Phenotypic Analysis of Mouse Hematopoietic Stem Cells Shows a Thy-1–Negative Subset

By Gerald J. Spangrude and Diane M. Brooks

Mouse hematopoietic stem cells can be identified and enriched from populations of normal bone marrow cells by immunofluorescent labeling of cell surface molecules followed by flow cytometric separation. We show here that the majority of hematopoietic stem cell activity, as defined by long-term competitive repopulation of irradiated animals and by a secondary transplant assay for spleen colony-forming units (CFU-S), could be localized in Ly-Ga haplotype cells, has very potent activity in most hematopoietic assay systems included in the Thy-1low population. In contrast, hematopoietic stem cell activity found in the bone marrow of Thy-1.2 genotype mouse strains was recovered in both the Thy-1low and Thy-1high populations. However, similar to Thy-1.1 strains, most activity was localized to the Ly-6A/E+ population of cells. The difference in Thy-1 phenotype of hematopoietic stem cell activity apparent between Thy-1.1– and Thy-1.2–expressing mouse strains was not caused by differences in the staining intensity of monoclonal antibodies (MoAbs) specific for the Thy-1 alleles. Furthermore, an antiframework MoAb that stains both alleles of Thy-1 separated hematopoietic stem cell activity from mice expressing the two alleles in the same manner as did allele-specific MoAb. The results of this study show that Thy-1 expression is not an invariant characteristic of mouse hematopoietic stem cells, and that mice expressing the Thy-1.1 allele are unique in that hematopoietic stem cell activity is found exclusively in the Thy-1low population.

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Hematopoiesis is an ongoing developmental process by which the cellular elements of the blood in an adult organism are continually replenished. One interesting aspect of hematopoiesis that has proven difficult to study is the primitive hematopoietic stem cell population that serves as the origin of all of the diverse end-stage cells found in normal blood. These cells have proven elusive because of their low frequency, because even within a rich source of hematopoietic repopulating activity such as the bone marrow they represent only a fraction of a percent of the total cells present. In recent years much progress has been made in designing methodologies to enrich hematopoietic stem cells from mouse1–4 and human5–7 hematopoietic tissues. However, it is likely that even the most enriched preparations of hematopoietic stem cells are not solely composed of the most primitive cells in the hematopoietic hierarchy,8,9 which include those cells capable of sustained production of mature progeny cells over a prolonged time period, eg, the life span of the organism. For modern applications of bone marrow transplantation (BMT) and for attempts to modify the genetic contents of hematopoietic repopulating cells with the aim to correct genetic abnormalities, it is interesting to distinguish and identify the sources of long-term repopulating activity. Further, in experimental systems that seek to quantitate the long-term repopulating compartment after manipulation of the mammalian host, eg, after application of chemotherapeutic or infectious agents, it is critical to develop reliable criteria to identify the primitive hematopoietic stem cells.

One approach to identifying hematopoietic stem cell populations uses monoclonal antibodies (MoAbs) to distinguish various subsets and lineages of hematopoietic cells. These subsets can be separated and isolated by flow cytometric methods for functional testing.2 By this approach, a population of mouse hematopoietic stem cells has been identified by virtue of expression of the Thy-1 and Ly-6A/E antigens in the absence of high-level expression of a variety of antigens characteristic of differentiated lineages of cells. This population, termed Thy-1lowLin−Ly-6A/E+, has very potent activity in most hematopoietic assay

MATERIALS AND METHODS

Animals. C57BL/Ka, C57BL/Ka-Thy-1.1, C57BL/10ScN, C57BL/6-Alpha-17, C57BL/6-Ly-5.1-Pepβ, AKR/J, and SJL/J

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mice were bred and maintained in our animal facility at Rocky Mountain Laboratories. DBA/2J mice were purchased through Jackson Labs (Bar Harbor, ME). All animals were maintained on acidified (pH 2.5) drinking water and autoclaved chow (Purina Mills Inc, St Louis, MO) ad libitum.

Magnetic particle-mediated depletions. In some experiments, BM suspensions were depleted of cells expressing the Ly-6A/E antigen by immunomagnetic particle methodology. For control depletions, the anti-CD5 MoAb 53-7-31313 (obtained from American Type Culture Collection [ATCC], Rockville, MD) was used because it is isotype matched to the anti-Ly-6A/E MoAb E13 161-719 (both are IgG2a) labels BM cells at approximately the same frequency (5-7% of the total number of cells), and does not react with long-term repopulating hematopoietic stem cells. All manipulations of BM cells were performed using Hanks' Balanced Salt Solution containing 3% fetal calf serum and 10 mmol/L HEPES buffer, pH 7.2. The cell suspensions were incubated with a saturating concentration of the relevant MoAb at a cell density of 5 × 10^6 cells/mL for 20 minutes on ice, followed by a wash. One millilitre of twice-washed immunomagnetic particles (Dynal Inc, Great Neck, NY; sheep antirat Ig specificity) was added to the washed cells and the mixture was incubated for 30 minutes at 4°C with constant mixing. The particles with attached cells were then removed from the solution by application of a magnetic field and the remaining cells were collected, concentrated by centrifugation, and the procedure was repeated. A small sample of the twice-depleted population was stained with a fluorescein-conjugated anti-Thy-1 reagent (Caltag Laboratories, South San Francisco, CA) and analyzed by flow cytometry to confirm depletion of the target population.

Hematopoietic stem cell enrichment. BM cells were prepared from 8 to 12 young adult mice (4 to 8 weeks old) by crushing femora and tibia with a mortar and pestle. Low-density cells were enriched by equilibrium centrifugation on a cushion of metrizamide (Nycomed analytical grade; Accurate Chemical and Scientific Corp, Westbury, NY) at a density of 1.085 g/mL. The low-density cells were reacted with a saturating solution of MoAb specific for CD2 (RM-221), CD3 (KT3-119), CD5 (53-7-313), CD8 (53-6-72), Mac-1 (M1/70.15.11.5.HL17), B220, Gr-1 (RA3-6B2 and RB6-8C5, respectively, obtained from Dr R. Coffman, DNAX Research Institute, Palo Alto, CA), and an erythroid marker (TER-119) obtained from Dr T. Kina, Kyoto University, Kyoto, Japan). Cells expressing any of these markers were removed by two cycles of immunomagnetic depletion as described above. Residual positive cells were labeled by staining with a fluorescent antirat Ig (Caltag). After a blocking step of 10 μg rat Ig (Pel-Freeze, Rogers, AR) for 5 minutes, cells were stained with phycoerythrin-conjugated anti-Ly-6A/E (E13 161-7, conjugated to phycoerythrin using reagents obtained from Molecular Probes, Inc, Eugene, OR) and a biotinylated anti-Thy-1 reagent. These included anti-Thy-1.2 (53-2-119) and 5A8, obtained from Caltag, anti-Thy-1.1 (19XE519), and anti-Thy-1 framework (31-119). The cell suspension was subsequently reacted with streptavidin-Red613 (Immunoselect, Life Technologies, Inc, Grand Island, NY), and dead cells were excluded from analysis by propidium iodide staining. Two-color flow cytometric analysis of 10,000 cells from each strain allowed direct evaluation of the relative contributions of the competing BM populations to the circulating pool of mature cells. At the termination of competitive repopulation experiments, the animals were killed and a similar analysis was performed on BM suspensions.

Pre-CFU-S assay. The pre-CFU-S assay detects very early stage cells in hematopoiesis which, unlike most CFU-S, have some ability to self-renew. It is useful for evaluating repopulating potential in a short-term assay, and in cases where strains of mice are to be tested for which no Ly-5 congenic strain exists. For these assays, 1 × 10^6 to 1 × 10^7 normal or immunomagnetic particle-depleted BM cells or 2 to 3 × 10^7 FACS-selected cells were transferred into lethally irradiated recipients. Thirteen days later, BM suspensions were prepared from these animals and transferred into secondary groups of irradiated animals. Usually each BM suspension (pooled from two primary recipients) was transferred into two or three groups of four mice, with each group receiving a fivefold dilution of the previous group’s marrow dose. Thirteen days later, the secondary groups of animals were killed and their spleens were removed and fixed in Telleyesniczky’s solution (70% ethanol-acetic acid-formalin, 20:1:1 by volume) before macroscopic surface colony count. The resulting colony counts were averaged and the data were calculated to reflect the number of CFU-S per femur of the primary recipient animal per 10^7 FACS-separated cells or 10^6 BM cells injected.
RESULTS

The majority of long-term repopulating cells express Ly-6A/E. While it is clear that one can enrich hematopoietic stem cell activity among Ly-6A/E+ cells; the possibility of Ly-6A/E- hematopoietic stem cells cannot be excluded. To determine whether the Ly-6A/E molecule is expressed by most long-term repopulating cells or by only a subset of them, experiments were performed in which immunomagnetic particles specifically removed BM cells bearing the Ly-6A/E surface marker. As a control, BM cells expressing CD5 were depleted in parallel samples. These depleted populations of cells were then tested in two hematopoietic assay systems, competitive repopulation and pre-CFU-S. As shown in Fig 1, depletion of Ly-6A/E-bearing cells markedly depressed the ability of the resulting BM population to competitively repopulate lethally irradiated recipients, even when the competing population consisted of 10-fold fewer normal cells. In contrast, CD5-depleted marrow under the same competitive conditions provided the majority of the long-term repopulating activity over the entire 16-week time span of the experiment. This result was observed whether total peripheral blood leukocytes or only circulating myeloid cells were assayed for donor or host origin. While the Ly-6A/E-depleted population produced a significant number of myeloid and nonmyeloid progeny detectable 4 weeks after the transplant, the contribution of these cells to the peripheral blood steadily decreased over the subsequent 12 weeks. The increase in donor-derived myeloid cells seen at the 16-week time point in Fig 1, when BM cells were analyzed, is most likely caused by the higher frequency of myeloid cells in BM compared with peripheral blood rather than a trend of increasing donor contribution to myeloid differentiation at this late time point. These results indicate that the majority of long-term repopulating activity present in the bone marrow of the tested mouse strains resides among Ly-6A/E+ cells, in agreement with the data of Uchida and Weissman.

The CD5- and Ly-6A/E-depleted populations were also tested using the pre--CFU-S assay. As shown in Table 1, this relatively short-term assay generally reflected the results obtained with the same two depleted populations in the long-term competitive repopulation assay. The number of pre--CFU-S was dramatically reduced in BM preparations depleted of Ly-6A/E+ cells, whereas CD5-depleted marrow contained fourfold to fivefold more pre--CFU-S activity relative to normal marrow. The enrichment of activity after CD5 depletion may have been caused by depletion of surface Ig+ B lymphocytes and CD5+ T cells by the anti-Ig immunomagnetic particles, or may indicate a negative regulatory influence that is removed by depletion of the lymphoid cells. In contrast to the pre--CFU-S data, Ly-6A/E-depleted BM contained the same number of primary CFU-S as did normal marrow (Table 1). However, the primary colonies produced by Ly-6A/E-depleted marrow were consistently smaller than those generated by the other two populations of cells (data not shown). These observations are consistent with the notion that the CFU-S assay measures late-stage progenitors in addition to more primitive cells, and that these late-stage cells do not necessarily express the Ly-6A/E antigen. The smaller size of spleen colonies generated by Ly-6A/E-depleted BM is consistent with in vitro observations of high proliferative potential colony-forming cells. CD5 depletion resulted in an increase in CFU-S frequency, although not to the same extent as seen with Ly-6A/E-depletion.

![Fig 1. Competitive repopulation by Ly-6A/E-depleted or control-depleted BM cells. After immunomagnetic depletion of cells expressing either Ly-6A/E or CD5, 10^6 depleted cells were injected along with 10^6 normal BM cells into lethally irradiated recipient animals. The normal cells and the recipient animals were genotypically Ly-5.2, while the depleted populations were derived from Ly-5.1 donor animals. At the indicated times after BMT, peripheral blood was analyzed for the presence of donor-derived cells by flow cytometric analysis, using MoAb specific for the two alleles of Ly-5 to discriminate donor from host cells. Donor-derived myeloid cells were identified in two-color analyses using the Ly-5 allelic MoAb in combination with MoAbs specific for myeloid cells. The data represent the results of one of four experiments, with four animals being represented in each group. Error bars represent standard deviations from the mean. The final analysis at 16 weeks is a staining of BM cells rather than peripheral blood.](image-url)
Table 1. Pre–CFU-S and CFU-S Content of BM Cells After Depletion With Anti-Ly-6A/E or Anti-CD5 MoAb

<table>
<thead>
<tr>
<th>Group</th>
<th>CFU-S/Femur/10^6 Cells* (pre-CFU-S)</th>
<th>CFU-S/S x 10^6 Cells† (day 13 CFU-S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal BM</td>
<td>50, 63, ND†</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>CD5 depleted</td>
<td>190, 350, 180</td>
<td>12 ± 5</td>
</tr>
<tr>
<td>Ly-6A/E depleted</td>
<td>3.1, &lt;0.3, &lt; 1.0</td>
<td>7 ± 4</td>
</tr>
</tbody>
</table>

*Primary animals for pre-CFU-S determinations received 10^6 cells per mouse (normal BM and CD5 depleted) or 10^7 cells per mouse (Ly-6A/E depleted). Thirteen days later, BM cells were harvested and transferred into secondary groups of animals for CFU-S determinations.
†BM cells treated as indicated were injected into lethally irradiated mice at a dose of 2.5 to 5 x 10^4 cells per mouse for CFU-S determination at day 13. Results are shown as the mean ± SD combined from three separate experiments.
‡Each number represents the average value obtained from three to six secondary recipients in a single experiment. ND, not determined.

Magnitude as was seen in the pre–CFU-S assay of the same population of cells.

Thy-1 antigen expression by long-term repopulating cells. To test whether long-term repopulating cells are exclusively included among those BM cells that express the Thy-1 antigen, Ly-6A/E+Lin^- BM cells were separated into two populations based on negative or low expression of the Thy-1 antigen (Fig 2). Isolated populations of cells expressing negative or low levels of the Thy-1 antigen were tested by both long-term competitive repopulation and short-term pre–CFU-S assays. By both assays, a striking difference was noted when comparing mouse strains expressing the Thy-1.1 allele with those expressing the Thy-1.2 allele. As shown in Fig 3A for the four animals from each group that had the greatest levels of donor-derived cells, competitive repopulating activity was present among Thy-1.1^-high but not Thy-1.1^-low cells. While six of six animals repopulated with 100 Thy-1.1^-low cells plus 10^6 normal BM cells contained greater than 5% congenic-derived myeloid cells in peripheral lymphocyte preparations at some point during the time course of the study, only 1 of 10 animals reconstituted in a similar competitive situation with Thy-1.1^-high cells contained greater than 5% congenic-derived myeloid cells. No animals in the group of 10 mice administered Thy-1.1^-high cells contained congenic-derived myeloid cells 20 weeks after the reconstitution. In contrast, two of six mice administered Thy-1.1^-low cells contained a large (> 60%) congenic contribution to the circulating myeloid compartment 20 weeks later.

Very different results were observed in recipients of 10^5 normal syngeneic BM plus 100 congenic cells of either the Thy-1.2^-high or Thy-1.2^-low phenotype. In each experimental group, 5 of 10 animals contained congenic-derived myeloid cells greater than 5% at some point during the time course, and in each group several animals continued to score positive 20 weeks after reconstitution. The majority (> 80%) of the circulating myeloid cells in two of these animals were descendants of the 100 sorted congenic cells (Fig 3B). Similar results were obtained when total nucleated peripheral blood cells were analyzed (data not shown); however, it is likely that long-term myeloid repopulation better reflects continuous input from a primitive progenitor because of the relatively rapid turnover of myeloid cells compared with lymphoid cells.

The results of pre–CFU-S assays performed on the Thy-1^-high and Thy-1^-low populations of Ly-6A/E+Lin^- BM cells from various strains of mice are shown in Table 2. In agreement with the long-term competitive reconstitution assay, very little activity was observed among Thy-1^-low populations from four mouse strains with Thy-1^-high populations from two different strains of mice, while Thy-1^-high populations from four mouse strains expressed as much or more activity than Thy-1^-low populations. Furthermore, the recovery of Thy-1^-low cells from Thy-1.2^-
expressing mouse strains was significantly lower than that from Thy-1.1-expressing strains; 59.4% ± 8.7% of Ly-6A/E+Linneg cells derived from Thy-1.1-expressing strains were among the Thy-1low subset, compared with 23.5% ± 6.9% derived from Thy-1.2-expressing strains. When the percentage of total pre-CFU-S activity recovered in the Ly-6A/E+Linneg fraction was broken down into the absolute percentage of activity included in the Thy-1low population, 98.7% ± 2.4% of the activity was recovered in Thy-1.1 mice while 19.2% ± 11.7% was recovered in Thy-1.2 mice. Thus, whereas the data shown in Table 2 indicate that the two subsets of cells isolated from Thy-1.2-expressing strains contain similar amounts of activity, consideration of the recovered number of each subset argues that the majority of pre-CFU-S activity within the Ly-6A/E+Linneg population of Thy-1.2 mice is contributed by cells that lack detectable expression of Thy-1.

Repopulating potential of Thy-1.2neg cells is not caused by antibody staining differences. Because two different MoAbs were used in these experiments to separate Thy-1.1- and Thy-1.2-expressing BM populations, it is possible that differences in species, subclass, or staining specificity between the two MoAbs accounted for the observed difference in biologic activity inherent in the Thy-1neg populations. A direct comparison of the Thy-1neg and Thy-1low populations isolated with each of the MoAbs showed that the final populations were very similar in terms of the level of Thy-1 expression (data not shown). In addition, staining of lymph node T lymphocytes by the two MoAbs showed that the anti-Thy-1.2 MoAb stained these cells about twofold brighter than did the anti-Thy-1.1 MoAb, arguing that the failure to recover long-term repopulating activity exclusively in the Thy-1low population was not because of staining problems intrinsic to the MoAb.

The variability of Thy-1 staining of BM populations with various MoAbs is shown stratified in Fig 4. Using several MoAbs specific for the Thy-1 allele, Ly-6A/E+Linneg BM cells were stained and the patterns of staining were compared. One MoAb (5a8) failed to detect Thy-1.2low expression among Ly-6A/E+Linneg BM cells. This MoAb also stained Thy-1.2high T cells in lymph node preparations to a lesser extent than did the other MoAb (Fig 4). An antiframework MoAb (31-11) that can be used to stain either allele of the Thy-1 molecule39 stained Thy-1.2 lymph node T lymphocytes at roughly 70% the intensity of the allele-specific anti-Thy-1.2 MoAb used in previous experiments (Fig 4). The 31-11 MoAb was similar to the allele-specific 53-2.1 MoAb in staining Thy-1low cells. To definitively address the question of whether staining with different antibodies contributed to the observed differences in functional activity, separations of BM cells were performed using the 31-11 antiframework MoAb to stain Thy-1 in each of the allelic forms. The results of pre-CFU-S assays on the separated populations (Table 3) were entirely consistent with what had been observed in previous experi-

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>CFU-S/Femur/10^9 Cells</th>
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<tbody>
<tr>
<td>Thy-1 Allele</td>
<td>Negative</td>
</tr>
<tr>
<td>C57BL/Ka-Thy-1.1</td>
<td>Thy-1.1</td>
</tr>
<tr>
<td>AKR/J</td>
<td>Thy-1.1</td>
</tr>
<tr>
<td>C57BL/Ka</td>
<td>Thy-1.2</td>
</tr>
<tr>
<td>C57BL/J-Ly-5.1-Pep^b</td>
<td>Thy-1.2</td>
</tr>
<tr>
<td>SJL/J</td>
<td>Thy-1.2</td>
</tr>
<tr>
<td>DBA/2</td>
<td>Thy-1.2</td>
</tr>
</tbody>
</table>

Ly-6A/E+Linneg BM cells separated from the indicated mouse strains into Thy-1neg and Thy-1low subsets as shown in Fig 2 were injected intravenously at a dose of 2 x 10^9 cells into lethally irradiated mice. Thirteen days later, BM was harvested from those mice and transferred into secondary recipients for CFU-S determinations. Values represent mean ± SD from four to seven secondary recipients in representative individual experiments.
ments using allele-specific MoAb. Virtually all pre–CFU-S activity detectable among Ly-6A/E+Linneg cells was included in the Thy-1.1low population, while the Thy-1.2neg and Thy-1.2low populations both contained significant levels of activity.

**DISCUSSION**

The prospect of studying the hematopoietic process in animal models has been greatly enhanced by the ability to identify and enrich primitive hematopoietic stem cells by virtue of MoAb staining of cell-surface antigens. However, to embark on any quantitative analysis of the compartment of primitive hematopoietic stem cells, eg, after treatment with myeloablative agents such as 5-fluorouracil or exposure to various infectious agents, some certainty must be invested in the phenotypic characteristics of the hematopoietic stem cell compartment. For this reason, we have carefully evaluated the functional characteristics of BM cells after separation into subpopulations based on MoAb staining, using two assay systems (competitive repopulation and pre–CFU-S) to detect primitive hematopoietic repopulating cells. The pre–CFU-S assay has previously been shown to be an excellent short-term in vivo assay in terms of correlation with long-term repopulating potential, a result substantiated by the experiments shown here. Therefore, the pre–CFU-S assay has great potential as a quantitative approximation of long-term repopulating potential in a short-term assay and offers the ability to screen putative hematopoietic stem cell populations before assay in the long-term competitive repopulating assay.

Both the competitive repopulation assay and the pre–CFU-S assay provided compelling evidence to suggest that, while a low frequency of Ly-6A/E+Linneg hematopoietic stem cells may exist, the majority of stem cells in the analyzed mouse strains express the Ly-6A/E antigen (Fig 1 and Table 1). This observation is only valid for mouse strains of the Ly-sb haplotype (eg, Am, C57BL, DBA, SJL) because Ly-6A/E is not expressed to any great extent by cells from mice of the Ly-ba haplotype without prior stimulation of the cells. Therefore, this cell surface marker has a limited application for mouse hematopoietic stem cell enrichments, because in many strains of mice it is not expressed on the population of interest (Spangrude GJ, Brooks DM: in preparation).

The ideal MoAb for enrichment applications such as those described here would have wide reactivity to hematopoietic stem cells of all mouse strains; several MoAbs have been described as being useful for stem cell enrichments; however, no broad screen of many mouse strains has been reported to date for these MoAbs. Lectins such as wheat germ agglutinin have been useful for stem cell enrichments and would be less likely to display species variability; however, populations enriched by this method

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**Table 3. Hematopoietic Reconstituting Activity of Thy-1 Subsets Isolated Using Several MoAbs**

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>MoAb</th>
<th>CFU-S/Femur/10^7 Cells</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Low</td>
</tr>
<tr>
<td>C57BL/Ka-Thy-1.1</td>
<td>19XE5</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>31-11</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td></td>
<td>53-2.1</td>
<td>75 ± 21*</td>
</tr>
<tr>
<td></td>
<td>31-11</td>
<td>88 ± 14</td>
</tr>
<tr>
<td>C57BL/Ka</td>
<td>3X53</td>
<td>45 ± 22</td>
</tr>
<tr>
<td></td>
<td>53-2.1</td>
<td>76 ± 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>92 ± 22*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>138 ± 52</td>
</tr>
</tbody>
</table>

BM subsets were isolated from the indicated mouse strains using either the allele-specific MoAb 19XE5 (anti-Thy-1.1) or 53-2.1 (anti-Thy-1.2) or the antiframework Thy-1 MoAb 31-11. Lethally irradiated primary recipient animals received an intravenous injection of 2 × 10^5 cells of the indicated subset. Thirteen days later, BM was harvested for CFU-S determination in lethally irradiated secondary recipients. Values represent the mean ± SD obtained from individual experiments, with each number representing three to eight spleens from secondary recipients.

*Values are from Table 2.
include relatively mature day 8 CFU-S unless additional selective criteria, such as rhodamine-123 staining, are also applied. The need for multiple selective criteria is a common feature of all stem cell enrichment protocols because no MoAb has been described that reacts exclusively with hematopoietic stem cells.

In the stem cell enrichment protocol used in these studies, the multiple selective criteria included Ly-6A/E and Thy-1 antigen expression as previously described. However, evaluation of Thy-1 expression by BM cells active in the competitive repopulation and pre-CFU-S assays produced a surprising result. By both assay systems, BM cells derived from Thy-1.2 genotype mice included significant activity among Thy-1.2low as well as Thy-1.2high cells (Table 2, Fig 3). In contrast, Thy-1.1 genotype mice included virtually all activity in the Thy-1.1low population, in agreement with a previous study. One possible explanation for this observation is that the MoAb used for separating the populations based on Thy-1.2 expression does not stain the Thy-1.2high population brightly enough to distinguish low-level staining from negative staining. However, the experiment that uses the antiframework MoAb to separate Thy-1.1high and Thy-1.1low cells from mouse strains expressing either allelic variant of Thy-1 (Table 3) eliminates the possibility that differences in MoAb staining account for the inability to isolate all hematopoietic repopulating activity in the Thy-1.2low population, and argues for a difference in the expression or configuration of the Thy-1 molecule in the mouse strains expressing the Thy-1.2 allele. Variations in tissue-specific expression of Thy-1 determinants with dependence on the allelic variant of the molecule have been reported previously.

Our results are consistent with those recently reported by Uchida and Weissman, who used competitive repopulation experiments to show that the majority of hematopoietic stem cell activity in one particular strain of mice (C57BL/Ka-Thy-1.1) was included among Thy-1.1lowLin-6A/E+ BM cells. We have extended those studies to show that the concept of a universal phenotypic description of mouse stem cells by these criteria is incorrect. From our results, less than half of the long-term repopulating activity found in normal BM (as estimated by the pre-CFU-S assay) is recovered among Thy-1.1low cells of mouse strains expressing the Thy-1.2 allele, which includes the majority of strains. It is likely that depletion of cells expressing lineage antigens remains a viable approach for enriching mouse hematopoietic stem cells, because most of these antigens are not known to exist in allelic forms and because the approach of lineage depletion has been adopted by many different laboratories using a wide variety of mouse strains. In addition to the study of Uchida and Weissman, Jordan et al have reported that virtually all hematopoietic stem cells present in mouse fetal liver tissue fail to express high levels of lineage markers.

The present study shows that Thy-1 expression is not a universal characteristic of hematopoietic stem cells in the mouse. In this regard, it is interesting to note the recent demonstration of Thy-1 expression on cells derived from human fetal liver that have many characteristics of hematopoietic stem cells. It is unclear how much variation will be observed between individual humans with regard to Thy-1 expression on these cells, and how the transition from fetal to adult tissue will affect the phenotype of the long-term repopulating cells. The fetal liver-derived hematopoietic stem cell population in the mouse shares the MoAb-defined phenotype of adult BM-derived cells; however, these experiments were performed using Thy-1.1 genotype animals. Furthermore, experiments designed to trace Thy-1 and Ly-6A/E expression by all assayable long-term repopulating cells in mouse fetal tissues derived from a variety of strains have not been reported. It is possible that the cell surface phenotype of these cells may change during ontogeny, as during recovery from chemically induced myeloablation. Therefore, it is difficult to predict, based on the data currently at hand, whether hematopoietic stem cells derived from all individual human fetal or adult sources will invariably express the Thy-1 antigen.

The demonstration here that many hematopoietic stem cells do not express the Thy-1 antigen argues against an obligatory role for this molecule in hematopoietic stem cell biology, at least when the cells are in a resting state. However, there may be redundancy in the molecules able to fill any putative role of the Thy-1 molecule. It is interesting to note that Müller-Sieburg et al found Thy-1 to invariably be expressed on cells capable of initiating Whitlock-Witte long-term BM cultures. These experiments were performed primarily in BALB/c mice, a strain that does not express Ly-6A/E on resting hematopoietic stem cells. Perhaps one of these two molecules is sufficient for any necessary functional role, and in the absence of Ly-6A/E the expression of Thy-1 may become critical. Experiments to test whether Thy-1 is invariably expressed on hematopoietic stem cells of Ly-6E mouse strains, which do not constitutively express Ly-6A/E, are currently under way.

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Phenotypic analysis of mouse hematopoietic stem cells shows a Thy-1- negative subset

GJ Spangrude and DM Brooks