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Enrichment of Functionally Distinct Mouse Hematopoietic Progenitor Cell Populations Using CD62L

Scott Cho* and Gerald J. Spangrude*†

The details of the bifurcation of the lymphoid and myeloid lineages following commitment by multipotent progenitor cells (MPP) remain a topic of controversy. We report that the surface glycoprotein CD62L can be characterized as a novel marker of this and other stages of early hematopoietic differentiation. Cell isolation and transplant studies demonstrated CD62Lneg/low long-term hematopoietic stem cells and CD62Lhigh MPP within the traditionally defined c-kitposLinnegSca-1pos stem/progenitor cell population. Within the MPP population, previously defined as c-kitposLinnegSca-1pos–Thy-1.1negFlt3pos, Sca-1 and CD62L resolved four populations and segregated Sca-1highCD62Lneg/low MPP from Sca-1highCD62Lhigh leukocyte-biased progenitors. Using a novel transplantation method that allows tracking of erythroid and platelet engraftment as an alternative to the classical method of in vitro colony formation, we characterized Sca-1highCD62Lneg/low cells as MPP, based on transient engraftment of these lineages. These data establish CD62L as a useful tool in the study of early hematopoiesis and emphasize the power of trilineage-engraftment studies in establishing the lineage potential of MPP subsets. The Journal of Immunology, 2011, 187: 000–000.

I
n adult mammals, all blood cells originate from a pool of hematopoietic stem cells (HSC) residing in the bone marrow. These adult stem cells possess the prototypical stem cell characteristics: the ability to self-renew through mitosis and the capacity to generate cells of all hematopoietic lineages (1). As HSC mature and differentiate into progeny cells, their self-renewal ability becomes limited, and their multipotency is lost through lineage commitment. The early events of hematopoietic differentiation have been described to occur within a subset of immature cells in the bone marrow identified by a shared expression pattern of surface markers: coexpression of stem cell-associated markers c-kit and Sca-1 and no or only low-level expression of the mature cell markers collectively known as Lineage (Lin) (2, 3). This subset of hematopoietic stem and progenitor cells is routinely termed the KLS (c-kitposLinnegSca-1pos) compartment.

Within the KLS compartment reside three distinct subpopulations that are considered to delineate early hematopoietic differentiation events. According to expression patterns of Flt3 and Thy1.1 surface markers, the three subpopulations are designated as Thy1.1posFlt3neg long-term HSC (LT-HSC), Thy1.1posFlt3pos short-term HSC (ST-HSC), and Thy1.1negFlt3pos multipotent progenitor (MPP) cells (4–6). Historically, Thy-1.1 expression levels have been termed Thy-1.1neg, Thy-1.1low, and Thy-1.1high to distinguish stem/progenitor cells (Thy-1.1neg and Thy-1.1low) from mature T cells (Thy-1.1high). To avoid confusion caused by this nomenclature, in this study we refer only to Thy-1.1neg and Thy-1.1pos cells, because it is not necessary to distinguish Thy-1.1low stem/progenitor cells from Thy-1.1high T cells. The LT-HSC subset includes the true HSC that initiates hematopoiesis. As LT-HSC differentiate, the Flt3 receptor is upregulated. Cells in the ST-HSC compartment are multipotent but possess a limited capacity for self-renewal, because transplantation studies showed the ST-HSC compartment to reconstitute the hematopoietic system of recipients only for ~6–12 wk (5, 6). Finally, the last stage within the KLS compartment is the MPP stage that has lost self-renewal capability, accompanied by the loss of Thy1.1, but maintains multipotency.

The functional heterogeneity within the MPP compartment, as defined by Flt3-expressing KLS cells, has been the focus of recent discussions (7–11), mainly triggered by a study describing the existence of lymphoid-primed MPP (LMP) (7). The study identified LMP in the HSC compartment as the population of cells that expresses the highest level of Flt3, constituting a significant fraction of MPP (approximately the top 25% of KLS cells for Flt3 expression). Unlike MPP cells, which have significant output in all hematopoietic lineages, LMP cells generated insignificant numbers of platelets and RBCs, suggesting the loss of erythro-megakaryocytic lineage (Meg/E) potential prior to cells exiting the HSC compartment and demonstrating the existence of oligopotent progenitors within the pool of true MPP. A subsequent study by another group showed that although LMP cells do have a detectable amount of Meg/E activity, it is significantly less than that of MPP, thereby contrasting the previous report’s claim of loss of Meg/E activity while confirming the existence of heterogeneity within the MPP population (9).

The MPP population has also been subfractionated using VCAM-1. In these studies, VCAM-1pos MPP generated cells of all lineages similar to traditional MPP cells, whereas VCAM-1neg MPP failed to generate Meg/E potentially as robustly as MPP cells

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or VCAM-1^pos MPP (10, 11). Consistent with the LMPP study, the investigators observed that VCAM-1^pos MPP cells expressed high levels of Flt3, whereas VCAM-1^pos MPP cells expressed both low and high levels of Flt3 (10). These observations suggested that Flt3 alone is insufficient to resolve committed subsets of MPP and that additional markers will be required to help identify functionally distinct subpopulations within MPP (8).

One issue with previous studies of the Meg/E potential of MPP is the prevalent use of the CD45 allelic system in transplant models since its introduction in 1988 (3). This model allows tracing of donor contributions to nucleated cell lineages by flow cytometry, a major advance over classical techniques that used electrophoresis to trace the origin of erythroid cells in transplant studies (12). More recent studies of Meg/E engraftment have used surrogate markers or progenitor cell assays to infer platelet and erythrocyte engraftment because CD45 is not expressed by these lineages (13). The use of GFP-transgenic mice allows lineage tracing of platelets (9); however, application of the GFP-transgenic model to erythroid chimerism has been problematic because of the failure of most GFP-transgenic mouse strains to express the transgene in the erythroid lineage (14). As a result, the contributions by the MPP subsets to persistent erythroid engraftment in comparison with HSC in a transplant setting remain to be determined.

Our laboratory previously reported that the CD62L adhesion molecule can be used to fractionate the Thy1.1^{pos} subset of KLS to identify a T cell-biased CD62L^{high} MPP and a CD62L^{neg/low} MPP with more generally distributed multipotency (15). These findings led us to hypothesize that CD62L is useful as an early marker of hematopoietic development. Our data demonstrated that, in a transplant setting, the CD62L^{neg/low} fraction of KLS contains highly enriched HSC, whereas the CD62L^{high} fraction contains MPP with a limited duration of output. We show that the CD62L^{neg/low} fraction contains HSC in both Sca-1^{high} and Sca-1^{low} subsets of KLS, with less HSC activity in the Sca-1^{low} subset, indicating a gradual output. We show that the CD62L^{neg/low} fraction of the now widely accepted Flt3^{neg}/Thy1.1^{pos} KLS population. Furthermore, we present evidence that CD62L and Sca-1 can be isolated to identify distinct subpopulations within the traditional MPP compartment, the Thy1.1^{pos}/Flt3^{pos} KLS population. Within this MPP compartment, the CD62L^{neg/low}/Sca-1^{high} KLS population contains the most primitive progenitor population, whereas the CD62L^{high}/Sca-1^{low} population contains the most mature progenitor population based on transplant studies that resolve trilineage engraftment. Fractionation of Meg/E potential from progenitors of nucleated lineages was achieved at the CD62L^{high}/Sca-1^{high} stage of development. These data indicated that CD62L is an effective marker for isolating functionally distinct MPP subpopulations, particularly in light of the restricted strain distribution of the Thy-1.1 allele (16) and the difficulty in confirming specificity of Flt3 staining because of the absence of sufficient numbers of Flt3^{pos} cells in normal mouse tissues.

Materials and Methods

Mice

Mice carrying homozygous Thy1^+ and Ly5^+ alleles on the C57BL background were generated and maintained in our animal facilities, as previously described (14). GFP-transgenic mice, generated by microinjection of C57BL/6 oocytes, were kindly provided by Dr. Masaru Okabe (Osaka University, Osaka, Japan) (17). These two strains were mated to generate C57BL mice with the GFP transgene on a Thy1^+ Ly5^+ background, which served as transplant donors in all experiments, except as shown in Fig. 3, where the donor strain had homozygous Thy1^+ and Ly5^+ alleles on the C57BL background but lacked the GFP transgene. Mice congenic for the diffuse allele of the hemoglobin-β (Hbb) chain on the B6 background (B6.Cg-Gpi1^Hbb^ H1^+D3H1) mice (18) were kindly provided by Dr. David Harrison (Jackson Laboratory, Bar Harbor, ME) and were used as transplant recipients. All mice were kept in the animal resources center at the University of Utah under institutional animal care and use committee-approved protocols.

Isolation of hematopoietic progenitor and stem cells

Bone marrow cells were harvested from young adult (6–12-wk-old) donor mice and incubated with a mixture of rat Abs to mature cell markers (CD2, CD3, CD5, CD8, CD11b, Ly-6G, TER119, B220, CD45R; RA3-6B2), and CD19 (1D3) were purified from the media of cultured hybridoma cell lines. PE-conjugated Sca-1 mAb was purchased from PharMingen (San Diego, CA). c-kit (SC11) mAb was purified and conjugated with Alexa Fluor 647 in our laboratory. CD4 and CD8 mAbs were purified and conjugated to allophycocyanin in our laboratory. Biotinylated Flt3, CD62L allophycocyanin-AF750, Thy1.1 PerCP-Cy5.5, Mac-1 PE, and Gr-1 PE Abs were purchased from eBioscience (San Diego, CA). Rat mAb 4A5, specific for mouse blood platelets (19), was kindly provided by Dr. S.A. Burstein (University of Oklahoma Health Sciences Center, Oklahoma City, OK) and was purified and conjugated to Alexa Fluor 647 in our laboratory.

Peripheral blood analysis

For posttransplant analysis, mice were anesthetized with isoflurane using the E-Z Anesthesia System (E-Z-Healthcare). Blood samples were collected into acid citrate dextrose anticoagulant solution from the retro-orbital sinus using heparinized capillary tubes. Immediately after the collection of blood samples, a volume of 10 μl blood/sample was added to diluted Alexa Fluor 647-conjugated 4A5 Ab, and an additional 10 μl sample was diluted in 100 μl M-2000 (1:10). The diluted samples were examined for 30 min at 20˚C. The RBC fraction was separated from the WBC fraction by Dextran T500 (Amersham Biosciences, Piscataway, NJ) in PBS and incubated at 37˚C for 30 min to separate the RBC and WBC fractions. WBC were stained with PE-conjugated Abs against Mac-1 and Gr-1 for myeloid WBC detection, biotinylated B220 or CD19 Ab with a subsequent labeling with Alexa Fluor 750-conjugated avidin for B cell detection, and CD4 and CD8 conjugated with allophycocyanin for T cell detection. Platelets and WBC were analyzed by a FACScan flow cytometer (BD Biosciences, San Jose, CA; modified by Cytek Development, Fremont, CA). Platelet analysis was performed by increasing forward and side scatter parameter gains until the platelet population, identified by 4A5 staining, could be gated to exclude debris. Donor WBC and platelets were identified by GFP fluorescence. Data are reported as the percentage donor cells of the indicated lineages or as calculated absolute cell numbers as indicated in the figure legends. Significance was determined using a one-tailed t test with equal variance (Excel; Microsoft, Bellevue, WA).

HPLC analysis of hemoglobin variants

An HPLC cation-exchange protocol was developed in our laboratory to discriminate and quantify Hbbα and Hbbβ in the peripheral blood samples (14). A stock solution of 100 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (Sigma-Aldrich, St. Louis, MO) was prepared by dissolving 100 mg DTNB in 2.5 ml DMSO, it was stored at −20˚C. The RBC fraction was derivatized by adding 5 μl the RBC fraction into 250 μl 40 mM NaCl and 2 mM DTNB and incubating at room temperature for 30 min. Following centrifugation at 12,000 × g for 2 min, the supernatant was ana-
alyzed using a VARIANT hemoglobin testing system (Bio-Rad Laboratories, Hercules, CA) with an optimized β-thalassemia short program.

**Repopulating unit calculation**

The repopulating unit (RU) calculation is a commonly-used method to quantify the frequency of repopulating cells in comparison with a known quantity, often competitor cells of whole bone marrow (12, 20). The following formula was used for RU calculation: donor RU = % donor cells × C/(100 − % donor cells), where C is the number of competing RU (12). One competing RU is assumed to be equivalent to 10⁵ whole bone marrow cells (21, 22).

**Results**

**CD62L- and Flt3-expression patterns in the KLS pool are analogous**

To test the hypothesis that CD62L is a useful marker of the early stages of hematopoietic differentiation, the KLS pool of early hematopoietic progenitors was analyzed for CD62L expression (Fig. 1). The expression pattern (Fig. 1A) revealed a bimodal distribution of CD62L among KLS cells, with one subset expressing an intensity of CD62L expression equal to or exceeding that seen on spleen cells (CD62L⁹ hereafter, representing 61 ± 2% of KLS cells) and a second subset ranging from negative to low (CD62L⁹/low hereafter, representing 39 ± 2% of KLS cells). Multiparameter flow cytometry comparing the distribution of CD62L with respect to Thy-1.1, which was shown to segregate Thy-1.1⁹ HSC from Thy-1.1⁹/low progenitor cells (16, 23), validated the distinction between the CD62L⁹/low and CD62L⁹/low subsets (Fig. 1B). Visual examination of the data indicated a clear discrimination of four distinct populations, analogous to what is seen using Thy-1.1 and Flt3 expression for the traditionally defined LT-HSC, ST-HSC, and MPP subsets (Fig. 1B). The fourth subset resolved by both marker sets as Thy-1.1⁹/low CD62L⁹/low or Thy-1.1⁹/low/Flt3⁹/low accounted for ~15% of KLS cells and was not evaluated further in these studies; however, we previously characterized the Thy-1.1⁹/low/CD62L⁹/low KLS subset as primarily a B lymphocyte progenitor population with some T lymphocyte and myeloid engraftment potential (15).

The CD62L⁹/low fraction of KLS contains the LT-HSC population

To investigate the presence of HSC in the CD62L⁹ high and CD62L⁹/low fractions in vivo, we performed a transplant experiment. GFP⁹/low/Hbb⁹/low mice were used as donors to allow for the tracking of platelets and WBC produced from the transplanted populations via flow cytometric analysis. RBC were tracked by the hemoglobin variant Hbb⁹ using HPLC analysis. For each transplant recipient, 1⁹ donor KLS cells were sorted according to CD62L expression alone (Fig. 1A), without selection for Thy-1.1 or Flt3 expression. The donor cells were transplanted into lethally irradiated Hbb¹⁺/id recipient mice along with 1⁹ recipient bone marrow cells serving as competitors.

The transplant data showed that all CD62L⁹/low cells reconstituted all three lineages of the hematopoietic system of the recipient mice strongly and persistently (Fig. 1C). In contrast, CD62L⁹ high cells failed to engraft persistently. Although cells of all three blood lineages were generated by CD62L⁹ high cells, the donor-derived cells diminished significantly during the weeks posttransplant. Donor-derived platelets were undetectable shortly after the transplant, whereas donor-derived RBC were not detected after week 9. WBC diminished to a very low level after initial engraftment but persisted throughout the entire observation period. The maximum number of RBC peaked prior to week 3 for CD62L⁹ high cells; however, CD62L⁹/low cells did not peak in RBC production until week 5 (Fig. 1C). Altogether, the data suggested that LT-HSC are restricted to the CD62L⁹/low population and are not present in the CD62L⁹ high population.

To confirm the usefulness of the CD62L⁹/low phenotype as a marker of LT-HSC, the bone marrow of the recipient mice was examined following the termination of the transplant experiment. Bone marrow samples harvested from recipients of CD62L⁹ high or CD62L⁹/low cells were Lin depleted and stained with c-kit and Sca-1 Abs to analyze the KLS compartment for GFP⁺ donor cells. Bone marrow cells of the CD62L⁹/low recipients showed a significant amount of GFP⁺ cells (54 ± 24% of KLS; range 35–89%), whereas in recipients of CD62L⁹ high cells only trace numbers of GFP⁺ cells were found (1.7 ± 0.6% of KLS; range, 1.1–2.4%; Fig. 2A). The GFP⁺ KLS cells were then isolated by FACS sorting and transplanted into another set of lethally irradiated hosts. Because

![FIGURE 1. CD62L expression pattern in early hematopoietic progenitors.](http://www.jimmunol.org/bj)
CD62Lhigh recipients generated only trace numbers of GFP+ cells in their KLS fractions, all GFP+ cells were pooled into one injection and given to one recipient. Five weeks later, peripheral blood samples were analyzed for donor-derived cells. Only mice receiving CD62Lneg/low donor-derived GFP+ KLS cells produced GFP+ progenies in the secondary transplant recipients (Fig. 2B). This indicated that the CD62Lhigh subset of KLS, which makes up <0.1% of the bone marrow, includes ~95% of the transient erythroid progenitor potential based on competition with 10^5 bone marrow cells. CD62Lhigh progenitors also peaked in RBC and WBC production earlier than CD62Lneg/low cells.

FIGURE 2. LT-HSC are confined to the CD62Lneg/low fraction of the KLS compartment. The transplant recipients shown in Fig. 1C were sacrificed 13 wk posttransplant, and their bone marrow cells were analyzed for the presence of donor-derived cells within the KLS population. A. Lin-depleted bone marrow prepared from each of the five transplant recipients in each group was evaluated for GFP+ cells within the KLS subset. One representative animal from each group is shown. Numbers represent mean ± SD values of each group. B. GFP+ KLS cells from each of five CD62Lneg/low transplant recipients were sorted and transplanted into one lethally irradiated secondary recipient. The trace numbers of GFP+ KLS cells in CD62Lhigh transplant recipients were pooled together and transplanted into one lethally irradiated secondary recipient. Five weeks posttransplant, peripheral blood analysis was performed. The average percentages of donor-derived cells are shown. UD, undetectable.

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The CD62Lhigh fraction of KLS contains the MPP population

In the primary transplant of the experiment shown in Fig. 2, we transplanted 10^5 bone marrow cells along with an equal number of 10^5 cells for both CD62Lneg/low and CD62Lhigh fractions. To evaluate the presence of LT-HSC, as defined by our ability to discriminate donor-derived cells in the peripheral blood of transplant recipients over time, we transplanted CD62Lhigh cells at a dose 10-fold greater than in the previous experiment (10^5 cells/recipient).

To evaluate the differential frequency of LT-HSC in the CD62Lneg/low fraction, these cells were transplanted at a limiting dose of 10^2 cells. As before, both populations were transplanted competitively with 10^5 bone marrow cells. Analysis of RBC engraftment in this experiment confirmed and extended our previous findings. Transplantation of 10^4 CD62Lhigh cells in competition with 10^5 bone marrow cells produced a wave of donor-derived RBC that diminished over time to a negligible percentage at week 16 (Fig. 3A). CD62Lhigh progenitors also peaked in RBC and WBC production earlier than CD62Lneg/low cells.

FIGURE 3. LT-HSC are enriched in the CD62Lneg/low fraction, and MPP are enriched in the CD62Lhigh fraction of KLS. A. The CD62Lneg/low fraction (10^5 cells) or the CD62Lhigh fraction (10^5 cells) of the KLS compartment were each transplanted into groups of five lethally irradiated recipient mice along with 10^5 normal bone marrow cells. Donor animals in this experiment lacked the GFP transgene, therefore only erythrocytes (RBC) and leukocytes (WBC) were tracked over time by peripheral blood sampling based on analysis of Hbb and CD45 allelic markers. B. Each CD62L fraction of the KLS compartment was transplanted into four lethally irradiated mice, at a dose of 500 cells/mouse, without added competitive bone marrow cells. Donor cells lacked the GFP transgene. All transplant recipient mice survived; however, only recipients of CD62Lneg/low cells showed persistent engraftment of both RBC and WBC lineages. Error bars indicate SEM.
of CD62L<sub>neg/low</sub> cells, whereas CD62L<sub>high</sub> cells provided only transient engraftment of erythroid and leukocyte lineages that was eclipsed by endogenous HSC activity by 16 wk posttransplant. Collectively, the data shown in Figs. 1–3 illustrate that CD62L expression levels can be a useful biomarker for separation of LT-HSC from MPP within the KLS bone marrow population.

The CD62L<sub>neg/low</sub> fraction of the KLS<sup>neg</sup> compartment includes most LT-HSC activity

Thy-1.1 and Flt3 have been characterized as markers for FACS sorting of LT-HSC. To evaluate the distribution of CD62L expression relative to LT-HSC potential among KLS cells in the context of Thy-1.1 and Flt3, we performed multiparameter flow cytometry to evaluate the Thy-1.1<sup>neg</sup>Flt3<sup>neg</sup> subset of KLS, previously shown to include LT-HSC, with respect to CD62L expression. Electronic gating on the Thy-1.1<sup>pos</sup>Flt3<sup>neg</sup> fraction of KLS (KLS<sup>neg</sup>) showed that the frequencies of the CD62L<sub>neg/low</sub> (78 ± 3%) and CD62L<sub>high</sub> (22 ± 3%) populations reversed with respect to that seen in the complete KLS population (Fig. 4A; compare with Fig. 1A). To evaluate LT-HSC activity, we transplanted 2 × 10<sup>3</sup> sorted cells (KLSF<sup>neg</sup>CD62L<sub>neg/low</sub> and KLSF<sup>neg</sup>CD62L<sub>high</sub>) into lethally irradiated recipients along with 10<sup>5</sup> normal bone marrow competitor cells.

Peripheral blood analysis for engraftment activity showed strong trilineage engraftment by the KLSF<sup>neg</sup>CD62L<sub>neg/low</sub> fraction, reconstituting ~90% of platelets, erythrocytes, and leukocytes, despite competition from a 50-fold excess of unseparated bone marrow cells.

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bers of platelets and erythrocytes compared with the other two populations, suggesting that they represent transitional populations between the less mature CD62L\textsuperscript{neg/low}Sca-1\textsuperscript{high} and the more mature CD62L\textsuperscript{high}Sca-1\textsuperscript{low} populations. The dramatic reduction of Meg/E potential observed as progenitors shifted from CD62L\textsuperscript{neg/low}Sca-1\textsuperscript{high} to CD62L\textsuperscript{high}Sca-1\textsuperscript{low} is consistent with the idea that the CD62L\textsuperscript{neg/low}Sca-1\textsuperscript{high} phenotype is uniquely associated with progenitor cells that retain Meg/E potential. This observation parallels other studies that also showed the reduction of Meg/E potential with the changing expression of other developmental Ags (Flt3 and VCAM-1) (7, 10).

The peripheral blood analysis of the transplant recipients also revealed differences in the numbers of WBC progenies generated from the subfractionated populations of the KLS MPP; however, these were only significant between subsets discriminated by Sca-1 expression levels and not by CD62L expression levels. Both Sca-1\textsuperscript{high} populations generated robust numbers of myeloid and B lymphocyte cells 2 wk after transplantation, with B lymphocytes persisting to a greater extent compared with myeloid lineage cells (Fig. 5C). A similar pattern was observed for T cell development, although donor-derived T cell numbers were low and not significant until 9 wk posttransplant (data not shown). At this time, the Sca-1\textsuperscript{high} subset generated $3.33 \pm 0.09 \times 10^5$ T cells/μL of peripheral blood, whereas the Sca-1\textsuperscript{low} subset generated $0.07 \pm 0.02 \times 10^5$ T cells/μL ($p = 0.007$). The observed difference in lineage potential seen in Fig. 5B and 5C suggests that the CD62L\textsuperscript{neg/low}Sca-1\textsuperscript{high} subset includes true MPP but that upregulation of CD62L expression is accompanied by a decrease in Meg/E potential and a maintenance of WBC potential. Subsequently, a further loss of progenitor cell potential is identified by downregulation of Sca-1 expression.

### Discussion

In this report, we characterized the usefulness of CD62L as a marker of hematopoietic differentiation. Although numerous markers for isolation of HSC subsets have been described, additional markers of differentiation add to our understanding of the complexity of the hematopoietic hierarchy. New advances in fluorescent probe and instrument technologies allow a deeper and more detailed view into the stages of development previously defined by a relatively small subset of surface Ags. The depth of our understanding of early hematopoietic development will depend on the availability and specificity of various markers. Additionally, the use of robust methods for distinction of transplanted donor cells from recipient cells is critical, because the inability to visualize engraftment in erythroid and platelet lineages using the CD45 allelic system has resulted in a general lack of good experimental evidence regarding the progenitor cells for these lineages. In vitro colony-forming assays have been used for this purpose, but these experiments are prone to artifacts and lack the in vivo relevance inherent in transplantation assays (24, 25).
The expression profile of Flt3 in conjunction with Thy1.1 has been useful in resolving the three early hematopoietic compartments (LT-HSC, ST-HSC, and MPP) that form the foundation of our current understanding of early hematopoietic events in adult mice (4–6). We have found that few commercially available anti-Flt3 reagents are suitable for high-quality resolution of LT-HSC from MPP, and the use of Flt3 is further complicated by the lack of suitable positive-staining control cell populations. As an additional marker, Thy1.1 is effective for the isolation of HSC within the KLS population but is limited to the few Thy1.1-expressing mouse strains because the more common Thy-1.2 allele is not expressed by most HSC (16). These issues emphasize the need for ongoing investigation of known markers, as well as exploration for new markers, to advance our ability to isolate HSC and to understand HSC biology. We presented in vivo evidence for CD62L as an alternative, as well as a useful supplemental Ag, for use with Flt3 to improve isolation of HSC subsets. Abs against CD62L are available in a wide variety of conjugates, and expression of CD62L in normal mouse spleen is robust (Fig. 1A). Our transplant data demonstrated that CD62L fractionation of the KLS population yields a CD62Lneg/low population containing LT-HSC and a CD62Lhigh population devoid of LT-HSC and containing MPP. Further fractionation of MPP, as defined by the phenotype KLS-Thy-1.1negFlt3neg, convincingly separates CD62Lneg/low from more restricted progenitor subsets lacking Meg/E potential that are CD62Lhigh. Differential temporal kinetics and persistence of engraftment are also consistent with the usefulness of CD62L expression as a marker of LT-HSC versus MPP and later stages of progenitor cells.

In this report, we expanded upon previous findings describing CD62L as an effective marker for dividing the lymphoid progenitor subset (KLS-Thy-1.1neg) of bone marrow into a CD62Lhigh fraction, which resembled an early T-lineage progenitor, and a CD62Lneg/low fraction, which resembled the traditional MPP (15). Bone marrow transplant results from previous studies were restricted to the characterization of WBC engraftment only, because the approach used to differentiate the donor-derived hematopoietic cells from the cells of the recipient mice was allele-specific Ab labeling of CD45. As a result, previous studies could not discriminate Meg/E potential in the two fractions of MPP, as we showed in this article. The data presented in this study demonstrated that fractionated subfractions of KLS MPP that are distinguishable by surface expression levels of CD62L and Sca-1 and are functionally distinct. Our in vivo transplant experiments demonstrated that the CD62Lneg/lowSca-1high KLS MPP population includes the most robust engraftment activity in all lineages, whereas the CD62Lhigh Sca-1low population exhibited significantly less production of all lineages. Interestingly, platelet and erythrocyte potential segregated from WBC potential at the CD62LhighSca-1low stage of development within the KLS MPP population. This finding is consistent with previous reports demonstrating that subpopulations within KLS MPP vary with respect to Meg/E activity.

Additional markers were reported to subfractionate the KLS compartment into distinct subpopulations. Several of the lineage markers that are typically used for depletion of mature hematopoietic cells have been applied in subfractionation strategies (26). Another set of markers, the Slam family proteins (CD150, CD244, and CD48), have also been reported to fractionate KLS into HSC and MPP (27, 28). A ligand for CD62L, the CD34 molecule, has also been used to isolate HSC and to fractionate KLS (29, 30). The coexpression patterns of these markers with CD62L is unknown. Future investigations into the relationship of CD62L expression with these other markers would be interesting and may yield new subpopulations that may represent acute transition stages, granting a better understanding of early hematopoietic events at higher resolution.

Collectively, our data demonstrated that CD62L, despite having an expression pattern that is similar to that of Flt3, is able to identify functionally distinct subpopulations of MPP that would be impossible to resolve using Flt3 alone. Furthermore, the data supported the model of heterogeneous KLS MPP that sheds Meg/E potential, marked by the upregulation of CD62L, prior to the lymphoid and myeloid lineage separation through the formation of lymphoid- and myeloid-restricted progenitor cells.

Disclosures
The authors have no financial conflicts of interest.

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