</td>
<td>10–60% (BM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>Newborn BALB/c</td>
<td>UCB</td>
<td>20–65% (SP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborn BALB/c Rag₂⁻/⁻ γ⁺⁻</td>
<td>&gt;60% (PBL, LiV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Newborn BALB/c Rag₂⁻/⁻ γ⁺⁻</td>
<td>FL</td>
<td>&gt;50% (SP, BM)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

[a] SPL, spleen.
[b] Percentage of huCD45⁺ cells in the PBL (or mentioned organs), 6–12 wk after transplantation.
[c] Presence of human hemopoietic-derived lineages: M, myeloid; B, B cells; and T, T cells.
[d] Engraftment of the murine thymus by human progenitors; when positive, absolute number of human (CD45⁺) thymocytes is mentioned.
the genetic background, and the direct comparison of the two existing strains, i.e., BALB/c vs C57BL/6 Rag2\(^{-/-}\)\(\gamma_c\) mice, will be extremely informative. Interestingly, differences were already noticeable after reconstitution of adult animals with human UCB cells: analysis of the BM showed that engraftment in the C57BL/6 background was extremely poor (<1% CD45\(^+\) cells), whereas NOD/SCID and BALB/c Rag2\(^{-/-}\)\(\gamma_c\) mice appeared to be similar (around 20% chimerism) (18, 38).

**T cell development in HIS mice**

All major compartments of the human innate and adaptive immune system develop de novo in HIS (BALB-Rag/γ) mice (39, 40). The murine thymus of HIS (BALB-Rag/γ) mice supports human Tαβ and Tγδ cell development. This gives rise to the establishment of cortex- and medulla-like regions within the thymic lobules of the mouse thymus. At the age of 8 wk, the proportion of double-positive CD4\(^+\)CD8\(^+\) thymocytes ranges from 50 to 80%, indicating that sustained thymopoiesis occurs in HIS (BALB-Rag/γ) mice. This percentage decreases in an age-dependent fashion, but a large proportion of CD4\(^+\)CD8\(^+\) thymocytes can still be observed 4–6 mo after inoculation of CD34\(^+\) FL cells. In young HIS (BALB-Rag/γ) mice (4–6 wk after injection of FL CD34\(^+\) cells), the number of human thymocytes ranges from 5 × 10\(^5\) to 15 × 10\(^5\) cells, as compared with 2–10 × 10\(^6\) cells in young adults (8–12 wk after inoculation) and 0.5–2 × 10\(^6\) cells in old animals (>4 mo after inoculation). Mature αβ T cells are 20–30 times more abundant than γδ T cells. Furthermore, human CD16\(^+\)CD56\(^+\) NK cells are also observed in the murine thymus.

Human mature T cells start seeding the peripheral lymphoid organs around 4–5 wk after reconstitution. They populate the BM (~1% of CD45\(^+\) human cells), spleen, liver, lungs, and peripheral blood (5–20%), with a CD4/CD8 ratio of 3:1–4:1. These T cells proliferate after in vitro stimulation with allogeneic human dendritic cells (39) or irradiated PBL in presence of PHA and huIL-2 (our unpublished observations). Furthermore, the number of mature T cells can be transiently enhanced in vivo by treatment with a superagonist anti-CD28 Ab (N. Legrand, T. Cupedo, A. U. van Lent, M. Ebeli, K. Weijer, T. Hanke, and H. Spits, submitted for publication), which has been described for its unique property of inducing human T cell activation independently of TCR stimulation (47). These observations indicate that human T cells developing in HIS (BALB-Rag/γ) mice are functional. Interestingly, a CD4\(^+\) CD25\(^+\) glucocorticoid-induced TNFR family-related gene subset of T cells, enriched for foxp3 expression, is also observed both in the thymus and in the periphery (39) (N. Legrand, T. Cupedo, A. U. van Lent, M. Ebeli, K. Weijer, T. Hanke, and H. Spits, submitted for publication), strongly suggesting that they represent human T cells exerting regulatory functions (48).

**Selecting MHC molecules of human T cells in HIS mice**

The fact that human T cells can populate the peripheral lymphoid organs of HIS (BALB-Rag/γ) mice implies that these cells undergo a process of positive selection. Our current findings with BALB/c nude-Rag2\(^{-/-}\)\(\gamma_c\) mice, which do not show any T cells after engraftment of CD34\(^+\) FL cells, strongly indicate that no thymus-independent T cell development is achieved in the HIS mice (our unpublished observations). In theory, developing human T cells in HIS (BALB-Rag/γ) mice could be positively selected both by thymic epithelial cells of the recipient mouse and by BM-derived human cells, especially in suboptimal conditions (49). Results obtained so far indicate that this dual selection could indeed occur, since MLRs show no/low T cell proliferation against BALB/c-derived or autologous human dendritic cells, i.e., tolerance/negative selection is achieved (39). Still, the absence of a reaction does not necessarily indicate that the circulating T cells in HIS mice are restricted to mouse or human MHC molecules without any bias, although the generated T cell repertoire is broad and indistinguishable from the repertoire of a normal donor (39, 40). For instance, after infection of HIS (BALB-Rag/γ) mice with influenza, we only observed T cells specific for viral epitopes in the context of murine MHC molecules (our unpublished observations). This result could reflect a biased T cell repertoire to mouse MHC molecules in the periphery, although thymic selection is performed practically in virtue of TCR affinities for both human and murine MHC molecules. Alternatively, the nature of the targeted tissues (mouse vs human) by the used infectious agents could result in the specific activation of T cell repertoires that were selected by mouse or human MHC molecules. In support of this argument, EBV infection of HIS (BALB-Rag/γ) mice was shown to give rise to human T cells that proliferate ex vivo when cultured on EBV-transformed B cells (39). This observation suggests that the generated T cells are specific for EBV epitopes presented by human B cells in the context of human MHC molecules.

In addition, it was previously reported that the half-life of T cells in the classical SCID-hu (Thy/Liv) model is ~24 h (50). This observation could be due to the lack of MHC elements supporting the peripheral survival of T cells selected by human MHC molecules in the thymic graft. Interestingly, we observed that human T cells in HIS (BALB-Rag/γ) mice also display a high turnover rate and are prone to apoptosis (N. Legrand, et al., submitted for publication), despite the fact that they are potentially selected by both human and murine MHC molecules. This observation could reflect a mismatch between central selection and peripheral survival requirements of these T cells, negative effects due to the murine innate immune system, and/or lack of factors involved in T cell homeostasis.

**Development of other hemopoietic lineages in HIS mice**

B cells are the majority of the human hemopoiesis-derived cells in HIS (BALB-Rag/γ) mice. The murine BM supports human B cell differentiation and contains mainly immature IgM\(^-\)CD10\(^-\)CD20\(^-\) B cells, whereas most B cells in the spleen were IgM\(^+\) IgD\(^-\)CD10\(^+\)CD20\(^+\) (39, 40). Human IgM and IgG produced by CD19\(^+\)CD27\(^+\)CD138\(^-\) plasma cells are gradually accumulating with time in the serum, demonstrating complete B cell maturation in this system, in contrast to previous results in NOD/SCID mice (32). HIS (BALB-Rag/γ) mice vaccinated with tetanus toxoid 12–17 wk after transplantation of CD34\(^+\) UCB cells could develop a specific IgG response, and memory B cells, although, for yet to be understood reasons, this was not the case if animals received tetanus toxoid only 8 wk after inoculation of human progenitors (39). Furthermore, the presence of B cells induces de novo generation of mouse follicular dendritic cells, presumably in a human lymphotxin-α-dependent manner, and formation of germinal center-like structures (39).
Other important lineages develop from the human progenitors in the HIS (BALB-Rag/γ) mice. We reported low frequencies of CD56<sup>+</sup> NK cells in all analyzed organs, the thymus being the organ where most NK cells are observed (40). Similarly, CD14<sup>+</sup> monocytes and CD11c<sup>+</sup> conventional dendritic cells, which can be used ex vivo as stimulators of allogeneic T cells, were also observed in low amounts, except in the BM and liver (39, 40). CD123/IL-3Rα<sup>+</sup>BDCA2<sup>+</sup>CD11c<sup>+</sup> plasmacytoid dendritic cells, also referred to as natural IFN-producing cells (51), are developing in HIS (BALB-Rag/γ) mice and accumulate in high frequencies in the BM and the liver. Upon ex vivo stimulation with influenza virus, these plasmacytoid dendritic cells produce high levels of IFN-α (39). Furthermore, the HIS (BALB-Rag/γ) mouse model has been used in our laboratory to demonstrate that the expression of the SpI-B transcription factor is needed for the correct development of human plasmacytoid dendritic cells by using a small interfering RNA “knockdown” approach (52).

Overall, the HIS (BALB-Rag/γ) mouse model supports the development of both the human innate and adaptive immune systems, and it can be manipulated to answer questions linked to developmental biology (40, 52) or treatment purposes.

Future prospects

HIS mice represent a major advance for the in vivo study of development and function of the human immune system, though this new model still appears to be suboptimal in some aspects, e.g., for T cell development. The combination of this approach with techniques permitting the manipulation of human stem cells for gene silencing and overexpression provides many perspectives to better understand the genetic basis of in vivo differentiation of human hematopoietic lineages.

Nonetheless, it is clear that Rag2<sup>−/−</sup>γ<sub>c</sub><sup>−/−</sup> mice are not the most optimal recipients to achieve fully operational human immune system. As a result of the disruption of the IL-7R signaling axis in these animals, functionality of CD3<sup>+</sup>CD4<sup>+</sup>IL-7R<sup>+</sup> lymphoid tissue inducer cells is compromised and formation of most lymph nodes is therefore impaired (53). Consequently, the search for a mouse strain lacking NK cells, but still harboring normal lymph node development is of interest. Rag2<sup>−/−</sup>IL-2Rβ<sup>−/−</sup> mice are good candidates since these mice lack T, B, and NK cells (54) but, in contrast to Rag2<sup>−/−</sup>γ<sub>c</sub><sup>−/−</sup> mice, this strain is expected to develop normal lymph nodes. Furthermore, while the original observations of improved engraftment after infusion of cytokine mixtures (7, 9, 34, 38) and the relative bias toward B cell differentiation in several SCID mouse models, it is suggested that murine GM-CSF, M-CSF, IL-3, and thrombopoietin (which support myeloid/erythroid differentiation) are not cross-reactive with human cells, whereas Flt3 ligand, IL-7 (which support lymphoid differentiation), and SCF are cross-reactive, at least to some extent. It may therefore be of interest to insert the genes encoding human IL-3, TPO, SCF, and/or GM-CSF into the host mice, as well as other cytokines, like IL-15 for improvement of NK cell development. As an alternative for this gene knock-in approach, infusion of molecules with increased stability can be used. Using a fusion protein between the Fc fragment of human Ig and IL-7, it was recently shown that the proportion of human cells in NOD/SCID/γ<sub>c</sub><sup>−/−</sup> mice engrafted with CD34<sup>+</sup> cells from human mobilized PBLS increased by a factor of 2–3 in thymus and blood (55). Molecules involved in the development of the innate immune system, e.g., TLRs, could also be targeted to improve its establishment in the mouse or to help for an optimal initiation of immune responses. Finally, by introducing human class I (HLA-A/B/C) and class II (HLA-DP/DQ/DR) molecules of the MHC, both central selection of human T cells and their peripheral survival capacity will certainly be improved. Several groups are currently working on these extremely challenging approaches, and exiting results should arise in the coming years. It is highly likely that these efforts will provide accurate models for in vivo preclinical studies for human infectious diseases and genetic disorders.

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References


