From progenitor cell to immune cell : cytokines and transcription factors in human hematopoietic development

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Role of IL-7 on human lymphoid development and homeostasis in "Human Immune System" $Rag2^{-/-}\gamma c^{-/-}$ mice

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Abstract

Interleukin-7 (IL-7) is a central cytokine in the development and maintenance of lymphoid cell populations, although interspecies discrepancies have been previously highlighted. We report here that murine IL-7 is around 100-fold less potent than human IL-7 for supporting human T cell development and expansion *in vitro*. We have studied the *in vivo* role of IL-7 in human hematopoiesis using an experimental model for development and function of the human immune system, namely newborn BALB/c $Rag2^{-/-}\gamma_c^{-/-}$ mice transplanted with human hematopoietic stem cells. We show that repeated injections of human IL-7 in young humanized mice give rise to a transient accumulation of thymocytes and plasmacytoid dendritic cells. Genetic manipulation of the transplanted human hematopoietic cells was performed using Bcl-2 and Bcl-XL, with positive effects on global human reconstitution and specific enhancement of T and NK cells, respectively. Over-expression of a chimeric murine/human IL-7Rα chain using the mouse extracellular domain shows positive outcome on all analyzed immune cell subsets, with the exception of T cells. Overall, IL-7 alone is probably not sufficient to fully support human T cell development and survival, and acts directly or indirectly as a positive factor on human hematopoiesis and lymphocyte survival, in synergy with other signals that are sub-optimal in humanized BALB/c $Rag2^{-/-}\gamma_c^{-/-}$ mice.

Introduction

Lymphoid development and survival are tightly regulated by several mechanisms, including intrinsic events, cell-to-cell interactions and soluble factors (reviewed in (1-3)). Among the latter, IL-7 has been described as a major extra-cellular ligand for the control of lymphocyte differentiation, maintenance of cell viability and regulation of lymphocyte homeostasis. The receptor for IL-7 consists of two chains, the IL-7 receptor alpha chain (IL-7Rα/CD127) and the common gamma chain (γ_c/ CD132), also shared by the receptors for IL-2, IL-4, IL-9, IL-15 and IL-21. IL-7 is described as being produced in a constitutive manner by stromal cells located in a large variety of non-lymphoid and lymphoid tissues, including thymus, spleen and guts (reviewed in (4)). In contrast, the expression of IL-7Rα is tightly regulated during development and maturation of lymphoid cells, leading to the concept that the amount of IL-7 is mostly influenced by consumption rather than production (5).

The expression of IL-7Rα is mostly restricted to hematopoiesis-derived cell lineages, including common lymphoid precursors, some stages of developing T and B cells, mature T cells and bone marrow-derived macrophages (4-7). The expression dynamics of the receptor for IL-7 is a major regulation key during hematopoiesis, and constitutive expression of IL-7Rα impacts negatively on lymphoid development, e.g. during thymopoiesis (8). Upon ligation of IL-7 to its receptor, the Janus kinase 1 (JAK1) and JAK3 are brought together and induce phosphorylation of the IL-7Rα chain, which leads to the recruitment of several signaling partners, including signal transducer and activator of transcription 5 (STAT-5), and downstream activation of signaling cascades, including STATs, PI3 kinase and Src kinase pathways (reviewed in (4, 9)). Consequently, IL-7 has been implied in various phenomena, such as induction of cell proliferation, control of cell metabolism, maintenance of cell survival – in particular through the induction of Bcl-2 family members – and antigen receptor V(D)J rearrangements in developing T and B cells (4, 5, 9). As far as lymphocyte survival is concerned, it was shown that IL-7 enhances Bcl-2 expression in freshly isolated T cells (10), and that IL-7 supplementation in T cell cultures protects them from dexamethasone-induced apoptosis, correlating with enhanced expression of Bcl-2 and Bcl-XL (11). The homeostatic maintenance of naïve T cells is also strongly dependent on IL-7 levels, although IL-7 acts in synergy with other partners – e.g. IL-15 or TCR/MHC interactions – and is not required to the same extent in survival vs. homeostatic proliferation, or in the case of naïve $CD4^+$ vs. $CD8^+$ T cells (12, 13).

Genetic ablation of IL-7 or γ_c expression in mice leads to severe reduction in numbers of thymocytes, B cell progenitors in the bone marrow, and consequently peripheral T and B cells (14, 15). The phenotype of mice deficient for the expression of IL-7Rα is more pronounced (16), probably due to the fact that another ligand of IL-7Rα, thymic stromal lymphopoietin (TSLP), can not partially overcome the lack of IL-7 signaling (17, 18). In contrast, human patients with deficiencies for the expression of γ_c (19), IL-7Rα (20) or JAK3 (21, 22) completely lack T cells, but exhibit normal or increased numbers of circulating B cells (23). These discrepancies between mouse and human studies therefore highlight major inter-species differences for the role of IL-7 in lymphocyte development and homeostasis. In order to elucidate these differences, which remain partly elusive, *in vitro* experimental models of human lymphopoiesis have been set up. For instance, addition of IL-7 to human T cell progenitors in fetal thymic organ cultures (FTOC) has been shown to improve TCRβ rearrangements (24), which correlates with the absence of such rearrangements in SCID-X1 γ_c-deficient patients (25), whereas IL-7-deficient mice exhibited TCRβ rearrangements (26). Alternatively, the role of IL-7 can be addressed *in vivo*, using mouse models with a human immune

system (HIS). We and others have constructed such HIS mice by transplanting human hematopoietic stem cells (HSC) into conditioned newborn BALB/c $Rag2^{-/-}\gamma_c^{-/-}$ recipient mice, and the resulting HIS (BALB-Rag/γ) mice exhibit multilineage reconstitution by human lymphoid and myeloid cells (27, 28).

In the present study, we used co-cultures of OP9-huDL1 bone marrow stromal cells and human post-natal thymocyte progenitors (29-31) to analyze the relative potency of murine and human IL-7 (muIL-7 and huIL-7 respectively) on the expansion and extent of T cell commitment. We addressed the *in vivo* role of IL-7 in lymphopoiesis by using HIS (BALB-Rag/γ) mice, by repeatedly inoculating huIL-7 at various ages. Alternatively, we genetically modified the human HSC before transplantation by retroviral transduction, so that the human cells would over-express Bcl-2, Bcl-XL or a chimeric mu/huIL-7Rα chain. Overall, our results indicate that mouse IL-7 is around 100-fold less efficient than human IL-7 in supporting human immune cell survival and differentiation, but that huIL-7 alone is not sufficient to restore full homeostasis *in vivo* in the HIS (BALB-Rag/γ) mouse model.

Materials and methods

Cell lines, constructs and production of viral supernatants. The murine bone marrow stromal OP9-control and OP9-huDL1 cell lines were previously described (29, 31). Cells were maintained in MEMα (Invitrogen, Carlsbad CA, USA) supplemented with 20% FCS (Hyclone, Logan UT, USA). The cDNA sequence encoding human IL-7, human IL-7Rα and a chimeric murine-human IL-7Rα were inserted into the multiple cloning site of the LZRS vector upstream of an internal ribosomal entry site and enhanced green (GFP) or yellow (YFP) fluorescent protein, respectively (32). Control vectors were empty LZRS IRES-GFP and LZRS IRES-YFP. Retroviral supernatants were produced as described (33) using the 293T-based Phoenix packaging cell line (34).

Isolation of human hematopoietic progenitors. Human hematopoietic progenitors were isolated from post-natal thymus (PNT) obtained from surgical specimens removed from children up to 3 years of age undergoing open heart surgery. Alternatively, human hematopoietic progenitors were isolated from fetal liver (FL) tissue samples obtained from elective abortions, with gestational age ranging from 14 to 20 weeks. The use of these human tissues was approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam (AMC-UvA) and was contingent on informed consent. Human hematopoietic progenitors were purified as follows. The PNT tissue was mechanically disrupted and pressed through a stainless steel mesh to obtain a single-cell suspension, which was left overnight at 4°C. The thymocytes were isolated the next day from a Ficoll-Paque™ Plus (GE-Healthcare, Chalfont St Giles, UK) density gradient. $CD34^+$ cells were enriched by immunomagnetic cell sorting, using direct CD34 human progenitor cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The $CD34^+$ thymocytes were stained with mAb against CD34, CD1a, CD56, and BDCA2. The $CD34^+CD1a^-CD56^-BDCA2^-$ (further referred to as $CD34^+CD1a^-$) population was sorted using a FACS Aria (BD Bioscience, Franklin Lakes NJ, USA), to purity always ≥ 99%. In the case of FL samples, magnetic enrichment of $CD34^+$ cells was performed by using the Indirect CD34 Human Progenitor Cell Isolation Kit (Miltenyi Biotech), after preparation of single cell suspension and isolation of mononuclear cells by density gradient centrifugation over Lymphoprep Ficoll-Hypaque (Nycomed Pharma). The SCID-repopulating progenitor cell population was further purified by sorting $CD34^+CD38^-$ cells, using an ARIA sorter (BD Bioscience) to purity always ≥ 99%.

Retroviral transduction. Human hematopoietic progenitors were transduced with control or gene of interest expressing vectors before OP9 co-cultures. The $CD34^+CD1a^-$ PNT progenitors were cultured overnight in IMDM (Invitrogen, Carlsbad CA, USA) supplemented with Yssel's medium (35), 5% normal human serum (NHS), 20 ng/ml human stem cell factor (huSCF; PeproTech, Rocky Hill NJ, USA), and 20 ng/ml human interleukin-7 (huIL-7; PeproTech). The following day, cells were incubated for 6 to 8 hours with virus supernatant in fibronectin-coated plates (30 µg/mL; Takara Biomedicals, Otsu, Shiga, Japan). The identical procedure was used with FL $CD34^+CD38^-$ cells, except that the medium was supplemented with 20 ng/mL human thrombopoietin (huTPO, PeproTech).

Co-cultures of human progenitor and OP9 cells. The *in vitro* development of human T cells was assessed by co-culturing $5x10^4$ PNT $CD34^+CD1a^-$ progenitor cells with $5x10^4$ OP9 or OP9-DL1 cells in MEMα (Invitrogen, Carlsbad CA, USA) with 20% FCS (Hyclone, Logan UT, USA), 5 ng/ml human FLT3L (huFlT3L, PeproTech) and variable amounts huIL-7 (PeproTech, Rocky Hill NJ, USA) or muIL-7. The co-

cultures were supplemented every 2 to 3 days with fresh medium, and progenitor cells were transferred to fresh stromal cells every 4 to 5 days of culture (29).

Generation of HIS (BALB-Rag/γ) mice. BALB/c Rag2$^{-/-}\gamma_c^{-/-}$ mice (36) were bred and maintained in self-ventilated isocages, and were fed autoclaved food and water. Mice with a human immune system (HIS (BALB-Rag/γ)) were generated with minor modifications as compared to previously described (27, 28). Newborn (<1week old) Rag2$^{-/-}\gamma_c^{-/-}$ mice received sub-lethal (3.5 Gy) total body irradiation with a X-ray Röntgen source, and were injected intra-hepatic (i.h.) with 5-10x10^5 sorted CD34$^+$CD38$^-$ human FL cells. The FL cells may have been previously transduced, and the cell bulk was then inoculated to the newborn recipients. All manipulations of HIS (BALB-Rag/γ) mice were performed under laminar flow.

Flow cytometry analysis for cell surface markers. Cell suspensions were labeled with FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-Cy7 or Alexa-700 coupled anti-human mAb targeting the following cell surface markers: CD3, CD4, CD8, CD19, CD45, CD123 (BD Bioscience, Franklin Lakes NJ, USA), CD11c, CD19 (Coulter-Immunotech, Luminy, France), CD1a (T6-RD1, Beckman Coulter, Fullerton CA, USA), CD38 (DAKO, Glostrup, Denmark) and BDCA2 (Miltenyi Biotec). Dead cells were excluded based on DAPI incorporation. All washings and reagent dilutions were done with PBS containing 2% FCS and 0.02% sodium azide (NaN_3). All acquisitions were performed with a LSR-II (BD Bioscience) cytometer interfaced to FACS Diva software system.

Statistical analyses. Data were subjected to two-tail unpaired Student's *t*-test analysis where indicated in the figure legends. The obtained p values were considered significant when $p<0.05$.

Results

Weak cross-reactivity of mouse IL-7 to the human IL-7 receptor

Mouse and human IL-7 are described as being cross-reactive to their respective IL-7 receptor counterpart (37), allowing some degree of functional redundancy in chimeric experimental systems such as chimeric fetal thymic organ cultures. We analyzed to which extent murine IL-7 was able to sustain development and survival of human lymphoid precursors in presence of mouse bone marrow stromal OP9 cells expressing the human Notch1 ligand DeltaLike1 (Fig.1A). It was already described that human $CD34^{+}CD1a^{-}$ PNT lymphoid progenitors cultured in these conditions are prone to expand extensively and progress towards the T cell lineage (29-31). We examined the outcome of OP9-huDL1/PNT co-cultures that were supplemented either with muIL-7 or with huIL-7, at concentrations ranging from 0.5 to 50 ng/ml. The use of muIL-7 only induced limited expansion of the human PNT progenitors in the co-culture assay over a 3-week period (Fig.1B). Furthermore, the accumulation of human T cell committed cells (expressing CD1a and/or CD3) and $CD3^{+}CD1a^{-}$ mature T cells in the co-culture assay was also reduced in presence of muIL-7, as compared to equivalent concentrations of huIL-7 (Fig.1C). Of interest, the extent of cell expansion and T cell differentiation was similar when 50 ng/mL muIL-7 and 0.5 ng/ml huIL-7 were used, suggesting that huIL-7 is around 100-fold more potent than muIL-7 to promote human PNT proliferation and differentiation.

Expression of the human IL-7 receptor in HIS (BALB-Rag/γ) mice

Development and peripheral accumulation of human lymphoid cells is known to be suboptimal in various models of mice humanized for the immune system, including the HIS (BALB-Rag/γ) mice (37-39). We questioned whether the poor cross-reactivity of muIL-7 *in vivo* could explain at least partially the suboptimal levels of human reconstitution in mouse recipients. For this purpose, we transplanted purified human hematopoietic stem cells (HSC) into sub-lethally irradiated severely immuno-compromised newborn BALB/c $Rag2^{-/-}\gamma_c^{-/-}$ mice (Fig.2A). The resulting HIS (BALB-Rag/γ) mice are repopulated in a multilineage fashion by human immune cells in all major primary and secondary lymphoid organs, although they never exhibit cellular repopulation to the extent of a wild-type mouse (27, 28, 38). We analyzed the expression of the human IL-7 receptor on human cells found in “young” (2 weeks after xenograft transplantation) and “adult” (≥ 10 weeks after xenograft transplantation) mice. In “young” HIS (BALB-Rag/γ) mice, human cells are mostly immature progenitors found in the liver and the bone marrow, and only limited human colonization of the mouse thymic rudiments is observed (data not shown). In “adult” HIS (BALB-Rag/γ) mice, human cells have actively colonized all mouse peripheral lymphoid organs, and IL-7Rα was found on the surface of immature thymocytes and mature T cells (Fig. 2B).

In vivo supplementation of young and adult HIS (BALB-Rag/γ) mice with human IL-7

Considering the role of IL-7 in maintenance of human HSC and during early human thymopoiesis, we repeatedly inoculated “young” HIS (BALB-Rag/γ) mice with huIL-7 over 4 weeks and we carefully analyzed the impact on the development and accumulation of human immune cells (Fig.3A). Thymus cellularity was increased around 3-fold on average after 2 weeks of treatment, and only transiently (Fig.3B). A more detailed analysis of thymic subsets revealed that the absolute number of $CD4^{-}CD8^{-}CD3^{-}$ triple negative thymocytes, i.e. the most early T cell progenitors, was

increased 3-fold after 1 week of huIL-7 treatment, and that most of the increase of thymus size at 2 weeks was due to selective and transient accumulation of their $CD4^+CD8^+$ double-positive thymocytes progeny (Fig.3C). This wave of immature thymocytes resulted eventually in accumulation of $CD3^+CD1a^-$ mature thymocytes 4 weeks after the onset of huIL-7 inoculation, i.e. in 6-7 weeks old HIS (BALB-Rag/γ) mice that do not usually exhibit peripheral T and B cell repopulation (Fig.3C). Interestingly, the accumulation of human mature thymocytes did not give rise to enhanced egress of mature T cells (data not shown).

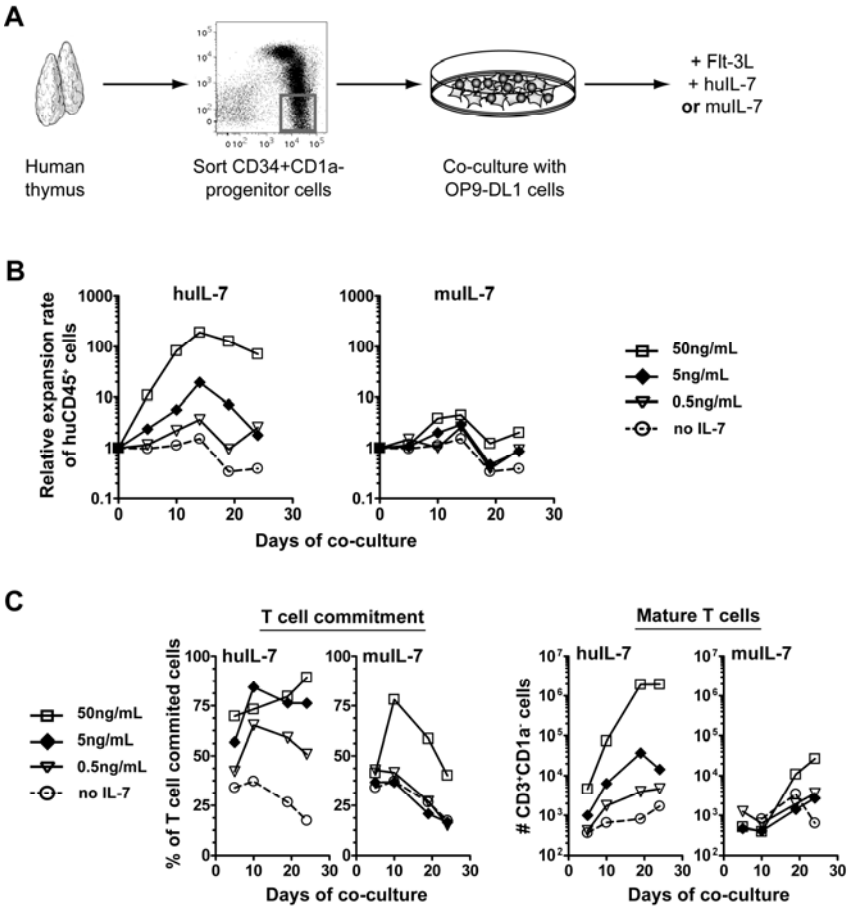

Figure 1. In vitro evaluation of muIL-7 cross-reactivity to the huIL-7 receptor. *(A) Human $CD34^+CD1a^-$ lymphoid progenitors were purified from post-natal thymocytes and co-cultured with stromal OP9 cells expressing the Notch ligand huDL1. The co-cultures were supplemented with huFLT3L and variable amounts of either huIL-7 or muIL-7. (B) The graphs show the global human cell expansion in the co-cultures over time, relative to the original $CD34^+CD1a^-$ PNT progenitors input ($50x10^3$ cells, rate=1). Typical cell expansions obtained with no IL-7 (open circles, dotted line), 0.5 ng/ml (open triangles), 5 ng/ml (closed diamonds) and 50 ng/ml (open squares) of either huIL-7 (left) or muIL-7 (right) are shown. (C) The fraction of human cells committed to the T cell lineage (expressing CD1a and/or CD3) was followed over time and is shown in the left graphs. The accumulation of mature T cells ($CD3^+CD1a^-$) is detailed in the right graphs.*

The analysis of other developing cell lineages was also performed. In the bone marrow, we could not observe any significant impact of repeated huIL-7 injection into "young" HIS (BALB-Rag/γ) mice (Fig.3D), consistent with studies indicating that human B cells do not require IL-7 for their proper development (20). In contrast, we observed an accumulation of human plasmacytoid dendritic cells (pDC) in the liver of huIL-7 treated HIS (BALB-Rag/γ) mice (Fig.3E). Interestingly, the effect was once again transient and peaked around 2-3 weeks of treatment.

Human lymphoid and myeloid cells are found in all major lymphoid organs in adult (≥ 10 weeks after xenograft transplantation) HIS (BALB-Rag/γ) mice. We repeatedly injected such "steady state" adult HIS (BALB-Rag/γ) mice for 2 weeks with high doses of huIL-7 and analyzed the effect on human cell development and peripheral homeostasis. The global human engraftment was not improved in huIL-7 treated animals (Fig.4A), and no significant effect was observed on T cells (Fig.4B), B cells, pDCs or any other immune cell lineage (data not shown). Of note, huIL-7 did not enhance human thymopoiesis when treatment was applied to adult HIS (BALB-Rag/γ) mice, in contrast to what we observed with "young" animals. Altogether, these results show that supplementation of "young" HIS (BALB-Rag/γ) mice with huIL-7 improves human T cell and pDC development, although transiently and moderately, whereas huIL-7 is not giving rise to major effects in adult animals.

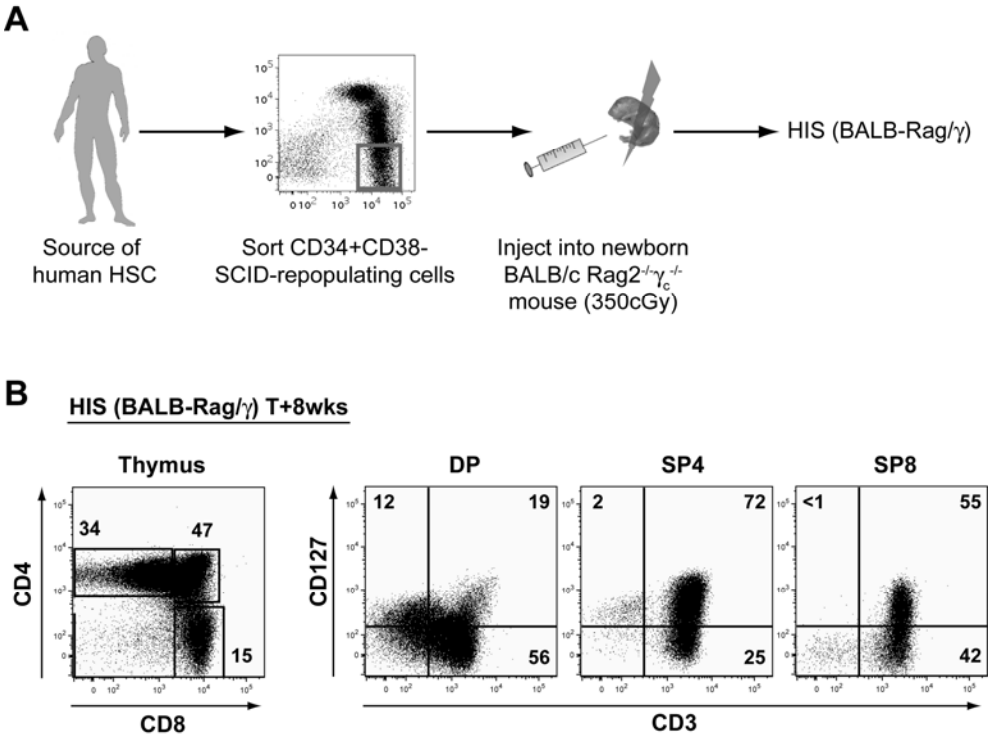

Figure 2. Production and characterization of HIS (BALB-Rag/γ) mice. *(A) Schematic representation of steps performed to produce mice with a human immune system, using newborn BALB/c Rag2$^{-/-}\gamma_c^{-/-}$ mice as hosts for human fetal liver CD34$^+$CD38$^-$ xenograft. (B) A detailed analysis of the human thymocytes was performed in adult (≥10 weeks after xenograft transplantation) HIS (BALB-Rag/γ) mice and plots show IL-7 receptor expression on the surface of double-positive (DP, CD4$^+$CD8$^+$) and single-positive (SP4, CD4$^+$CD8$^-$ and SP8, CD4$^-$CD8$^+$) cells.*

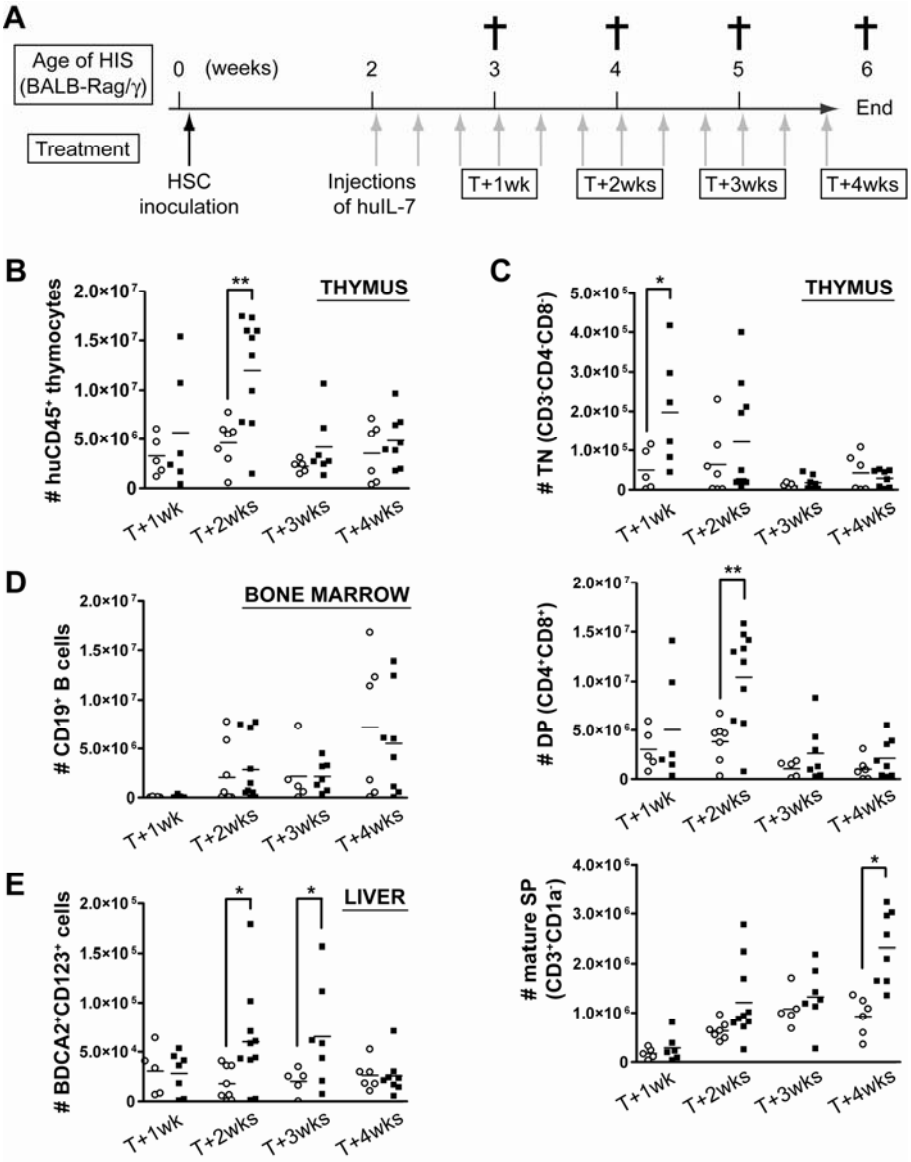

Figure 3. Injection of huIL-7 into "young" HIS (BALB-Rag/γ) mice. *(A) Schematic representation of the experimental set-up. Two weeks after human HSC transplantation, the animals received every other day 1 µg of huIL-7 i.p., over a 4-week period. The control animals received the same volume of PBS. Some animals were sacrificed and analyzed every week after the onset of the treatment. (B) The number of human thymocytes was compared between PBS (open circles) and huIL-7 treated (closed squares) HIS (BALB-Rag/γ) mice. (C) A detailed analysis of human thymopoiesis was performed for the immature triple-negative (TN, $CD3^-CD4^-CD8^-$), immature DP and mature ($CD3^+CD1a^-$) SP sub-populations. (D) B cell development and (E) the accumulation of human plasmacytoid dendritic cells were analyzed in the bone marrow and in the liver, respectively. Three independent experiments were performed and the results were pooled to perform the statistical analysis. We used a total of 5-7 PBS-treated HIS (BALB-Rag/γ) mice and 6-10 huIL-7-treated HIS (BALB-Rag/γ) mice per time point. Student's t-test analysis: * p<0.05; ** p<0.01.*

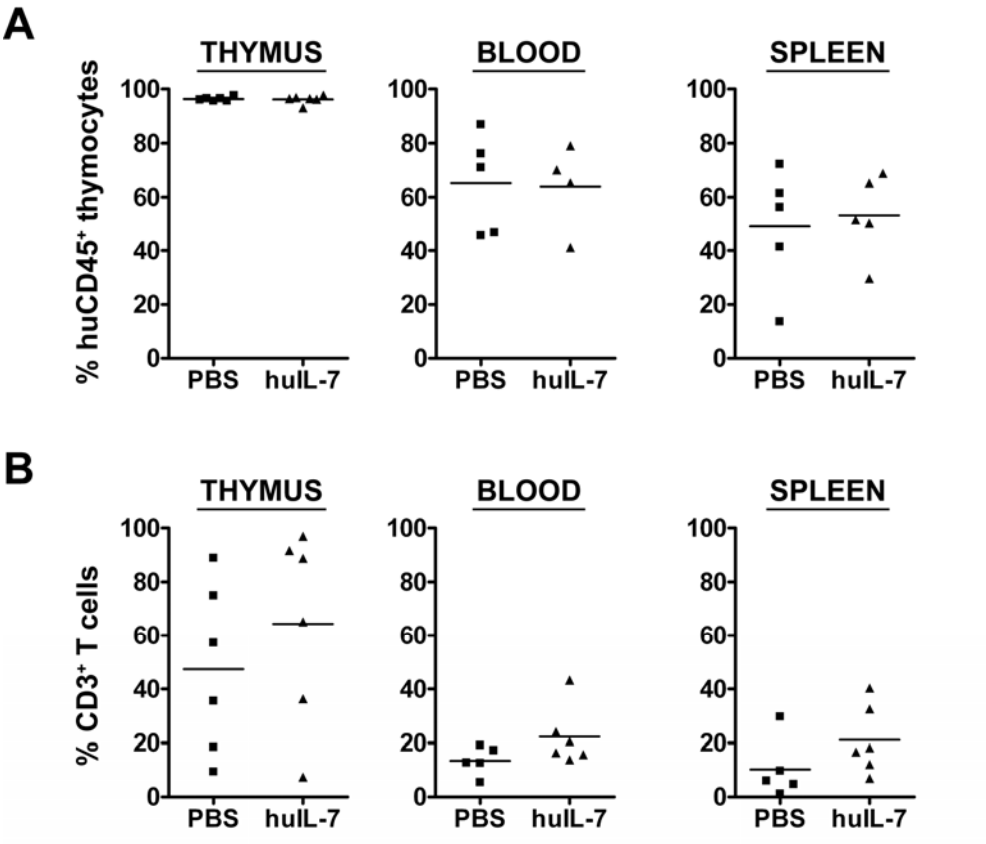

Figure 4. Injection of huIL-7 into "adult" HIS (BALB-Rag/γ) mice. *Adult HIS (BALB-Rag/γ) mice were treated for two weeks with PBS or 5 µg of huIL-7 every other day, and the two groups were eventually analyzed. (A) The level of human engraftment (% huCD45$^+$) is plotted for the thymus, the blood and the spleen. (B) The graphs show the frequency of mature T cells (CD3$^+$CD1a$^-$) in these lymphoid organs. No statistical difference could be observed.*

Poor human peripheral T cell survival despite anti-apoptotic gene expression

IL-7 is known to induce the expression members of the Bcl-2 family (4, 9), and the balance between pro- and anti-apoptotic factors of this family directly influences lymphocyte survival. Interestingly, over-expression of Bcl-2 in IL-7Rα deficient animals was shown to rescue partially the defective lymphopoiesis normally observed in this genetic background (40, 41). Furthermore, induction of apoptosis in T cells is counteracted by IL-7, which induces expression of Bcl-2 and Bcl-XL (10, 11). We therefore investigated the impact of Bcl-2 or Bcl-XL over-expression on human hematopoiesis in the HIS (BALB-Rag/γ) mouse model. Prior to xenograft transplantation into newborn recipients, we transduced the human HSC with retroviral vectors to over-express the genes of interest (Fig.5A). The over-expression of Bcl2 tended to favor the survival of human cells, and this effect did not result in differentiation bias (Fig.5B). In contrast, over-expression of Bcl-XL strongly gave rise to T cell lineage commitment, resulting into increased thymus size which was almost fully repopulated by GFP$^+$ cells. As a consequence, peripheral T cells were derived from the Bcl-XL

expressing cells, although there was no difference in terms of number of human cells in peripheral lymphoid organs (Fig.5C). Of note, we previously described that NKp46^{+} NK cell numbers are also increased in the thymus and the spleen of Bcl-XL HIS (BALB-Rag/γ) mice (ND. Huntington, N. Legrand, K. Weijer, NL. Alves, A. Plet, Y. Jacques, H. Spits and JP. Di Santo, manuscript submitted). Overall, Bcl-2 is supporting survival of human cells, whereas Bcl-XL promotes thymopoiesis without supporting peripheral T cell homeostasis.

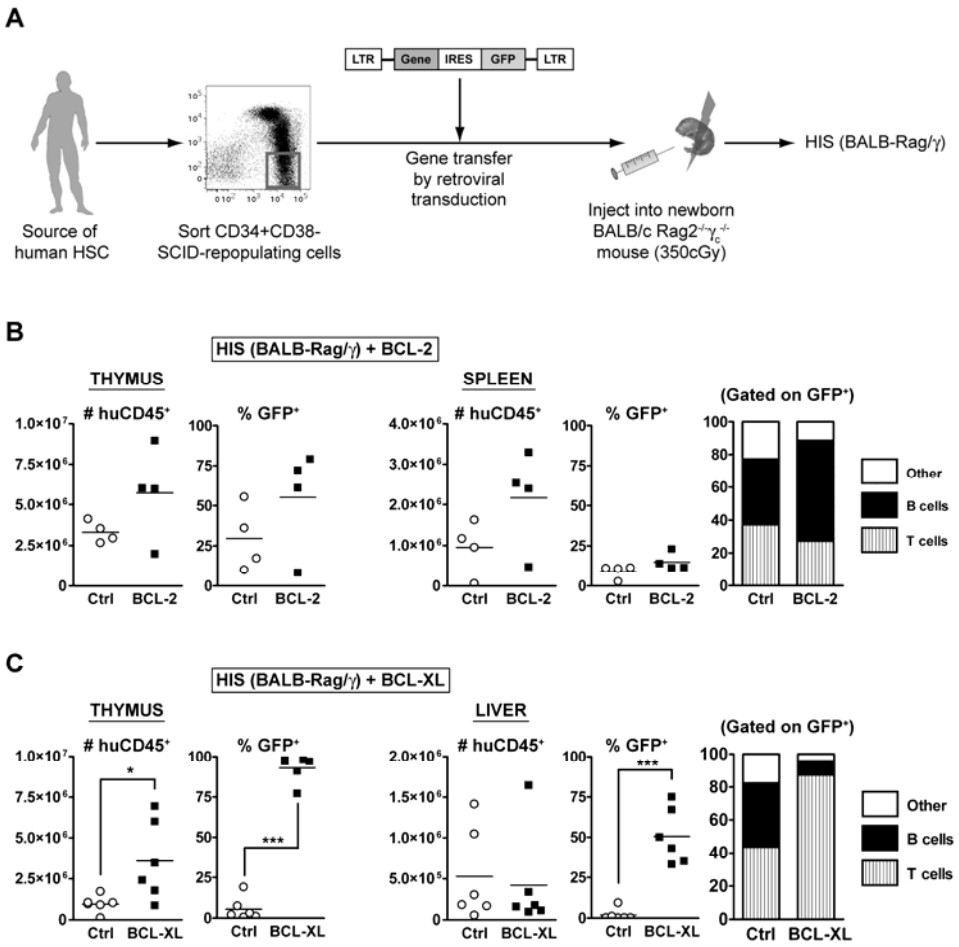

Figure 5. Over-expression of BCL family members in HIS (BALB-Rag/γ) mice. *(A) Experimental scheme including HSC manipulation by retroviral transduction prior to xenograft transplantation. (B) Over-expression of Bcl-2. The two left graphs show the number of human cells and the fraction of GFP^{+} cells recovered from the thymus of adult animals (≥10weeks post-transplantation). The three graphs on the right show the number of human cells, the fraction of GFP^{+} cells and the relative lineage distribution within GFP^{+} cells in the spleen of the same animals (C) The same analysis was performed with HIS (BALB-Rag/γ) mice containing Bcl-XL over-expressing human cells. Student's t-test analysis: * p<0.05; *** p<0.001.*

Functional complementation of human cells with murine/human chimeric IL-7 receptor

After providing exogenous huIL-7 or survival advantage by a genetic delivery system to the human cells in the HIS (BALB-Rag/γ) mice, we took advantage of the fact that muIL-7 is produced in these animals but not "consumed" (5), due to the absence of murine lymphocytes. Following a similar strategy as previously described for Bcl-2 family members, we provided a chimeric IL-7 receptor to the human HSC by retroviral transduction before transplantation into newborn BALB/c $Rag2^{-/-}\gamma_c^{-/-}$ mice. The chimeric mu/huIL-7Rα chain used is composed of the extracellular domain of the muIL-7Rα chain and the transmembrane and cytoplasmic domains of huIL-7Rα chain, and therefore insures proper binding of muIL-7 and optimal signal transduction within human cells.

The resulting HIS (BALB-Rag/γ) mice were analyzed around 10 weeks after xenograft transplantation, once human cell colonization in peripheral lymphoid organs was optimal, and we observed that human reconstitution was significantly improved in the spleen (Fig.6A), bone marrow, liver and peripheral blood (not shown) of mu/huIL-7Rα transduced HIS (BALB-Rag/γ) mice, but not in the thymus (Fig.6A). This improvement was mostly due to the human cells expressing the chimeric IL-7Rα chain, each injected mu/huIL-7Rα GFP^+ human progenitor giving rise to 5-10 fold more human cells than their control GFP^+ counterpart (being empty vector or huIL-7Rα expressing vector). The relative accumulation of human cells expressing the chimeric mu/huIL-7Rα chain was heterogeneous, since the relative numbers of human B cells, $BDCA2^+$ pDC but also conventional $CD11c^+BDCA2^-$ dendritic cells (cDC), were increased in the bone marrow, spleen, liver and blood of these animals (Fig.6B/C and data not shown), whereas T cell numbers were similar between HIS (BALB-Rag/γ) mice produced using HSC transduced either with the chimeric mu/huIL-7Rα chain, the huIL-7Rα chain or the corresponding control GFP expressing vectors (Fig.6D).

Overall, increasing the accessibility of human cells to the environmental IL-7 (muIL-7) globally enhances the levels of human reconstitution by B cells, pDC and cDC/monocytes, but not T cells.

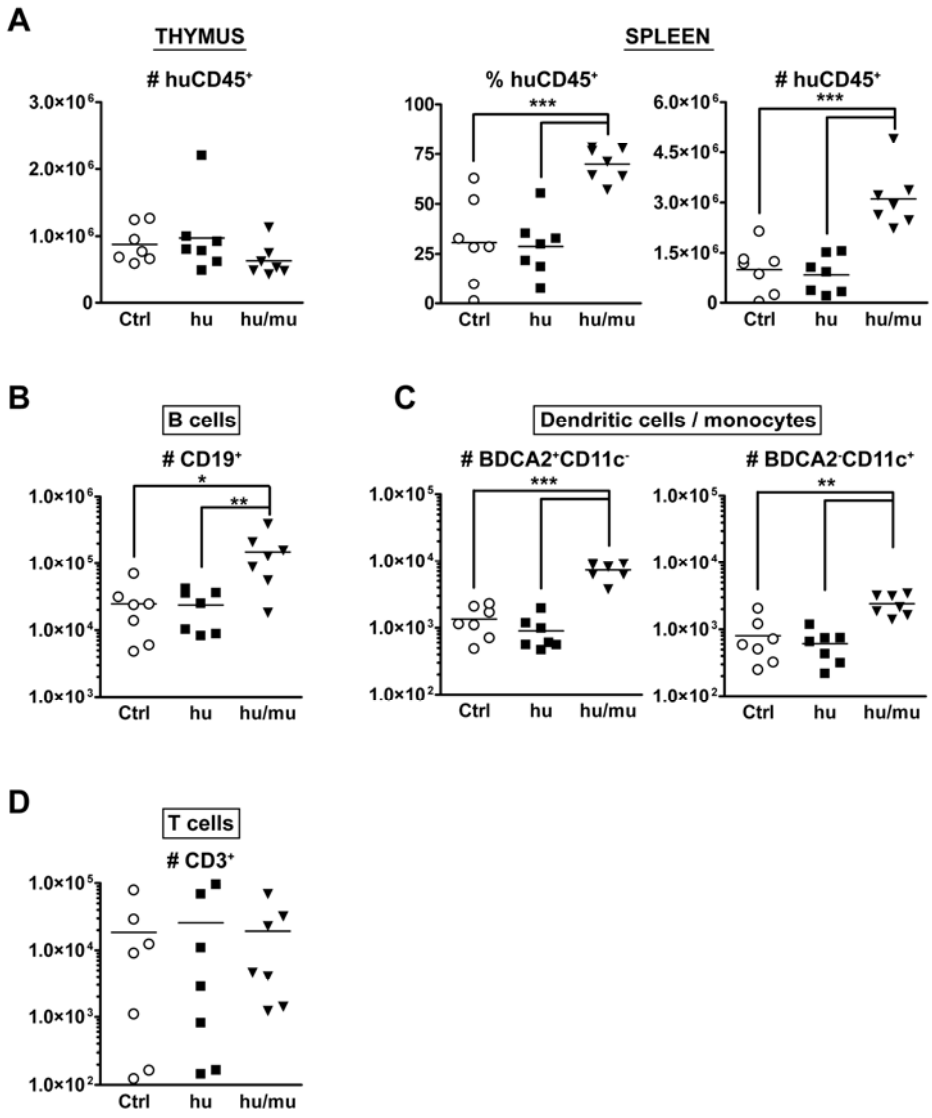

Figure 6. Improved muIL-7 usage in HIS (BALB-Rag/γ) mice with enforced expression of a mu/huIL-7Rα chimeric chain. *(A) The left graph shows the number of human thymocytes harvested from adult HIS (BALB-Rag/γ) mice (≥10weeks post-transplantation) produced either with control-transduced, huIL-7Rα-transduced or chimeric mu/huIL-7Rα-transduced human HSC. The frequency of huCD45$^+$ cells in the thymus was always ≥95%. The two graphs on the right show the frequency and number of human cells in the spleen. (B-D) We measured the number of (B) B cells (data from the bone marrow), (C) plasmacytoid DC (left) and conventional DC / monocytes (right; data from the liver), and (D) T cells (data from the spleen) generated by transduced progenitors. The results show a normalized amount of cells generated from a fixed amount of GFP$^+$ progenitors, in order to correct for variations in transduction efficiency. Student's t-test analysis: * p<0.05; ** p<0.01; *** p<0.001.*

Discussion

The role of IL-7 in human hematopoiesis and lymphocyte homeostasis is still not fully resolved. Considering the various cell lineages and developmental stages where IL-7 receptor expression is detected, and keeping in mind major discrepancies between mouse and human studies, it is likely that several aspects of human IL-7 biology remain elusive. In this study, we explored human hematopoiesis and its dependency of IL-7, using an experimental system mimicking a human immune system *in vivo*. In adult HIS (BALB-Rag/γ) mice, the major human lymphoid and myeloid cell lineages are observed, although the reconstitution levels are far from optimal. For instance, the amounts of human T cells found in the thymus and peripheral lymphoid organs are around 1% of what one could expect in a wild-type mouse. Interestingly, we observed *in vitro* that muIL-7, although being cross-reactive to the human IL-7 receptor (37), is around 100-fold less potent than huIL-7 to induce proliferation, survival and differentiation of human PNT progenitors in the OP9-huDL1 co-culture system. We therefore questioned whether this low efficacy was particularly detrimental in an *in vivo* model of human hematopoiesis.

Repeated injections of huIL-7 into HIS (BALB-Rag/γ) mice between week 2 and 6 after xenograft transplantation improved thymopoiesis and pDC accumulation, but only in a transient fashion peaking at two weeks after the onset of the treatment. Inoculation into adult animals did not exhibit any notable effect on human cell differentiation, especially T cell development in the thymus, and only minor T cell accumulation was noticed in peripheral lymphoid organs. These results suggest that a transient wave of human cell accumulation can be induced by exogenous huIL-7 around 2 weeks after xenograft transplantation, potentially through the enforced differentiation of huIL-7-sensitive human progenitors that are lost or becoming huIL-7 insensitive in adult HIS (BALB-Rag/γ) mice. This may be the consequence of a competitive selection process of human progenitors that do not require fully optimal huIL-7 induced signals for their survival, i.e. the most fit cells in a muIL-7 saturated environment. In such condition, as observed in IL-$7^{-/-}$ or IL-$7R\alpha^{-/-}$ mice (15, 16), only limited lymphopoiesis can be achieved. Human B cell differentiation is not induced by exogenous huIL-7 in HIS (BALB-Rag/γ) mice, consistent with previous human studies focusing on patients deficient for components of the IL-7 receptor and its signaling cascade such as IL-7Rα, JAK3 and γ_c (19-22).

One of the strengths of a humanized mouse model such as HIS (BALB-Rag/γ) mice resides in the possibility to prospectively analyze cellular factors involved in human immune system development and function through human HSC genetic manipulation. When a survival advantage was provided to the HIS (BALB-Rag/γ) human cells through Bcl-XL over-expression, which would be expected to decrease T cell susceptibility to apoptosis (11), human thymocytes and, to a lesser extent, NK cells were indeed selectively favored. Despite increased T cell production in the thymus, Bcl-XL expressing human T cells did not accumulate significantly in peripheral lymphoid organs, highlighting the fact that other survival signals are required for optimal peripheral T cell survival. In contrast, Bcl-2 over-expression induced human cell accumulation in a non-specific manner, i.e. without significantly favoring any immune cell lineage. Optimized usage of muIL-7 by the human cells, by enforced expression of a chimeric mu/huIL-7Rα chain, clearly showed global improvement of human engraftment level in HIS (BALB-Rag/γ) mice, except for the T cell lineage. It was already shown in mouse that over-expression of IL-7Rα is impairing the “altruistic” down-regulation of the IL-7 receptor on DP thymocytes, and that this effect could not be solely explained by lower Bcl-2

expression (8, 42). A similar phenomenon could partly explain the lack of significant effect on T cell development and homeostasis observed in mu/huIL-7Rα chain transduced cells.

By using these three different approaches, we could never significantly improve the peripheral human T cell reconstitution in HIS (BALB-Rag/γ) mice, despite consistent improvements of human thymopoiesis in the murine thymus. Overall, these results therefore indicate that the survival defect of human cells, and particularly T cells, at the periphery of HIS (BALB-Rag/γ) mice is probably multi-parametric, and can not be overcome by providing a single survival agent, either by exogenous supplementation or by genetic means. For instance, IL-15 is also know as a major cytokine for survival of naïve $CD8^+$ T cells, memory T cells and NK cells (12) and we have also observed positive impact of injections of huIL-15/IL-15Rα-Fc complex on human $CD8^+$ T cells and NK cells in HIS (BALB-Rag/γ) mice (ND. Huntington, N. Legrand, K. Weijer, NL. Alves, A. Plet, Y. Jacques, H. Spits and JP. Di Santo, manuscript submitted).

There are several lines of observations clearly highlighting the T cell homeostasis defect in HIS (BALB-Rag/γ) mice, the most obvious being the level of human thymocytes and peripheral T cell numbers as compared to wild-type mice. We previously described the fact that human T cells in HIS (BALB-Rag/γ) mice are actively cycling and dying, as a majority of T cells incorporate BrdU within 24h and stain positive for Annexin-V (43). Furthermore, depletion of T cells in HIS (BALB-Rag/γ) mice using an anti-CD28 superagonist antibody (43) or an anti-CD4 antibody (N. Legrand, unpublished observation) is never recovered up to 8 weeks after treatment. There are several potential causes for the defective T cell homeostasis in HIS (BALB-Rag/γ) mice, in a non-exclusive way. To some extent, as we showed here for IL-7, cross-reactivity between mouse cytokines and the corresponding receptor is likely not optimal (37). Actually, such interspecies incompatibilities may apply to any receptor/ ligand system, such as TCR-MHC interactions, molecules involved in cell circulation (e.g. chemokines and their receptors), in cell adhesion, in co-stimulation or in cross-talk with epithelial and stromal components. Altogether, these limitations are likely to negatively impact on HSC survival and subsequently hinder immune cell development, e.g. poor entry of early thymic progenitors *in situ* would result in limited thymopoiesis (44).

Overall, we show in this study that IL-7 enhances human T cell differentiation and positively influences human hematopoiesis, which can be mimicked by over-expression of the anti-apoptotic factors Bcl-2 and Bcl-XL. Although IL-7 is necessary for improved human hematopoiesis and immune cell survival in HIS (BALB-Rag/γ) mice, it is not sufficient by itself, and future generations of HIS mice will probably have to combine the expression of several human gene products, both soluble and on stromal cellular components, in order to ensure improved levels of cellular reconstitution by human immune cells and their proper functional activity.

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