CD34 expression on long-term repopulating hematopoietic stem cells changes during developmental stages

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The CD34 antigen serves as an important marker for primitive hematopoietic cells in therapeutic transplantation of hematopoietic stem cells (HSC) and gene therapy, but it has remained an open question as to whether or not most HSC express CD34. Using a competitive long-term reconstitution assay, the results of this study confirm developmental changes in CD34 expression on murine HSC. In fetuses and neonates, CD34 was expressed on Lin−c-Kit+ long-term repopulating HSC of bone marrow (BM), liver, and spleen. However, CD34 expression on HSC decreased with aging, and in mice older than 10 weeks, HSC were most enriched in the Lin−c-Kit−CD34− marrow cell fraction. A second transplantation was performed from primary recipients who were transplanted with neonatal Lin−c-Kit+ CD34high HSC marrow. Although donor-type HSC resided in CD34-expressing cell fraction in BM cells of the first recipients 4 weeks after the first transplantation, the stem cell activity had shifted to Lin−c-Kit−CD34− cells after 16 weeks, indicating that adult Lin−c-Kit+CD34− HSC are the progeny of neonatal CD34-expressing HSC. Assays for colony-forming cells showed that hematopoietic progenitor cells, unlike HSC, continue to express CD34 throughout murine development. The present findings are important because the clinical application of HSC can be extended, in particular as related to CD34-enriched HSC and umbilical cord blood HSC. (Blood. 2001;97:419-425)

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decrease in CD34 expression on HSC occurs with a similar time course, even in older recipients engrafted with neonatal Lin−c-Kit+CD34+ HSC. The present findings have important implications for the clinical applications of HSC, in particular CD34-enriched HSC transplantation and umbilical cord blood transplantation.

**Materials and methods**

**Mice**

C57BL/6 Ly-5.1 mice were kindly provided by Dr Koichi Ikuta (Kyoto University, Kyoto, Japan) and C57BL/6 Ly-5.2 mice were obtained from Shizuku Laboratory Animal Center (Shizuoka, Japan). These mice were bred and maintained in a specific pathogen-free microisolator environment. For experiments on fetuses, one or two female mice were caged with a male for 2 hours late in the afternoon and then examined for vaginal plugs. The appearance of the vaginal plug was designated as day 0 of gestation. Mice within 24 hours from birth were used as neonatal mice. In the transplantation experiments, recipient mice were given neomycin (1.1 g/1000 mL) in the drinking water for the first month after irradiation and transplantation.

**Antibodies**

The antibodies used in immunofluorescence staining included 49E8 (anti-CD34, kindly provided by Dr Hirohumi Hamada, Cancer Chemotherapy Center, Tokyo, Japan), ACK45 (anti-c-Kit), A20 (anti-Ly-5.1), and 104 (anti-Ly-5.2). Lineage marker antibodies included RB6-8C5 (anti-Gr-1), M1/70 (anti-Mac-1), RA3-6B2 (anti-CD45R/B220), 30-H12 (anti-Thy-1.2), L3T4 (anti-CD4), 53-672 (anti-CD8a), and TR-119 (anti-TER-119). All the antibodies except 49E8 were purchased from Pharmingen (San Diego, CA). Antibodies for Mac-1 and Thy-1, markers for macrophage/monocyte lineage cells and T lymphocytes, respectively, were omitted from the cocktail of lineage marker antibodies, as their expression on HSC has been reported.22,25 All antibody incubations were carried out for 30 minutes on ice.

**Cell preparation**

The BM cells were flushed from femurs and tibiae of fetal, neonatal, and adult mice. Liver and spleen cells were obtained by rubbing tissue between 2 pieces of glass and repeated pipetting. Cell suspensions were then filtered through a sterile 40-μm Cell Strainer (No. 2340; Falcon, Lincoln Park, NJ), stained with biotinylated antilineage markers, and enriched for cells not expressing the lineage markers (Lin−), using streptavidin-conjugated magnetic beads (PerSeptive Biosystems, Framingham, MA). Lin− cells were then stained with fluorescein isothiocyanate (FITC)–anti-CD34 and phycoerythrin (PE)–anti-c-Kit, and sorting was performed on a FACSVantage (Becton Dickinson, Mountain View, CA).26,27 In second transplantation experiments, Lin− cells prepared by a method using immunomagnetic beads coated with sheep antirat IgG (Dynal AS, Oslo, Norway)28,29 were stained with biotinylated anti-Ly-5.2, followed by FITC–anti-CD34, PE–anti-c-Kit, and PE–cyanine 5–succinimidylester-streptavidin.

**Transplantation and analysis of recipients**

Varying numbers of sorted cells from Ly-5.2 mice were injected into sublethally irradiated Ly-5.1 mice together with 1 × 10^5 unfractionated BM cells from Ly-5.1 mice. In a preliminary experiment, we determined that 1 × 10^5 BM cells was the minimum dose of cells required for more than 95% recipient survival. Eight to 10 weeks after transplantation, peripheral blood (PB) was collected from the tail veins of the recipient mice. Red blood cells were removed, and the nucleated PB cells were stained with FITC–anti-Ly-5.2 and PE-antimyeloid cells (Mac-1 and Gr-1), anti-B lymphocytes (B220), or anti-T lymphocytes (Thy-1), and analyzed on a FACScan (Becton Dickinson). The mice in which donor-derived (Ly-5.2+) cells made up more than 1% of all B220+, Thy-1+, and Mac-1+/Gr-1+ cells in PB were scored as positive for successful reconstitution. Stable chimerism was maintained for over 6 months in all engrafted mice, although the reconstitution of T lymphocytes was slightly late compared with that of B lymphocytes or myeloid cells. The secondary transplants into Ly-5.1 mice were carried out using Ly-5.2 cells sorted from BM cells of the primary recipients (Ly-5.1 mice) 4 and 16 weeks after the first transplantation of Ly-5.2 mouse HSC.

**Assay for colony-forming cells**

Clonal cell culture was done in triplicate, as described.30,31 Briefly, 1 mL culture mixture containing 2.5 × 10^5 cells sorted from BM cells of Ly-5.2 mice at various developmental stages, α-modified Eagle medium (Flow Laboratories, Rockville, MD), 1.2% methylcellulose (Shinetsu Chemical, Tokyo, Japan), 30% fetal bovine serum (Hyclone Laboratories, Logan, UT), 1% deionized fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO), 10−4 M mercaptoethanol (Eastman Organic Chemicals, Rochester, NY), 100 ng/mL rat stem cell factor (SCF; Amgen, Thousand Oaks, CA) and human interleukin (IL)-6 (Tosho, Kanagawa, Japan), 20 ng/mL mouse IL-3 (Kirim Brewery, Tokyo, Japan) and human thrombopoietin (Tpo) (Kirim), 2 U/mL of human erythropoietin (Epo) (Kirim), and 10 ng/mL of human granulocyte colony-stimulating factor (G-CSF) (Kirim) was plated in each 35-mm suspension culture dish (No. 171099; Nunc, Naperville, IL), which was incubated at 37°C in a humidified atmosphere flushed with 5% CO2 in air. Colony types were determined on days 7 to 14 of incubation by in situ observation using an inverted microscope and according to the criteria described.30,32 Abbreviations for the colony types are as follows: GM, granulocyte and/or macrophage colonies; E, erythroid bursts; MK, megakaryocyte colonies; and Mix, mixed hematopoietic colonies.

**Results**

**CD34 expression on HSC in neonates**

Most stem cell activity resides in the Lin−c-Kit+ cell fraction in the murine fetus19,20 and adult,33 although a small population of c-Kit−dormant HSC in adult BM has been reported.10 Therefore, we first examined CD34 expression on Lin−c-Kit+ HSC in neonatal BM cells of Ly-5.2 mice. Figure 1A shows a flow cytometric analysis of c-Kit and CD34 expression on Lin− BM cells of a murine neonate. Although most of the Lin−c-Kit− cells (57.2%) did not express CD34, Lin−c-Kit+ cells (42.8%) revealed various levels of CD34 expression. The Lin−c-Kit+ cells were fractionated into 3 subsets on the basis of CD34 expression; CD34− (6.6%, the average of 4 mice), CD34low (58.3%), and CD34high (35.1%) (R1, 2, and 3, respectively, in Figure 1A). Cells from CD34−, CD34low, and CD34high fractions of Ly-5.2 mouse BM cells were injected into Ly-5.1 recipients together with 1 × 10^5 unfractionated BM cells of Ly-5.1 mice. Eight to 10 weeks later, PB of the recipients was analyzed for Ly-5.2−expressing Gr-1+/Mac-1+ myeloid cells, B220+ B lymphocytes, and Thy-1+ T lymphocytes. Figure 1B shows the results of transplantation experiments. All 8 mice transplanted with 1 × 10^5 Lin−c-Kit+CD34high cells and 2 of 4 mice transplanted with 1 × 10^2 Lin−c-Kit+CD34high cells had Ly-5.2+ myeloid and lymphoid cells in the PB. Figure 1C shows a representative PB profile of a mouse transplanted with 1 × 10^3 Lin−c-Kit+CD34high cells (R3), where 84.8% of Gr-1+/Mac-1+ cells, 98.4% of B220+ cells, and 41.3% of Thy-1+ cells were Ly-5.2+. Although the proportion of Ly-5.2+ cells depended on the number of cells injected into the recipient mice, all the mice had a higher proportion of Ly-5.2+ cells in B220− B lymphocytes than in other lineages.

In the transplantation of Lin−c-Kit+CD34low cells, 1 of 5, and 1 of 2 mice injected with 1 × 10^3, and 5 × 10^3 cells, respectively, had Ly-5.2+ myeloid and lymphoid cells. By contrast, all 6 mice
transplanted with $1 \times 10^5$ Lin$^{-}$c-Kit$^+$CD34$^-$ cells had no detectable Ly-5.2$^+$ PB leukocytes. Thus, long-term repopulating HSC were most enriched in the Lin$^{-}$c-Kit$^+$CD34$^{high}$ cell fraction in neonatal BM cells. Because CD34$^+$ cells accounted for only one fifteenth of the neonatal Lin$^{-}$c-Kit$^+$ BM cells, these results indicate that most neonatal BM HSC express CD34.

We then examined CD34 expression on HSC existing in neonatal liver and spleen. As shown in Figure 2, although the proportion of CD34$^{high}$ cells in Lin$^{-}$c-Kit$^+$ cells was smaller in neonatal liver or spleen than in BM, most long-term repopulating HSC were also included in the CD34$^+$ expressing cell fraction of neonatal liver and spleen cells, indicating that neonatal HSC express CD34, irrespective of the hematopoietic tissue in which they reside.

### CD34 expression on HSC in fetus

Next, CD34 expression on HSC in fetal liver at 14, 16 and 18 dpc was analyzed (Figure 2). Although the proportion of CD34$^{high}$ cells in Lin$^{-}$c-Kit$^+$ cells was larger in 14 and 16 dpc fetal than in neonatal livers, Lin$^{-}$c-Kit$^+$ cells of 18 dpc fetal liver had a distribution of CD34$^+$ expressing cells similar to that in neonatal liver (CD34$^{high}$ cells, 31.9%, 28.6%, 17.1%; CD34$^{low}$ cells, 53.9%, 52.8%, 72.1%; and CD34$^-$ cells, 14.2%, 18.6%, 10.8%, in 14, 16 and 18 dpc fetal livers, respectively). In transplantation of the 3 fractions from 14, 16, and 18 dpc fetal liver cells, all 7 mice transplanted with over $1 \times 10^5$ Lin$^{-}$c-Kit$^+$CD34$^{high}$ cells had Ly-5.2$^+$ myeloid and lymphoid cells at levels that depended on the number of cells injected. In mice injected with Lin$^{-}$c-Kit$^+$CD34$^{low}$ cells, only one recipient receiving $2 \times 10^4$ cells from 18 dpc fetal liver showed engraftment of Ly-5.2$^+$ cells, and 6 with less than $1 \times 10^4$ cells did not. No mouse had Ly-5.2$^+$ PB leukocytes among the 8 mice transplanted with $1 \times 10^2$ to $2 \times 10^4$ Lin$^{-}$c-Kit$^+$CD34$^-$ cells. We also carried out the transplantation using 18 dpc BM cells, in which CD34 expression in Lin$^{-}$c-Kit$^+$ cells revealed a similar distribution to that of neonatal BM cells (CD34$^{high}$ cells, 49.4%; CD34$^{low}$ cells, 42.6%; and CD34$^-$ cells, 8.0%). In the mouse injected with $3 \times 10^5$ Lin$^{-}$c-Kit$^+$CD34$^{high}$ BM cells, 60% of myeloid cells, 84% of B lymphocytes, and 37% of T lymphocytes expressed Ly-5.2. The mouse injected with $3 \times 10^5$ Lin$^{-}$c-Kit$^+$CD34$^{low}$ or Lin$^{-}$c-Kit$^+$CD34$^-$ cells had no Ly-5.2$^+$ PB leukocytes. These results indicate that most stem cell activity in the fetus at late gestational stages, as well as in neonates, resides in the CD34$^+$ expressing cell fraction.

### Change of CD34 expression on HSC with aging

Competitive long-term reconstitution assays were done using BM cells sorted from mice of various ages, the objective being to examine developmental changes in CD34 expression on HSC.

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**Figure 1.** Flow cytometric analysis and transplantation results. (A) Expression of c-Kit and CD34 on Lin$^{-}$ BM cells of a neonatal mouse (Ly-5.2). Lin$^{-}$c-Kit$^+$ cells were fractionated into CD34$^-$ (R1), CD34$^{low}$ (R2), and CD34$^{high}$ (R3) cells on the basis of CD34 expression. The averages of the proportions of the 3 fractions in Lin$^{-}$c-Kit$^+$ cells (n = 4) are shown in each window. (B) The percentages of Ly-5.2$^-$ cells in B220$^-$ cells (B), Thy-1$^-$ cells (T), and Gr-1/Mac-1$^-$ cells (M) in Ly-5.1 recipient PB 8 to 10 weeks after transplantation. The recipients were transplanted with CD34$^-$ (R1), CD34$^{low}$ (R2), and CD34$^{high}$ (R3) cells ($1 \times 10^6$ in B220$^-$, $1 \times 10^4$ in each of the other windows) in BM of neonatal mice (Ly-5.2). (C) A representative PB profile of a recipient mouse (Ly-5.1) engrafted with $1 \times 10^5$ Lin$^{-}$c-Kit$^-$CD34$^{high}$ cells (R3) sorted from Ly-5.2 BM. Ly-5.2$^-$ cells were present in all of B220$^-$ cells, Thy-1$^-$ cells, and Gr-1/Mac-1$^-$ cells in the PB.

**Figure 2.** CD34 expression on HSC in fetal and neonatal liver and spleen. Expression of c-Kit and CD34 on Lin$^{-}$ BM cells of neonatal liver, neonatal spleen, and fetal liver cells (16 dpc) of Ly-5.2 mice, and the percentages of Ly-5.2$^-$ cells in B220$^-$ cells (B), Thy-1$^-$ cells (T), and Gr-1/Mac-1$^-$ cells (M) in Ly-5.1 recipient PB 8 to 10 weeks after transplantation. The recipients were transplanted with CD34$^-$ (R1), CD34$^{low}$ (R2), and CD34$^{high}$ (R3) cells ($1 \times 10^5$ in B220$^-$, $1 \times 10^4$ in each of the other windows) in Ly-5.2 neonatal liver, neonatal spleen, and fetal liver cells (14-18 dpc). The averages of the proportions of the 3 fractions in Lin$^{-}$c-Kit$^+$ cells of neonatal spleen, neonatal liver, and fetal liver cells (n = 2, 2, and 4) are shown in each window in the flow cytometry graphs.
CD34 and c-Kit expression in BM cells of 1-, 4-, 8-, and 16-week-old mice are shown in Figure 3. Although the proportion of CD34<sup>−</sup> cells in BM Lin<sup>−</sup> c-Kit<sup>−</sup> cells showed no remarkable changes during aging, CD34<sub>low</sub> cells decreased and CD34<sub>high</sub> cells increased in BM of mice over 4 weeks of age. When transplanted with 1 × 10<sup>5</sup> Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>high</sub>, CD34<sub>low</sub>, and CD34<sup>−</sup> cells obtained from 1-week-old mouse BM cells, 8 of 8, 5 of 8, and 0 of 8 mice, respectively, showed successful engraftment, indicating that HSC were most enriched in Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>high</sub> fraction. The recipients transplanted with Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>high</sub> and Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>low</sub> cells sorted from 4-week-old mouse BM cells revealed a similar engraftment rate. Transplantation using 8-week-old mouse BM cells showed that, although most HSC still expressed CD34, they were more enriched in Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>low</sub> cells than in Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>high</sub> cells. In contrast to results with BM cells obtained from mice younger than 8 weeks, HSC were found in Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sup>−</sup> in addition to Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>low</sub> cell fractions in BM from 10- to 16-week-old mice. When 1 × 10<sup>5</sup> Ly-5.2+Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>high</sub> cells in 10- to 16-week-old mouse BM, which was the largest population in Lin<sup>−</sup> c-Kit<sup>−</sup> cells, were transplanted into 4 recipients, none was successfully engrafted with Ly-5.2<sup>+</sup> cells. By contrast, only 1 to 5 × 10<sup>2</sup> Ly-5.2<sup>+</sup>Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sup>−</sup> cells could repopulate in 5 of 8 recipients. Because the number of Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sup>−</sup> cells was only one twentieth that of Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>high</sub> cells in 10- to 16-week-old mouse BM, the result indicates that most hematopoietic repopulating ability is present in Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sup>−</sup> cell fraction in 10- to 16-week-old mice. Thus, CD34 expression on murine HSC decreases with aging.

Second transplantation of donor-derived HSC from the primary recipients

To confirm that adult Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sup>−</sup> HSC are the progeny of neonatal CD34-expressing HSC, we carried out a second transplantation of Ly-5.2<sup>−</sup> cells sorted from BM of Ly-5.1 primary recipients who were transplanted with 1 × 10<sup>5</sup> BM Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>high</sub> cells from Ly-5.2 neonates. CD34 expression on Ly-5.2<sup>−</sup>Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sup>−</sup> BM cells of the primary recipients 4 and 16 weeks after the first transplantation of Lin<sup>−</sup> c-Kit<sup>−</sup>CD34<sub>high</sub> cells from Ly-5.2 neonatal BM was similar to that for 4- and 16-week-old mice, respectively (Figures 4A and 5A, and compare Figure 3). We then sorted...
Ly-5.2\(^+\)Lin\(^-\)c-Kit\(^+\)CD34\(^{high}\), CD34\(^{low}\), and CD34\(^-\) cells from the primary recipients and transplanted them into Ly-5.1 secondary recipients. As shown in Figure 4B, most of the stem cell activity of Ly-5.2\(^+\) cells resided in the CD34-expressing cell fraction in BM cells of the primary recipients 4 weeks after the first transplantation. Figure 4C shows a representative PB profile of a mouse transplanted with 2 \(\times 10^3\) Ly-5.2\(^+\)Lin\(^-\)c-Kit\(^+\)CD34\(^{high}\) cells (R3) from a first recipient. As shown in Figure 5B, however, the stem cell activity was found in the Lin\(^-\)c-Kit\(^+\)CD34\(^{+}\) cell fraction in BM cells of the primary recipient 16 weeks after transplantation. A representative PB profile of a mouse transplanted with 1 \(\times 10^5\) Lin\(^-\)c-Kit\(^+\)CD34\(^{+}\) cells (R3) sorted from the first recipient. Ly-5.2\(^+\) cells were present in B220\(^+\) cells, Thy-1\(^+\) cells, and Gr-1/Mac-1\(^+\) cells in the PB.

**CD34 expression on colony-forming cells**

Finally, we examined CD34 expression on murine HPC, using a methylcellulose clonal culture assay. Lin\(^-\)c-Kit\(^+\)CD34\(^{high}\), CD34\(^{low}\), and CD34\(^-\) cells (2.5 \(\times 10^5\) cells) sorted from BM cells of mice at various developmental stages (18 dpc to 15 weeks old) were cultured in the presence of SCF, IL-3, IL-6, G-CSF, Epo, and Tpo. As shown in Table 1, Lin\(^-\)c-Kit\(^+\)CD34\(^{high}\) cells produced the largest number of hematopoietic colonies, whereas no colonies were generated from Lin\(^-\)c-Kit\(^+\)CD34\(^-\) cells at any developmental stage, indicating that HPC, unlike HSC, continue to express CD34 throughout development.

**Discussion**

Despite the clinical importance of CD34 antigen as a marker for primitive hematopoietic cells for HSC transplantation or gene therapy, it has been controversial whether or not all HSC express CD34 in mice or humans. We here demonstrated developmental changes in CD34 expression on murine HSC. In fetal, neonatal, and younger adult hematopoietic tissues, most of Lin\(^-\)c-Kit\(^+\) HSC expressed CD34. However, the CD34 expression on HSC decreased with aging, and HSC were relatively enriched in the Lin\(^-\)c-Kit\(^+\)CD34\(^-\) cell fraction in mice over 10 weeks of age. The evidence for a decrease of CD34 expression on HSC during development was strengthened by the second transplantation experiments showing that donor-type HSC were present in the CD34\(^-\) cell fraction in recipients engrafted with neonatal Lin\(^-\)c-Kit\(^+\)CD34\(^{high}\) HSC 16 weeks after the first transplantation. This developmental change in CD34 expression on murine HSC may explain the contradictory data in previous reports regarding CD34 expression. Although Morel and coworkers\(^8\) noted the presence of CD34 antigen on HSC in 4- to 6-week-old mice, Osawa and colleagues\(^9\) showed, using older mice, that Lin\(^-\)c-Kit\(^+\)Sca-1\(^+\)CD34\(^{low}\) cells rather than Lin\(^-\)c-Kit\(^+\)Sca-1\(^+\)CD34\(^{high}\) cells possessed stem cell activity. There remains a possibility that CD34 varies among different strains of mice, since we, as well as Morel and associates and Osawa and colleagues, used C57BL/6 mice.

The function of CD34 in hematopoiesis has been elusive,
although potential adhesive functions of CD34 have been reported.\textsuperscript{24,25} Recently, 2 groups of investigators reported on hematopoiesis in CD34-deficient mice.\textsuperscript{36,37} One group noted a decreased number of HPC in 10.5 dpc yolk sac, 14.5 dpc fetal liver, adult BM, spleen, and PB, and a poor response of adult HPC to cytokines, which suggested the involvement of CD34 in fetal and adult hematopoiesis.\textsuperscript{36} The present observation that HPC, unlike HSC, expressed CD34 throughout murine development from fetus to adult is consistent with their results. However, neither group discussed the biologic activity of long-term repopulating HSC of CD34-deficient mice. Therefore, the function of CD34 on HSC still remains unclear. An analysis of differences in biologic activities between neonatal Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{high}\) HSC and adult Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{-}\) HSC should be instructive to define the function of CD34 on HSC.

We found a difference in differentiation potential between neonatal Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{high}\) cells and adult Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{-}\) cells. Mice transplanted with neonatal Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{high}\) cells showed a predominant reconstitution of B lymphocytes, compared to adult Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{-}\) cells. The predominant reconstitution of B lymphocytes was also found in engraftment of Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{high}\) cells from fetal liver. A similar observation was made in the mice reconstituted by fetal liver Thy-1\(^{low}\)-Sca-1\(^{-}\)-Lin\(^{-}\)-Mac-1\(^{-}\)-CD4\(^{-}\)-c-Kit\(^{-}\) HSC.\textsuperscript{38} Therefore, the predominant reconstitution of B lymphocytes may result from a strong B-lymphoid potential of HSC from fetal, neonatal, and younger adult cells.

We found no difference in proliferation potentials between neonatal Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{high}\) HSC and adult Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{-}\) HSC. Although our experiments were not designed to evaluate the proliferative potential of single HSC, stable chimera was maintained for over 6 months in all recipients engrafted with the respective HSC phenotypes. Morrison and coworkers\textsuperscript{39} noted indistinguishable proliferation activities of single HSC between young and old mice, yet it was also reported that the proliferation potential of HSC isolated from fetal liver is higher than that from adult BM.\textsuperscript{24,39} Further studies are needed to clarify the difference in proliferation potentials between neonatal Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{high}\) HSC and adult Lin\(^{-}\)-c-Kit\(^{+}\)CD34\(^{-}\) HSC.

Aside from the evidence for developmental changes in CD34 expression on HSC, the present study has important implications for the clinical application of HSC. Because CD34-enrichment procedures are used to prevent graft-versus-host disease or for the purging of tumor/leukemic cells in therapeutic HSC transplantation, and are also used as a target cell population for gene therapy, it is an extremely crucial issue whether human HSC express CD34. Based on the present findings, we consider that more attention should be directed to age of the donor in discussing this issue. In addition, the present findings also suggest that fetal and neonatal HSC have characteristics different from those of adult HSC. Accordingly, more detailed characterization of umbilical cord blood HSC may contribute to further development of cord blood transplantation, which is now increasingly used as an alternative to BM transplantation.

References


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