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Characterization of hematopoietic progenitor cells that express the transcription factor SCL, using a *lacZ* "knock-in" strategy

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ABSTRACT **Gene targeting experiments have demonstrated that the transcription factor SCL is essential for primitive and definitive hematopoiesis in the mouse. To study the functional properties of hematopoietic cells expressing *SCL*, we have generated mutant mice ($SCL^{lacZ/w}$) in which the *Escherichia coli lacZ* reporter gene has been "knocked in" to the *SCL* locus, thereby linking β-galactosidase expression to transcription from the *SCL* promoter. Bone marrow cells from heterozygous $SCL^{lacZ/w}$ mice were sorted into fractions expressing high, intermediate and low levels of β-galactosidase (designated $lacZ^{high}$, $lacZ^{int}$, and $lacZ^{neg}$). Cells that were $lacZ^{high}$ or $lacZ^{int}$ were enriched for day 12 spleen colony-forming units and myeloid and erythroid colony-forming cells (CFCs). These fractions included >99% of the erythroid and >90% of the myeloid CFCs. Culture of sorted bone marrow populations on stromal cells secreting interleukin-7 or in fetal thymic organ cultures showed that B and T lymphoid progenitors were also present in the $lacZ^{high}$ and $lacZ^{int}$ fractions. These data provide a functional correlation between *SCL* expression and colony-forming ability in immature hematopoietic cells. Our data also suggested that expression of *SCL* was transient and confined to hematopoietic stem and/or progenitor cells, because the differentiated progeny of most lineages (except the erythroid) were β-galactosidase-negative.**

Hematopoiesis in the developing mouse embryo is critically dependent on the expression of the basic helix–loop–helix gene *SCL/TAL1*, a gene initially identified as a partner in the t(1;14) translocation associated with human T cell leukemia (1). Indications that *SCL* might play a role in normal hematopoiesis came from Northern blot analysis of mRNA from hematopoietic cells that showed that *SCL* was selectively expressed in early myeloid, erythroid, megakaryocytic, and mast cell lineages (2–4). Reverse transcription–PCR analyses demonstrated *SCL* expression in $CD34^+$ bone marrow cells (5, 6), which suggested that *SCL* might be expressed in hematopoietic progenitor cells.

The essential role played by *SCL* in hematopoietic development became apparent when it was shown that embryos in which the locus was ablated by homologous recombination failed to develop yolk sac hematopoiesis (7, 8). *SCL*-null embryonic stem (ES) cells did not generate hematopoietic colonies *in vitro* and did not contribute to hematopoiesis in chimeric mice, implying that *SCL* was also critical for definitive hematopoiesis (9, 10). Molecular analysis of differentiating *SCL*-null embryoid bodies revealed that they failed to express any hematopoietic-specific genes, although induction of genes coexpressed in endothelium was normal (11). These data supported a model in which *SCL* was absolutely required for the commitment of a putative hemangioblast to the hematopoietic lineage but was dispensable for initial differentiation into endothelium.

However, these gene ablation studies could not verify by functional assays the expression of *SCL* in hematopoietic progenitor and stem cells suggested by mRNA and reverse transcription-PCR studies. To address this issue, we have generated mutant mice ($SCL^{lacZ/w}$) in which the *Escherichia coli lacZ* reporter gene has been "knocked in" to the *SCL* locus. This paper focuses on an analysis of the functional properties of β-galactosidase-expressing cells in heterozygous $SCL^{lacZ/w}$ mice.

MATERIALS AND METHODS

Construction of the *SCL-lacZ* Targeting Vector. The 5′ arm of *SCL* homology, consisting of a 6-kb genomic fragment containing exon Ia–exon III, was cloned into the vector β-gal (12), which contained the *E. coli lacZ* gene coupled to the rabbit β-globin polyadenylation sequence. The 3′ homology arm of the targeting vector, comprising the *PGK* promoter-driven neomycin resistance gene coupled to a 1.75-kb fragment from the 3′ untranslated region of exon VI of *SCL,* was cloned downstream of the rabbit β-globin polyadenylation sequence.

Isolation of Targeted ES Cell Clones and Production of Chimeric Mice. The ES cell line W9.5 was maintained, electroporated, and selected with G418 (GIBCO) as described (7). Homologous recombinants were identified by Southern hybridization of DNA isolated from individual clones. Three targeted clones were injected into $(C57BL/6 \times C57BL/10)F_2$ blastocysts from female mice 3.5 days post coitum. Chimeras were mated to C57BL/6 mice to produce heterozygotes, which were identified by Southern hybridization of tail DNA.

Fluorescence-Activated Cell Sorter β-Galactosidase (FACS-Gal) Analysis. The FACS-Gal assay was performed basically as described (13). Bone marrow cells harvested from $SCL^{lacZ/w}$ or $SCL^{wt/wt}$ mice were washed in Hepes and potassium phosphate-buffered balanced salt solution supplemented with 5% fetal calf serum. The erythrocytes were lysed by incubation in 156 mM ammonium chloride, pH 7.3, at 37°C for 3 min. Nucleated cells were pelleted and resuspended in 150 μl of balanced salt solution/5% fetal calf serum and warmed to 37°C. Hypotonic loading was accomplished by diluting the cells 1:1 with warmed 2 mM fluorescein di-β-D-galactopyranoside (FDG; Sigma) and incubating at 37°C for 2 min. Three milliliters of cold balanced salt solution/5% fetal calf serum was added to stop loading and intracellular hydrolysis of FDG to fluorescein catalyzed by β-galactosidase was allowed to

Abbreviations: CFU, colony-forming unit; CFU-E, erythroid CFU; BFU-E, erythroid burst-forming unit; SCF, stem cell factor; FACS, fluorescence-activated cell sorter; IL, interleukin; Epo, erythropoietin; ES, embryonic stem; CFC, colony-forming cell; CFU-S, spleen CFU.

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proceed on ice for 3 h. Propidium iodide was added to a final concentration of 1 μg/ml before analysis. Cells were analyzed and sorted on a FACStar Plus (Becton Dickinson).

Clonogenic Assays for Hematopoietic Progenitors. Fractionated and unfractionated bone marrow cells from *SCL*$^{lacZ/w}$ or *SCL*$^{w/w}$ mice were cultured in 0.3% agar for myeloid colony formation or in 0.9% methylcellulose for erythroid colony formation as described (14). Day 7 myeloid colonies were stimulated with 1,000 units/ml interleukin (IL)-3 or 100 ng/ml stem cell factor (SCF); day 7 erythroid burst-forming units (BFU-E), with the combination of 1,000 units/ml IL-3 and 4 units/ml human erythropoietin (Epo); and day 2 erythroid colony-forming units (CFU-E), with 4 units/ml Epo. Differential colony counts were determined from agar cultures that had been fixed, dried, and stained with hematoxylin and luxol fast blue, and tested for acetylcholinesterase activity as described (14).

Limiting Dilution Analysis of Pre-B Cells. Between 10 and 3,000 fractionated and unfractionated bone marrow cells were deposited into individual wells of 96-well plates seeded with irradiated (30 Gy) IL-7-expressing NIH 3T3 cells (a gift from A Rolink, Basel Institute for Immunology). Wells were scored for growth after 7 days and cells pooled from multiple wells were stained with May–Grünwald–Giemsa and analyzed by flow cytometry by using directly conjugated antibodies to Mac-1 and B220 antigens as described (15).

Fetal Thymic Organ Culture. Embryonic day (E)15.5 thymic lobes from 129/Sv mice were depleted of endogenous lymphoid precursors by organ culture in medium containing 1.35 mM deoxyguanosine (Sigma) for 5–7 days as described (16). Individual lobes were then reconstituted by cocultivation for 24 h in hanging drops (17) with fractionated and unfractionated bone marrow cells from syngeneic *SCL*$^{lacZ/w}$ mice, followed by an additional 3-week period of organ culture. Cell suspensions of reconstituted thymic lobes were stained with May–Grünwald–Giemsa and analyzed by flow cytometry using directly conjugated antibodies to Thy-1, CD4, CD8, TCRβ, TCRγ, Mac-1, and B220 antigens as described (15).

Day 12 CFU-S Assay. Fractionated and unfractionated bone marrow cells from *SCL*$^{lacZ/w}$ mice were injected into the retro-orbital venous plexus of irradiated C57BL/6 recipients (two doses of 5.5 Gy separated by 3 h). Spleens were harvested after 12 days, weighed, and fixed in a 100:5:12 (vol/vol) ratio of 70% ethanol/glacial acetic acid/10% neutral buffered formalin. The macroscopic colonies were counted.

RESULTS

Generation of *SCL*$^{lacZ/w}$ Mutant Mice. The *E. coli lacZ* reporter gene was inserted by homologous recombination into the 5′ untranslated region of the mouse *SCL* gene, placing it under the control of *SCL* regulatory elements. The targeting event simultaneously deleted all *SCL* coding sequences, thereby also creating an *SCL*-null allele (Fig. 1*A*). This strategy ensured that *SCL* regulatory regions upstream of exon III and downstream of exon VI were left intact (18). Correctly targeted ES cell clones were identified by Southern hybridization of DNA using the 3′ flanking probe A. The structure of the targeted allele was verified by using a 5′ flanking probe and single integration of the targeting vector was confirmed with a *neo* probe (data not shown).

Three targeted ES cell clones were used to generate independent lines of mice. These were genotyped by Southern hybridization of tail DNA (Fig. 1*B*) or using a PCR-based strategy, as described previously (7). As expected, mice heterozygous for the *SCL-lacZ* allele (denoted *SCL*$^{lacZ/w}$) appeared normal but homozygous *SCL*$^{lacZ/lacZ}$ embryos died by E9.5 with absent yolk sac hematopoiesis, a phenocopy of the *SCL*$^{-/-}$ embryos previously described (7, 8).

Histochemical staining of *SCL*$^{lacZ/w}$ embryos for β-galactosidase activity revealed *lacZ* expression in the fetal liver, circulating blood cells, endothelium, and the developing brain, consistent with the sites of *SCL* mRNA and protein expression detected in previous studies (refs. 4 and 19 and data not shown). The same pattern was observed in all three targeted lines. This indicated that the expression pattern of the *SCL*-targeted *lacZ* gene reliably reflected endogenous *SCL* gene expression.

Characterization of β-Galactosidase-Expressing Cells in the Bone Marrow of Adult *SCL*$^{lacZ/w}$ Mice. β-Galactosidase-expressing hematopoietic cells in *SCL*$^{lacZ/w}$ mice were identified by using the FACS-Gal technique (13). This highly sensitive method permitted the recovery of viable populations of cells for morphological and functional evaluation. Because FACS-Gal fluorescence is proportional to the number of β-galactosidase molecules per cell (13), the level of fluorescence reflected *SCL* gene expression.

Bone marrow cells from adult *SCL*$^{lacZ/w}$ mice were separated into those expressing low (lacZ^{neg}; 93.3 $\pm$ 1.3%, mean $\pm$ SD derived from 13 experiments), intermediate (lacZ^{int}; 5.2 $\pm$ 0.8%), and high (lacZ^{high}; 1.5 $\pm$ 0.6%) levels of β-galactosidase activity (Fig. 2*A*). A small subset of wild-type (*SCL*$^{w/w}$) bone marrow cells (1.5 $\pm$ 0.4%) expressed intermediate levels of

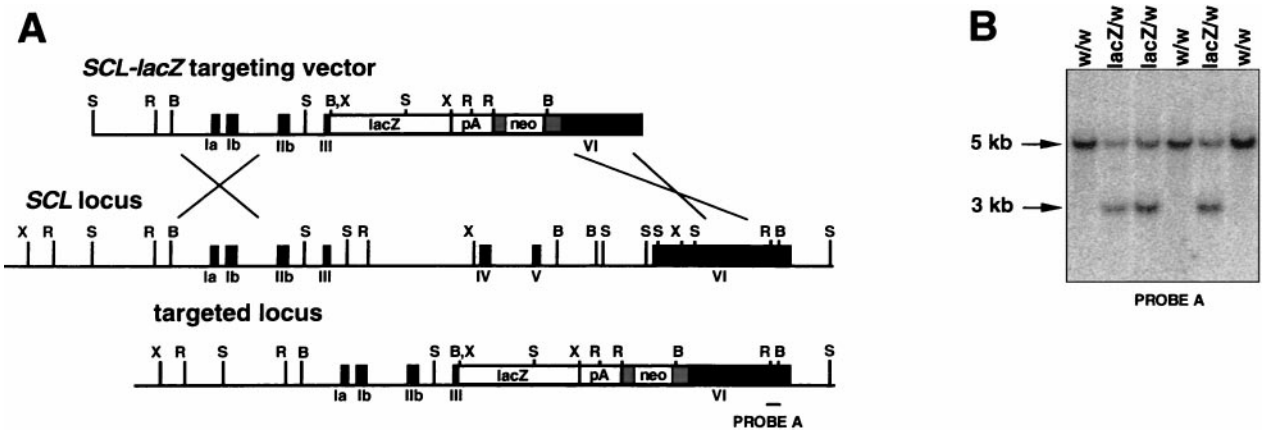

FIG. 1. *SCL-lacZ* gene targeting strategy. (*A*) The *SCL-lacZ* targeting vector is shown above the map of the *SCL* genomic locus. Regions of homology where recombination occurred are indicated as crosses and the correctly targeted locus is depicted below. Exons are shown as numbered, solid boxes. The positions of the *E. coli lacZ* gene (lacZ), the polyadenylation signal (pA), and the *PGKneo* cassette (neo) are indicated. Abbreviations: B, *Bam*HI; R, *Eco*RI; S, *Sac*I; X, *Xba*I. (*B*) Southern hybridization of *Bam*HI-digested DNA from wild-type (w/w) and heterozygous (lacZ/w) mice hybridized with probe A, indicating germ-line (5 kb) and targeted (3 kb) alleles.

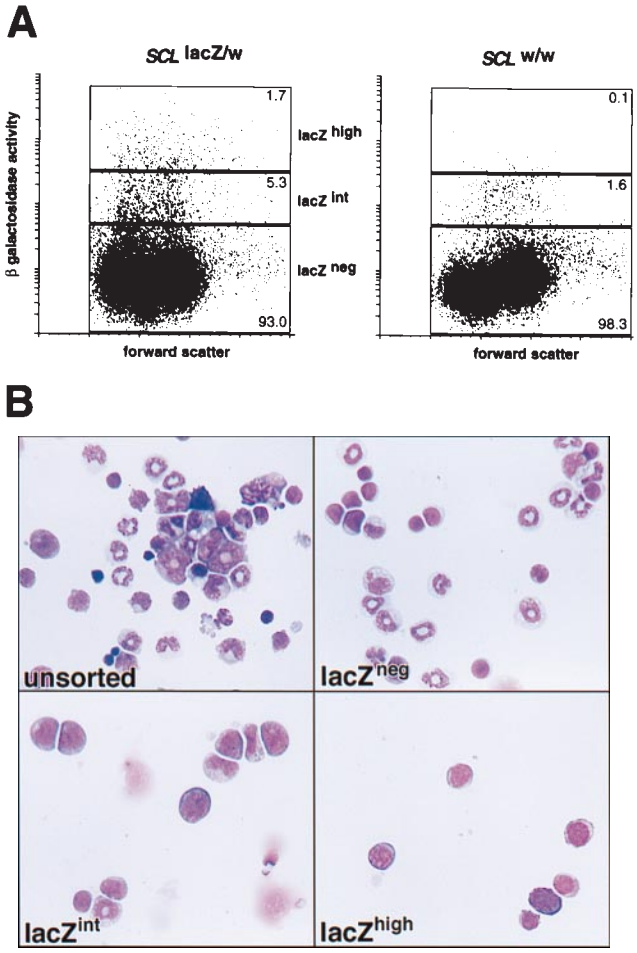

FIG. 2. Expression of β-galactosidase in $SCL^{lacZ/w}$ bone marrow cells. (*A*) Representative FACS profiles of forward scatter plotted against β-galactosidase activity (measured as fluorescence) for FACS-Gal-labeled bone marrow cells from $SCL^{lacZ/w}$ and $SCL^{w/w}$ mice. Gating windows and the percentage of cells in each window are shown for $lacZ^{neg}$, $lacZ^{int}$, and $lacZ^{high}$ fractions. (*B*) May-Grünwald-Giemsa stained cytocentrifuge preparations of unsorted and sorted bone marrow fractions from $SCL^{lacZ/w}$ mice.

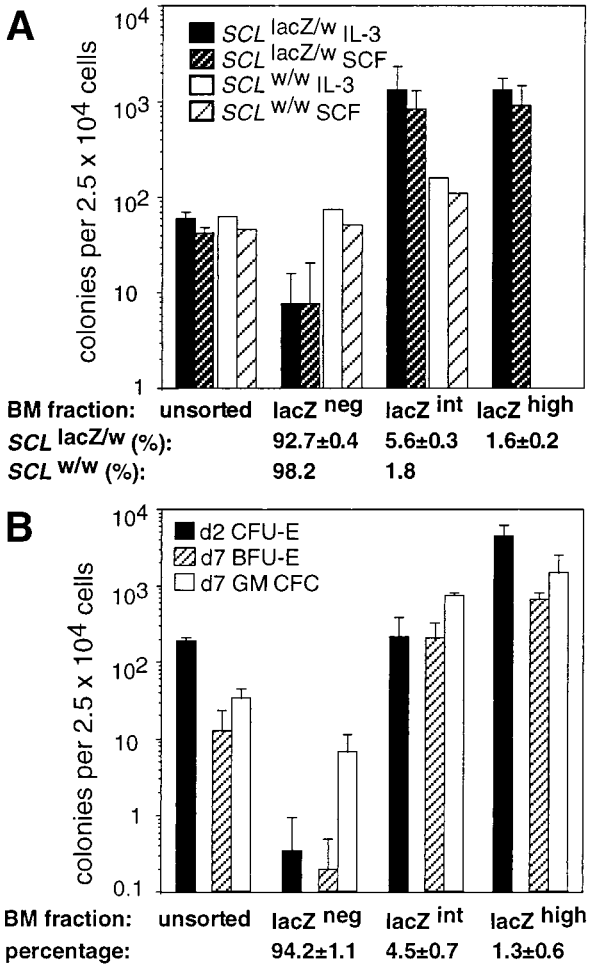

FIG. 3. Enrichment for myeloid and erythroid CFCs in the lacZ-positive bone marrow fractions of $SCL^{lacZ/w}$ mice. (*A*) Frequency of day 7 myeloid CFC in agar cultures of unsorted and sorted bone marrow fractions from $SCL^{lacZ/w}$ and $SCL^{w/w}$ mice cultured in IL-3 or SCF. Values represent the mean ± SD from five experiments for $SCL^{lacZ/w}$ and the mean from two experiments for $SCL^{w/w}$ bone marrow cells. The percentage of bone marrow cells that were sorted into each fraction is indicated. (*B*) Frequency of day 2 CFU-E, day 7 BFU-E, and myeloid (GM) CFC in methylcellulose cultures of unsorted and sorted bone marrow fractions from $SCL^{lacZ/w}$ mice cultured in Epo (for day 2 CFU-E) or IL-3/Epo (for day 7 BFU-E and GM CFC). Values represent the mean ± SD from three experiments. The percentage of bone marrow cells that were sorted into each fraction is indicated.

β-galactosidase activity, but very few cells (0.1 ± 0.1%) were $lacZ^{high}$. Undifferentiated blast cells were more frequent in the $lacZ^{int}$ (22 ± 3%) and $lacZ^{high}$ populations (32 ± 5%) than in the $lacZ^{neg}$ fraction (3 ± 1%) and nucleated erythroblasts were also increased in the $lacZ^{high}$ fraction (Fig. 2*B* and data not shown). This latter fraction was depleted of mature granulocytic and monocytic cells. These data were consistent with previous observations linking *SCL* expression to the erythroid lineage and to immature hematopoietic cells.

Myeloid and Erythroid Progenitor Cells Are Enriched in the β-Galactosidase-Positive Fraction from $SCL^{lacZ/w}$ Bone Marrow. Fractionated bone marrow cells from $SCL^{lacZ/w}$ and control $SCL^{wt/wt}$ mice were cultured in agar. As shown in Fig. 3*A*, there was a >100-fold enrichment of myeloid progenitor cells in the $lacZ^{int}$ and $lacZ^{high}$ fractions compared with $lacZ^{neg}$ bone marrow cells from $SCL^{lacZ/w}$ mice. In contrast, there was little difference in the frequency of progenitor cells in the $lacZ^{int}$ vs. $lacZ^{neg}$ fractions of wild-type bone marrow cells. Microscopy of stained agar cultures revealed granulocyte, macrophage, eosinophil, and megakaryocyte colonies in the cultures derived from $lacZ^{int}$ and $lacZ^{high}$ $SCL^{lacZ/w}$ bone marrow cells in the same proportions as were observed in cultures of unfractionated bone marrow cells (data not shown). There was an increased frequency of blast cell colonies in the $lacZ^{high}$ fraction (33 ± 6%) compared with unfractionated bone marrow cells (21 ± 8%) in cultures stimulated with SCF. Mast cell progenitors, detected by culturing bone marrow cells in the presence of IL-3, were also enriched in the β-galactosidase-positive fractions (data not shown).

The frequency of erythroid precursors in bone marrow populations from $SCL^{lacZ/w}$ mice was determined by culturing cells in methylcellulose. The enrichment for CFU-E and BFU-E in the $lacZ^{int}$ and $lacZ^{high}$ bone marrow cells was even greater than for the myeloid colony-forming cells (CFCs), with the CFU-E being particularly numerous in the $lacZ^{high}$ fraction (Fig. 3*B*). The degree of myeloid CFC enrichment observed in the β-galactosidase-positive populations was similar to that seen in the agar cultures, indicating that the greater enrichment for erythroid progenitors in the β-galactosidase-positive fractions was not simply due to the different culture methods.

When the absolute number of clonogenic cells in each fraction was calculated, >90% of the myeloid progenitor cells in $SCL^{lacZ/w}$ bone marrow agar cultures were $lacZ^{int}$ or $lacZ^{high}$. Conversely, only 5% of the clonogenic cells in $SCL^{w/w}$

Table 1. Distribution of clonogenic cells in sorted $SCL^{lacZ/w}$ bone marrow fractions

Genotype* (*n*)	Stimulus†	Colony type	Progenitor yield‡		Colony distribution, %§		
			Unsorted	Sorted	$lacZ^{neg}$	$lacZ^{int}$	$lacZ^{high}$
Agar cultures							
$SCL^{lacZ/w}$	IL-3	Day 7 myeloid	59 ± 11	102 ± 32	5 ± 4	64 ± 22	31 ± 26
(5)	SCF	Day 7 myeloid	42 ± 6	69 ± 25	8 ± 12	63 ± 14	29 ± 23
$SCL^{w/w}$	IL-3	Day 7 myeloid	63	76	95	5	NA¶
(2)	SCF	Day 7 myeloid	47	53	95	5	NA
Methylcellulose cultures							
$SCL^{lacZ/w}$	Epo	Day 2 CFU-E	189 ± 20	70 ± 43	0.3 ± 0.5	16 ± 14	84 ± 14
(3)	Epo/IL-3	Day 7 BFU-E	13 ± 11	18 ± 7	0.6 ± 1	50 ± 22	49 ± 22
		Day 7 myeloid	34 ± 12	60 ± 21	10 ± 5	60 ± 19	30 ± 17

*Bone marrow cells from mice of the indicated genotype were cultured in duplicate in agar or methylcellulose in the presence of growth factors for 2 or 7 days and the colonies counted. The number of experiments is shown in parentheses.
†IL-3 was used at 1,000 units/ml, SCF at 100 ng/ml, and Epo at 4 units/ml.
‡The number of CFCs in cultures of 2.5×10^4 bone marrow cells (unsorted) compared with the calculated recovery of CFCs from the sorted fractions. This figure was calculated for each experiment by summing (progenitor frequency × % of cells) for each fraction and then determining the mean for each group of experiments. Values given represent the mean ± SD. The mean progenitor frequency and the mean % cells in each fraction are shown in Fig. 3.
§The distribution of CFCs in the sorted bone marrow fractions given as a percentage.
¶NA, not applicable, since all lacZ-positive $SCL^{w/w}$ bone marrow cells were $lacZ^{int}$.

bone marrow were in the $lacZ^{int}$ fraction (Table 1). Comparing the number of myeloid progenitor cells observed in unsorted bone marrow and the yield calculated by summing the sorted fractions indicated that there was no significant progenitor cell loss during the sorting procedure (Table 1). Similarly, when the distribution of progenitor cells in methylcellulose cultures was compared, >99% of the CFU-E and BFU-E and 90% of the myeloid CFCs were β-galactosidase-positive. The level of β-galactosidase activity in the CFU-E was the highest, because 84% were $lacZ^{high}$, followed by the BFU-E (49% $lacZ^{high}$) and myeloid CFCs (30% $lacZ^{high}$). The calculated recovery of CFU-E after sorting was only 37% of the input number (compare "sorted" with "unsorted" columns in Table 1), whereas the calculated recovery of day 7 CFCs was in excess of 100%. This suggested that day 2 CFU-E may have been sensitive to the sorting procedure and that fractionation actually removed cells inhibitory for day 7 CFCs.

Summing the numbers of IL-3/Epo-responsive myeloid and erythroid colonies in methylcellulose cultures gave an estimated frequency of clonogenic precursors of 4.6% for $lacZ^{int}$ cells and 26% for $lacZ^{high}$ cells.

Day 12 CFC-S Are Enriched in β-Galactosidase-Positive Cells from $SCL^{lacZ/w}$ Bone Marrow. The genetic background of the $SCL^{lacZ/w}$ mice precluded experiments to examine long-term repopulating stem cells. However, we found that a more mature multipotential precursor cell, the day 12 CFU-S, displayed a degree of enrichment in the $lacZ^{int}$ and $lacZ^{high}$ fractions similar to the day 7 myeloid colony forming cells, being 50- to 100-fold more frequent than in the $lacZ^{neg}$ population (Table 2). As was the case with the myeloid CFCs, most of the day 12 CFU-S resided in the $lacZ^{int}$ fraction.

Table 2. Frequency of day 12 CFU-S in $SCL^{lacZ/w}$ bone marrow

Bone marrow* fraction	Cells injected per recipient	Spleen† colonies	Spleen weight, mg
Uninjected (4)	0	0.3 ± 0.5	20 ± 1
Unsorted (4)	5×10^4	10 ± 2	94 ± 33
$lacZ^{neg}$ (5)	5×10^4	1 ± 2	28 ± 17
$lacZ^{int}$ (5)	6×10^3	50 ± 43	62 ± 35
$lacZ^{high}$ (5)	6×10^3	103 ± 27	151 ± 37

*Bone marrow cells from $SCL^{lacZ/w}$ mice were injected into irradiated recipients. The number of recipients for each fraction is given in parentheses.
†Spleens were harvested after 12 days and macroscopic colonies counted. Values represent the mean ± SD colony numbers calculated per 5×10^4 cells injected.

B and T Lymphoid Progenitors Are Present in the β-Galactosidase-Positive Fractions of $SCL^{lacZ/w}$ Bone Marrow. FACS-Gal staining of thymocytes or splenocytes from $SCL^{lacZ/w}$ and $SCL^{w/w}$ mice did not reveal any $SCL^{lacZ/w}$ lymphoid populations with increased β-galactosidase activity (data not shown), consistent with the absence of *SCL* expression in these cell types (3, 5). However, this analysis did not address whether more primitive lymphoid precursors expressed *SCL*.

Bone marrow fractions were cultured in limiting dilution on stromal layers of IL-7-expressing NIH 3T3 cells to detect the growth of pre-B cells (20, 21). In each of three experiments, clonogenic cells were present in the β-galactosidase-positive as well as -negative fractions at a frequency of ≈1:100 (data not shown). However, the populations of hematopoietic cells that were evident by 7 days of culture were heterogeneous, especially in wells into which $lacZ^{int}$ or $lacZ^{high}$ cells had been sorted.

Examination of cytocentrifuge preparations of cells grown from unsorted bone marrow or $lacZ^{neg}$ seeded wells revealed a predominance of lymphocytes, but there were also some macrophages (data not shown). Approximately one-third of the cells in $lacZ^{int}$ and $lacZ^{high}$ wells were lymphocytes and the remaining cells were macrophages or immature myeloid cells. Flow cytometry of the harvested cells labeled with antibodies recognizing the B cell antigen, B220, and the myeloid antigen, Mac-1 confirmed these lineage assignments (Fig. 4). It is likely that the enrichment for myeloid precursors in the β-galactosidase-positive fractions contributed to the dominance of macrophages in cultures initiated with lacZ-positive cells.

We also assessed the ability of the different bone marrow fractions to reconstitute T lymphopoiesis in deoxyguanosine-treated fetal thymic lobes. As detailed in Table 3, visible enlargement of thymic lobes after a 3-week culture period correlated with colonization of the thymic rudiment. Both the frequency of thymic lobe enlargement and the cellular recovery were greater in lobes reconstituted with β-galactosidase-positive than β-galactosidase-negative cells, indicating that T lymphoid precursors were enriched in $lacZ^{int}$ and $lacZ^{high}$ bone marrow fractions of $SCL^{lacZ/w}$ mice. Examination of cytocentrifuge preparations of cells recovered from the reconstituted lobes revealed an identical lymphoid morphology in all cases (data not shown). Over 95% of cells stained brightly for Thy-1, confirming the T cell lineage of the recovered cells. Most cells (65–90%) were immature $CD4^-8^-$ thymocytes and only 15–25% expressed T cell receptors on their surface (Fig. 5). The same immunophenotype was observed regardless of the bone marrow fraction used to reconstitute the thymic lobes. This suggested that thymic precursors with similar kinetics of

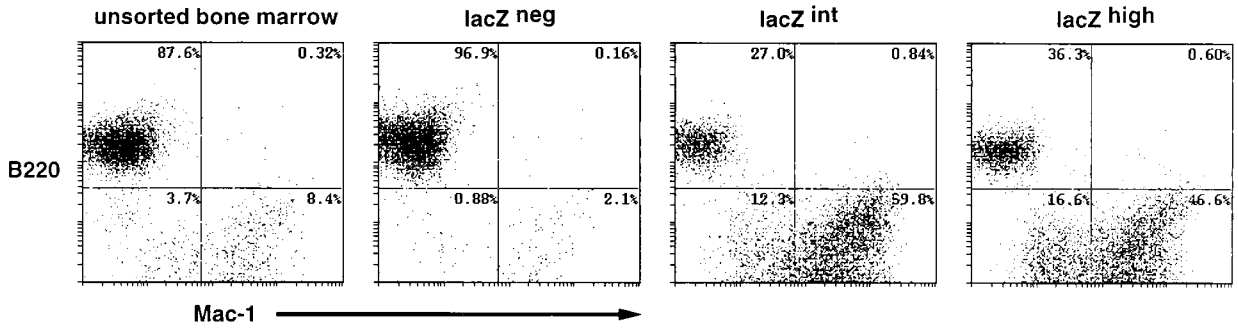

FIG. 4. B lymphoid progenitor cells are found in lacZ-positive and -negative bone marrow fractions of $SCL^{lacZ/w}$ mice. Flow cytometric profiles of cells harvested from IL-7/NIH 3T3 cultures seeded with unsorted or sorted bone marrow fractions and labeled with directly conjugated antibodies against the B lymphoid antigen, B220, and the myeloid antigen, Mac-1.

differentiation were responsible for reconstituting the thymic lobes in all cases.

DISCUSSION

To investigate the functional attributes of *SCL*-expressing hematopoietic cells, we have used homologous recombination to "knock-in" the *E. coli lacZ* gene into the murine *SCL* locus, thereby linking the production of β-galactosidase to transcription from the *SCL* promoter. The expression pattern of the *SCL*-targeted *lacZ* gene mirrored known sites of *SCL* gene expression, indicating that β-galactosidase activity in $SCL^{lacZ/w}$ animals represented a valid facsimile of endogenous *SCL* expression. We employed the FACS-Gal technique to identify and recover viable hematopoietic cells with different levels of β-galactosidase activity from $SCL^{lacZ/w}$ mice.

A major finding from these experiments was that myeloid and erythroid progenitor cells were enriched in the lacZ-positive bone marrow fractions from $SCL^{lacZ/w}$ mice. Interestingly, there was a correlation between the level of β-galactosidase activity and the type of colony observed (see Fig. 3*B* and Table 1). The highest lacZ expression levels correlated with enrichment for day 2 CFU-E, which were 20-fold more abundant in $lacZ^{high}$ than in $lacZ^{int}$ populations. Although >99% of day 7 BFU-E were β-galactosidase-positive, their frequency was only 3-fold greater in $lacZ^{high}$ than in $lacZ^{int}$ cells. As a group, day 7 myeloid progenitors expressed lower levels of β-galactosidase. In fact, ≈60% of these cells were $lacZ^{int}$ and 10% were $lacZ^{neg}$. However, examination of fixed, stained agar cultures of the myeloid colonies revealed that blast cell colonies were more frequent in the $lacZ^{high}$ fraction. Similarly, day 12 CFU-S, the most primitive multipotential precursor assayed in our experiments, were also enriched in the $lacZ^{high}$ populations (Table 2).

From these data, we can propose a model in which *SCL* is expressed in multipotential and myeloid progenitor cells but is rapidly extinguished once cells begin to differentiate and lose proliferative potential. The pattern differed in the erythroid lineage, where levels of *SCL* rose during initial differentiation from multipotential progenitor to day 2 CFU-E. As judged by the distribution of nucleated erythroblasts between the β-galactosidase-positive and -negative populations, it is likely that only a subset of these cells continued to express *SCL*. This model is consistent with the results of *in situ* hybridization that demonstrated *SCL* transcripts in a subset of $CD34^+$ cells and immature erythroblasts (5) and analyses that revealed more abundant *SCL* expression early in erythroid colony development (6, 22, 23). *SCL* transcripts were detected at lower levels in early progeny of myeloid precursors and were lost as cells differentiated (23). We have demonstrated that there are clonogenic lymphoid as well as myeloid progenitors that express *SCL*. Although the majority of lymphoid precursors that grow on stromal cell layers in the presence of IL-7 are $B220^+$ Kit^+ pre-B-I cells (24), more primitive progenitors have also been identified, including a common lymphoid progenitor with T, B, and natural killer cell reconstituting capacity (25). Thus, the presence of B lymphoid progenitors in the β-galactosidase-negative and -positive bone marrow fractions from the $SCL^{lacZ/w}$ mice may indicate that the expression of *SCL* varies within the hierarchy of B lymphoid progenitor cells.

T lymphoid precursor cells were selectively enriched in the β-galactosidase-positive vs. β-galactosidase-negative bone marrow fractions. It is likely that the $lacZ^{int}$ and $lacZ^{high}$ bone marrow cells that seeded the thymic rudiment were at least oligopotential lymphoid precursors if not multipotential lymphomyeloid stem cells, because the spectrum of bone marrow-derived cells capable of reconstituting the thymus is restricted to primitive and more mature multilineage hematopoietic stem cells (26) and common lymphoid progenitors (25). Indeed, the

Table 3. T cell progenitors are enriched in lacZ-positive cells from $SCL^{lacZ/w}$ bone marrow

Bone marrow fraction*	Input cells per lobe†	Lobe enlargement‡	Cells recovered§		Fold increase¶
			Per lobe	Per 5×10^4 cells	
No cells	0	0/5	5.5×10^2‖		
Unsorted (100%	5×10^4	8/8	1.6×10^4	1.6×10^4	1.0
lacZ neg (89%)	5×10^4	1/8	1.4×10^3	1.4×10^3	0.1
lacZ int (7.5%)	2×10^3	6/7	1.8×10^4	4.5×10^5	27.5
lacZ high (3.5%)	1×10^3	6/8	1.3×10^4	6.3×10^5	38.5

*Bone marrow cells from $SCL^{lacZ/w}$ mice were cocultivated with deoxyguanosine-treated thymic lobes for 24 h prior to organ culture. The percentage of bone marrow cells gated into each fraction is given in parentheses. Results are given for one representative experiment of two performed.
†The number of cells cocultivated with each thymic lobe.
‡Visible lobe enlargement was assessed after a 3-week culture period.
§Cellularity of pooled thymic lobes expressed per lobe or per 5×10^4 input cells.
¶Ratio of cells recovered compared to unsorted bone marrow per 5×10^4 input cells.
‖Only stromal cells were recovered.

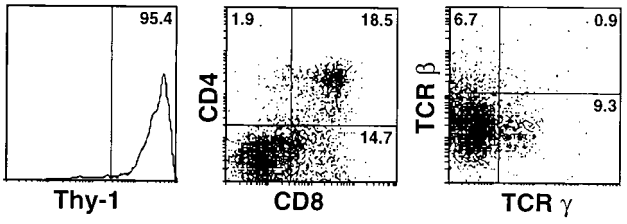

FIG. 5. T lymphoid cell surface markers expressed by the progeny of bone marrow cells reconstituting fetal thymic lobes in organ culture. Identical flow cytometric profiles were obtained regardless of whether β-galactosidase-positive or -negative bone marrow fractions were used to repopulate the thymic lobes.

earliest intrathymic precursors still retain the ability to differentiate along T, B, and natural killer cell lineages (26).

Fluorescence-activated cell sorting has permitted purification and characterization of hematopoietic stem and progenitor cells largely based on their expression of a diverse range of cell surface proteins including class I histocompatibility antigens, Thy-1, Ly-6A/E (Sca-1), c-*kit*, CD4, and CD34 (27). Unfortunately, with the exception of c-*kit*, very little is known about the role that any of these molecules plays in hematopoietic stem cell development. This study has succeeded in stratifying hematopoietic cells into functional groups based on their expression of the developmentally "relevant" transcription factor, *SCL*. The association of *SCL* expression with a broad range of lymphoid and myeloid hematopoietic progenitors, including the earliest precursors assayed, complements prior gene ablation studies and is consistent with the postulated role of *SCL* as a "master regulator" of hematopoiesis.

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