Stem Cell Factor Consistently Improves Thymopoiesis after Experimental Transplantation of Murine or Human Hematopoietic Stem Cells in Immunodeficient Mice

Evert-Jan Wils, Elwin J. C. Rombouts, Irene van Mourik, Hergen Spits, Nicolas Legrand, Eric Braakman and Jan J. Cornelissen

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Deficient thymopoiesis is a pivotal determinant of impaired immune competence in the later time period after allogeneic hematopoietic stem cell transplantation (HSCT). Stem cell factor (SCF) is essentially involved in early thymopoiesis. We evaluated whether SCF administration would improve recovery of thymopoiesis following HSCT in immunodeficient mice receiving: 1) bone marrow (BM) transplantation of congenic mice; or 2) human fetal liver HSCT in the human immune system mouse model. Following murine BM transplantation, SCF significantly enhanced thymopoiesis and peripheral T cell recovery in lymph nodes and spleen. SCF did not affect BM lymphoid progenitor recovery and/or expansion. Median thymic cellularity increased from 0.9 in PBS- to 266 × 10^4/thymus in SCF-treated mice (p = 0.05). Following human HSCT in human immune system mice, higher thymic cellularity was observed in SCF-treated mice. Double-negative and early double-positive thymocyte subsets increased, but especially late double-positive, CD4 single-positive, and CD8 single-positive thymocyte subsets were significantly enhanced (p < 0.05). These results show that exogenous supply of SCF may significantly improve murine and human posttransplant thymopoiesis, for which the effect is probably exerted by directly promoting T cell development intrathymically rather than by enhanced entry of prethymically expanded lymphoid progenitors.


Evert-Jan Wils,*† Elwin J. C. Rombouts,*† Irene van Mourik,* Hergen Spits,**‡ Eric Braakman,* and Jan J. Cornelissen*

Abbreviations used in this article: BDCA2, blood dendritic cell Ag 2; BM, bone marrow; BMC, bone marrow cells; BMT, bone marrow transplantation; CLP, common lymphoid progenitor; DN, double-negative; DP, double-positive; EL, early lymphoid progenitors; ELP, early progenitor with lymphoid and myeloid potential; FL, fetal liver; FLt3L, FLt3 ligand; HIS, human immune system; HSCT, hematopoietic stem cell transplantation; Lin, lineage; LN, lymph node; LSK, lineage-negative sca1–positive c-Kit–positive cells; LT-HSC, long-term hematopoietic stem cell; MPP, multipotent progenitors; pDC, plasmacytoid dendritic cells; rh, recombinant human; rr, recombinant rat; SCF, stem cell factor; SP, single-positive; ST-HSC, short-term hematopoietic stem cell.

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Materials and Methods

**Mice**

RAG-2−/− common cytokine γ-chain (IL-2Rγc)−/− mice on a mixed background (originally bred at The Netherlands Cancer Institute, Amsterdam, The Netherlands) were inbred on a BALB/c background. RAG-1−/− (CD45.2 on C57BL/6 background) and C57BL/6 (CD45.1) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were bred under specific pathogen-free conditions at the Erasmus Medical Center Experimental Animal Center or in individual ventilated cages in the Animal Biosafety Level 2 animal facility of the Academic Medical Center of the University of Amsterdam (Amsterdam, The Netherlands). Food and water were available ad libitum. Housing, care, and all animal experiments were performed in accordance with Dutch legal regulations, which include approval by an ethical committee.

**Transplantation of murine bone marrow and human FL cells**

Bone marrow cells (BMC) obtained from crushed femurs and tibias of donor mice were depleted of T cells by incubation with rat anti-mouse CD4 (YTS191, YTA312) and rat anti-mouse CD8 (YTS169) mAbs for 30 min on ice, followed by a wash and immunomagnetic depletion using goat-anti-rat IgG microbeads and the autoMACS (Miltenyi Biotec, Bergisch Gladbach, Germany). The efficacy of T cell depletion was estimated prior to transplantation for each experiment and estimated at >2 log (2 to 3 logs). Tn-12 wk-old BALB/c mice were 3 GY irradiated ([137Cs] γ-rays; Gammacell; Atomic Energy of Canada, Ottawa, Ontario, Canada) and received 2 × 107 T cell-depleted C57BL/6 (CD45.1) congenic BMC by tail vein infusion.

The HIS mice model was generated as described previously (25, 26). In short, newborn (days 3–7) RAG-2−/− IL-2Rγc−/− mice were 3.5 Gy irradiated and transplanted with 5–10 × 106 CD34+CD38−/low FL cells intrahepatically. FL tissue samples were obtained from elective abortions, with a gestational age of 14–18 wk. The use of these human tissues was approved by the Medical Ethical Committees of the Academic Medical Center of the University of Amsterdam (Amsterdam, The Netherlands). Food and water were available ad libitum. Housing, care, and all animal experiments were performed in accordance with Dutch legal regulations, which include approval by an ethical committee.

**Cytokine administration**

Recombinant rat (r) and recombinant human (rh) SCF were kindly provided by Amgen (Thousand Oaks, CA). Recipient mice of murine BMC received PBS or rrSCF (100 μg/kg/injection) by s.c. injection three times a week from day 1 until the end of the experiment. In HIS mice, PBS or rhSCF (100 μg/kg/injection) was administered i.p. three times weekly as of day 14 following transplantation.

**Flow cytometric analysis**

At different time points following murine HSCT and human into mouse HSCT; bone marrow (BM), liver, thymus, peripheral blood, lymph node (LN), or spleen was harvested and analyzed using flow cytometry.

**Murine BM model.** Absolute numbers of subsets of peripheral blood leukocytes were determined by single-platform flow cytometry as described previously. mAbs against murine epitopes used for flow cytometric analysis were: anti-CD3, anti-CD4, anti-CD8, anti-CD3, anti-CD19, anti-CD45.1, and anti–Gr-1 (BD Pharmingen, San Jose, CA). Thymic and BM subsets were defined and analyzed as previously described (16). Thymic double-negative (DN) cells were defined as Lineage− (Lin−), CD4−, CD8−, and CD5−. Thymic double-positive (DP) and single-positive (SP) cells were defined as CD45+CD3+CD4+CD8+ and CD45+CD3+CD4−CD8− (CD4 SP) or CD4−CD8+ (CD8 SP). Long-term hematopoietic stem cells (LT-HSC; lineage-negative sca-1–positive c-Kit–positive cells [LSK]flt3−/−Lin−), short-term HSC (ST-HSC; LSKflt3+CD34+), multipotent progenitors (MPP; LSKflt3−/−Lin−CD127+), early lymphoid progenitors (ELP; LSKflt3−/−Lin−CD127−), common lymphoid progenitors (CLP; Lin−CD127+ sca-1−/−c-Kit−/−IL-7Raa1−/−CD43+CD11b+); and early progenitor with lymphoid and myeloid potential (EPLM; Lin−, B220−, CD127+, c-Kit+, CD34−/low) (CD34−/low). In BM samples were determined using previously published FACS criteria (27, 28). In brief, BMC were stained with a mixture of biotin-conjugated lineage panel (NK1.1-bio, MAC-1-bio, Gr-1-bio, TER-119-bio, CD3e-bio, and CD19-bio). All subsequent cells were washed, and Lin− cells were visualized by streptavidin-conjugated Pacific Orange. Subsequently, cells were also stained using Alexa 700-conjugated anti-sca-1, alophycocyanin-H7–conjugated anti–c-Kit, PE-conjugated anti-flt3, PeCy7-conjugated anti–CD127, PerCP-Cy5.5 anti-CD93, CD34-Alexa 647, elilow 450–anti-B220, and FTTC-conjugated anti–CD45.1.

**HIS model.** Peripheral blood and lymphoid organs were harvested and analyzed at different time points following transplantation. Mice were excluded from further analysis when BM human chimerism was <2.5%. To obtain a single-cell suspension, organs were minced and passed through a nylon mesh. Only the liver-cell suspensions were floccled, and mononuclear cells were isolated. Cells were washed and counted using a Casy counter (Roche Diagnostics, Almere, The Netherlands). Absolute numbers of cell subsets in organs were determined by multiplying the number of nucleated cells by the percentage of positive cells for the indicated surface-marker(s). mAbs against human epitopes used for flow cytometric analysis were CD45 (2D1), CD33, CD34 (8G12), CD38 (HB7), c-Kit (CD117), CD3 (SK7), CD4 (SK3), CD8 (SK7), CD14 (M52E), CD19 (HIB19), HLA-DR (BD Biosciences), blood dendritic cell Ag 2 (BDC2), AC141 (Miltenyi Biotec), and CD1a (T6-RD1; Beckman Coulter). Immature human subsets in BM and liver were defined as follows: stem cell-like, CD34+CD38−/low, myeloid-like progenitors, CD34+CD38−/low–c-Kit−/−CD33−; and lymphoid-like progenitors, CD34+CD38−/low–c-Kit−/−CD33− (Fig. 2A). Human thymocyte subsets were first gated as human CD45− cells and further defined as follows: DN, CD3−CD4+CD8−; early DP, CD3−CD4+CD8+; late DP, CD3+CD4+CD8−; and SP, CD3+CD4−CD8− or CD3−CD4+CD8− (Fig. 2B). Peripheral mature human cell subsets were first gated as human CD45+ cells and further defined as follows: B cells, CD19−HLA-DR−; plasmacytoid dendritic cells (pDC), BDC2+HLA-DR−; monocytes, CD14+BDC2+; and T cells, CD3+HLA-DR−. All cells were analyzed on a flow cytometer (LSRII or FACSaria; BD Immunocytometry Systems, San Jose, CA) using FlowJo software (Tree Star).

**Statistical analysis**

Statistical comparisons of experimental data between recipients of PBS or SCF groups were performed with a two-sided Mann–Whitney U test for unpaired data. The p values >0.05 were considered significant. Recovery data of groups of mice are presented as box-whisker plots, showing median, 25–75th percentiles (box), 10–90th percentiles (whiskers), and outliers (asterisks).

**Results**

SCF improves thymopoiesis and T cell recovery after murine HSCT

We first evaluated whether SCF administration would affect thymic recovery following murine T cell-depleted (CD45.1) BMT in the congeneric RAG-1−/− (CD45.2) mice. Mice were treated three times weekly with PBS or rhSCF s.c. as of day 1 until 4 or 6 wk posttransplantation. Thymic cellularity and thymocyte subsets of donor origin were determined in thymi of SCF− (n = 5) and PBS-treated (n = 5) BMT mice at 4 wk posttransplantation. As depicted in Fig. 1A, median numbers of total donor-derived thymocytes measured 9 × 103 in PBS-treated mice and 2660 × 103 in SCF-treated animals (p = 0.05). Although all thymocyte subsets increased following SCF, significant increases were observed in the DP, CD4 SP, and CD8 SP thymocyte subsets. In PBS-treated mice, the median number of DP, CD4 SP, and CD8 SP thymocytes measured 0.5, 0, and 0 × 103 per thymus, respectively. Mean numbers and ranges of the DN, DP, and SP subsets estimated, 3 × 103 (0–15 × 103), 49 × 103 (0–2458 × 103), and 11 × 103 (0–47 × 103), respectively. The recovery of the DP and SP thymocyte subsets appeared enhanced in SCF-treated mice with median numbers of DP, CD4 SP, and CD8 SP thymocytes estimated at 2400, 66, and 31 × 103 per thymus, respectively (Fig. 1B–E). Mean numbers and ranges of the DN, DP, and SP subsets of SCF-treated mice were estimated at 16 × 103 (0–45 × 103), 2451 × 103 (577–4154 × 103), and 95 × 103 (13–201 × 103), respectively. Next, we assessed whether SCF-induced improved thymopoiesis would translate into improved peripheral T cell recovery. Absolute numbers of donor-derived newly developed CD4+ and CD8+ T cells were very low in mice treated with PBS or SCF until 4 wk after transplantation in spleen and LN (Table 1). Therefore, evaluation of peripheral T cell recovery was also performed in mice treated for 6 wk. T cell numbers increased
significantly between week 4 and 6 after transplantation. Median CD4+ T cell numbers in LN of PBS-treated mice measured $0.23 \times 10^4$ at 4 wk and $253 \times 10^4$ at 6 wk posttransplantation. In SCF-treated mice, T cell numbers showed improved recovery in LN and spleen at 6 wk as compared with PBS-treated mice. Median LN CD4+ T cell numbers in SCF-treated mice measured $0.53 \times 10^4$ at 4 wk ($p = 0.64$) and $923 \times 10^4$ at 6 wk ($p < 0.05$) posttransplantation. Median numbers of newly developed splenic CD4+ T cells measured 594 and $1502 \times 3 \times 10^4$ in PBS- and SCF-treated mice, respectively ($p < 0.05$). Similar results were obtained for CD8+ T cell recovery. Peripheral blood T cell recovery at that time point showed a nonsignificant trend toward improved T cell recovery. Of note, donor B cell, NK cell, dendritic cells, and myeloid recovery as measured in peripheral blood, spleen, and LN were similar between PBS- and SCF-treated mice (data not shown). Furthermore, SCF administration did not affect overall donor chimerism (Supplemental Fig. 1).

As c-Kit is expressed on hematopoietic progenitor cells in BM, including myeloid precursor cells and progenitors with lymphoid and thymic-seeding potential, SCF may exert its beneficial effect directly by improving thymocyte proliferation and differentiation or alternatively by expansion of lymphoid progenitors at the BM level. Therefore, we addressed the question whether SCF would expand prethymic BM progenitors, including LT-HSC, ST-HSC, MPP, ELP, EPLM, CLP1, and CLP2. These subsets were quantified in PBS- and SCF-treated BMT mice after 4 wk of treatment (Table II). Absolute numbers of LT-HSC were higher in SCF-treated mice as compared with PBS-treated mice. Median numbers of LT-HSC measured $0.3 \times 10^4$ and $0.6 \times 10^4$ per two femurs in PBS- and SCF-treated mice ($p = 0.03$). However, the numbers of ST-HSC, MPP, ELP, EPLM, CLP1, and CLP2 were not increased following SCF treatment. Median numbers of ST-HSC, MPP, ELP, EPLM, CLP1, and CLP2 measured 10 versus 15, 3.5 versus 5.5, 6.6 versus 7.5, 0.2 versus 0.2, 1.1 versus 2.5, and 1.8 versus $1.3 \times 10^4$ per two femurs in PBS- versus SCF-treated mice, respectively ($p > 0.05$).

Collectively, these results suggested that SCF improves thymopoiesis by a direct effect at the thymus level rather than by enhanced entry of lymphoid progenitors, as we earlier observed in mice treated by FLT3L following murine BMT (16). To address the question of whether our results with SCF might be translated to a human setting, we next studied the effect of SCF in our recently developed HIS mouse model, as this humanized model may closely resemble human thymopoiesis (25).

<table>
<thead>
<tr>
<th>Table I. Donor T cells in PBS- or SCF-treated BMT mice</th>
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<tr>
<td><strong>CD4+ T Cell</strong></td>
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Median absolute numbers (range) of CD4+ and CD8+ T cells in spleen ($\times 10^4$), LN ($\times 10^4$), and peripheral blood (per microliter of blood) of mice receiving a congenic BMT and were treated with PBS ($n = 4$) or SCF ($n = 5$) for 4 or 6 wk (PBS, $n = 8$, versus SCF, $n = 10$).

*p < 0.05 comparing PBS versus SCF.

ND, not determined.
SCF improves human thymopoiesis in HIS mice

The 3.5 Gy-irradiated newborn RAG-2+/-IL-2Rγc-/- mice were transplanted intrahepatically with 5–10 × 10^6 CD34+CD38low-selected human FL cells and treated with rhSCF or PBS three times weekly i.p. as of week 2 for 2, 4, or 6 wk. Thymic cellularity and human thymocyte subsets were quantified in thymi of SCF- and PBS-treated HIS mice at 4, 6, and 8 wk after transplantation as outlined in the Materials and Methods section (Fig. 2B). Overall, human thymic cellularity was significantly increased in SCF-treated mice at both 6 and 8 wk posttransplantation (Fig. 3). Median thymic cellularity measured 176 and 326 × 10^3/thymus at 6 wk (p = 0.04) and 123 and 585 × 10^3/thymus at 8 wk posttransplantation (p = 0.01) in PBS- and SCF-treated HIS mice, respectively. Although DN and early DP thymocyte recovery were not significantly enhanced in SCF-treated HIS mice (Fig. 4A, 4B), late DP, CD4 SP, and CD8 SP thymocyte subsets were significantly increased in thymi of SCF-treated as compared with PBS-treated HIS mice (Fig. 4C–E). Median human late DP thymocyte numbers measured 7.6 and 156 × 10^3/thymus, median human CD4 SP thymocyte numbers 0.2 and 139 × 10^3/thymus, and median human CD8 SP thymocyte numbers 3.2 and 80 × 10^3/thymus in PBS- and SCF-treated HIS mice, respectively. Mean values and ranges of DN, DP, and SP human thymocyte subsets were estimated at 1.5 × 10^3 (0.7–5 × 10^3), 94 × 10^3 (0.255 × 10^3), and 57 × 10^3 (0.645 × 10^3), respectively, in PBS-treated HIS mice, whereas the respective values in SCF-treated mice were 8.4 × 10^3 (0.57 × 10^3), 242 × 10^3 (0.835 × 10^3), and 463 × 10^3 (0.615 × 10^3).

Peripheral T cell recovery in HIS mice

We recently observed impaired peripheral human T cell survival in the HIS model (26), thereby allowing us to study peripheral recovery only to a limited extent. Therefore, we also evaluated whether the observed enhancement of thymopoiesis would result in better T cell recovery in peripheral organs. Absolute numbers of T cells, but also B cells, pDCs, and monocytes were determined in spleen and liver of HIS mice at weeks 4 and 8. Results are shown in Table III. In PBS-treated HIS mice, human leukocyte recovery in spleen and liver increased from week 4 to week 8. Median splenic B cell numbers measured 8.7 × 10^3/spleen at 4 wk and 2361 × 10^3/spleen at 8 wk (p = 0.01). A concurrent increase in cell numbers was also observed for splenic T cells, pDCs, and monocytes and for T cells, B cells, and monocytes in the liver. Administration of SCF resulted in a minor increase in recovery of hepatic pDC, B and T cells, and splenic T cells as compared with PBS-treated HIS mice at weeks 4 and 8. However, statistical significance was only obtained for hepatic B cell numbers 4 wk after transplantation. Median B cell numbers measured 25 and 78 × 10^3 in PBS- and SCF-treated HIS mice, respectively (p < 0.05).

SCF transiently improves human progenitor cell recovery

Given the observed improved B cell recovery, the improved thymopoiesis, and the well-known expression of c-Kit on human hematopoietic progenitor cells, we evaluated whether administration of SCF would affect engraftment and recovery of human progenitors in BM and liver of HIS mice. Results are depicted in Table IV. Overall progenitor cell engraftment was assessed by numbers of human CD34+ progenitor cells in both liver and BM. It appeared that overall human progenitor cell engraftment in BM increased in time, irrespective of SCF administration. Median numbers of CD34+ progenitor cells in BM increased from 130 to 1550 × 10^3/femur between week 4 and 8 posttransplantation in PBS-treated HIS mice (p < 0.01). Human progenitor cell engraftment in the liver did not significantly increase in time and was not affected by SCF administration. In more detail, progenitor cells characterized by CD34+CD38low, CD34+CD38high cells, myeloid, and lymphoid-like progenitors (CD34+CD38highc-Kit+, myeloid: CD33+, lymphoid: CD33−) were determined in liver and BM of SCF- and PBS-treated HIS mice. Absolute numbers of CD34+CD38low and CD34+CD38high human progenitor cells in the liver did not increase in time in PBS-treated HIS mice. Median CD34+CD38low cell numbers measured 4 and 2 × 10^5/liver in the liver at weeks 4 and 8, respectively. In contrast, absolute numbers of hepatic lymphoid and myeloid progenitor-like cells decreased in time. Median lymphoid-like progenitor cell numbers measured 3.2 and 0.6 × 10^5 per liver at weeks 4 and 8, respectively (p < 0.01). Administration of SCF appeared to be associated with
improved recovery of all human progenitor subsets evaluated in the liver at 4 wk after transplantation as compared with PBS-treated mice. Significance was obtained for lymphoid-like progenitor cell recovery. Median lymphoid progenitor-like cell numbers measured 10.5 (range 5.6–25.2) in SCF-treated mice compared with 3.2 $\times$ 10^3/liver (range 0.9–9.5) in PBS-treated mice 4 wk after transplantation ($p = 0.02$).

In BM, absolute numbers of CD34$^+$CD38$^{high}$ human progenitor cells increased in time in PBS-treated HIS mice. Median CD34$^+$CD38$^{high}$ cell numbers measured 119 and 1545 $\times$ 10^3/femur at weeks 4 and 8, respectively ($p < 0.01$). CD34$^+$CD38$^{low}$, lymphoid, and myeloid progenitor-like cells did not significantly increase in time. Administration of SCF resulted in a nonsignificant improved recovery of all evaluated human progenitor subsets in BM at 4 and 8 wk posttransplantation.

**Discussion**

Deficient thymopoiesis is currently considered the most important determinant of impaired immune competence in human recipients of allogeneic stem cell grafts in the later time period after transplantation (1, 3). Although severely compromised in older transplant recipients, the thymus still retains the ability to support T cell development, even in patients $>40$ y of age (29, 30). However, recapitulation of thymopoiesis may take many months if not years, which may confer a substantial risk for opportunistic infections and death in older transplant recipients (11, 31, 32). Therefore, new approaches to restore or boost thymopoiesis are currently receiving considerable attention, including the application of cytokines that are critically involved in thymopoiesis. We and others experimentally explored the cytokines IL-7 (33–37) and FLT3L (15, 16), and these studies fueled their potential for further clinical development. Although IL-7 and FLT3L have been studied more elaborately, the early acting hematopoietic cytokine SCF has so far received relatively little attention (24). Apart from its role in the earliest phases of hematopoiesis, SCF is also critically involved in thymopoiesis with expression of its receptor on developing thymocytes before the expression of the Ag-specific TCR (17, 18). It provided the rationale to study SCF in a murine BMT model as well as in humanized mice as a preferable translational model.

In the current study, we demonstrate that exogenous administration of SCF significantly improves thymopoiesis in both mod-

![FIGURE 3](http://www.jimmunol.org/)

**FIGURE 3.** Overall human thymopoiesis and subset distribution in HIS mice. Median human thymic cellularity and subset distribution in murine thymi 4 ($n = 5$ versus 6), 6 ($n = 11$ versus 15), and 8 wk ($n = 10$ versus 13) posttransplantation in HIS mice receiving PBS or SCF, respectively. The $p$ values for human thymic cellularity between SCF and PBS are indicated.

![FIGURE 4](http://www.jimmunol.org/)

**FIGURE 4.** SCF improves thymic recovery following human SCT. Newborn RAG-2$^{-/-}$γc$^{-/-}$ mice were 3.5 Gy irradiated and received 5–10 $\times$ 10^4 CD34$^+$CD38$^{low}$ FL cells intrahepatically. Mice were treated with PBS ($n = 10$) or SCF ($n = 13$), and thymi were harvested 8 wk posttransplantation. Data are presented as box-whisker plots of DN (CD3$^-$CD4$^-$CD8$^-$) (A), early DP (CD3$^+$CD4$^+$$CD8^+$) (B), late DP (CD3$^+$CD4$^+$$CD8^+$) (C), CD4 SP (CD3$^+$CD4$^+$$CD8^-$) (D), and CD8 SP (CD3$^+$CD4$^-$CD8$^+$) (E) human thymocytes shown in mice treated with PBS and SCF. Outliers are indicated by asterisks (*). The $p$ values between SCF and PBS are indicated.
Intrathymically, c-Kit+ early thymic progenitors develop subsets have been shown to efficiently seed the murine thymus. Of note, only the lymphoid-primed MPP and CLP that predominantly give rise to B cells and show low c-Kit expression (38). Previous studies have shown that exogenous administration of FLT3L did expand MPP not affected by SCF administration. Previously, we and others showed that exogenous administration of FLT3L did expand MPP and CLP, which preceded an increase of thymocytes and enhanced thymopoiesis (16, 43). That observation and the absence of SCF-mediated expansion of BM progenitors with thymic seeding potential suggest that it is unlikely that enhanced thymic seeding has accounted for improved thymopoiesis in our murine model.

Murine models may mirror human hematopoiesis remarkably well, but important differences remain that hamper the proper translation of murine findings into potential human applications (44). To bridge the translational gap, HIS mice have been developed that allow the study of the human immune physiology. In addition, the HIS model allows for a more rapid and cost-effective evaluation of promising candidates to manipulate human hematopoiesis and lymphopoiesis, as in the current study. Of note, human T cell lymphopoiesis differs considerably from murine lymphopoiesis. Human HSC are enriched in Lin-CD34+CD38low/Lin-negative subsets (LT-HSC) that develop via ST-HSC and MPP into progenitor cells with lymphoid potential. LSK cells and the MPP (LSKflt3+) subset may develop into CLP1 and CLP2 that predominantly give rise to B cells and show low c-Kit expression (38). Of note, only the lymphoid-primed MPP and CLP subsets have been shown to efficiently seed the murine thymus (39–41). Intrathymically, c-Kit+ early thymic progenitors develop via DN2 into DN3 and lose c-Kit expression (17). TCR-positive thymocytes that mature into CD4SP or CD8SP thymocytes lack c-Kit expression, which remains so after egress from the thymus and during the development of the peripheral immune response. In the current study, exogenous SCF significantly enhanced murine thymopoiesis after T cell-depleted BMT with most pronounced expansion of TCR-positive thymocytes. The DN subset was only marginally affected, suggesting that c-Kit+ thymocytes rapidly complete their rearrangement and express the TCR upon SCF stimulation, followed by strong expansion of the DP subset. In line with the earlier observed impaired thymopoiesis in c-kit−/− and SCF−/− thymi (19, 20, 42), our results underscore the essential role of SCF signaling in early thymopoiesis and suggest that both acceleration and enhancement of thymopoiesis may be achieved by exogenous SCF. Our results compare well to those of Chung et al. (24), who recently observed a comparable enhancement of thymopoiesis following congenic murine BMT by SCF, especially when combined with IL-7.

A modest expansion of the early c-Kit+, Flt3+ Lin-negative subsets (LT-HSC) was observed in the BM of SCF-treated mice, but the lymphoid-biased c-Kit+ subsets, including (lymphoid-primed) MPP and CLP with thymic seeding potential, were not affected by SCF administration. Previously, we and others showed that exogenous administration of FLT3L did expand MPP and CLP, which preceded an increase of thymocytes and enhanced thymopoiesis (16, 43). That observation and the absence of SCF-mediated expansion of BM progenitors with thymic seeding potential suggest that it is unlikely that enhanced thymic seeding has accounted for improved thymopoiesis in our murine model.

**Table III. Human leukocyte subsets in spleen and liver of PBS or SCF-treated HIS mice**

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<th>PBS</th>
<th>SCF</th>
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<tr>
<td><strong>Spleen</strong></td>
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<tr>
<td>T cells</td>
<td>0.0 (0.0–0.0)</td>
<td>0.0 (0.0–0.0)</td>
<td>35 (0–161)</td>
<td>76 (1.4–113)</td>
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<tr>
<td>B cells</td>
<td>8.7 (2.8–20)</td>
<td>26 (4.1–39)</td>
<td>2361 (432–5348)</td>
<td>1071 (561–2317)</td>
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<td>pDC</td>
<td>2.6 (0.7–3.5)</td>
<td>4.0 (0.4–8.5)</td>
<td>28 (0–61)</td>
<td>16 (4.4–24)</td>
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<td>Monocytes</td>
<td>0.2 (0.04–0.4)</td>
<td>0.5 (0.03–0.6)</td>
<td>10 (2.8–23)</td>
<td>5.8 (2.9–61)</td>
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<td><strong>Liver</strong></td>
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<tr>
<td>T cells</td>
<td>0.04 (0.0–0.4)</td>
<td>0.2 (0.0–1.3)</td>
<td>4.3 (0–22)</td>
<td>20.1 (2.4–121)</td>
</tr>
<tr>
<td>B cells</td>
<td>25 (7.0–45)</td>
<td>78 (37–100)*</td>
<td>323 (52–360)</td>
<td>363 (126–607)</td>
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<tr>
<td>pDC</td>
<td>28 (8.6–51)</td>
<td>54 (23–97)</td>
<td>37 (3.5–68)</td>
<td>41 (31–54)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.7 (0.2–6.2)</td>
<td>2.8 (1.1–5.1)</td>
<td>13 (2.8–37)</td>
<td>8.7 (3.0–22)</td>
</tr>
</tbody>
</table>

Median absolute numbers ($\times 10^3$ range) of human T cells (CD3+HLA-DR-), B cells (CD19+HLA-DR+), pDC (BDCA2+ HLA-DR+) and monocytes (CD14+ BDCA2+) in spleen and liver at 4 and 8 wk posttransplantation in HIS mice treated with PBS (n = 8 and 10) or SCF (n = 10 and 13).

*p < 0.05 comparing PBS versus SCF.

**Table IV. Human progenitor cell subsets in BM and liver of HIS mice**

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>SCF</th>
<th>PBS</th>
<th>SCF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+</td>
<td>130 (9–467)</td>
<td>299 (15–925)</td>
<td>1550 (53–4039)</td>
<td>2181 (124–5522)</td>
</tr>
<tr>
<td>CD34+CD38low</td>
<td>10 (0–29)</td>
<td>12 (0–33)</td>
<td>6 (0–51)</td>
<td>16 (0–58)</td>
</tr>
<tr>
<td>CD34+CD38high</td>
<td>119 (9–449)</td>
<td>281 (15–903)</td>
<td>1545 (51–4005)</td>
<td>2160 (124–5514)</td>
</tr>
<tr>
<td>CD33+c-Kit*</td>
<td>9.3 (2.1–20.7)</td>
<td>15.7 (1.2–49.2)</td>
<td>8.3 (0.7–15.3)</td>
<td>8.8 (1.6–58.5)</td>
</tr>
<tr>
<td>CD33- c-Kit*</td>
<td>2.2 (1.0–7.8)</td>
<td>5.2 (0.7–15.6)</td>
<td>8.0 (0.4–15.3)</td>
<td>8.9 (1.6–40.7)</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+</td>
<td>37 (0–164)</td>
<td>83 (3–330)</td>
<td>137 (9–594)</td>
<td>503 (17–736)</td>
</tr>
<tr>
<td>CD34+CD38low</td>
<td>4 (0–29)</td>
<td>14 (0–49)</td>
<td>2 (0–6)</td>
<td>2 (0–9)</td>
</tr>
<tr>
<td>CD34+CD38high</td>
<td>33 (0–135)</td>
<td>70 (3–281)</td>
<td>135 (9–588)</td>
<td>500 (17–727)</td>
</tr>
<tr>
<td>CD33+c-Kit*</td>
<td>1.1 (0.2–4.1)</td>
<td>3.0 (2.5–8.2)</td>
<td>0.4 (0.0–1.8)</td>
<td>0.6 (0.0–3.0)</td>
</tr>
<tr>
<td>CD33- c-Kit*</td>
<td>3.2 (0.9–9.5)</td>
<td>10.5 (5.6–25.2)*</td>
<td>0.6 (0.1–3.0)</td>
<td>0.6 (0.0–3.0)</td>
</tr>
</tbody>
</table>

Median numbers of human progenitor subsets (range) $\times 10^3$/organ at week 4 and 8 posttransplantation in HIS mice treated with PBS (n = 8 and 10) or SCF (n = 10 and 13).

*p < 0.05 comparing PBS versus SCF.
entiated CD34⁺CD38⁻ progenitors. CD33 upregulation is associated with a bias toward myelorethroid potential (46), and CD10 or CD7 expression is associated with lymphoid and thymic seeding potential (47–50). Human thymocytes develop into mature T cells via a highly ordered process within the human thymus (49). Similar to murine thymocytes, c-Kit is expressed on the earliest human DN thymocytes (18, 51, 52). In contrast, c-Kit is more abundantly expressed on primitive murine hematopoietic progenitor cells, whereas human c-Kit is preferentially expressed on myeloid and also lymphoid-committed progenitors (48, 53). These differences of expression by hematopoietic progenitors may at least in part explain the SCF-mediated expansion of LT-HSC after murine BMT, whereas such an effect was absent in the HIS model. In line with previous in vitro studies (54, 55), the human CD34⁺CD38⁻ subset was not affected by SCF. Although total CD34⁺CD38bright and CD34⁺CD38bright⁺c-Kit⁺ progenitor cell numbers were also not affected by SCF, a modest increase in CD34⁺CD38bright⁺CD33⁻ c-Kit⁺ progenitor cells, which may harbor progenitors with high lymphoid development potential, was observed in HIS mice. In addition, SCF resulted in an early increase of human B cells that may have originated from the latter subset. It seems, however, unlikely that expansion of the CD34⁺CD38bright⁺CD33⁻ c-Kit⁻ subset accounts for the observed improvement of thymopoiesis. First, the increase of B cell recovery appeared relatively modest and was only observed at week 4, whereas B cells are the preferred leukocyte subset that rapidly develops following engraftment in HIS mice. Second, other lymphoid cells like NK cells, and pDCs were not enhanced in SCF-treated HIS mice, arguing against an effect on a CLP. Third, in contrast to the effect of FLT3L in our murine model (16), SCF selectively enhanced arginating against an effect on a CLP. Third, in contrast to the effect of FLT3L in our murine model (16), SCF selectively enhanced thymocyte development into mature T cells predominantly caused an improved peripheral T cell recovery, although we cannot exclude that peripheral expansion may have contributed to some extent. Despite a similar increase in thymic recovery, T cell numbers were not increased in SCF-treated HIS mice. HIS mice recapitulate the development of hematolymphoid development, but homostatic support of mature T cells in peripheral organs has remained rather poor. HIS mice remain a hybrid human–mouse system in which cytokine receptor compatibility between species and MHC-HLA mismatch may compromise mature T cell survival (56, 57). As SCF selectively affects T cell progenitor cells, in contrast to IL-7 (26, 58) and IL-15 (59), improved survival of mature T cells following rhSCF was not expected. However, further study of functional immune competence in humanized mice would require optimized peripheral T cell homeostasis and survival (60). Despite the abundant presence of human APCs (B cells, monocytes), the survival and expansion of newly developed human T cells needs to be improved. We recently showed no significant effect of IL-7 on peripheral T cell numbers in HIS mice (26), possibly suggesting that more complete human donor chimerism should be pursued, instead of a persisting and even declining situation of mixed and split chimerism of the various hematopoietic lineages. Nevertheless, HIS mice do allow for studying possible thymopoiesis-stimulating agents in a translational perspective. Apart from human FL-derived HSC, also clinically used stem cell sources such as umbilical cord blood may be used to further bridge a translational gap.

In summary, exogenous SCF significantly enhanced both murine and human thymopoiesis in translational models of severe lymphopenia, as can be observed clinically following HSCT. These results justify further preclinical and toxicology studies aimed at the potential application of SCF after human HSCT for improving thymopoiesis.

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Disclosures
The authors have no financial conflicts of interest.

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