Thymus Reconstitution by c-kit-expressing Hematopoietic Stem Cells Purified from Adult Mouse Bone Marrow

By Peter de Vries,* Kenneth A. Brasel,* Hilary J. McKenna,† Douglas E. Williams,* and James D. Watson‡

From the *Department of Experimental Hematology, Immunex Corporation, Seattle, Washington 98101; and the †Department of Molecular Medicine, University of Auckland School of Medicine, Auckland, New Zealand

Summary

The introduction of clonal assays and long-term culture systems has resulted in considerable progress in the understanding of the early events that control self-renewal and commitment to differentiation of pluripotent hematopoietic stem cells (PHSC). Relatively little is known about the factors that control the commitment of PHSC to the lymphoid lineages, especially the T cell lineage. In the present study, the expression of the proto-oncogene c-kit was used to isolate and study the capacity of highly purified day 14 colony-forming units-spleen (CFU-S) to reconstitute the thymus of sublethally irradiated Thy-1 congenic recipient mice. We demonstrate here that one c-kit positive (c-kit+) stem cell upon intrathymic transfer can effectively reconstitute the thymus of a sublethally irradiated recipient. After a lag phase of 15 d, high levels of donor-derived thymocytes (Thy-1.1+) could be detected until 65 d after transplantation in Thy-1.2+ host mice. Donor-derived cells were only detected in the lobe of the thymus in which cells were previously injected and not in the noninjected lobe. These data suggest that c-kit+ stem cells do not migrate from one lobe to another and that they do not re-seed the thymus after having migrated to the bone marrow. The level and duration of reconstitution was found to be cell dose dependent, suggesting that, over time, endogenous stem cells compete with donor stem cells for available sites in the thymus microenvironment. The data presented in this paper demonstrate that commitment of purified adult bone marrow-derived c-kit+ stem cells to the T cell differentiation pathway can occur in the thymus and does not have to happen in the bone marrow.

Hematopoiesis is a dynamic, complex process by which large numbers of mature blood cells with specific functions and limited life spans are generated (for review see reference 1). All mammalian blood cell types originate from a common, very small population of pluripotent hematopoietic stem cells (PHSC), found predominantly in the bone marrow (BM) (2). PHSC are characterized by their capacity for self-renewal and for generation of committed progenitors of at least eight identified hematopoietic lineages (1, 3).

The regulation of hematopoiesis is dependent on complex interactions among hematopoietic cells, cytokines, and stromal elements (1, 3–5). Progress in the understanding of early events in hematopoiesis has resulted from clonal assays for (committed) stem cells both in vivo and in vitro (1, 6–8) and the identification and molecular cloning of a wide variety of cytokines which are essential for the survival (in vitro) of colony-forming cells (1, 4, 9). The introduction of long-term BM cultures for myeloid cells and B lymphoid cells (5, 10–12) showed the importance of stromal elements for hematopoiesis.

Little is known about the factors that control the commitment of PHSC to lymphoid lineages, the T cell lineage in particular. It is still not clear whether thymic stem cells enter the thymus as uncommitted PHSC or as committed T lineage stem cells. In other words, does commitment of PHSC to the T cell lineage occur in the BM or thymus?

To address this question we purified stem cells (day 14 colony forming units-spleen [CFU-S]), (6) from adult mouse BM using a modification of an earlier described purification procedure (13). Instead of using the mitochondrial dye rhodamine 123, stem cells have been purified on the basis of the expression of the proto-oncogene c-kit by using its biotinylated ligand called SLF (steel factor), which was recently characterized at the cDNA and protein level (14–16). Isolated
c-kit<sup>mm</sup> cells were highly enriched for day 14 CFU-S. One c-kit<sup>mm</sup> cell could give rise to one day 14 spleen colony if a spleen-seeding efficiency (17, 18) of 10% is assumed. Although the purification of day 14 CFU-S has been the end point in many purification protocols for PHSC (14, 19–28) it is now widely accepted that perhaps only a subpopulation of day 14 CFU-S are truly PHSC. The majority of day 14 CFU-S are often considered to be myeloid-restricted stem cells (19, 24, 26, 28–30). This is supported by the fact that erythroid, granulocytic, megakaryocytic, and monocytic cells are consistently present in spleen colonies, either as pure populations or in varying mixtures (31–33). However, cells of the T lymphocyte lineage have never been demonstrated in spleen colonies (1), and the presence of B lymphoid cells has only rarely been reported (34, 35).

In the present study we tested the capacity of the purified c-kit<sup>mm</sup> stem cells to reconstitute the thymus of sub lethally Thy-1 congenic recipient mice upon intrathymic (IT) transfer (36). It was found that in four out of nine mice, one purified c-kit<sup>mm</sup> cell could reconstitute a thymic lobe. Upon IT transfer of 10<sup>3</sup> c-kit<sup>mm</sup> cells, progeny of the c-kit<sup>mm</sup> cells were first detected 15 d after transplantation, and were still detected 65 d after IT transfer. The level and duration of reconstitution were shown to correlate with the number of cells injected.

These data, together with the fact that the c-kit<sup>mm</sup> cell fraction forms spleen colonies on day 14 (day 14 CFU-S) with unit efficiency, demonstrate that commitment of the c-kit<sup>mm</sup> stem cells to the T cell differentiation lineage can take place in the thymus microenvironment.

**Materials and Methods**

**Mice.** 6–10-wk-old female B6.PL-Thy-1<sup>+</sup> (Thy-1.1) mice (The Jackson Laboratory, Bar Harbor, ME) were used as BM donors in purification experiments. 8–10-wk-old female C57BL/6 (Thy-1.2) (Charles River Breeding Laboratories, Inc., Wilmington, MA) or C57BL/10SN (Thy-1.2) (The Jackson Laboratory) were used as recipients of purified BM stem cells in IT transfer experiments (36). In these experiments, recipient mice were exposed to 750 rad of gamma irradiation from a 137Cs source (Mark 1 irradiator; Shepard and Associates, Glendale, CA) at a dose rate of 93 rad per min, and were transplanted 1–4 h after irradiation.

**Preparation of Cell Suspensions.** BM cell suspensions were obtained by flushing the femoral and tibial shafts with 1 ml ice cold Hepes buffered (10 mM, pH 6.9) HBSS without phenol red (HH; Gibco Laboratories, Grand Island, NY). The cells were passed through a 88-μm pore size filter (Nitex; Small Parts Inc., Miami, FL) to remove cell clumps. The single cell suspensions were washed twice in HH and kept at 4°C.

Thymic lobe single cell suspensions were obtained by grinding the tissue between the wetted (PBS) frosted ends of two glass slides. Cells were washed once in HH containing 5% heat-inactivated FCS (Intergen Company, Purchase, NY) and 0.02% sodium azide (HHSA; HH plus serum plus azide), before immunofluorescence staining.

**Immunofluorescence Staining.** Determination of donor (Thy-1.1) and/or host (Thy-1.2) origin of thymocytes was performed using an optimal amount of culture supernatant of anti-Thy-1.1 producing clone 22.1.D1, and purified anti-Thy-1.2/FITC (Becton Dickinson Immunocytometry Systems, San Jose, CA), respectively. Cells were incubated for 30 min at 4°C, followed by one wash in HHSA and a second 30-min, 4°C incubation with an optimal amount of FITC-conjugated goat F(ab')<sub>2</sub> anti-mouse IgM μ chain (Tago Inc., Burlingame, CA) (anti-Thy-1.1 only). Cells were analyzed using an unmodified FACScan<sup>®</sup> (Becton Dickinson & Co.).

To determine the cellular composition of (reconstituted) thymus lobes, the following mAbs were used: anti-CD4/FITC (clone GK 1.5), anti-CD8/FITC (Becton Dickinson & Co.), anti-CD3 (α/β chains)/FITC (PharMingen, San Diego, CA), anti-Mac-1/FITC (Boehringer Mannheim Corp., Indianapolis, IN), anti-Gr-1/FITC (PharMingen), and 15-1.4/FITC (13). Cells were incubated for 45 min at 4°C, washed once in HHSA, and analyzed on the FACScan<sup>®</sup>.

**Stem Cell Purification.** Stem cells were purified from adult BM according to a modification of a previously described procedure (13, 20). Briefly, after a discontinuous density gradient separation and simultaneous labeling with Texas red conjugated wheat germ agglutinin (WGA/TxR), the low density cells were stained with FITC-conjugated mAb Ab 15-1.4.1 (15-1.4.1/FITC) (37) and an optimal amount of biotinylated murine rSLF (steel factor, Immunex Corporation, Seattle, WA). SLF was biotinylated as described by Armitage et al. (38). In some experiments, the cells were also labeled with an optimal dose of anti-B220/FITC antibody (CD45R; Pharmingen). After 1 h at 4°C, the cells were washed once in HHSA and then incubated with PE-conjugated streptavidin (SA/PE; Becton Dickinson & Co.) according to the manufacturer's recommendations. After 30 min on ice, the cells were washed once and resuspended in HH plus 5% FCS at 2–4 × 10<sup>6</sup> cells/ml, analyzed, and sorted using an unmodified dual laser equipped flow cytometer (FACStar Plus<sup>®</sup>; Becton Dickinson & Co.).

The cells of interest were selected in the following way: first, the WGA/TxR<sup>+</sup> positive (WGA<sup>+</sup>) cells were selected by setting electronic gates around these cells using the LYSYSII software program (Becton Dickinson & Co.). Second, the "blast cells" were selected by gating out the WGA<sup>+</sup> cells with intermediate forward light scatter and low to intermediate side light scatter intensities (13, 20). Then the 15-1.4.1<sup>+</sup>/WGA<sup>+</sup> blast cells, or the 15-1.4.1<sup>+</sup>/B220<sup>+</sup>/WGA<sup>+</sup> blast cells were selected. Finally, the c-kit<sup>+</sup> and c-kit<sup>−</sup> WGA<sup>+</sup>/15-1.4.1<sup>+</sup>/B220<sup>+</sup> blast cells were selected. These cells will be further abbreviated as c-kit<sup>+</sup> and c-kit<sup>−</sup> cells. The selected c-kit<sup>+</sup> and c-kit<sup>−</sup> cells were then sorted (normal-R mode). During the sort the cells were kept at 4°C.

**IT Injection.** Sublethally irradiated mice were anesthetized by intraperitoneal injection of a combination of Ketaset (ketamine HCl; Aveco Co., Inc., Fort Dodge, IA) and Rompun (xylazine; Mobay Corporation, Shawnee, KS) at 132 μg/g body weight, and 8.8 μg/g body weight, respectively. The sternum was exposed, and the anterior bisected. The indicated numbers of purified c-kit<sup>+</sup> cells were then injected in 10–15 μl volumes into one thymic lobe using a 50-μl syringe (Hamilton Co., Reno, NV) and a 30-gauge needle, and the incision was closed (36). At various times after transfer of the cells, the mice were killed, both thymic lobes isolated separately, and single cell suspensions prepared for immunofluorescence staining.

**Results**

**Purification of Hematopoietic Stem Cells.** With the purification procedure described above, which is not MHC class I, Thy-1, or Ly-6A/E (Sca-1) haplotype-dependent and therefore widely applicable (13, and de Vries, P., unpublished ob-
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Days after (IT) transplantation

Figure 1. Kinetics of thymic lobe reconstruction by $10^3$ purified $c$-kit$^+$ stem cells. Single thymus lobes of sublethally irradiated (750 rad) C57BL/6 (Thy-1.2) mice were injected intrathymically with $10^3$ $c$-kit$^+$ stem cells purified from B6.PL-Thy-1$^+$ (Thy-1.1) BM. At the indicated times, the lobes were isolated and stained for the presence of donor- (Thy-1.1$^+$) and host- (Thy-1.2$^+$) derived thymocytes. The results depicted represent the mean of three experiments in which three IT injected mice were analyzed.

servations), both $c$-kit$^+$ and $c$-kit$^-$ cells can be isolated. The ratio of $c$-kit$^+$/$c$-kit$^-$ cells is dependent upon whether or not the anti-B220 mAb is included in the selection procedure. It changes from 1.8:1 to 1:4, respectively, without or with the anti-B220 mAb. However, the frequency of $c$-kit$^+$ cells does not change and remains $0.04 \pm 0.04\%$ of total nucleated cells in unseparated BM, whereas the frequency of $c$-kit$^-$ cells changes from $0.07 \pm 0.03\%$ to $0.01 \pm 0.01\%$.

The $c$-kit$^+$ cell fraction contained two times more day 14 CFU-S per $10^3$ injected cells than unseparated BM (96 $\pm$ 22 vs 41.3 $\pm$ 5.1). In contrast, the $c$-kit$^-$ cell fraction was 260-fold-enriched for day 14 CFU-S to unit purity, if a spleen-seeding efficiency (17, 18) of 10% is assumed (10,747 $\pm$ 1,029 day 14 CFU-S per $10^3$ nucleated cells). The frequency of day 14 CFU-S in the $c$-kit$^+$ and $c$-kit$^-$ fraction was not influenced by the inclusion of the anti-B220 mAb in the isolation procedure.

In the present study, we focused on the $c$-kit$^+$ cells only. Additional biological properties of the $c$-kit$^+$ and $c$-kit$^-$ cells will be published elsewhere (de Vries, P., K. Brasel, D. Williams, H. Shilling, and F. Fletcher, manuscript in preparation).

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Table 1. Purified $c$-kit$^+$ Cells Give Rise to a Phenotypically Normal Thymus upon IT Transfer

| Percent positive cells | Thy-1.1 | Thy-1.2 | CD4 | CD8 | CD3 | Gr-1 | Mac-1 | 15-1.4.1 |
|------------------------|---------|---------|-----|-----|-----|------|-------|----------|
| Untreated control*     | 1 $\pm$ 1 | 95 $\pm$ 1 | 93 $\pm$ 1 | 85 $\pm$ 5 | 88 $\pm$ 8 | 8 | 0 | 19 |
| Reconstituted lobes†    | 2 $\pm$ 1 | 92 $\pm$ 7 | 91 $\pm$ 8 | 87 $\pm$ 8 | 70 $\pm$ 18 | 8 $\pm$ 3 | 0 | 11 $\pm$ 1 |

B6.PL-Thy-1$^+$ (Thy-1.1) mice were injected IT with $3 \times 10^5$ $c$-kit$^+$ cells isolated from C56BL/6 (Thy-1.2) BM. After 22 d, the mice were killed. Single cell suspensions of thymic lobes were stained with the indicated mAbs and examined by flow cytometry.

* Data represent the mean $\pm$ SD of two, or the percentage of positive cells from one thymus (Gr-1, Mac-1, 15-1.4.1) in untreated C57BL/6 mice.

† Data represent the mean $\pm$ SD of four reconstituted lobes.

Figure 2. Reconstitution of the injected thymus lobe by purified $c$-kit$^+$ stem cells. Fluorescence distribution of cells harvested from the intrathymically-injected (thick line) and noninjected lobe (thin line) of a sublethally irradiated C57BL/6 (Thy-1.2) mouse given $4.8 \times 10^5$ $c$-kit$^+$ cells isolated from B6.PL-Thy-1$^+$ (Thy-1.1) BM (A) Staining with Thy-1.1- (donor) specific antibody. (B) Staining with Thy-1.2- (host) specific antibody.

Kinetics of Thymus Reconstitution. We were first interested to see if $c$-kit$^+$ cells could reconstitute the thymus of sublethally irradiated recipient mice upon IT transfer. Therefore, $10^3$ $c$-kit$^+$ (Thy-1.1) stem cells were injected into thymus lobes of sublethally irradiated Thy-1.2 recipient mice. At different time points after transplantation, both injected and noninjected thymus lobes were removed and examined for the presence of donor- (Thy-1.1$^+$) and host- (Thy-1.2$^+$) derived cells.

The data presented in Fig. 1 show that $c$-kit$^+$ cells can reconstitute the thymus of sublethally irradiated recipients. Thy-1.1$^+$ progeny derived from $10^3$ injected $c$-kit$^+$ cells first appeared in the injected thymus lobe after 15 d (Fig. 1). Within 7 d the percentage of donor-derived cells increased from 17% to 82 $\pm$ 42%. This number stayed more or less the same until day 65, at which point 92% of the thymocytes in the injected thymus lobe were donor derived and the experiment was terminated. During the course of the

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experiment there were two points at which the percentage of donor-derived cells dropped: days 33 and 54 after injection. However, the biological relevance of this drop in donor-derived cells is more than likely minimal considering the rather large SE observed at these days: 26 ± 42% at day 33, and 39 ± 45% at day 54.

Reconstituted thymus lobes showed the same percentage of helper-inducer T cells (CD4+), cytotoxic/suppressor T cells (CD8+), mature TCR (α/β)-expressing T cells (CD3+), granulocytes and monocytes/macrophages (mAbs Gr-1+, Mac-1+, and 15-1.4.1+) as thymi from untreated controls (Table 1), suggesting that the c-kitpos cells give rise to a normal thymus.

During the course of the experiments, reconstitution of the noninjected lobe by donor cells was not often observed (<37% of the animals). A typical result obtained after IT injection of Thy-1.1 c-kitpos cells is shown in Fig. 2. Only the lobe that was injected with Thy-1.1 c-kitpos cells contained Thy-1.1+ cells, whereas the noninjected lobe did not contain Thy-1.2pos cells. This means that there was no migration from one lobe to the other, and that there was no reseeding by Thy-1.1pos stem cells from progenitors that might have migrated outside the thymus, e.g., to the BM (36, 39, 40). These data also suggest that the commitment of c-kitpos cells to thymocyte differentiation can take place in the thymus microenvironment, and does not necessarily occur outside the thymus, e.g., in the BM.

**Frequency of Thymus Lobe Reconstitution in the c-kitpos Cell Fraction.** Given that c-kitpos cells could reconstitute the thymus of sublethally irradiated recipients upon IT injection, it was interesting to determine the frequency of cells with thymus reconstitution capacity in the c-kitpos cell fraction. We have here and previously (41) shown that, assuming a spleen-seeding efficiency of 10% for c-kitpos cells, one c-kitpos cell can give rise to one day 14 CFU-S. Mice were injected intrathymically with decreasing numbers of c-kitpos cells, and after 28 days their thymus lobes were analyzed for the presence of donor- and host-derived cells using Thy-1.1- and Thy-1.2-specific antibodies. The results are shown in Table 2. In 44% (four of nine) of the mice that were injected with a single c-kitpos cell, the injected lobe contained donor-derived cells. None of the contralateral lobes were reconstituted by donor cells in these nine animals. When mice were injected with 200 or more c-kitpos donor cells, 100% donor cell reconstitution was observed.

A wide range of donor cells was observed in most of the groups of reconstituted mice ranging from <10% to >95% donor reconstitution (Table 2). The large range of donor-derived cells observed in most of the groups seemed to be independent of the injected number of cells. In addition, at 28 d after IT injection, a clear correlation between the percentage of donor-derived cells and the number of c-kitpos cells injected was not observed.

**Cell Dose and Thymus Reconstitution.** Fig. 3 shows the results of an experiment designed to examine the effect of graded c-kitpos cell doses on the kinetics and duration of thymus reconstitution. The rate of reconstitution seemed to be cell dose independent. Donor-derived thymocytes were first detected 22 d after injection no matter if 3 × 10^2, 10^3, or 300 c-kitpos cells were injected. However, in this experiment it was found that the higher the cell number injected, the larger the percentage of donor-derived cells. With 3 × 10^3 cells injected on average, 94 ± 2% of the thymocytes were of donor origin throughout the course of the experiment. With 10^3 c-kitpos cells injected, the average percentage of donor-derived thymocytes was 25% lower (69 ± 22%) than in the previous group. When 300 cells were injected, a maximum percentage of Thy-1.1pos cells was observed.

![Figure 3. The effect of the purified c-kitpos cell dose on the level and duration of thymic lobe reconstitution. Sublethally irradiated C57BL/6 and/or C57BL/10SNJ (Thy-1.2) were injected intrathymically with 300 (○), 10^3 (△), or 3 × 10^3 (●) purified c-kitpos stem cells isolated from the BM of B6.PL-Thy-1.1 (Thy-1.1) mice. At the indicated time points, thymic lobes were isolated and stained for the presence of donor- and host-derived thymocytes. The data represent the mean percentage Thy-1.1pos thymocytes in treated lobes harvested from three mice.](image-url)
Discussion

In this report we demonstrate that bone marrow-derived, purified stem cells expressing the proto-oncogene c-kit can efficiently reconstitute the thymus of sublethally irradiated mice after IT transfer (Figs. 1 and 2 and Tables 1 and 2).

Thymocytes harvested from thymic lobes of mice injected intrathymically with c-kit\textsuperscript{m} cells showed the same phenotype as those from unperturbed mice (Table 1), indicating that injected c-kit\textsuperscript{m} cells can give rise to normal thymocyte development. However, we did not compare the functional capacities of the thymocytes in reconstituted lobes with those of untreated mice.

One c-kit\textsuperscript{m} stem cell could reconstitute the thymus (Table 2). On average, more than half of the resultant thymocytes (59 ± 42%) were of donor origin 28 d after injection of a single c-kit\textsuperscript{m} cell (Table 2). At this time point, the number of thymocytes observed in irradiated and intrathymically-injected animals was roughly the same (4-5 × 10\textsuperscript{7} per lobe) as in unperturbed animals (H. McKenna, unpublished results). From these figures it can be calculated that c-kit\textsuperscript{m}-derived cells on average replicated once every 24 h after IT transfer.

We have demonstrated that one c-kit\textsuperscript{m} stem cell can also give rise to one day 14 CFU-S (this report, and 41), generally considered to be an erythromyeloid-restricted stem cell (19, 24, 26, 28-30). The data presented in this communication suggest that at least a subpopulation of c-kit\textsuperscript{m} cells are truly pluripotent stem cells, and also that commitment to the T cell lineage can take place in the thymus.

In contrast to c-kit\textsuperscript{m} cells, c-kit\textsuperscript{p} cells are not enriched for day 14 CFU-S and 3 × 10\textsuperscript{3} c-kit\textsuperscript{m} cells are needed for thymic lobe reconstitution after IT transfer (data not shown). These data illustrate the usefulness of c-kit expression as a selectable marker in the isolation of stem cell subsets.

It has been reported that as thymic stem cells differentiate, they lose their capacity to reconstitute (36, 39, 40, 42). We have found in adoptive transfer experiments, in which cells from a thymic lobe originally injected with 3 × 10\textsuperscript{3} c-kit\textsuperscript{m} cells, at several time points after transplantation were injected into thymic lobes of secondary recipients, that the capacity to reconstitute a secondary thymus lobe was lost between 6 and 18 h after the primary IT injection (data not shown). In these experiments, we did not investigate if c-kit\textsuperscript{m} cells were present in the primary injected thymic lobe. The rapid loss of reconstitution capacity is most likely due to commitment of the c-kit\textsuperscript{m} stem cells to the T cell lineage (36, 39, 40, 42), and might explain the observed lack of migration between thymic lobes (Fig. 2) or the lack of re-seeding of both lobes by donor-derived Thy-1.1\textsuperscript{m} cells that homed to other tissues (BM) after IT injection. If in long-lasting experiments like the one shown in Fig. 1 the thymus was reconstituted by a recirculating Thy-1.1\textsuperscript{m} PHSC coming from somewhere else like the BM, both thymus lobes would have been reconstituted (36, 39).

We also have examined the presence of donor-derived cells in BM, spleen, and lymph nodes after IT injection. Donor-derived Thy-1.1\textsuperscript{m} cells were not found in these organs. These findings might suggest that some degree of BM "processing" may be necessary to enable these primitive c-kit\textsuperscript{m} cells to migrate properly from the thymus to distant sites once they have differentiated into CD4\textsuperscript{m} or CD8\textsuperscript{m} thymocytes. However, the present studies do show that c-kit\textsuperscript{m} cells are capable of giving rise to T lymphocytes upon IT transfer. In addition, upon intravenous injection into SCID mice or lethally irradiated normal mice, c-kit\textsuperscript{m} cells were also capable of thymus reconstitution 2-6-mo after transplantation (P. de Vries, unpublished observations).

Progeny of the c-kit\textsuperscript{m} stem cells was first detected 15 d after transplantation (Fig. 1). After 22 d the average percentage of donor-derived thymocytes reached 82 ± 24% and remained high (76 ± 17%) until day 65 when the experiment ended. Apparently, the thymus microenvironment is very well suited to harbor (the progeny of) c-kit\textsuperscript{m} stem cells. It is not clear what factors are involved in the commitment to differentiation along the T cell lineage of c-kit\textsuperscript{m} stem cells. However, it is known that thymic epithelial and stromal cells constitutively express messenger RNA for IL-7 (43) and SLF (Dr. Phil Morrissey (Immunex Corp.), personal communication), suggesting a potential role for the SLF/c-kit ligand/receptor pair.

A similar lag period in thymic reconstitution as observed in the present studies has been reported by others after IT injection of unseparated adult BM cells or thymocytes (39). In contrast, Spangrude and Scollay (44) found both myeloid and T lymphoid donor-derived progeny 7 d after IT injection of 200 Thy-1\textsuperscript{m} Lin\textsuperscript{m} Sca-1\textsuperscript{m} (22) stem cells. Given the different methods employed to isolate the reconstituting cell populations, it is difficult to compare these data with ours, because overlapping but slightly different cells might be isolated by both procedures. In addition, Spangrude and Scollay (44) used a different detection system than we used in the current report (mAbs specific for Ly-5 alleles rather than mAbs specific for Thy-1 alleles, respectively).

The level and duration of thymus reconstitution by c-kit\textsuperscript{m} cells appeared to be cell dose dependent (Fig. 3). When 300 cells were injected, a decline in donor-derived cells was observed at time points longer than 33 days after IT cell transfer, whereas such a decline was not seen when 10\textsuperscript{3} or 3 × 10\textsuperscript{3} c-kit\textsuperscript{m} cells were transplanted. The decline in donor-derived cells was always accompanied by a comparable increase in host-derived thymocytes. One possible explanation for this observation is that with the higher cell numbers, most if not all sites capable of supporting engraftment are occupied by donor cells and that endogenous stem cells have to compete for these sites. When 10\textsuperscript{3} or 3 × 10\textsuperscript{3} donor cells are injected, host stem cells may have a proliferative disadvantage and be unable to out-compete donor stem cells. When 300 cells are injected, donor cells may be eventually out-competed by host stem cells.
The results obtained in this study strongly suggest that commitment to differentiation of pluripotent hematopoietic stem cells (c-kit+ cells) to the T cell pathway can occur in the thymus. The current studies document the ability of at least a subpopulation of the c-kit+ cell fraction to give rise to cells of either the erythromyeloid or (T) lymphoid series under the appropriate microenvironmental influences. This cell population gives rise to multipotent day 14 CFU-S at unit efficiency, and at a minimum, at least 1 in 25 of these cell population gives rise to multipotent day 14 CFU-S at least a subpopulation of the commitment to differentiation of pluripotent hematopoietic stem cells which may be amenable for use in studies of lineage commitment.

In conclusion, these data suggest that the c-kit+ cell fraction contains a population of primitive myelolymphoid hematopoietic stem cells which may be amenable for use in studies of lineage commitment.

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Address correspondence to Dr. Peter de Vries, Department of Experimental Hematology, Immunex Corporation, 51 University Street, Seattle, WA 98101.

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