

Evidence that hematopoietic stem cells express mouse *c-kit* but do not depend on steel factor for their generation

(self-renewal/bone marrow transplantation/growth factor)

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ABSTRACT The interaction of the mouse *c-kit* receptor, designated Kit receptor, and steel factor promotes the proliferation and differentiation of hematopoietic progenitor cells. Monoclonal antibodies against the extracellular portion of the mouse Kit receptor were established. Five percent to 10% of total bone marrow cells expressed the Kit receptor, and half of them lack the expression of lineage markers. The Kit receptor was expressed on 70–80% of Thy-1.1^{lo} Lin⁻ Sca-1⁺ cells, which express Thy-1.1 antigen at a low level and constitute ≈0.05% of adult bone marrow and fetal liver; by previous studies, these cells have been shown to be highly enriched for multipotent hematopoietic stem cells (HSCs) and are the only hematopoietic cell subset with this activity. Spleen colony formation and long-term multilineage reconstitution activities were contained in the Kit⁺ but not in the Kit⁻ subpopulations of Thy-1.1^{lo} Lin⁻ Sca-1⁺ cells from adult bone marrow, suggesting that the Kit receptor is expressed on HSCs from the earliest stage—i.e., pluripotent HSCs. The role of steel factor in the development and self-renewal of HSCs was tested with *Sl/Sl* homozygote fetuses, which lack genes to encode functional steel factor. They were shown to have 30–40% of the number of HSCs on days 13–15 when compared with normal littermates. However, the absolute number of HSCs increased during fetal development in the *Sl/Sl* mice. The results suggest that the Kit receptor–steel factor interaction may not be essential for the initiation of hematopoiesis and the self-renewal of (at least) fetal HSCs.

Hematopoietic stem cells (HSCs) play an essential role in hematopoiesis. It is generally accepted that HSCs have two major characteristics: the capacity for self-renewal and the capability to differentiate into all lineages of hematolymphoid cells. However, the process by which this occurs is not yet well understood. HSCs have been enriched from adult bone marrow (1–3) and fetal liver (4). By combination of (i) Thy-1 antigen expression at low levels (Thy-1^{lo}), (ii) the absence or low expression of lineage markers [TER-119 (erythroid), B220 (B cell), Mac-1 (monocyte), Gr-1 (granulocyte), CD4, and CD8 (T cell) (Lin⁻)], and (iii) Sca-1 expression (Sca-1⁺), Thy-1.1^{lo} Lin⁻ Sca-1⁺ cells were isolated from adult bone marrow and fetal liver and shown to be highly enriched for multipotent HSCs (5, 6). This population was also shown to be capable of long-term multilineage reconstitution of lethally irradiated mice (5, 7).

Mutations at the steel (*Sl*) or the dominant spotting (*W*) loci give rise to a similar set of defects in mice. These are characterized by a lack of pigmentation and gametogenesis and a reduction in hematopoietic activity that leads to anemia and a deficiency of mast cells (8). It has been shown that the wild-type *Sl* gene encodes a growth factor protein (9–16). The *W* locus encodes a tyrosine kinase family protooncogene, mouse *c-kit* designated Kit, which functions as a cell surface

receptor (17–20); this receptor can bind the steel factor (SIF) (9, 14, 15). SIF shows strong synergistic actions with other growth factors. SIF has profound effects on the erythroid lineage in costimulation assays with erythropoietin (12, 13). It has been also reported that SIF acts as the most potent comitogen yet tested on HSCs, especially in combination with interleukins 3, 6, or 1 (IL-3, IL-6, or IL-1) (21, 22). However, it is still unknown whether SIF is a self-renewal factor for HSCs (22). To test whether SIF is necessary for the fetal generation of HSCs, we first analyzed the expression of Kit on the surface of hematopoietic stem cells with an anti-Kit monoclonal antibody and then characterized the HSC population in *Sl/Sl* fetuses; *Sl/Sl* mice lack functional SIF because of a large deletion in the coding sequence (14, 15). Our results suggest that the Kit receptor is expressed on HSCs. Although somewhat diminished in number, HSCs are present and can multiply in the absence of SIF in *Sl/Sl* mice.

MATERIALS AND METHODS

Animals. C57BL/6J, C57BL/Ka, C57BL/Ka-Thy-1.1 (Thy-1.1, Ly-5.2), C57BL/6J-Ly-5.1-Pep^{3b} (Thy-1.2, Ly-5.1), and WC/Re-*Sl*/+ mouse strains were bred and maintained in the animal facility at Stanford University School of Medicine. All mice were regularly maintained on acidified water. The age of embryos and fetuses was determined by scoring for the appearance of a vaginal plug and taking as day 0 the morning on which the mating plug was observed.

Establishment of Monoclonal Antibodies. Adult bone marrow or newborn liver cells were cultured in RPMI medium supplemented with 10% (vol/vol) fetal calf serum, 10% (vol/vol) WEHI-3 culture supernatant, 50 μM 2-mercaptoethanol, 2 mM glutamine, 1× nonessential amino acids (Irvine Scientific), 1 mM sodium pyruvate, 100 units of penicillin per ml, and 100 μg of streptomycin per ml for more than 4 weeks as described (23). Wistar rats were immunized three times with 1–2 × 10⁷ IL-3-dependent mast cells derived from normal bone marrow. Rat spleen cells were fused with the mouse myeloma FOX-NY (24), and the culture supernatants of hybridoma clones were screened by fluorescence-activated cell sorting for their reactivity to IL-3-dependent mast cells derived from WB/ReJ-+/? and -W/W newborn mouse liver [+/? and +/? mast cells (+/? cells) are Kit⁺, while W/W mast cells are Kit⁻ (25)]. Two clones, 2B8 and 3C11, which stained the cell surface of +/? but not of W/W mast cells, were isolated and characterized. Both of these monoclonal antibodies immunoprecipitated a protein of about 150 kDa on mast cells. These results indicate that 2B8 and 3C11 antibodies recognize the mouse Kit molecule. 2B8 and 3C11 antibodies are both IgG2b (κ light chain).

Antibodies, Immunofluorescence Staining, and Analysis. Monoclonal antibodies used for cell surface staining have

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Abbreviations: HSCs, hematopoietic stem cells; Thy-1^{lo}, Thy-1 antigen expression at a low level; SIF, steel factor; IL-3, -6, and -1, interleukins 3, 6, and 1; CFU-S, spleen colony-forming unit(s).

been described (5, 6) and listed as follows: TER-119, anti-mouse erythroid lineage; 19-IXE5, anti-Thy-1.1; 53-2.1, anti-Thy-1.2; RA3-6B2, anti-B220; M1/70, anti-Mac-1; RB6-8C5, anti-Gr-1; GK1.5, anti-mouse CD4; and 53-6.7, anti-mouse CD8. The anti-CD5 (Ly-1) antibody used was 53-7.3 (ref. 26). Preparation of antibodies, cell-surface staining, and fluorescence-activated cell sorting analysis were carried out as described (6).

Enrichment of HSCs. Adult bone marrow cells of C57BL/Ka-Thy-1.1 mice were stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (145-2C11) and a mixture of lineage markers (TER-119, anti-B220, anti-Mac-1, anti-Gr-1, anti-CD4, anti-CD8, and anti-Ly-1 antibodies), followed by phycoerythrin-conjugated goat anti-rat IgG (5, 6). CD3⁺ cells were negatively selected by magnetic separation with paramagnetic beads coupled to sheep antibodies to fluorescein. The remaining CD3⁻ cells were stained with FITC-anti-Thy-1.1, and Thy-1⁺ cells were enriched with paramagnetic beads. The cells were incubated with normal rat serum and stained with biotin-conjugated anti-Sca-1 antibody, followed by Texas red-conjugated avidin and allophycocyanin-conjugated anti-Kit (2B8) antibody.

Day 14 fetal liver cells of C57BL/Ka-Thy-1.1 mice were stained with TER-119 antibody. The cells were washed and incubated with paramagnetic beads coupled to sheep antibodies to rat IgG (Dynal, Great Neck, NY) at a cell-to-beads ratio of 1-4 for 30 min at 4°C. The labeled TER⁺ cells were removed by magnetic separation and discarded. The negatively selected cells were incubated with beads, and the TER⁺ cells were removed again. The remaining cells were stained with a mixture of antibodies to lineage markers (TER-119, anti-B220, anti-Mac-1, anti-Gr-1, anti-CD4, and anti-CD8), followed by phycoerythrin-conjugated goat anti-rat IgG. The cells were incubated with normal rat serum and stained with biotin-anti-Sca-1 and FITC-anti-Thy-1.1 antibodies, followed by Texas red-avidin and allophycocyanin-anti-Kit (2B8) antibody.

Spleen Colony Assay. The spleen colony assay was performed as described (6, 27). Spleen colonies were counted 8 or 12 days after injection.

Long-Term Multilineage Reconstitution. Thy-1^{lo} Lin⁻ Sca-1⁺ Kit⁺ or Kit⁻ cells were isolated from C57BL/Ka-Thy-1.1 mice. Limiting numbers of cells were injected into lethally irradiated C57BL/6J-Ly-5.1-Pep^{3b} mice together with 2 × 10⁵ unirradiated syngeneic host bone marrow cells. After 10 or 15 weeks, ≈0.5 ml of peripheral blood was collected into 0.5 ml of phosphate-buffered saline (PBS) containing 3 mg of EDTA (pH 7.2) per ml. The sample was added with 0.5 ml of 2% Dextran T500 in PBS and incubated for 30 min at 37°C to let erythrocytes clump and settle. The clear upper layer was removed and centrifuged, and the cell pellet was treated with hypotonic shock. The isolated peripheral blood leukocytes were stained with either anti-Mac-1 plus anti-Gr-1, anti-B220, or FITC-anti-Thy-1.1 plus FITC-anti-Thy-1.2 antibodies, followed by FITC-goat-anti-rat IgG (in cases of anti-Mac-1 plus anti-Gr-1, and anti-B220), biotin-anti-Ly-5.2 antibody, and Texas red-avidin. The mice in which the Ly-5.2⁺ Lin⁺ (Mac-1/Gr-1⁺, B220⁺, or Thy-1⁺) donor-derived cells made up >1% of each Lin⁺ cells were scored as positive for the reconstitution of each cell lineage.

Isolation and Staining of *Sl/Sl* Fetal Liver Cells. WC/Re-*Sl*/⁺ female mice were mated with WC/Re-*Sl*/⁺ male mice. On days 13-15 of gestation, each fetus was isolated. *Sl/Sl* fetuses were easily distinguished by their anemic appearance and decreased number of fetal liver cells. This was confirmed by PCR amplification of genomic DNA with SIF primers. High molecular weight DNA from the tissues from each +/+ (+/+ or +/*Sl*) or *Sl/Sl* fetus was isolated as described (28). The DNA was amplified for 40 cycles by PCR (6). The SIF primers (5'-primer, 5'-TCTCCGAAGAGGCCAGAAAC-3';

3'-primer, 5'-CTCGGGACCTAATGTTGAAG-3') do not span introns (13). The predicted size of amplified DNA fragments was 141 base pairs (bp). The DNA of *Sl/Sl* fetuses did not give rise to the PCR fragment of the correct size. Fetal liver cells of +/+ or *Sl/Sl* fetuses were stained with anti-Thy-1.2, anti-Sca-1 antibodies, and antibodies to lineage markers as described (6).

RESULTS

Establishment of Anti-Kit Monoclonal Antibody. Because the *W* allele has a deletion in transmembrane domain of Kit mRNA, *W/W* mast cells cannot express the Kit receptor on their surface (25). By cell surface staining, 2B8 and 3C1 antibodies showed differential staining between +/? and *W/W* mast cells (Fig. 1). Both 2B8 and 3C1 antibodies immunoprecipitated cell surface molecules with molecular weights of ≈150 kDa (data not shown), consistent with the results reported previously (29). These results suggested that 2B8 and 3C1 recognize the extracellular portion of the Kit molecule.

Expression of Kit Receptor on Bone Marrow Cells and HSCs. Adult bone marrow cells were stained with an anti-Kit antibody and antibodies to lineage markers (TER-119, B220, Mac-1, and Gr-1) (Fig. 2). The cells expressing Kit made up about 5-10% of bone marrow cells. About half of them lacked expression of the lineage markers; the rest of them expressed the lineage markers at low levels (especially the Gr-1 marker). These results are consistent with results reported previously (30). Next, the expression of Sca-1 and Kit on Thy-1^{lo} Lin⁻ cells was analyzed (Fig. 3). Nineteen percent of Thy-1⁺-enriched cells were Lin⁻ (Fig. 3A *Left*). When only Lin⁻ cells were analyzed, 77% of the cells were Thy-1⁻. The Thy-1⁻ Lin⁻ population was divided into either Kit⁺ (35%) or Kit⁻ (42%) subpopulations. The Thy-1^{lo} Lin⁻ population (15.1%) was further divisible into three major subpopulations, Kit⁺ (53%), Sca-1⁺ Kit⁻ (8.7%), and Sca-1⁻ Kit⁻ (38%) (Fig. 3B *Left*). The Kit⁺ cells were further divided into Sca-1⁺ (31%) and Sca-1⁻ (22%) subpopulations, although it could be argued that Sca-1 staining is unimodal rather than bimodal. To compare Kit expression on candidate HSCs between fetal and adult, Thy-1^{lo} Lin⁻ cells in day 14 fetal liver were also analyzed (Fig. 3B *Right*). Although the pattern of Kit and Sca-1 expression on Thy-1^{lo} Lin⁻ cells from day 14 fetal liver was basically similar to that of adult bone marrow, Kit⁺ cells were concentrated more on the Sca-1^{lo} side of the Sca-1⁺/Sca-1⁻ border than that found in adult bone marrow.

Stem Cell Activities of Kit-Defined Thy-1^{lo} Lin⁻ Sca-1⁺ Subpopulations. Thy-1^{lo} Lin⁻ Sca-1⁺ cells from both fetal and adult mice contained highly enriched day 12 colony-forming units in the spleen (CFU-S) (5, 6), a measure of primitive myeloerythroid progenitors. The CFU-S in the subpopulations of Thy-1^{lo} Lin⁻ cells in adult bone marrow were analyzed (Table 1). The Sca-1⁺ Kit⁺ cells gave rise to 1

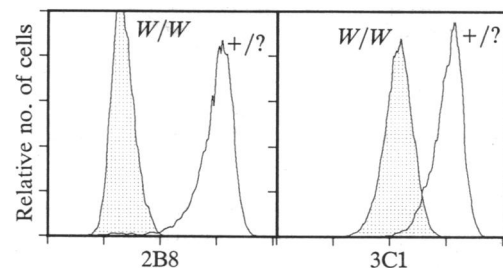


FIG. 1. Surface staining of mast cells with anti-Kit monoclonal antibodies. IL-3-dependent mast cells derived from +/? or *W/W* newborn liver were stained with monoclonal anti-Kit antibodies (2B8 or 3C1), followed by FITC-anti-rat IgG.

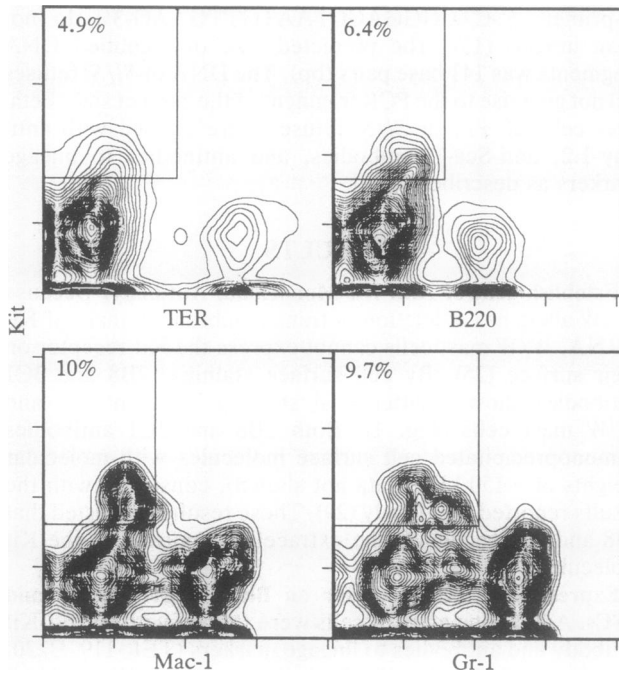


FIG. 2. Expression of Kit receptor on bone marrow cells. Total bone marrow cells were stained with TER-119, anti-B220, anti-Mac-1, or anti-Gr-1 antibody and anti-Kit antibody (2B8). The cells expressing Kit receptor were boxed.

CFU-S out of 8.3 cells at day 12, while Sca-1⁺ Kit⁻ cells gave rise to one colony out of 455 cells. It was previously reported that Thy-1^{lo} Lin⁻ Sca-1⁺ cells give rise to 1 CFU-S (day 12) out of 10 cells injected (5). These results suggest that the Thy-1^{lo} Lin⁻ Sca-1⁺ Kit⁺ cells contain most of the CFU-S activity detected in the Thy-1^{lo} Lin⁻ Sca-1⁺ population. This also suggests that the staining with the anti-Kit antibody

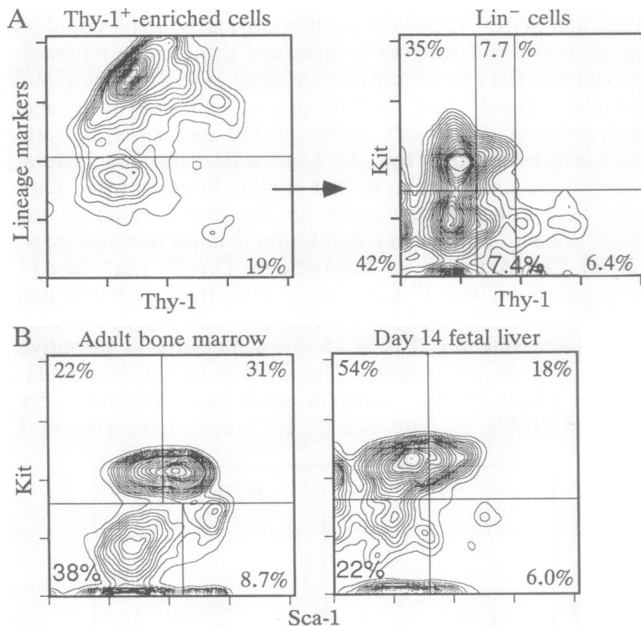


FIG. 3. Expression of Kit receptor on hematopoietic stem cells. (A) Isolation of Thy-1^{lo} Lin⁻ cells. Nineteen percent of Thy-1⁺-enriched cells were Lin⁻ (Left). After only Lin⁻ cells were analyzed, Thy-1^{lo} cells were 15% of Lin⁻ cells (Right). About 43% of Lin⁻ cells expressed the Kit receptor. (B) The expression of Sca-1 and Kit on Thy-1^{lo} Lin⁻ cells from adult bone marrow (Left) and day 14 fetal liver (Right). Kit was expressed on 78% and 75% of the Thy-1^{lo} Lin⁻ Sca-1⁺ cells in adult bone marrow and day 14 fetal liver, respectively.

probably did not inhibit or promote colony formation in the spleen. The Sca-1⁻ Kit⁻ cells gave rise to one colony out of 850 cells. These results suggest that the Kit⁻ subpopulations of Thy-1^{lo} Lin⁻ cells have very low CFU-S activity. In contrast, Sca-1⁻ Kit⁺ cells gave rise to 1 CFU-S out of 44 cells injected at day 12 and 1 CFU-S per 47 cells injected at day 8. This result is consistent with the committed myeloerythroid but non-stem-cell nature of this subset. The ratio of CFU-S on day 12 to that on day 8 is 9.5 for Sca-1⁺ Kit⁺ cells and 1.1 for Sca-1⁻ Kit⁺ cells, consistent with the result of Thy-1^{lo} Lin⁻ Sca-1⁺ and Sca-1⁻ cells reported previously (5). These results suggest that the Kit⁺ subset of both Thy-1^{lo} Lin⁻ Sca-1⁺ and Thy-1^{lo} Lin⁻ Sca-1⁻ cells is highly enriched for clonogenic myeloerythroid progenitor activity.

It has been shown that Thy-1^{lo} Lin⁻ Sca-1⁺ cells contain HSCs with long-term multilineage reconstitution activity but that Thy-1^{lo} Lin⁻ Sca-1⁻ cells do not (5, 7). To check whether HSCs express Kit or not, the Kit⁺ and Kit⁻ subpopulations of Thy-1^{lo} Lin⁻ Sca-1⁺ cells were tested for their long-term reconstitution activity in an assay where they were not required for radioprotection (Table 2). Limiting numbers of Thy-1^{lo} Lin⁻ Sca-1⁺ Kit⁺ or Kit⁻ cells from C57BL/Ka-Thy-1.1 mice were injected into lethally irradiated Ly-5.1 congenic mice together with 2 × 10⁵ Ly-5.1 bone marrow cells. This assay enables one to check long-term multilineage reconstitution without requirement for short-term radioprotection. After 10 or 15 weeks, peripheral blood leukocytes were analyzed for Ly-5.2⁺ donor-derived cells. At 10 weeks, 4 of 19 mice injected with 20 Thy-1^{lo} Lin⁻ Sca-1⁺ Kit⁺ cells (the limit number for one or two responding cells) (ref. 7; N. Uchida and I.L.W., unpublished data) showed donor-derived cells in the myeloid, B, and T lineages. An additional 11 of 19 mice showed reconstitution in one or two of the three lineages. In total, 15 of 19 mice were positive for donor-derived cells. At 15 weeks, 5 of 18 and 4 of 16 mice injected with 20 and 10 Thy-1^{lo} Lin⁻ Sca-1⁺ Kit⁺ cells, respectively, showed donor-derived cells in all three lineages, with a grand total of 15 of 18 (20 cells injected) and 8 of 16 (10 cells injected) hosts showing reconstitution in one, two, or all three of the lineages. Elsewhere we have demonstrated that donor-derived myeloid cells may be present but may disappear in mice later expressing T and T + B cells (ref. 7; N. Uchida and I.L.W., unpublished data). Thus, multilineage outcomes are likely from 11 of 19 20-cell-injected mice at 10 weeks, 11 of 18 20-cell-injected mice at 15 weeks, and 7 of 16 10-cell-injected mice at 15 weeks. On the other hand, Thy-1^{lo} Lin⁻ Sca-1⁺ Kit⁻ cells gave rise to no donor-derived cells at either 10 or 15 weeks. These results suggest that Thy-1^{lo} Lin⁻ Sca-1⁺ Kit⁺ cells contain HSCs with long-term reconstitution activity. All of these data suggest that HSCs express Kit on their surface.

Development of HSCs in *Sl/Sl* Fetal Liver. It has been shown that SIF has profound effects on hematopoietic progenitor cells in combination with other growth factors (13, 16, 22). In our hands SIF is a potent comitogen for Thy-1^{lo} Lin⁻

Table 1. Frequency of CFU-S activity in selected subpopulations of adult bone marrow Thy-1^{lo} Lin⁻ cells

Subpopulation	1/Frequency of CFU-S activity*		Ratio (day 12/day 8)
	Day 8	Day 12	
Sca-1 ⁺ Kit ⁺	79	8.3	9.5
Sca-1 ⁺ Kit ⁻	>1280†	455	NA
Sca-1 ⁻ Kit ⁺	47	44	1.1
Sca-1 ⁻ Kit ⁻	>3410†	853	NA

NA, not applicable.

*Mean from one to four experiments.

†No colonies were observed after total 1280 or 3410 cells were injected.

Table 2. Long-term reconstitution with selected subpopulations of adult bone marrow Thy-1^{lo} Lin⁻ cells

Population	Cells injected, no. per mouse	Time of PBL analysis, wk	Total mice	Mice with donor-derived cells in myeloid (M), B, and T lineages, no.					
				M+B+T	M+B	B+T	B	M	None
Sca-1 ⁺ Kit ⁺	20	10	19	4	4	3	4	0	4
	20	15	18	5	3	3	4	0	3
	10	15	16	4	0	2	1	1	8
Sca-1 ⁺ Kit ⁻	10	10	18	0	0	0	0	0	18
	10	15	17	0	0	0	0	0	17

M, Ly-5.2⁺ Mac-1/Gr-1⁺ monocytes and granulocytes; B, Ly-5.2⁺ B220⁺ B lymphocytes; T, Ly-5.2⁺ Thy-1.1/Thy-1.2⁺ T lymphocytes; PBL, peripheral blood leukocytes.

Sca-1⁺ cells but by itself is not capable of inducing proliferation by these cells (S. Heimfeld, J. Friedman, I.L.W., unpublished data). However, it is not yet clear whether SIF has an indispensable role in the development and self-renewal of HSCs. Because Kit is expressed on HSCs, we next attempted to infer the effect of SIF on HSC development *in vivo* by comparing the appearance of Thy-1^{lo} Lin⁻ Sca-1⁺ cells in normal and in *Sl/Sl* homozygote fetuses, which lack functional SIF (14, 15). Because it has been reported that *Sl/Sl* fetuses die at around days 15–16 of gestation (31), we checked the fetuses between days 13 and 15. The total number of fetal liver cells in non-*Sl/Sl*-fetuses (designated +/?) increased by 2- to 5-fold each day between days 13 and 15 of gestation (Table 3). Although the cell number in *Sl/Sl* fetal livers was 18–24% of that of +/? fetal livers during this period, it increased at the same rate as in +/? fetal liver. On days 13 and 14, the +/? fetal liver contained 1200 and 2300 Thy-1^{lo} Lin⁻ Sca-1⁺ cells, respectively, at a relative constant percentage of 0.03%. Most significantly, the number of Thy-1^{lo} Lin⁻ Sca-1⁺ cells in *Sl/Sl* fetal liver was 470 on day 13 and 780 on day 14. These Thy-1^{lo} Lin⁻ Sca-1⁺ cells are more highly represented in *Sl/Sl* fetal liver (0.05%) than +/? littermates, perhaps because of diminution of these cells at a later stage of expansion in +/? mice. These results show that the Thy-1^{lo} Lin⁻ Sca-1⁺ cells, as a population, increased in number in *Sl/Sl* fetal liver between days 13 and 14, doubling at about the same rate as in +/? fetal livers. But are these functional multilineage progenitors? We compared the total number of CFU-S in +/? and *Sl/Sl* fetal liver (Table 4). The number of day 8 and day 12 CFU-S in +/? fetal liver increased by 3- to 5-fold during days 13–15 of gestation. In *Sl/Sl* fetal liver, the number of CFU-S was 31–43% of that in +/? fetal liver, but it also increased by 3- to 5-fold between days 13 and 15. These data suggest that SIF is probably not essential for the initiation of hematopoiesis in the mouse embryo and that HSCs can multiply in number in its absence.

DISCUSSION

These studies strongly suggest that the Kit receptor is expressed on HSCs and other myeloerythroid progenitors in

adult bone marrow and fetal liver. In fact, isolated Thy-1^{lo} Lin⁻ Sca-1⁺ cells respond to SIF as a comitogen, resulting in extensive myeloerythroid proliferation and differentiation (J. Friedman and I.L.W., unpublished data), albeit without a concurrent increase in HSC activity or Thy-1^{lo} Lin⁻ Sca-1⁺ cell numbers. This set of findings is consistent with a model wherein SIF is an important stem cell-acting factor but one that might not be involved in HSC self-renewal. Recently, it has been shown, using anti-Kit monoclonal antibodies, that the Kit receptor is expressed on at least one set of hematopoietic progenitors (30). *In vivo* injection of an anti-Kit antibody with blocking activity resulted in the elimination of most CFU-S activity as well as hematopoietic progenitors responsive to IL-3 and to granulocyte/macrophage and macrophage colony-stimulating factors. The *in vivo* fate of HSCs as measured by radioprotection and lymphoid as well as myeloerythroid differentiation was not assessed. Taken together, these results suggest that the Kit receptor is expressed on pluripotent and multipotent HSCs and myeloerythroid progenitors.

The Kit receptor was expressed on HSCs in fetal liver and adult bone marrow. Therefore, it may serve as a useful marker to isolate HSCs from a variety of sources, such as yolk sac and *in vitro* differentiating embryonic stem cell cultures (32–34).

In this study the numbers of Thy-1^{lo} Lin⁻ Sca-1⁺ cells and CFU-S increase in *Sl/Sl* fetal liver during development, suggesting that at least the fetal HSC population expands in number in the absence of SIF, although the possibility of the HSC influx into fetal liver from sources such as the yolk sac cannot be completely excluded. Although these studies on HSCs show that hematopoiesis can take place in the absence of SIF, it is possible that there may be some unknown factor(s) or mechanisms that, alone or with SIF, may play a crucial role in the initiation of hematopoiesis. It is conceivable that fetal HSCs are less dependent on SIF for their generation and/or maintenance than adult HSCs, a postulate that may be difficult to test experimentally. Rather, it seems more likely that SIF may act as a comitogen that promotes differentiation mainly along the erythroid lineage (unpub-

Table 3. Developmental change in the number and the percentage of HSCs in +/? and *Sl/Sl* fetal liver

Day of gestation	Total cells, no. × 10 ⁻⁶ per fetus			No. of Thy-1 ^{lo} Lin ⁻ Sca-1 ⁺ cells per fetus			% of Thy-1 ^{lo} Lin ⁻ Sca-1 ⁺ cells in fetal liver		
	+/?*	<i>Sl/Sl</i> *	Ratio (<i>Sl/+</i>)	+/?†	<i>Sl/Sl</i> †	Ratio (<i>Sl/+</i>)	+/?†	<i>Sl/Sl</i> †	Ratio (<i>Sl/+</i>)
13	2.8 ± 1.6	0.55 ± 0.22	0.24	1240	470	0.38	0.027	0.057	2.1
14	7.2 ± 3.7	1.3 ± 0.38	0.18	2330	780	0.33	0.025	0.052	2.1
15	32 ± 7.3	6.9 ± 2.9	0.22	ND	ND	ND	ND	ND	ND

Fetal liver cells of +/? or *Sl/Sl* fetus were stained with anti-Thy-1.2, anti-Sca-1 antibodies, and antibodies to lineage markers. The number of Thy-1^{lo} Lin⁻ Sca-1⁺ cells was calculated from the percentage in fetal liver. Numbers per fetus of total fetal liver cells and Thy-1^{lo} Lin⁻ Sca-1⁺ cells are shown. The ratio of the number in *Sl/Sl* to that in +/? fetal liver is also shown. ND, not done.

*Mean ± SEM from two litters.

†Mean from one litter.

Table 4. Developmental change in the number of CFU-S in +/- and *Sl/Sl* fetal liver

Day of gestation	Day 8 CFU-S			Day 12 CFU-S		
	+/? no./fetus	<i>Sl/Sl</i> no./fetus	Ratio (<i>Sl</i> /+)	+/? no./fetus	<i>Sl/Sl</i> no./fetus	Ratio (<i>Sl</i> /+)
13	ND	ND	ND	83	27	0.33
14	700	300	0.43	450	140	0.31
15	2570	840	0.33	1310	420	0.32

Average numbers per fetus of CFU-S are shown from one litter. The ratio of the number in *Sl/Sl* to that in +/- fetal liver is also shown. ND, not done.

lished data) rather than HSC self-renewal and/or commitment to the lymphoid lineages. In that view, it is possible that HSCs may be acted on not only by SIF but also by one or more self-renewal factors, perhaps more tissue-specific in their expression. It will be important to define (i) whether SIF, either in soluble or membrane-bound form, alone or in combination with other factors, can in any circumstances lead to the generation and self-renewal of HSCs; and (ii) what microenvironmental factors exist that cause the appearance and generation of large numbers of HSCs in the mouse fetus.

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